Air Toxics Hot Spots

Risk Assessment Guidelines

Technical Support Document

For the Derivation of

Noncancer Reference Exposure Levels

Air Toxicology and Epidemiology Branch

Office of Environmental Health Hazard Assessment

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Executive Summary

This document describes the methodology used in developing acute, 8-hour and chronic health protective levels (Reference Exposure Levels or RELs) for use in California's Air Toxics Hot Spots and Toxic Air Contaminants programs. The basic methodology for REL development used previously by the Office of Environmental Health Hazard Assessment (OEHHA) and other agencies undertaking public health risk assessment remains unchanged. This consists of identification of a point of departure, such as a level identified in an animal experiment or an epidemiological study at which no adverse effects (or at least minimal adverse effects) are observed, or a benchmark dose (a statistical estimate of a threshold in the dose response relationship for the chemical of concern). Extrapolation from this point of departure to a health protective level for the target human population is by means of explicit models where possible, but more often by means of uncertainty factors.

This document addresses the SB 25 mandate to ensure OEHHA's risk assessment methods adequately protect infants and children, and incorporates scientific advances since the methodologies for acute and chronic RELs were first developed. Methods are added for developing 8-hr RELs in addition to the traditional acute REL for infrequent 1-hr exposures, and the chronic REL for long-term, continuous exposures. We harmonize the methodologies for acute, eight-hour and chronic RELs to the extent possible. This document also defines special procedures for derivation of RELs based on certain toxicological endpoints such as trigeminal mediated irritancy. The following notes summarize the specific changes in methodology relative to the previous version of the Hot Spots guidelines for evaluation of noncancer health effects.

Use of Specific Models Rather than Uncertainty Factors when Possible

There is considerable inadequacy in the data available for evaluating noncancer health impacts of chemicals and setting appropriate RELs. The standard approach has been to use uncertainty factors (UFs) to help ensure that public health is protected. In recent years such techniques as the benchmark dose method (BMD) and physiologically based pharmacokinetic (PBPK) modeling have taken advantage of available data to quantitatively address uncertainties in the standard approach to noncancer REL derivation, namely dividing an animal no-observed-adverse-effect-level (NOAEL) by uncertainty factors (UFs). OEHHA recommends the use of these techniques wherever possible in order to address quantitatively the adequacy of acute and chronic RELs to protect the health of both children and adults. However, in some cases the available dose-response data are not suitable for application of the benchmark dose approach. Furthermore, it must be noted that data are not available for many chemicals to use PBPK modeling. Thus, while PBPK is a useful tool, the traditional paradigm (e.g., NOAEL or BMD divided by UFs) will still be most frequently used.

Value of Default Intraspecies Uncertainty Factor (UF_H)

The uncertainty factor used to account for intraspecies (inter-individual) variability in the human population (UF_H) has previously been assigned a default value of 10. Inter-individual variability has been modeled indicating a distribution that ranges from 1 to 720 for a specified set of chemicals. The value of 10 represented the 85th percentile in this analysis. Investigators have

proposed subdividing the intraspecies uncertainty factor into toxicokinetic (UF_{H-k}) and toxicodynamic (UF_{H-d}) subfactors; an initial analysis was consistent with subdivision of the 10-fold factor into two equal subfactors, both thus having a default value of the square root of 10 ($\sqrt{10}$) which is equal to 3.16. However, it appears that a default toxicokinetic value of $\sqrt{10}$ may not be adequate for all chemicals, routes of elimination, or for the entire population, in particular the subpopulation of infants and neonates. A toxicokinetic subfactor of 10 is therefore recommended to protect infants and neonates, unless data are available to indicate that this subpopulation is not at higher risk due to differences in toxicokinetics. There may also be cases where a toxicokinetic subfactor larger than 10 is warranted based on chemical-specific factors. Currently, there are scant data available to indicate whether or not the toxicodynamic subfactor of $\sqrt{10}$ adequately protects infants and children. It is known that the developing organism can be many-fold more sensitive to toxicants such as lead (Pb), mercury (Hg), and diethylstilbestrol (DES) than a mature organism. Differentiating the contribution of toxicokinetic (TK) and toxicodynamic (TD) differences is difficult. Where data indicate, a larger toxicodynamic uncertainty factor (UF_{H-d}) than the traditional value of $\sqrt{10}$ may be used.

Additional Three-fold Database Deficiency Factor

The United States Environmental Protection Agency (U.S. EPA) applies a general UF to Reference Concentrations (RfCs) and Doses (RfDs) for chemicals lacking adequate toxicological studies. Although this was not used in the previous version of the Hot Spots guidance, OEHHA now recommends an additional three-fold UF to chemicals with substantial toxicological data gaps, including, but not limited to, developmental toxicity. In some cases, it may be appropriate to apply a database deficiency factor larger than three-fold. This partially addresses the mandate under SB 25 to ensure that acute, chronic and eight-hour RELs are protective of infants' and children's health. The need for the additional database deficiency UF will be evaluated on a chemical-by-chemical basis.

One particular concern, based on SB 25, is to account for potential effects on developing organisms, particularly for chemicals lacking adequate developmental toxicity assessment. Potential "warning signs" or triggers that may indicate the need for the use of an UF to account for developmental toxicity include: neurotoxicity and neurobehavioral deficits, effects on the thyroid, mutagenicity, carcinogenicity, immunotoxicity, structure activity relationship models, endocrine agonist or antagonist activity, changes in cell proliferation, alteration of signal transduction and/or gene expression, or disruptions in maternal homeostasis. In addition, it is important to recognize impacts on the respiratory system by compounds that induce or exacerbate asthma (e.g., ozone), as children are more impacted by asthma than adults, and allergic asthma may be viewed as resulting from impacts on the developing immune and respiratory system.

Available data on mechanisms of toxicity, reactivity, potential for systemic distribution, and structure activity relationships (e.g., with known developmental toxicants), will be considered in the decision to apply the additional database deficiency UF. In addition, where evidence of effects at lower levels exists but available data are inadequate to use for a quantitative dose-response assessment, consideration will be given to applying a database deficiency UF. The product of all UFs applied usually will not exceed $\sqrt{10} \times 10^3$ (or 3000 after rounding to one

significant figure). However, there may be exceptional cases where a larger UF would be justified.

Use of Modified Haber's Law for duration adjustments

Previously, OEHHA adjusted the lowest observed adverse effect level (LOAEL) or no observed adverse effect level (NOAEL) observed in a study by means of the modified Haber's Law procedure if the exposure duration of the study was different from the exposure duration of concern for an acute (1-hour) REL. OEHHA continues to recommend this adjustment, where appropriate, in developing acute RELs (and eight-hour RELs based on acute studies) for systemic toxicity and other endpoints where cumulation over time is anticipated. The current recommendation is to use a default value of 3 for the concentration exponent (*n*), rather than 2 as before, in the absence of compound-specific information.

The most sensitive health endpoint for a number of acute RELs and some chronic RELs is trigeminal mediated irritancy of the eyes, nose or upper respiratory system. Trigeminal mediated irritancy is a receptor-mediated mechanism, sometimes referred to as the common "chemical sense." It appears that Haber's Law does not apply to trigeminal irritancy effects, so OEHHA will not use Haber's Law adjustments for instances in which a trigeminal mechanism for eye, nasal and respiratory irritancy can be determined for the chemical and concentration of concern.

U.S. EPA Human Equivalent Concentrations (HEC) Procedure and Interspecies Extrapolation

OEHHA's previous guidelines endorsed the use of the U.S. EPA Human Equivalent Concentration (HEC) procedure for chronic RELs. This procedure adjusts the internal dose in an animal inhalation study to the human equivalent dose taking into account, for example, differences in breathing rates, surface area of the respiratory tract and deposition. The U.S. EPA HEC procedure has been used in the past instead of the toxicokinetic component of the interspecies uncertainty factor, resulting in a total UF_A of $\sqrt{10}$. However, the HEC procedure only adjusts the exposure and does not account for other aspects of kinetics such as absorption, distribution, metabolism and excretion. OEHHA will continue to use the U.S. EPA HEC procedure when appropriate, but will retain a toxicokinetic factor of 2 as part of the overall interspecies uncertainty factor (i.e., a total UF_A of 6.32) when it is used, to reflect the greater uncertainty involved in this procedure than in full compound- and species-specific pharmacokinetic models.

Physiologically Based Pharmacokinetic Modeling (PBPK)

PBPK modeling provides a scientific methodology for assessing interspecies differences and intraspecies variability. The PBPK approach requires chemical specific information or estimation techniques for parameters such as blood:air partition coefficients. The PBPK approach is potentially more accurate than other approaches such as the U.S. EPA's HEC procedure because it takes into account more parameters and uses chemical specific information. However, it requires much more data and therefore cannot be applied in all cases. The PBPK approach involves greater uncertainty when required parameters are estimated rather than measured. Validation of PBPK models with independent data is required for them to be used

with confidence. OEHHA will use this methodology when possible, instead of the default application of the pharmacokinetic portions of the intraspecies and interspecies uncertainty factors and in preference to the HEC procedure. This change will apply to all three types of Reference Exposure Levels.

PBPK analysis has been used to determine the equivalent tissue dose that an adult human would receive at the air concentration used in an animal study. PBPK analysis can also be used to determine the tissue dose in children. It can replace the $\sqrt{10}$ (= 3.16) value for the pharmacokinetic interspecies uncertainty factor and possibly part or all of the 10-fold pharmacokinetic intraspecies uncertainty factor, if the necessary physiological parameters and physiochemical characteristics are available for the population type, age group and chemical in question. The acute, chronic, or eight-hour REL will be based on the most sensitive age group (or other specifically modeled individuals).

Exposure Duration Adjustments for Developmental Toxicity Data

Developmental toxicity presents difficulties as a critical endpoint for REL development. Developmental toxicants can act during narrow temporal windows of fetal and postnatal development, often with multiple and/or poorly understood mechanisms. These chemicals can cause very specific anatomical lesions, more general impacts such as growth retardation, or functional deficits (e.g., behavioral changes). The tissue dose during the window of vulnerability may be the critical factor rather than the total dose, or vice versa. Developmental toxicants that bind to hormone receptors, for example, may only need to be present at a tissue dose threshold concentration during a brief window of vulnerability in order to exert a profound developmental effect. The developmental studies available to determine an acute, eight-hour or chronic REL may provide little clue as to the mechanism, window of temporal vulnerability, or relative importance of total dose versus peak tissue concentration as a determinant of toxicity.

In order to ensure that RELs based on developmental endpoints are protective, OEHHA will not use Haber's Law to adjust the NOAEL or LOAEL in an animal developmental study where the exposure duration of the study and REL are different. Instead, since the actual timing and duration of the sensitive period are unknown, the NOAEL (with appropriate uncertainty factors) is regarded as an indication of a level on which to base the REL. This should help ensure that the REL will be protective if the tissue or air concentration during a particular temporal window is more of a determinant of developmental toxicity than total dose.

Eight-hour Reference Exposure Levels

OEHHA has developed a methodology for a new class of Reference Exposure Levels for eight-hour exposure. This new type of REL is needed for the Hot Spots Program in order to refine the risk assessment approach for the large number of facilities that emit chemicals for 8 hours per day, 5 days per week and to utilize the advanced features in air dispersion modeling capabilities. The air dispersion modeling in the Hot Spots Program has traditionally modeled such emissions as if they were uniformly emitted over 24 hours a day, 7 days a week, 365 days a year. Advances in computer capabilities have made it feasible to model more accurately the ground level concentrations of these emission scenarios by using meteorology obtained during the time when the facilities are actually operating (generally daytime). The majority of the highly

populated areas in California have significant diurnal-nocturnal meteorological differences that can affect the magnitude of the modeled risk and location of receptors.

The chronic noncancer health impacts of offsite workers have been traditionally assessed with the 24-hour chronic RELs. Because offsite workers generally work 8 hours not 24, the eight-hour RELs will ensure a more accurate assessment of the health impacts of their exposures. The eight-hour RELs will also be useful for assessing the health impacts of exposure of children in schools who can be exposed for up to 8 hours. The eight-hour RELs should be protective against repeated eight-hour exposures, so the pharmacokinetics will need to be carefully considered when setting the standards. The accumulation of the chemical or cumulative injury from repeated daily exposures will need to be considered if either the total dose or the area under the curve is the determinant of toxicity (reflecting a possible role of cumulative injury). Therefore, some, but not all eight-hour RELs will have the same basis as the corresponding chronic REL.

Children's Health in Relation to Chronic and Eight-hour RELS

Children and infants show biochemical, physiological and behavioral differences from adults, which can result in higher tissue doses for some chemicals. The significance of the higher tissue doses can vary considerably, particularly for infants, depending on the mechanism of toxicity. This becomes especially important when developing RELs for eight-hour repeated or chronic continuous exposures. The chronic and eight-hour RELs are intended to be "safe levels" of lifetime or repeated intermittent eight-hour exposure to a particular chemical. The dose received by inhalation or by non-inhalation routes can be greater for infants and children at the same air concentration or contaminated media concentration than it would be for an adult. Similarly, the internal dose and the toxicological consequences of an internal dose may be different for infants and children than it is for adults. For some chemicals it may be appropriate to use infant or child parameters for PBPK modeling, when PBPK modeling can be used for a chronic or eight-hour REL. For other chemicals, it may be more appropriate to take into consideration the greater exposure of children as part of the total lifetime daily dose, and adult parameters for PBPK modeling may be the most appropriate.

Chronic Oral Reference Exposure Levels (RELs) and Children's Health

Some chemicals in the Air Toxics Hot Spots Program are evaluated for multi-pathway exposures; in particular, non-volatile chemicals that deposit on surfaces such as soil or food crops, and/or bioaccumulate in breast milk. Oral RELs are used in risk assessment to address these pathways. Oral RELs are expressed in terms of a "safe" dose in mg/kg body weight (BW)-day. Thus, the higher doses in mg/kg that children receive from oral or dermal pathway exposure, relative to adults, can be directly assessed, when appropriate, by using the appropriate exposure variables for children (e.g., soil ingestion rates). There are some chemicals for which cumulative lifetime dose is an important determinant of toxicity (e.g., cadmium). The oral dose for these chemicals can be determined using lifetime average daily consumptions rates, for example for homegrown produce. These lifetime average daily consumption rates are simulated over 70 years and incorporate the greater consumption rates and lower body weights for the period that a person is a child. The major determinant of toxicity for other chemicals may be tissue dose or cumulative dose during childhood or a portion of childhood.

In addition to the higher doses in mg/kg BW-day that children receive, the pharmacokinetic and pharmacodynamic differences between children and adults that have been previously discussed may make children more or less vulnerable to chemical exposure. Whenever possible, OEHHA will assess these differences using PBPK modeling. As in the case of the inhalation RELs, an intraspecies UF of 30 ($\sqrt{10} \times 10$) instead of 10 may be considered in some cases to protect children's health when insufficient data exist for PBPK modeling. Likewise, an additional UF may be considered where there are substantial data gaps, e.g. the absence of developmental data.

1 Introduction

Hazardous substances are routinely released into the environment as a result of predictable continuous, intermittent or short-term emissions from facilities and predictable process upsets or leaks. As a result, the public living or working in communities surrounding industrial facilities is at risk of being exposed to airborne toxicants. Local air pollution control officers, industrial facility operators, and others have a need for clear guidance regarding the acute and chronic health effects of hazardous substances emitted into the air.

Under the Air Toxics Hot Spots Act of 1987 (Senate Bill 1731, Statutes of 1992), the Office of Environmental Health Hazard Assessment (OEHHA) was required to develop risk assessment guidelines for stationary sources of airborne toxicants. In an initial response to this mandate, OEHHA followed the recommendations of a then current National Academy of Sciences/ National Research Council review (NRC, 1994) of risk assessment practices by establishing uniform, science-based guidelines to be used in the derivation of acute and chronic Reference Exposure Levels (RELs) applicable to the general public exposed routinely to hazardous substances released into the environment. The products of this original guidelines development process were presented in the previous versions of the Technical Support Documents (TSDs) comprising the Air Toxics Hot Spots Risk Assessment Guidelines (OEHHA, 1999a; 2000a). Application of these RELs in risk assessments is described in the Air Toxics Hot Spots Risk Assessment Guidance Manual (OEHHA, 2003).

This document builds on that earlier effort by updating the methodology for developing RELs for noncancer health endpoints and incorporating additional information that has since become available. In particular, it will address the mandate of the Children's Environmental Health Protection Act (Senate Bill 25, Escutia; Chapter 731, Statutes of 1999; "SB 25") to consider children specifically in evaluating the health effects of Toxic Air Contaminants (TACs). In recent years, there have been growing concerns regarding children's exposure to environmental chemicals; the California Legislature passed SB 25 to help address these concerns. OEHHA's initial response was to develop a prioritization rationale for identifying those TACs most likely to show differential health impacts on infants and children, and to identify the five highest priority TACs as mandated by the statute (OEHHA, 2001). The present document continues the process by presenting revised methodology for the development of RELs for use in the Air Toxics Hot Spots program that takes into account possible differential impacts of TACs or other Hot Spots chemicals on children's health. This document also presents updates to the methodology for REL development that reflect advances in the science of risk assessment since OEHHA's methodologies for acute and chronic RELs were previously presented. These advances include issues explored in various reports by the U.S. EPA (U.S. EPA, 2000; 2002; 2004; 2006a; 2006b; 2006c; 2007a) and by committees of the National Academy of Sciences/National Research Council (NRC, 2001; 2007) issued since OEHHA completed development of the previous guidelines, as well as a variety of research papers and reviews published in the general scientific literature.

Quantitative risk assessment is used to derive noncancer health values including acute, chronic and the newly defined eight-hour RELs. OEHHA has aimed for consistency between the recommended methods for developing acute, eight-hour and chronic RELs, and therefore has

consolidated the previously separate guidance for acute and chronic RELs into a single TSD covering all three current types of RELs. The use of benchmark dose methodology, presented as an option in a previous document, is now recommended as the default method when data permit. Additional discussion of pharmacokinetic methods for interspecies extrapolation is presented, and, most importantly, these guidelines respond to new scientific understanding of the diversity in exposure and sensitivity of the human population, especially infants and children.

1.1 Objective

The objective of this document is to present our revised methods for deriving acute, eight-hour and chronic inhalation RELs for hazardous airborne substances. RELs are used in risk assessments to evaluate the potential for adverse public health impacts from facility emissions or similar localized sources in the Air Toxics Hot Spots Program, and from widespread exposures in the Toxic Air Contaminants program. The REL is an exposure at or below which adverse effects are not expected to occur in a human population, including sensitive subgroups (e.g., infants and children), exposed to that concentration for a specified duration. These health-based RELs are applicable to risk characterization of air releases, defined in Health and Safety Code (H&SC) Section 44303, as:

> "*including actual or potential spilling, leaking, pumping, pouring, emitting, emptying, discharging, injecting, escaping, leaching, dumping, or disposing of a substance into the ambient air and that results from routine operation of a facility or that is predictable, including, but not limited to continuous and intermittent releases and predictable process upsets or leaks*."

These health guidance values are designed to be protective against the noncancer health effects of exposure to airborne chemicals. OEHHA has also developed or endorsed cancer potency factors that can be used to protect the general public against the cancer causing effects of carcinogenic TACs (OEHHA, 2005a). In some cases the same chemical can cause noncancer health impacts and also cause cancer and thus may have both types of health values. The acute RELs are designed to protect against a 1-hour exposure duration occurring infrequently (e.g., no more than once every two weeks) (see Section 5.4.1). Chronic RELs are designed to protect against exposure for 24 hours a day for at least a significant fraction of a lifetime, defined as 12% of a standard lifetime of 70 years, i.e., 8.4 years. This document updates our procedures for acute and chronic RELs in order to ensure that the potentially greater vulnerability of children and infants is taken into consideration. In addition, OEHHA is developing eight-hour RELs, designed to protect against routine exposures of that duration (which approximates an average workday) that could occur as often as daily, but are not expected to do so for an entire lifetime.

Existing risk assessment methodologies use an uncertainty factor of 10 for intraspecies (inter-individual) variability in the human population (UF_H) which is assumed to protect children as well as other sensitive subpopulations. Such assumptions are crude but data have generally been lacking to use anything other than this assumption for most chemicals. This document evaluates available information in order to determine if more specific guidance for protecting children can be incorporated into our standard procedures for determining noncancer guidance values protective of children. This revised guidance document incorporates scientific developments since our last procedures were developed (OEHHA, 1999a; 2000a).

One of the challenges in developing a standard public health protective approach for acute, eight-hour and chronic RELs is the variability in available data for different chemicals. Some well-studied chemicals have human data, extensive animal data, and data necessary for pharmacokinetic modeling of tissue dose, while other chemicals may have limited data based on animal studies. OEHHA needs an approach that allows development of a public health protective acute, eight-hour or chronic REL accounting for the potential greater vulnerability of children. Child protective default procedures need to be available in the absence of chemical specific information. However, more scientifically sophisticated methods can be employed when chemical specific data are available.

1.2 Legislative Mandates

OEHHA is responsible for conducting health effects assessments of airborne chemicals, including chemicals listed under Health and Safety Code (H&SC) Section 44321, that are used by the California Air Resources Board in its risk management activities. As defined under the Air Toxics "Hot Spots" Information and Assessment Act of 1987 (Assembly Bill 2588; Chapter 1252, Statutes of 1987; California H&SC Section 44300 *et seq*., as amended), a risk assessment includes a comprehensive analysis of the dispersion of hazardous substances in the environment and the potential for human exposure, and a quantitative assessment of both individual and population-wide health risks associated with those levels of exposure. This document establishes a standardized procedure for generating the health-based values (acute, eight-hour and chronic RELs) used for assessing noncancer risks within the risk assessment process.

In preparing this document, OEHHA is responding to state legislation enacted in 1992 and 1999. SB 1731 (Chapter 1162, Statutes of 1992) required OEHHA to develop risk assessment guidelines for implementing the "Hot Spots" Act. The original guidelines were published as Technical Support Documents in 1999 – 2000 and as a condensed Guidance Manual in 2003 (OEHHA, 2003). This revision is in response to the Children's Environmental Health Protection Act (SB 25), which requires the California Environmental Protection Agency (CalEPA) to consider children specifically in setting Ambient Air Quality Standards (AAQS) and in developing criteria for Toxic Air Contaminants (TACs). SB 25 requires OEHHA to consider the following in its health effects assessments and recommendations:

(1) exposure patterns among infants and children that result in disproportionately high exposure,

(2) special susceptibility of infants and children,

(3) effects of simultaneous exposures to compounds with the same mechanisms of action, and

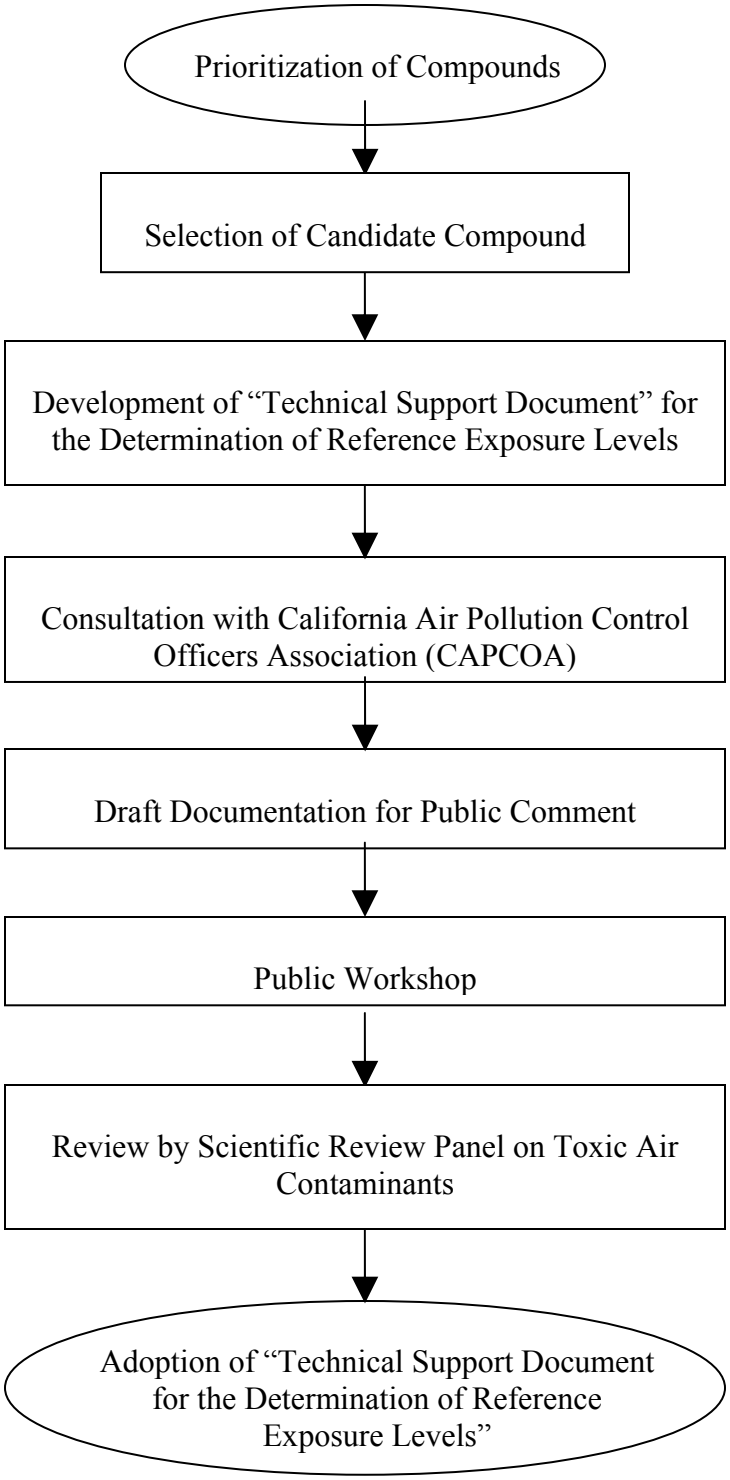
(4) any interactions of air pollutants.

The law requires OEHHA to evaluate available information on the Ambient Air Quality Standards (AAQS) and to develop a list of Toxic Air Contaminants (TACs) that "may cause infants and children to be especially susceptible to illness". OEHHA developed the document *Prioritization of Toxic Air Contaminants under the Children's Environmental Health Protection Act* (OEHHA, 2001) to address the identification of the first five TACs with special impacts on infants and children. That document underwent public comment and peer review by the State's Scientific Review Panel (SRP) on Toxic Air Contaminants. The statute requires OEHHA to evaluate 15 toxic air contaminants per year to ensure that our health effects assessments are

adequate to protect infants and children, and use these assessments to update the list of TACs that may cause infants and children to be especially susceptible to illness. To help meet the requirements of SB 1731 and SB 25, OEHHA in this document describes and evaluates the methodology to estimate RELs that explicitly consider infants and children, and derives such levels for specific chemicals.

OEHHA and the California Air Resources Board (CARB) have set up a procedure to facilitate the public comment and peer review process necessary for implementation of SB 1731 and SB 25 (Figure 1-1). This process includes internal OEHHA review, consultation with the California Air Pollution Control Officers Association (CAPCOA) and the California Air Resources Board (CARB), a public comment period, and public workshops. In addition, the Scientific Review Panel (SRP) on Toxic Air Contaminants, administered by the CARB, will review this document. OEHHA staff will respond to public comments on the changed methods and chemical-specific RELs, and update and revise the document as appropriate. The SRP will review the revised document, public comments and OEHHA's responses, and provide scientific input, which will be incorporated into the final draft.

FIGURE 1-1. PUBLIC AND PEER REVIEW PROCESS FOR ESTABLISHING REFERENCE EXPOSURE LEVELS



1.3 Summary of the Methodological Changes for Developing Acute, Eight-hour and Chronic RELs

- If sufficient data are available for REL development, then a benchmark dose approach will be preferred over the traditional NOAEL/UF approach, unless some specific feature or limitation of the data makes this impossible.

- If sufficient data are available, PBPK modeling (Appendix E) will be used to determine the tissue dose in a default human model if an animal toxicity study is used. The relationship determined by the PBPK model will replace the pharmacokinetic portion (UF_{A-k}) of the interspecies uncertainty factor (UF_A). In cases where a PBPK model is not available, UF_{A-k} is assigned a default value of $\sqrt{10}$.

- The HEC procedure (Appendix F) may be used in place of a part of the UF_{A-k}, leaving a residual value of 2 for this sub-factor (since the default value of the toxicodynamic component interspecies uncertainty factor, UF_{A-d}, is $\sqrt{10}$, this results in an overall value of UF_A= 6.32 when a HEC calculation is used, or 10 where no model of any kind is applied) (OEHHA, 2003). A modified HEC procedure using children's physiological values may be used as appropriate.

- The mechanism of toxicity and pharmacokinetics for chemicals will be evaluated when developing chronic and 8-hr RELs in order to help determine if long-term total dose or shorter-term tissue concentration is the predominant factor for toxicity. Infants and children are potentially more vulnerable because of pharmacokinetic, pharmacodynamic, and exposure differences. Therefore if PBPK modeling is used to determine human equivalent concentration from an animal study, children's or infants' physiological and biochemical parameters may be used in the modeling as appropriate.

- Since children may also be more vulnerable because of metabolism (activation or deactivation), excretion differences, or special sensitivities of developing organs and tissues, PBPK modeling may be needed to model children's tissue doses when a worker study is used to determine a LOAEL or NOAEL.

- When an uncertainty factor approach is used due to the lack of data for compound-specific models of toxicokinetics and toxicodynamics, an overall intraspecies uncertainty factor (UF_H) of 30 rather than 10 (toxicokinetic component, UF_{H-k} =10; toxicodynamic component, $UF_{H-d} = \sqrt{10}$) will be used as a default procedure to protect infants' and children's health in cases where metabolism is important in the activation or elimination of the compound and where renal and hepatic activity is key to the toxicological activity. For direct-acting chemicals whose site of action is the point of first contact a UF_{H-k} of $\sqrt{10}$ may be sufficient. Where significant concern for toxicodynamic differences larger than three-fold is present, a larger UF_{H-k} may be applied.

- The LOAELs or NOAELs for RELs based on trigeminally-mediated irritancy will no longer be adjusted using modified Haber's Law when the exposure duration of the study is different from the exposure duration for which the REL is intended to be protective. In

other cases, where the modified Haber's law procedure is applicable, the default value of the concentration exponent used when chemical specific information is unavailable will be 3, rather than 2 as previously.

- If the available toxicology literature does not include developmental studies for a chemical, or has substantial toxicological data gaps, the toxicological endpoints associated with the chemical, the chemical's reactivity, mechanism of toxicity, structure activity relationships and other factors, will be evaluated to decide if an additional database deficiency factor, with a default value of 3, will be applied. A larger value may, in some extenuating circumstances, be applied.

2 Reference Exposure Level (REL) Development Process

Reference Exposure Levels (RELs) are concentrations or doses at or below which adverse health effects are not likely to occur in the general human population, including sensitive subpopulations, for specified exposure durations. A central assumption is that a population threshold exists below which adverse effects will not occur in a population; however, such a threshold is not observable and can only be estimated. Areas of uncertainty in estimating effects among a diverse human population are addressed using extrapolation and UFs.

RELs are based on the most sensitive relevant health effect reported in the medical and toxicological literature (see Section 4.0). One of the issues in selecting the appropriate health end point for REL development is the difficulty in distinguishing "adverse" effects which should be of concern for the health of any individual experiencing such an effect from "precursor" effects which may be purely biochemical changes indicative of initial events in a chain of consequences which might (or perhaps might not, depending on individual circumstances) result in disease, and "adaptive" responses which reflect impacts of a biological process which is not of itself harmful or is within the capacity of normal biochemistry and physiology to accommodate without impairment of overall function of the organism. NRC (2007) has considered this issue and points out the need for a health-protective and case-specific approach to selection of endpoints, noting that the concept of "adversity" is actually a continuum ranging from initial events such as contact and uptake of the toxicant, through distribution, metabolism and contact with the target tissue, to various grades of precursor event leading up to changes which would be regarded as frankly pathological. They point out also that the degree of response over this continuum at a specific dose level may vary widely between individuals, depending on their age, genetic constitution, nutrition, prior health status and many other factors, not all of which can necessarily be predicted. OEHHA concurs in recommending a cautious selection of endpoint, emphasizing the importance of considering precursor events and individual variability: this issue is discussed further in Section 4.3.2.

RELs are designed to protect the most sensitive individuals in the population including infants and children by the selection of appropriate toxicological endpoints and extrapolation models, and by the inclusion of margins of safety in the form of various UFs. Since uncertainty factors are incorporated to address data gaps and uncertainties, exceeding the REL does not automatically indicate an adverse health impact.

Figure 2-1 depicts the steps involved in developing RELs. A complete literature search is conducted for each chemical, but the chemical summaries in Appendix D may only cite those studies contributing to the REL development and reflecting relevant routes of exposure.

FIGURE 2-1. REL DEVELOPMENT PROCESS

Conduct literature search

⇓

choose best study, emphasizing human data

⇓

identify critical biological endpoint

⇓

estimate threshold for effect (benchmark or NOAEL)

⇓

temporal/dosimetric adjustments (time extrapolation, HEC, Children's HEC, PBPK)

⇓

account for uncertainties in data

(extrapolating animal data to humans; occupational studies to sensitive subgroups)

⇓

Reference Exposure Level (REL)

(listed in Appendix B & Appendix D)

2.1 List of Substances Considered

All substances listed by the California Air Resources Board (CARB), whose emissions must be quantified for the Air Toxics "Hot Spots" Program, are considered for evaluation and development of RELs. The substances included on the Air Toxics "Hot Spots" Program List are those substances found on lists developed by the International Agency for Research on Cancer (IARC), the U.S. Environmental Protection Agency (U.S. EPA), the U.S. National Toxicology Program (NTP), the CARB (*i.e.* the list used in the Toxic Air Contaminant Program), the Hazard Evaluation System and Information Service (State of California), or on the Proposition 65 list of carcinogens and reproductive toxicants (State of California). The complete list of substances whose emissions must be quantified is contained in Appendix C.

2.2 Hazard Index Approach

The Air Toxics "Hot Spots" Program uses RELs as indicators of potential adverse health effects. A "hazard index" (HI) approach is used to estimate potential health effects resulting from hazardous substances by comparing measured or modeled exposure levels with RELs. (For a detailed description of this method, please refer to the document entitled *The Air Toxics Hot Spots Program Guidance Manual for Preparation of Health Risk Assessments* (OEHHA, 2003) which is available online at:

http://www.oehha.ca.gov/air/hot_spots/pdf/HRAguidefinal.pdf

Cumulative exposure (to multiple chemicals and from multiple sources) has become a concern in recent years. For many facilities a large number of chemicals may be emitted or may be present in the air at the location of the individual receptor or exposed population. To assess the cumulative impact of several chemicals present at the same time, it is important to consider the interaction of effects of the toxicants. These interactions may result in an overall effect that is equal to, less than, or greater than predicted from the effects observed with exposures to the individual chemicals (Ikeda, 1988; Jonker et al., 1990; DeVito et al., 2000). The potential for synergistic or antagonistic interactions has only been investigated for a small fraction of the millions of possible combinations of chemicals with potential human exposure. Effects of multiple chemical exposures on human health remain an area for future study.

One of the specific mandates of SB25 is the consideration of cumulative exposures to toxic chemicals, which has become a core concern for environmental justice in view of the propensity for disadvantage communities to be located in areas with a high density of pollutant-emitting facilities, and of freeways and other heavily used traffic corridors with attendant local peak concentrations of mobile-source-related pollutants. They thus receive higher local pollutant exposures compared to the State-wide average. This adverse environmental milieu is often compounded by the presence of population factors tending towards more adverse health impacts, such as less access to health care, poor nutrition and poor housing. Cumulative exposures may consist of multiple sources of the same chemical impacting a single receptor, multiple chemical exposures from the same source, or combinations of these situations. The hazard index approach is useful in addressing the predicted effects of such cumulative exposures (Salmon, 2007)

In risk assessments using the HI approach for either acute, eight-hour or chronic exposures, the impacts of exposures to multiple chemicals which impact the same target organ are treated as additive by default. The concept of a threshold underlies the assumption of additivity in chemical interactions. Exposure to a single chemical in the air may not result in a toxic response if it is below the threshold necessary to elicit a response. However, simultaneous exposure to two similar chemicals at sub-threshold levels may result in a toxic response. This is taken into account by adding together the individual ratios of the modeled concentration to the REL for all chemicals that impact the same target organ or system.

This may underestimate the effect in cases in which interactions are synergistic, or overestimate it if the effects are either not additive or antagonistic. There are a few cases where synergism between different toxic chemical exposures has been identified, but most multiple exposure situations which have been examined quantitatively in fact do show simple additivity, at least at low to moderate doses. This is well illustrated by the work on PCBs (DeVito et al., 2000; Crofton et al., 2005), which also shows that synergism or antagonism, perhaps related to enzyme induction or inhibition may be observed at higher doses. Additivity was also seen for phthalates causing male reproductive tract malformations in rats following co-exposure to di(n-butyl) phthalate (DHP) and diethylhexyl phthalate (DEHP) during sexual differentiation in rats (Howdeshell et al., 2007). These observations support the use of the additivity assumption by default for low-dose exposures, but also indicate the need to consider synergistic or antagonistic interactions between chemicals in those special cases where they have been identified.

Another limitation of the HI approach to assess the potential for health effects is the fact that different RELs have vastly different cumulative uncertainty factors depending on the quality of

the data. Thus the probability of adverse health impacts when the REL or HI is exceeded may be quite different with different chemicals.

For a particular target organ or system, the HI is calculated as follows (U.S. EPA, 2004):

$$HI = C_1 / REL_1 + C_2 / REL_2 + \ldots C_i / REL_i$$

where for i substances with the same toxicological endpoint,

HI = hazard index

C_i = concentration for the ith substance

REL_i = REL for the ith substance

Advances in computer capabilities and the development of software programs, such as the Air Resources Board's Hot Spots Analysis Reporting Program (HARP), have made assessment of chemical exposure from multiple stationary facilities more practical. However, it should be borne in mind that both the cumulative exposures and the health impacts in children may be different from those in adults, because of physiological, biochemical and behavioral factors (Hattis, 1996a; OEHHA, 2001; Miller et al., 2002).

3 Populations of Concern

RELs developed since the beginning of the Hot Spots Program are intended to protect the individuals who live or work in the vicinity of emissions of potentially toxic substances. The general population consists of individuals with a wide range of susceptibility. An individual's current level of susceptibility may be transitory or long lasting, and innate or induced by some prior exposure or event. The general population includes some people who are likely to be especially susceptible to developing adverse effects (e.g., infants, children, the elderly, pregnant women and those with acute or chronic illnesses). Individuals in the general population who may be at greater risk for developing adverse effects following chemical exposure include:

- those with increased exposure (e.g., infants, children, adults engaged in physical activity),
- those undergoing physiological change (e.g., infants, children and adolescents; pregnant women and their fetuses),
- individuals with impaired physiological conditions (e.g., elderly persons, persons with existing diseases; persons who are immunosuppressed),
- individuals with lower levels of protective biological mechanisms due to genetic variability within the population (U.S. EPA, 1994a) and
- members of communities impacted by multiple sources of exposure.

Less susceptible individuals are healthy adults without any genetic or biological predisposition that may increase sensitivity to the chemical of concern. RELs are intended to protect both individuals at low risk for chemical injury as well as identifiable sensitive subpopulations (groups of more highly susceptible individuals) from adverse health effects in the event of exposure. OEHHA has been mandated under SB 25 to ensure that RELs are also protective of infants and children, and other sensitive subpopulations. More useful scientific information has become available in the last few years to evaluate the special vulnerabilities of infants and children and to thus ensure that the RELs are protective.

Because the true range of variability is unknown, there may be a proportion of the population for whom the RELs will not be fully protective. It is OEHHA's intent that the levels will protect nearly all individuals, including those who are identifiable at the high end of susceptibility. However, they may not protect hypersensitive individuals (i.e., those who exhibit extremely rare or idiosyncratic responses that could not have been predicted from studying the health effects of the substance in animal studies, or population-based epidemiological studies of reasonable size).

While OEHHA has attempted to identify specific sensitive subgroups for each substance from the literature, it is not possible to identify all conditions predisposing toward adverse health effects following exposure to toxic substances. Because RELs pertain to inhalation exposures, the lungs are often the major target organ of toxicity, and asthmatics are frequently identified as a sensitive subgroup. For most compounds, the range of inter-individual variability is poorly characterized. An exception is sulfur dioxide, which has been studied in both normal as well as

asthmatic individuals. In a study of asthmatic subjects, there was a 7-fold distribution in the range of sulfur dioxide concentrations required to produce bronchoconstriction (Horstman et al., 1986). Thus, it is reasonable to conclude that asthmatics may be at least seven times as sensitive to the effects of sulfur dioxide as non-asthmatic individuals.

Hattis has presented an analysis of human variability in threshold responses in pharmacodynamic and toxicological studies (Hattis, 1996a; 1996b; Hattis et al., 1999). In many cases the variability in response in the general human population appears to be continuous and is well modeled by a log-normal distribution. The magnitude of the variability depends greatly on the endpoint and on the slope of the dose-response curve. In their analysis, some human threshold responses ranged over more than three orders of magnitude. Such continuous variability is hypothesized to reflect cumulative influences of a number of polymorphisms. These may be at various genetic loci, all of which individually have a small impact on the degree of dose dependence of the response, or there may be several polymorphisms at a single locus, associated with a variety of different levels of response. In other cases the response may be primarily influenced by a single gene, with two relatively common variants having markedly different properties. In this case the observed variability may appear to be heterogeneous, and is better described by a bimodal distribution or separate distributions.

3.1 Children as a Population of Concern

In response to the potentially greater vulnerability of infants and children to chemical exposure compared to adults (reviewed by OEHHA 2001), revised procedures for development and reassessment of noncancer health standards are outlined here. These are intended to ensure that age-related sensitivities are taken into consideration as far as possible in the development of noncancer health standards.

Many of the issues, which arise in ensuring adequate protection of children's health, are the same for acute, eight-hour, and chronic RELs. In this document, our approach in addressing these issues will be two-fold.

1. We have developed changes in the methodology for REL derivation in order to ensure that these health values are protective of children's health.
2. We have applied the changes in the methodology to individual RELs.

Over time, we will add new RELs, and address the adequacy of existing RELs that were developed with the initial methodologies. In recent years new methodologies have been developed to address uncertainties more quantitatively in determining health-protective levels and to ensure public health protection. OEHHA recommends expanding the use of techniques such as the benchmark dose method and physiologically based pharmacokinetic (PBPK) modeling wherever possible in order to improve the protection of public health with acute, eight-hour and chronic RELs for all members of the population, and for infants and children in particular. As noted in the introduction, data gaps will prevent PBPK modeling for many chemicals, and in some cases, will make benchmark dose analysis difficult. Thus, the traditional UF approach remains an important method.

3.2 Differences between Children and Adults

Children's potential vulnerability to toxic effects from chemicals can result from differences in exposure. For example:

- Children breathe more than adults on a per kg body weight basis and thus inhale more of the pollutant.
- For non-inhalation routes of exposure, children are also more exposed on a per kg body weight basis than adults because they eat more food, drink more water, have more dermal contact with soil, and have higher rates of inadvertent soil ingestion than adults.
- Children may consume more of one type of food than adults and have a less diverse diet.

Children may differ in terms of pharmacodynamics, particularly since more cell division and differentiation are occurring in children than in adults due to growth and organ development.

- Undifferentiated cells may be more prone to injury than are differentiated cells.
- Major organ development occurs in utero and during early postnatal development.
- Disruption of migration and differentiation of one type of cell may alter that of another.
- Some structures (e.g., the brain) continue to develop through adolescence.

Children and particularly neonates can be quite different pharmacokinetically from adults. Factors that affect absorption, distribution, metabolism, and elimination (ADME) of toxicants, often differ by age. Such factors include:

- Lung surface area available for absorption of gases, and airflow dynamics that alter deposition of inhaled particulates.
- Activating or detoxifying enzymes such as cytochrome P450 enzymes and Phase II conjugating enzymes are known to be present in infants in amounts and ratios different from adults. Fetal forms of cytochrome P450 are present and may differ in activity towards many substrates compared with the adult forms.
- Consequently, xenobiotic metabolism may occur through different pathways, be apportioned differently between competing pathways, and occur more slowly than in adults.
- Elimination may occur faster or more slowly than in adults.

These factors influencing vulnerability in children may change rapidly and may be present only for a period of weeks to months. In the case of prenatal development, periods of vulnerability could be as short as hours or days. In neonates, the variability in developmental stage appears to be wide for some processes including xenobiotic metabolism and renal clearance (Islam and Schlipkoter, 1989). Consequently inter-individual variability in susceptibility in a particular age range may be much greater than in adults, and thus harder to characterize. OEHHA addressed some of the behavioral, physiological and biochemical differences between children and adults in OEHHA (2001) and in Miller et al. (2002).

3.2.1 Pharmacokinetic Differences

3.2.1.1 Absorption

3.2.1.1.1 Inhalation

The lungs are the major route of entry of volatile airborne environmental pollutants and the majority of airborne semivolatile and nonvolatile pollutants. Exposure to environmental pollutants during lung development has the potential to significantly affect the overall growth and function of the respiratory system in children. The effects of exposure are likely to be different during each phase of development. Recent studies have found links between air pollution and preterm birth (Ritz and Yu, 1999; Sagiv et al., 2005; Wilhelm and Ritz, 2005), infant mortality (Loomis et al., 1999; Conceicao et al., 2001; Ha et al., 2001; Woodruff et al., 2006), deficits in lung function growth (Kunzli et al., 1997; Galizia and Kinney, 1999; Gauderman et al., 2004), and possibly, development of asthma (Gauderman et al., 2005; McConnell et al., 2006). The lungs are structurally immature in neonates and continue to mature throughout childhood (Fanucchi and Plopper, 1997; Plopper and Fanucchi, 2004; Fanucchi et al., 2006).

Lung development is a long-term, continuous process, and lung growth continues for up to eight years after birth. The development of the human lung can be divided into six stages: embryonic, pseudo-glandular, canalicular, saccular, alveolar, and vascular maturation (Zeltner and Burri, 1987). The first four stages occur prior to birth during fetal development. By birth the alveolar stage has begun, and the newborn infant has about 10 million alveoli. By approximately age eight, when adult numbers of alveoli have developed, there are about 300 million alveoli. Concomitantly the alveolar surface area increases from about 3 m^2 at birth to about 75 m^2 in adults leading to an air-tissue gas exchange area some 25-fold larger in adults (Islam and Schlipkoter, 1989).

During the maturation time following birth, there are multifold increases in overall lung size, active cellular differentiation, cell division, branching morphogenesis, and alveolar formation (Tyrala et al., 1977; Harding et al., 2004). Episodic exposure to environmental pollutants, specifically ozone, compromises postnatal morphogenesis of tracheobronchial airways in the monkey (Fanucchi et al., 2006).

Airways change in size and shape with maturation, altering deposition patterns. In animal models, exposure to environmental ozone during the early postnatal period alters the development of the distal pulmonary airways (Fanucchi et al., 2006). Lung function also continues to change, increasing until late adolescence in both males and females. Chronic airway disease and decreased lung function in children exposed to ambient air pollution may be due to repeating cycles of injury and repair altering normal lung maturation (Smiley-Jewell et al., 2000; Fanucchi et al., 2006).

Respiratory minute ventilation is increased in infants and children, resulting in a greater exposure per unit time on a weight basis and per unit surface area of lung, compared to adults. For inhalation exposures to equivalent chemical concentrations, both indoor and outdoor, infants and

children are at the same or greater risk of exposure based on their much higher minute ventilation on a body weight and lung surface area basis (i.e., mL/kg/m^2/min) (Snodgrass, 1992).

The deposition of inspired particles in the lungs is dependent on particle size and anatomical features of the respiratory tract. Deposition occurs primarily through impaction for coarser particles and through Brownian motion for finer particles. Greater fractions of inhaled particles less than five μm in diameter reach the distal airways compared to larger particles. Particle deposition tends to be greater in children because of the smaller diameters of the airways compared to adults (Snodgrass, 1992).

3.2.1.1.2 Other Routes

Ingestion is a major route by which infants and children are exposed to environmental chemicals (U.S. EPA, 2000). A number of factors may result in significant differences in the absorption of environmental pollutants by infants and children vs. adults. Most drugs administered by the oral route are absorbed into the systemic circulation by passive diffusion. The two factors most affecting this process are gastric pH and emptying time (Milsap and Jusko, 1994). Both processes vary with age from birth through infancy and childhood. At birth the gastric acidity is neutral (pH 6-8) due to the presence of amniotic fluid in the stomach (Avery et al., 1966). Following birth, gastric acid appears in the first one to two days of life and increases during the first weeks to months approaching adult levels by three months of age (Miller, 1941). Premature infants may continue to have lower gastric acidity due to immature acid secretion. The pH of the stomach influences the absorbed dose of ionizable chemicals, thus altering the potential dose to the infant (Agunod et al., 1969).

Gastric emptying time influences the fraction of an oral dose which is absorbed into the systemic circulation. The gastric emptying rate in neonatal infants is variable and prolonged and is affected by both gestational and postnatal age (Signer, 1975; Siegel et al., 1984). Absorption rates for several chemicals (e.g., phenobarbital, digoxin, arabinose and xylose) increase throughout the first year of life. While delayed absorption seen in neonates is partially due to slower gastric emptying and gastrointestinal motility, other factors such as lower pancreatic enzyme function and bile acid secretion as well as a higher gastrointestinal pH also play a role (Alcorn and McNamara, 2003).

Not all absorption processes depend on passive diffusion: some toxic materials (especially metals) are carried by, or interfere with the operation of, physiological active transport processes, which show age-dependent changes. For instance, the decline in the absorption of lead by the gastrointestinal tract from 45% in children to 10% in adults probably reflects decreases in calcium receptors with age (Heath et al., 2003).

Skin permeability and hence dermal absorption change as the skin develops. Dermal absorption may be significantly higher in neonates due to an immature epidermis and increased skin hydration. The surface area/bodyweight ratio is also much higher in infants and children than adults (0.067 to 0.033 m^2/kg *vs*. 0.025 m^2/kg in adults)(Snodgrass, 1992). Severe toxicity has been observed in infants following topical application of hexachlorophene (Tyrala et al., 1977) and isopropanol (McFadden and Haddow, 1969) due to higher absorbed dose in infants relative

to adults on a body weight basis. Alternatively, dermal administration may be the preferred therapeutic route in certain situations (e.g., theophylline in premature infants)

3.2.1.2 Distribution

Our knowledge of the distribution of chemicals in the body is perhaps best developed for pharmaceuticals, while much less is known about chemical distribution following environmental exposures. For this reason, the discussion that follows relies heavily on the pharmaceutical literature.

The distribution of absorbed chemicals in the infant and child is affected by a number of factors, primarily the concentration and types of plasma proteins and the relative size of fluid, fat and tissue compartments of the body (Milsap and Jusko, 1994). Total body water may be as high as 85 percent by weight in premature infants and 78 percent in full-term neonates versus 50-60 percent in adults (Friis-Hansen, 1961; 1971). The percentage of body weight that is body water affects the volume of distribution of absorbed drugs and other chemicals. The apparent volume of distribution (Vd, measured in L/kg body weight) relates the amount of drug in the body to its plasma concentration. Chemicals that are water-soluble have higher volumes of distribution, while those that are lipophilic have lower volumes of distribution in infants. For example, gentamycin, theophylline and phenytoin show two to three-fold higher Vds in neonates than in adults. Conversely, the Vd in neonates for diazepam, which is more lipophilic, is only one-third that of the adult value (Milsap and Jusko, 1994).

The binding of absorbed chemicals to plasma proteins depends upon the quantity of binding proteins available, the binding or affinity constant of the chemical for the protein(s), the number of available binding sites, and the presence of pathophysiological conditions, which may alter the binding interaction (Besunder et al., 1988). The affinity of plasma albumin for acidic drugs increases along with total plasma protein concentration from birth into early infancy (Morselli et al., 1980). The reduced plasma protein binding of drugs in newborns is probably due to lower total plasma protein concentration as well as such qualitative differences as persistence of fetal albumin with lower affinity for drugs and lower levels of γ-globulins and lipoproteins (Morselli et al., 1980). During early infancy plasma albumin, total protein concentrations, and α1-acid glycoprotein are lower and don't reach adult values until one year of age (Brodersen et al., 1983; Herngren et al., 1983). Three of the drugs noted above (theophylline, diazepam and phenytoin) all exhibit lower protein binding (1/3 to 1/10) in neonates versus adults (Morselli, 1976; Rane and Wilson, 1976; Morselli et al., 1980). In addition to the quantitative and qualitative differences in plasma proteins during early development, disturbances in acid-base balance and increased blood concentrations of endogenous substances such as free fatty acids and bilirubin can affect protein binding of drugs or the release (i.e., displacement) of bound drugs or other exogenous chemicals. Lower levels of albumin and elevated free fatty acids and bilirubin in the neonate may result in a larger Vd of and higher unbound concentrations of trichloroacetic acid (TCA), a metabolite of perchloroethylene (PCE) and trichloroethylene (TCE), in the blood (Muller et al., 1972; Ginsberg, 2000).

For some chemicals, the route of exposure affects the form of the chemical that appears in the blood. This in turn affects the binding and distribution of the absorbed chemical. For example, manganese appears to be absorbed from the gut primarily in the divalent form, with

approximately 80% of it subsequently bound in plasma to $ß_1$-globulin and albumin (Foradori et al., 1967). These manganese-protein complexes are efficiently removed from the blood during the first pass through the liver and returned to the gut in bile for elimination, thus limiting their distribution. By contrast, the pulmonary route of exposure results in much higher blood levels of manganese which are more widely distributed in the body (Roels et al., 1997). In the blood, unbound manganese may be converted by ceruloplasmin to the trivalent cation which is then bound by transferrin. Transferrin-manganese complexes are much less efficiently removed by the liver and thus survive first pass elimination to circulate to other tissues throughout the body (Gibbons et al., 1976).

Other factors that affect tissue distribution of toxicants include higher organ to body weight ratios in infants and children than adults, and the lack of a mature blood:brain and other tissue:blood barriers. Morphine is three to ten times more toxic to newborn than to adult rats due primarily to the higher permeability of the newborn brain to morphine (Rozman and Klaassen, 1996), and the distribution of methylmercury to the brain is greater if exposure occurs in utero or neonatally (Ballatori and Clarkson, 1982). Brain size in infants and children is much greater than in adults relative to body weight. A newborn's brain is one-third the size of an adult brain while its body weight is only about four percent that of adults (i.e., 3.0 vs. 70 kg) (Snodgrass, 1992). Cerebral blood flow is greater per brain weight in children vs. adults. A five-year old child has a cerebral flow of about 71 ml/min-100 g vs. 51 ml/min-100 g in an adult (Chiron et al., 1992). Thus not only is there a relatively greater brain mass to body weight ratio in children, but the brain receives a higher blood flow.

Dosing of infants and children based on surface area instead of body weight is generally preferred although this applies mainly to drugs that are distributed in extracellular body water. Environmental chemicals, which are often lipophilic, may pose a greater risk to children due to the possibility of enhanced dermal uptake due to increased skin surface area and permeability. In addition, lipophilic agents would have decreased volumes of distribution in infants due to their lower body fat content compared to typical adults.

3.2.1.3 Metabolism

The development of xenobiotic metabolizing enzymes in utero and after birth affects the rates of activation of chemicals to toxic intermediates, and the detoxification and ultimately clearance of xenobiotic compounds. Recent reviews have highlighted available information on the ontogeny of the Phase I enzymes (Cresteil, 1998; Hines and McCarver, 2002). The metabolic capacity of the liver (as a function of body weight) generally increases rapidly to near adult levels early in life, but significant qualitative and quantitative differences exist in newborns and neonates. Most of the microsomal enzyme systems responsible for drug metabolism are present at birth and their activities increase with postnatal age (Morselli et al., 1980). Liver Phase I reactions (oxidation, reduction, hydroxylation) develop rapidly during infancy (Stromland et al., 1994). The total cytochrome P450 (CYP) content of human liver microsomes is unchanged from fetal life through the first year of postnatal life and is approximately one-third the total adult content (Streissguth et al., 1991). There are fetal isoforms of the CYP enzymes, and the exogenous chemical substrate kinetics of these forms are not well characterized. In general, the level of inducibility of fetal CYP forms is unknown (Pineiro-Carrero and Pineiro, 2004). The postulated development of individual cytochrome P450 (CYP) forms during infancy and childhood has been summarized in a previous document (OEHHA, 2001, see Table 5). Except for CYP3A4 and CYP3A7 these data are based on immunological analyses for the presence of CYP mRNA and protein rather than on the metabolism of specific chemical substrates. In general, three groups of CYP P450 could be described:

(1) CYP3A7 and CYP4A1 present in fetal liver and active on endogenous substrates;

(2) an early neonatal group including CYP2D6 and CYP2E1 which surge within hours of birth; and

(3) a later developing neonatal group, CYP3A4, CYP2C's, and CYP1A2 (Cresteil, 1998).

Treluyer et al. (1997) observed that treatment of infants with barbiturates resulted in induction of CYP2C activity and increased metabolism of diazepam and tolbutamide. Total CYP3A protein, a major cytochrome P450 enzyme responsible for biotransformation of many exogenous compounds, is relatively constant in neonates and adults, but the isoforms change. CYP3A7 expression peaks in the neonatal liver and declines over time to undetectable levels in adult liver; CYP3A7 appears to be responsible for aryl hydrocarbon hydroxylase (AHH) activity in the fetus (Hakkola et al., 1998). CYP3A4 is the primary adult hepatic form of the 3A series, and is very low in neonatal liver increasing slowly after birth; at one month there is about one-third of the CYP3A4 activity as an adult liver (Rice and Barone Jr., 2000). CYP2E1 is an important enzyme catalyzing metabolism of a number of environmental contaminants including benzene, trichloroethylene and toluene. Following birth hepatic CYP2E1 increases gradually reaching about one-third of adult levels by one year of age and is essentially 100% of adult levels by 10 years of age (Rice, 1996). CYP2D6 levels are low in fetal hepatic tissue and rise after birth reaching about two-thirds of the adult levels in infant's age one month to five-year old children (Treluyer et al., 1991). CYP2C9 and CYP2C19, the most abundant CYP 2 enzymes in adult human liver, appear in the week after birth, and are about 30% of adult levels up to one year of age (Treluyer et al., 1997). CYP1A2 appears between one and three months of age, and reaches about half of adult levels at one year (Hines and McCarver, 2002). Evidence from both substrate

activation and immunological detection indicates CYP1A1 is expressed in fetal liver, where it can activate such xenobiotics as benzo[a]pyrene and aflatoxin B1 (Hines and McCarver, 2002). CYP1A1 appears to be less important in adult liver but present in inducible form in extrahepatic tissues (Hakkola et al., 1998), while CYP1B1 is present in both adult and fetal extrahepatic tissues. Studies of pharmaceutical clearance demonstrate the ontogeny of cytochrome P450 in infants and children, including gender-based differences (e.g., caffeine demethylation) (Kearns et al., 2003).

While there has been some study of the development of human CYP P450 enzymes and of the Phase II conjugation enzymes in the liver (reviewed in Cresteil, 1998; Kearns et al., 2003; Hines and McCarver, 2002; McCarver and Hines, 2002), there is less information about the timing of development of activity in other tissues. Watzka et al. (1999) observed sex- and age-related differences in CYP1A1 activity in the human brain, where enzyme activity increased dramatically from birth and reached adult levels by puberty. In the lung, animal studies have shown that exposure to environmental toxicants (sidestream tobacco smoke) can induce cytochrome P450 enzymes resulting in earlier activity (Gebremichael et al., 1995). The impacts of toxicity from activation of compounds by cytochrome P450 enzymes in early life depends on the rate of detoxification as well as capacity for repair. In neonatal rabbits, repair of injury to pulmonary Clara cells by toxicants activated by cytochrome P450 enzymes is decreased resulting in permanent structural changes in the adult animal (Miles, 1983).

The activity of phase II conjugation reactions, which are usually detoxifying, is generally lower at birth (Goldstein, 1983). Hence, there is concern that detoxification and elimination of chemicals are slower in infants. Conjugation with glucuronic acid is significantly lower at birth with activity 2.5-fold below adult levels (Lehman and Fitzhugh, 1954). Glucuronidation generally matures to adult levels in two months, although glucuronidation of some drugs does not reach adult levels until puberty (Calabrese, 1983). Reduced glucuronidation would result in slower removal of aniline, N-hydroxyarylamines, phenol, and benzene metabolites in neonates. Acetylation and sulfation reactions are generally high in newborn infants and rapidly reach adult levels. Thus, neonates may conjugate drugs or environmental chemicals with sulfate rather than glucuronic acid (e.g., acetaminophen). Acetylation by the N-acetyltransferases and sulfation by sulfotransferases are generally high in newborn infants and somewhat comparable to adult levels, although it may vary by tissue and by specific sulfotransferase (Renwick et al., 2000; McCarver and Hines, 2002). Infants are less susceptible to acetaminophen acute toxicity than adults due to their more active sulfation (Levy et al., 1975). There are several forms of glutathione (GSH) sulfotransferases (GSTs) with GST-P_i prevalent in the fetus and decreasing postnatally. GST-alpha and GST-mu are detected in fetal liver and increase over the first few years of life to adult levels (McCarver and Hines, 2002). GST-mu is involved in arene oxide detoxification. GST-alpha is two-fold more active in children 0.5 to 4 years of age than in adults. Plasma glutathione (GSH) is similar in children 0.5 to four years of age and in adults (U.S. FDA, 2006). Blood esterase activity, which is less than half that of adults at birth, is more depressed in premature infants than in full-term infants and doesn't reach the latter's activity for ten to twelve months. Esterase activity in newborns is two to ten-fold lower than the adult level. Low esterase activity coupled with lower volume of distribution may account for prolonged effect of local anesthetics observed during delivery (Ecobichon and Stephens, 1973). Neonates and premature infants may be deficient in the detoxification of organophosphorus (OP) insecticides (Augustinsson and Barr, 1963; Cole et al., 2003; Costa et al., 2003; Costa et al., 2005). Epoxide hydrolase, important in

detoxifying reactive epoxide metabolites, is present in fetal liver although at much reduced activity relative to adults (McCarver and Hines, 2002).

As a result of differing enzyme activity, some chemicals are metabolized by wholly different metabolic pathways depending on age. In infants, theophylline is N-methylated to caffeine, a minor pathway for adults in whom the majority of theophylline dose is N-demethylated or C-oxidized to monomethylxanthines or methyl-uric acid. Several studies have evaluated age-related pharmacokinetic differences in humans using information about drug disposition (Renwick and Lazarus, 1998; Renwick et al., 2000; Ginsberg et al., 2002; Hattis et al., 2003). Calculation of internal doses as the area under the blood concentration times time curve (AUC) for the same doses (mg/kg) indicated that the major difference from adults occurs in preterm and full-term neonates and young infants (Renwick et al., 2000). Higher AUC internal doses in neonates and young infants versus adults were noted for seven drugs which are substrates for glucuronidation, one with substrate specificity for CYP1A2, and four with substrate specificity for CYP3A4 metabolism, and inter-individual variation in elimination by these detoxification pathways did not differ by age group (Renwick et al., 2000). Ginsberg et al. (2002) evaluated pharmacokinetic information on 45 drugs in children and adults metabolized by different cytochrome P450 pathways, Phase II conjugations, or eliminated unchanged by the kidney. These authors noted half-lives in infants three to nine-fold longer than those of adults. In evaluating the inter-individual variability by age, Hattis et al. (2003) noted that the largest inter-individual variability occurred in the youngest children, apparently due to variability in development of critical metabolism and elimination pathways. Notably, these authors observed that for risk assessment modeling, unimodal distributions may be inadequate for young children and for overweight older children.

While the variations in pharmacokinetics with age are an important consideration in risk assessment, additional complexity is overlain by genetic polymorphisms rendering some individuals more susceptible than others. Perera et al. (1999) showed that:

1. There is significant transplacental transfer of polycyclic aromatic hydrocarbons (PAHs) and environmental tobacco smoke constituents from mother to fetus.
2. PAH-DNA adducts in maternal and newborn white blood cells are increased from environmental exposure.
3. The fetus is more sensitive to genetic damage than the mother.
4. Newborns with a specific restriction fragment length polymorphism (RFLP), CYP1A1 Msp1, had elevated numbers of adducts compared to those without the RFLP.

The report of Canalle et al. (2004) indicated that genetic variants in xenobiotic metabolizing genes may play a significant role in the susceptibility to acute lymphoblastic leukemia (ALL), the most frequent malignancy in children. The case-control study involved 113 Brazilian children with ALL and 221 matched controls. Carriers of the rare GSTP1 Val allele were at higher risk of ALL (odds ratio (OR) = 2.7; 95% CI = 1.1-6.8; p = 0.04). No difference was seen for GSTM1, GSTT1 or for the mutant CYP1A1*2 and CYP2E1*3 variants. However, when the mutant CYP variants were analyzed in combination with GSTM1 and the risk elevating GSTP1, the risk of ALL was increased (OR = 10.3; 95% CI = 1.0-111.8; p = 0.05) suggesting a combined effect.

3.2.1.4 Excretion

Many drugs are more slowly eliminated in neonates and infants than in adults (Kearns et al., 2003). A classic example is the toxicity associated with chloramphenicol in neonates resulting from a longer half-life (10 hr) compared to adults (2-5 hr) (Miles, 1983). This difference would also apply to some environmental contaminants in the drinking water. Differences in volume of distribution, metabolism, and the maturity of renal and hepatic elimination processes all play a role. Premature infants may have very low glomerular filtration rates (GFRs) (0.6 to 0.8 mL/min) (Plunkett et al., 1992; Milsap and Jusko, 1994). Studies with a large number of drugs have shown that the large majority of these agents are more slowly eliminated in neonates and infants than in adults. While this may be partly due to an increased volume of distribution for water-soluble drugs and their metabolites, additional factors may also be involved. At birth, glomerular function is more developed than tubular function and this persists for six months (Guignard et al., 1975; Hook and Hewitt, 1977; Arant Jr., 1978). At birth, the GFR is lower (2-4 ml/min), increases in the first few days (8-20 ml/min) and slowly increases to adult values by 8-12 month old infants (Robillard et al., 1999; Kearns et al., 2003). Over the first two to three weeks of life, Guignard et al. (1975) reported a two-fold increase in insulin clearance. The GFR values in neonates ranged from 6.0 to 32.2 mL/min/m^2 with a mean value of 10.8 ± 1.0 mL/min/m^2, n = 22. Premature infants may have GFRs as low as 0.6 to 0.8 mL/min (Plunkett et al., 1992; Milsap and Jusko, 1994). Early increases in GFR are related to: increases in cardiac output, decreases in peripheral vascular resistance, increases in mean arterial pressure, increased surface area of the kidney for filtration, and increased membrane pore size (Morselli et al., 1980; Plunkett et al., 1992). For the first two to three months of life, GFR is lower than that of adults. Clearance values at 10 to 30 times lower than seen in adults have been reported for some compounds (e.g., aminoglycoside antibiotics) (Morselli et al., 1980; Morselli, 1989). These age-related changes in renal function lead to decreased body clearance and prolonged blood half-lives in neonates for any chemical that relies upon renal excretion for elimination.

Studies in animals have shown that newborn and young animals have a reduced capacity to excrete chemicals into the bile compared to adult animals. Klaassen (1972) observed that the blood concentration of ouabain, a drug whose primary route of excretion is the bile, was seven-fold higher in seven-day old rats than in 39-day old rats. Ouabain is 40-fold more toxic in newborn than adult rats. Similarly both indocyanine green and sulfobromophthalein and their respective glucuronides were excreted more slowly in the bile of neonates than in adult rats (Klaassen, 1973). Ballatori and Clarkson (1982) found that the long half-life of methylmercury in neonatal rats was due to their inability to excrete the chemical in the bile, which is the main elimination route in adults. It is anticipated that these findings are indicative of likely deficiencies in the biliary excretion of certain environmental chemicals in human neonates and young children (Hattis, 1996a)

The use of various toxicokinetic factors in assessing children's risk is the subject of a recent comprehensive review (Ginsberg et al., 2004c). These authors conclude that children are more toxicokinetically variable than adults because of their variable growth and maturation rates, in addition to the factors that create toxicokinetic variability in adults that relate to genetics, nutrition, disease, body composition, and prescription (and other) drug use. Children also present the problem of variable growth rates, which can make even small age bins (subdivisions) relatively heterogeneous, especially if one in concerned about protecting the tails of the

distribution (e.g., 90th percentile). With greater variability, it is also more likely that a substantial fraction of a certain age group will lie outside the half-log toxicokinetic variability range we normally allocate to the adult defaults.

3.2.2 Pharmacodynamic Differences

Much more information exists for pharmacokinetics as a function of age than for pharmacodynamics. Many changes in pharmacodynamics occur with age and the mechanisms underlying toxic effects are of great interest to toxicologists, however, quantitative data are sparse.

The maturation of various systems, including the immune system, the nervous system, the reproductive system, the digestive system, and the blood-brain barrier, reflects qualitative and quantitative changes in receptors with age. Age-related differences in the LD_{50}, which reflect changes in the dose-response curve with age, may be related to receptor changes. Examples of pharmacodynamic differences in the pharmaceutical literature include development of Reye's syndrome following aspirin administration in children but not adults (Belay et al., 1999), and greater risk for developing hypersensitivity reactions, including Stevens-Johnson syndrome, when treated with lamotrigine (Guberman et al., 1999). Windows of susceptibility to various toxicants are seen during development. In humans, autism has been associated with thalidomide exposure during days 20 to 24 after conception (Stromland et al., 1994). Diphenylhydantoin, an anticonvulsant used to treat epilepsy, may cause human malformations including craniofacial anomalies and growth abnormalities (Eluma et al., 1984; Orup Jr. et al., 2003). In mice it causes different types and frequencies of malformations depending on when during gestation it is administered (Eluma et al., 1984; Buehler et al., 1994). Other agents known to cause human developmental toxicity include aminopterin, diethylstilbestrol (DES), ethyl alcohol in alcoholic beverages, etretinate, isotretinoin (retinoic acid), lead, and methylmercury.

In the case of ethyl alcohol, exposure in utero is associated with Fetal Alcohol Syndrome (FAS), a distinctive constellation of abnormalities at birth. In its more severe form, the syndrome is characterized by dysmorphogenesis of the head, heart, limbs and brain, deficits in IQ and memory, and behavioral problems such as hyperactivity and adolescent antisocial behavior (Streissguth et al., 1991). Similarly, lowered IQ and behavioral problems manifesting during childhood and later have also been associated with early life exposures to lead (Needleman et al., 1990) and cigarette smoke (Williams et al., 1998; Maughan et al., 2001; Yolton et al., 2005). As a result, the insidious nature of exposure to toxic agents during development may become more evident later in life, years after cessation of the toxic exposure. Delayed neurotoxicity has been seen both in humans following developmental exposure to methylmercury in contaminated seafood, and in monkeys experimentally exposed to methylmercury in utero through puberty. Among humans with developmental exposure to methylmercury, the manifestations of Minamata disease were delayed for several years in some individuals following cessation of exposure, and got progressively worse in others (Harada, 1995). Compared to an unexposed cohort, individuals exposed to methylmercury 20-30 years previously experienced significant deficits in motor function and impairments in visual, auditory and somatosensory systems (reviewed in Rice and Barone (2000). Similarly in monkeys, significant impairments of somatosensory and motor functions were observed up to 11 years following cessation of methylmercury exposure compared to controls (Rice, 1996).

While the specifics of the mechanisms underlying the toxicodynamic responses to these compounds have not been elucidated in most cases, the data generally indicate a significantly greater vulnerability of developing versus mature systems. In addition, it is important to recognize that the deleterious effects of exposure may not be immediately apparent, and in fact, may not be evident until much later in life.

3.3 Risk Assessment Considerations

The U.S. EPA recently developed a document entitled: *A Framework for Assessing Health Risks of Environmental Exposures to Children* (U.S. EPA, 2006c). This document presents considerations and questions for the risk assessor when evaluating the effects of life-stage at exposure on the toxic response or health outcome. A number of useful questions that the risk assessor should consider when evaluating the database on any chemical are discussed in the framework document. It should be noted that for the majority of chemicals in the Air Toxics Hot Spots Program, there will be limited data and this matrix of questions will likely remain unanswered for the risk assessor. Some of the considerations for dose-response assessment are noted below:

- Are there life-stage specific outcomes in the database for the chemical, particularly those with adequate information to conduct a quantitative does-response assessment?
- What is the most susceptible life-stage evident in the available data or by inference from other information?
- Are there known windows of susceptibility or developmental processes likely to be susceptible to the agent?
- Are there differences in the mechanism of toxicity in immature and mature animals or humans?
- Are there known toxicodynamic differences by life-stage (for example, receptors, repair mechanisms, enzymes or processes involved in signal transduction or other key developmental processes)?
- Are there likely differences in toxicokinetics that can be modeled or estimated? Do those differences change over life-stages and how? Are there data to describe toxicokinetic differences coinciding with the most susceptible life-stage?

4 REL Development Methodologies

OEHHA has developed uniform approaches for acute and chronic Reference Exposure Level (REL) development. The first descriptions of these approaches appeared in the *Air Toxics Hot Spots Program Risk Assessment Guidelines, Part III Technical Support Document for the Determination of Noncancer Chronic Reference Exposure Levels, April 2000* (OEHHA, 2000a) and the *Air Toxics Hot Spots Program Risk Assessment Guidelines. Part 1: The Determination of Acute Reference Exposure Levels for Airborne Toxicants* (OEHHA, 1999a).

4.1 Criteria for Studies Utilized to Identify Adverse Health Effects

Although a wide variety of information may be reviewed, only certain key studies are used to develop RELs. The following criteria are used to determine the relevance and quality of data used for REL development.

Peer reviewed papers published in the open scientific literature are the usual and preferred source of the data used in REL development, but other sources that may be used include government reports such as National Toxicology Program (NTP) studies, full laboratory reports of Good Laboratory Practices (GLP) compliant or otherwise well-conducted studies, and documents that have been reviewed by other impartial organizations but have not themselves been published in the literature, such as doctoral dissertations. These alternative sources are normally only used if they, or at least an appropriate summary of them (such as the toxicity summaries prepared by California Department of Pesticide Regulation (DPR) from laboratory reports submitted confidentially under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA)), are publicly available online or by request. Review papers are not used for REL development as they are considered secondary sources. Studies involving a single chemical are given preference over those with multiple simultaneous exposures, especially if these are not quantified. Studies using multiple exposure doses and clearly indicating dose-response information are preferred, but in some cases an inadequate toxicology database may necessitate the use of older studies in which such information is unclear. Such studies are used only if there is no other relevant study available, and they are consistent with the general toxicology database.

4.1.1 Selection of Key Studies

An important step in the development of any REL is the identification of peer-reviewed research studies that contribute most significantly to the weight of evidence as to the degree of hazard presented to humans by a particular substance. The studies may involve a human population studied in an epidemiological, clinical, or experimental exposure setting, or they may involve experimental studies with animals. These key studies are given greatest weight in estimating the dose-response relationship (by determination of a LOAEL, NOAEL, or benchmark dose) for adverse effects, and in identifying the nature of the critical adverse effect.

4.1.1.1 Human Data

Human data are logically most relevant to assessing human health effects associated with chemical exposures. Principles for evaluating human exposure studies for use in determining

health-based exposure levels have been discussed (OEHHA, 1999a; 2000a; 2003; 2005a). Whenever possible, RELs have been based on human data with relevant routes of exposure (principally inhalation). Of the 51 acute RELs originally developed, 36 were based on human health effects (OEHHA, 1999a), while of the first 80 chronic RELs, 22 were based on human data (OEHHA, 2005a).

Human studies used in assessing health effects of chemicals have included epidemiological studies, controlled exposure experiments, and case reports. Each of these three study types can provide important information needed to protect public health. When using these studies for risk assessment, several factors are important in evaluating their quality and in determining the level of certainty associated with their use. It should be noted that controlled studies of exposure in infants and children are rare outside of clinical trials.

4.1.1.1.1 Epidemiological Data

Epidemiological studies generally produce data on effects of chemical exposure to a large number of persons. Areas of concern when interpreting epidemiological studies include exposure measurement, health effects measurement, and accounting for co-variables and confounding variables (Rothman and Greenland, 2005). The population studied may consist of the general public or employees exposed in the workplace to varying concentrations of airborne chemicals.

Exposure measures frequently represent the greatest weakness of available epidemiological studies. Continuous, long-term exposure monitoring of individual subjects is rarely available. Frequently it is necessary to use limited, short-term, exposure monitoring data, which in many cases are not specific to the individuals under study, in order to derive an estimate of what the individual exposures may have been. Occupational exposures may vary over time as industrial hygiene practices change and individuals change jobs. Also, analytical methods have changed and in many cases improved over the years, and earlier measurements may be much less accurate, or not comparable to more recent data (due to different techniques and equipment for trapping and quantifying, especially for particles and fibers, and recent development of biological monitoring methods). Thus, estimating exposure levels that existed in workplaces many years ago is difficult, and exposure measurements in population-based environmental studies may be even more problematic. Nevertheless, the degree to which air concentrations can be adequately estimated is critical in determining the usefulness of an epidemiological study.

Health effect measures in epidemiological studies also frequently differ from those reported in experimental animal studies and must be carefully examined. Measurements of human health effects generally consist of recording observable effects, including clinical reports of disease or disability. If tests are conducted specifically for a study, those tests are usually non-invasive, but standard clinical hematology, X-rays and other standard medical results may also be available. Health effects data are compared with those compiled from a non-exposed group and may be presented as incidence, standardized mortality ratios (SMR), odds ratio (OR) or relative risk (RR) ratios. Health effects with a long latency may be missed if the exposure duration or length of follow-up in the study is inadequate.

For epidemiological studies to be useful, co-variables and confounding variables need to be controlled or removed from the study. Co-exposure to other chemicals is also an important concern as a potentially confounding effect. Occupational studies raise an additional concern in that generally healthy workers may be less sensitive to the adverse effects of chemical exposures than some others in the general population, including children, the elderly, carriers of genetic polymorphisms, which affect sensitivity, and persons with preexisting medical conditions. Gender-specific effects may be obscured by bias that may be present where a workplace is disproportionate by gender (NRC, 1986a).

"Negative" epidemiological studies (i.e., those not presenting an outcome different from the null hypothesis, properly described as "non-positive") present additional difficulties in interpretation. Estimating the power of the study to detect adverse effects is important to indicate the maximum incidence consistent with the observed null result. In addition, statistical confidence limits can be put around an observed null result. Although non-positive epidemiological studies may be useful as supporting evidence in favor of a REL derived from another data set, it is unlikely that such studies would themselves provide useful data for deriving a REL.

4.1.1.1.2 Controlled Human Exposure Studies

Controlled exposure studies have the advantages of having quantified exposure concentrations and of being conducted with human subjects, thus combining two important features of human epidemiological and animal toxicity studies (Hackney and Linn, 1983). The limitations of such studies include:

(1) involve small sample sizes,

(2) have a very short exposure duration,

(3) use narrowly focused response measurements that might miss significant health effects and

(4) usually only involve relatively healthy adults.

In spite of these potential shortcomings, controlled studies in human subjects, especially in sensitive subpopulations such as asthmatics, are given preference over animal studies in the REL development process, particularly for acute RELs. Human studies were used only if they were consistent with the standard ethical practices of investigation at the time they were conducted. The preferred study is a modern, ethical study approved by an Institutional Review Board for Human Studies. Although data from exposure of infants and children would help OEHHA address its mandate to protect children's health, controlled studies of exposures to infants and children are rare outside the context of clinical trials of potentially beneficial pharmaceuticals. Controlled exposures of children or infants to environmental chemicals are difficult to justify ethically, even at presumably safe levels, because their lack of maturity may result in increased and unpredicted susceptibility, and also limits or prevents their free and informed consent.

4.1.1.1.3 Case Reports

Individual case reports of adverse effects associated with exposures to a chemical can be useful, especially as qualitative confirmation that effects observed and quantified in animals also occur

in exposed humans. Multiple case histories with the same endpoint are especially relevant. However, these reports are generally not appropriate for quantitative analysis because of the very small sample size and the usually unquantified exposures (Goldstein, 1983). Only rarely would case reports be used as the basis for a proposed REL.

4.1.1.2 Animal Data

Animal toxicity studies are the most widely available source of data for REL development. However, studies that address all the toxic endpoints of interest, which include, specifically, pre- and postnatal developmental toxicity as well as effects on adults, are not always available, and studies with the specific exposure periods of one hour, eight hours, or with chronic exposure may not have been done.

Identification of the most appropriate animal species requires consideration of all available data relevant to prediction of human effects from animal observations. Studies of the most sensitive species have frequently been selected as key studies. Such an approach has the advantage of offering maximal protection, especially since humans may be more sensitive than laboratory animals in response to chemical exposure (Lehman and Fitzhugh, 1954). However, the animal species most sensitive to a substance is not necessarily that most similar to humans in developing adverse effects from a particular exposure. In general, of the animals used in laboratory studies, non-human primates are considered to be the most similar in response to exposures to toxic substances, but to date only the acute REL for hydrogen cyanide was developed using data from a controlled exposure of a non-human primate (the cynomolgus monkey, *Macaca fascicularis*). Such studies are relatively rare; those that are conducted typically involve less than lifetime exposure and a small number of individuals and thus have limited statistical power. This reflects the high cost and long duration inevitably associated with studies in non-human primates, and also ethical concerns that such animals should not be subjected to experimentation unless absolutely necessary.

Selection of the animal model and key study can be influenced by what is known about human health effects, and relevant areas of similarity and dissimilarity between humans and the test animal species (Calabrese, 1983). Comparison of human and animal pharmacokinetics and metabolism may be useful in selecting the relevant animal model for predicting human health effects. For example, hamsters and rabbits have much greater metabolic rates than monkeys (Plopper et al., 1983). This may increase or decrease their susceptibility to toxic substances relative to humans. However, in most instances it is not possible to determine which species responds most like humans.

An increasing number of environmental air contaminants (see Appendix E for examples and commentary) have physiologically-based pharmacokinetic (PBPK) models available to aid in extrapolating doses from animals to humans. The ratio of human to animal internal chemical concentration obtained from a validated PBPK model can replace the toxicokinetic component of the interspecies uncertainty factor ($UF_{A\text{-}k}$, see Table 4.1.1). These PBPK models are also useful in examining human inter-individual pharmacokinetic differences, including those between infants, children and adults.

TABLE 4.1.1. UNCERTAINTY FACTORS USED IN DERIVING ACUTE, 8-HOUR AND CHRONIC RELS

Method or Factor	***Values Used***	***REL types***
LOAEL uncertainty factor (UF_L)	1 NOAEL or benchmark used 6 LOAEL, mild effect 10 LOAEL, moderate to severe effect	A, 8, C A, 8, C A, 8, C
Combined interspecies uncertainty factor (UF_A)	1 human observation √10 animal observation in nonhuman primates 10 where no data are available on toxicokinetic or toxicodynamic differences between humans and a non-primate test species	A, 8, C
Interspecies uncertainty factor, toxicokinetic component ($UF_{A\text{-}k}$)	1 where animal and human PBPK models are used to describe interspecies differences 2 for residual toxicokinetic differences in studies of non-primate species using the HEC approach √10 non-primate studies with no chemical- or species-specific kinetic data	A, 8, C
Interspecies uncertainty factor, toxicodynamic component ($UF_{A\text{-}d}$)	1 where animal and human mechanistic data fully describe interspecies differences. (*This is unlikely to be the case.)* 2 for residual susceptibility differences where there are some toxicodynamic data √10 non-primate studies with no data on toxicodynamic interspecies differences	A, 8, C
Intraspecies uncertainty factor, toxicokinetic component ($UF_{H\text{-}k}$) for systemic toxicants	1 human study including sensitive subpopulations (e.g., infants and children) 1 where a PBPK model including measured inter-individual variability is used √10 for residual susceptibility differences where there are some toxicokinetic data (e.g., PBPK models for adults only) 10 to allow for diversity, including infants and children, with no human kinetic data	A, 8, C

TABLE 4.1.1. UNCERTAINTY FACTORS USED IN DERIVING ACUTE, 8-HOUR AND CHRONIC RELS

Method or Factor	***Values Used***	***REL types***
Intraspecies uncertainty factor, toxicodynamic component ($UF_{H\text{-}d}$)	1 Human study including sensitive subpopulations (e.g., infants and children) √10 All other cases including human studies with normal adult subjects only	A, 8, C
Subchronic uncertainty factor (UF_S)	1 Study duration >12% of estimated lifetime √10 Study duration 8-12% of estimated lifetime 10 Study duration <8% of estimated lifetime	C
Database deficiency factor (UF_D)	1 No substantial data gaps √10 Substantial data gaps including, but not limited to, developmental toxicity	A, 8, C

Notes for Table 4.1.1.:
A = acute REL; 8 = eight-hour REL; C = chronic REL. Individual UFs are rounded after multiplication, so two factors of √10 cumulate to 10, but one is rounded down to 3. Cumulative UF values are normally limited to between 1 and 3,000: if the latter value is exceeded it is generally taken to indicate that the source data are insufficient to support derivation of a REL. The table presents suggested default values in particular situations; these may be modified in either direction by more specific data relating to the test and target populations considered.

An experimental study should have a clear rationale and protocol, use Good Laboratory Practice (GLP) standards (or equivalent), and use appropriate analysis methods, including statistical analysis (U.S. FDA, 2006). Experimental study designs and criteria recommended by the NTP have been reviewed (Chhabra et al., 1990). However, the goal of protecting public health must be weighed with experimental design so that important endpoints are not missed and responses of relevant species are not ignored.

Previously some chronic RELs (e.g., naphthalene and 1,3-butadiene) were calculated using data from the standard 2-year NTP chronic toxicity/carcinogenicity study (OEHHA, 2000a). In many cases shorter screening studies, used for determining appropriate dose levels for the two-year study, are also reported and may contain additional useful information on noncancer toxic endpoints. In an NTP study using the inhalation route, groups of 50 rats and mice (6-8 weeks old) of both sexes are exposed six hours a day, five days a week for two years to two or three concentrations of a chemical. At necropsy the animals are examined for changes in approximately 30 organs or systems. Incidences of adverse effects at each dose in each sex and species are tabulated. A clear positive dose-response is regarded as an important indicator that a reported effect is dose related rather than incidental. Although the NTP study has been considered a gold standard for assessing chronic adverse effects, the protocol specifies young adult (8-10 week old rats or mice) animals as the starting point, so it does not include exposures of fetal, neonatal, pre-pubertal or adolescent animals. While this study design is useful to detect changes in adults, it may miss or underestimate effects in young animals. Two current animal

tests most relevant to children's health are (1) the developmental toxicity study, in which pregnant females are exposed during specific periods of gestation, and (2) the two-generation reproductive study, in which parents and offspring are exposed to the chemical. The absence of one or both of these studies from a chemical's database creates a serious data gap relating to children's environmental health and may result in the application of a data deficiency uncertainty factor (UF_D, typically $\sqrt{10}$) to address a lack of developmental data (Table 4.1.1. and Section 4.4.9.1).

4.2 Weight of Evidence Evaluations and Criteria for Causality

A "weight-of-evidence" approach is generally used to describe the body of evidence on whether or not exposure to a chemical causes a particular effect. Under this approach, the number and quality of toxicology and epidemiological studies, as well as other sources of data on biological plausibility, are considered in making a scientific judgment (OEHHA, 2005b). OEHHA has not adopted a categorical ranking of the weight-of-evidence, but provides a descriptive analysis of strengths and uncertainties of the evidence considered as part of the toxicity review supporting each REL. The U.S. EPA on the other hand has used a formal scheme of this type for their RfCs (U.S. EPA, 1994a).

Many of the proposed criteria for determination of causality are based on analyses of epidemiological studies (OEHHA, 2005b). These same criteria are however of general applicability to animal toxicology studies, although the degree of emphasis and extent of likely problems differ between these two data types. In analyzing animal studies, the nature and extent of the exposure and the characteristics of the exposed animals are generally well controlled. Under these circumstances, issues such as observation of a dose-response relationship, reproducibility of findings, and mechanism of action (including consideration of its relevance to humans) are key elements of the weight-of-evidence. On the other hand, for epidemiological studies the nature and extent of the exposure is often uncertain quantitatively and even qualitatively, and the exposed population is substantially more diverse than in a controlled animal experiment. Methodological issues that are considered in the review of the epidemiologic literature include:

1) the sample size of the study, which affects the power to detect an effect;

2) the extent to which the analysis or design takes into account potential confounders, or other risk factors;

3) selection bias, or whether the study groups were comparable; and

4) the potential for bias in ascertaining exposure.

Criteria for evaluating associations between exposure and health effects have been recommended by the International Agency for Research on Cancer (IARC, 2006) (http://monographs.iarc.fr/ENG/Preamble/index.php), and standard epidemiology texts (Lilienfeld and Lilienfeld, 1980; Rothman and Greenland, 1998). Much discussion has ensued over the last two centuries on causal inference. Most epidemiologists utilize similar sets of causal guidelines, proposed by Hill (1971), which OEHHA has employed (OEHHA, 2005b).

It should be noted that the causal criteria are guidelines for judging whether a causal association exists between a factor and a disease, rather than hard-and-fast rules. Lilienfeld and Lilienfeld (1980) note that:

> "*In medicine and public health, it would appear reasonable to adopt a pragmatic concept of causality. A causal relationship would be recognized to exist whenever evidence indicates that the factors form part of the complex of circumstances that increases the probability of the occurrence of disease and that a diminution of one or more of these factors decreases the frequency of that disease. After all, the reason for determining the etiological factors of a disease is to apply this knowledge to prevent the disease.*"

Commonly used causal criteria, based on those of Hill (1971), are described briefly below. These considerations are described in more detail in Rothman and Greenland (1998), the Surgeon General's Reports on Smoking (U.S. DHHS, 2004), and OEHHA's environmental tobacco smoke (ETS) document (OEHHA, 2005b).

4.2.1 Strength of Association

A strong association between a factor and a disease (historically considered to be a relative risk or odds ratio ≥ 2; and statistically significant) makes alternative explanations for the disease less likely. Small magnitude associations (i.e., risk estimate > 1 but ≤ 2) make alternative explanations (undetected biases or confounders) more likely. However, such small magnitude associations do not necessarily indicate lack of causality and are relatively common in environmental epidemiology. For example, the widely accepted associations between air pollution and cardiovascular/pulmonary mortality, and passive smoking and lung cancer, are considered small magnitude associations (risk estimate >1 and < 2). It is important to avoid confusing small magnitude of association with statistical insignificance. From a public health perspective, such small magnitude associations for a common disease can mean large numbers of people affected by the health outcome when exposure is frequent and widespread.

4.2.2 Consistency of Association

If several investigations find an association between a factor and a disease across a range of populations, geographic locations, times, and under different circumstances, then the factor is more likely to be causal. Consistency argues against hypotheses that the association is caused by some other factor(s) that varies across studies. Unmeasured confounding is an unlikely explanation when the effect is observed consistently across a number of studies in different populations.

Relevant observations include similarity of effects noted in different studies. For example, if an effect was noted in only one of many studies of a particular strain of laboratory rodent, or in only one of many epidemiological studies, evidence for a causal association between the chemical exposure and the effect is weakened. Associations that are replicated in several studies of the same design and among different populations (or species for animal studies) or geographical regions, using different epidemiological approaches, or considering different routes or sources of exposure are more likely to represent a causal relationship than isolated observations from single studies (IARC, 2006). If there are inconsistent results among investigations, possible reasons are

sought (such as adequacy of sample size or control group, methods used to assess exposure, range in levels of exposure), and results of studies judged to be rigorous are emphasized over those of studies judged to be methodologically less rigorous. For example, studies with the best exposure assessment are more informative for assessing the association than studies with limited exposure assessment, all else being equal.

4.2.3 Temporality

Temporality means that the factor associated with causing the disease occurs in time prior to development of the disease. The adverse health effect should occur at a time following exposure that is consistent with the nature of the effect. For example, respiratory irritation immediately following exposure to an irritant vapor is temporally consistent, whereas effects noted years later may not be. On the other hand, tumors, noted immediately following exposure, might be temporally inconsistent with a causal relationship, but tumors arising after a latency period of months (in rodents) or years (in rodents or humans) would be temporally consistent. An issue of temporal association that is sometimes difficult to clarify is the distinction between an effect due to chronic exposure and an acute effect due to repeated acute exposures. It may be inappropriate to develop a chronic REL based on an endpoint that is essentially an acute health effect seen repeatedly with daily workplace exposure.

4.2.4 Coherence and Biological Plausibility

A causal interpretation cannot conflict with what is known about the biology of the disease. The availability of experimental data or mechanistic theories consistent with epidemiological observations strengthens conclusions of causation. For example, the presence of known carcinogens in tobacco smoke supports the concept that exposure to tobacco smoke could cause increased cancer risk. Similarly, if the mechanism of action for a toxicant is consistent with development of a specific disease, then coherence and biological plausibility can be invoked.

4.2.5 Dose-response

A basic tenet of toxicology is that increasing exposure or dose generally increases the response to the toxicant. A progressively increasing response with increased exposure makes it difficult to argue that the factor is not associated with the disease. To argue otherwise necessitates that an unknown factor varies consistently with the dose of the substance and the response under question. While increased risk with increasing levels of exposure is considered to be a strong indication of causality, absence of a graded response does not exclude a causal relationship (IARC, 2006). The dose-response curves for specific toxic effects may be non-monotonic. For instance, where the dose response shows saturation in an observable or experimentally achievable exposure range, the effect of exposures in this range could be nearly maximal, with any additional exposure having little or no effect. Further, there are examples of U-shaped or inverted U-shaped dose-response curves, (e.g., for endocrine disrupters) (Almstrup et al., 2002; Lehmann et al., 2004).

4.2.6 Specificity

Specificity is generally interpreted to mean that a single cause is associated with a single effect. It may be useful for determining which microorganism is responsible for a particular disease, or associating a single carcinogenic chemical with a rare and characteristic tumor (e.g., liver angiosarcoma and vinyl chloride, or mesothelioma and asbestos). But it is not helpful when studying diseases that are multifactorial, or toxic substances that contain a number of individual constituents, each of which may have several effects and/or target sites.

4.2.7 Experimental Evidence

While experiments are often conducted over a short period of time or under artificial conditions (compared to real-life exposures), experiments offer the opportunity to collect data under highly controlled conditions that allow strong causal conclusions to be drawn. Experimental data that are consistent with epidemiological results strongly support conclusions of causality. There are also "natural experiments" that can be studied with epidemiological methods, such as when exposure of a human population to a substance declines or ceases; if the effect attributed to that exposure decreases, then there is evidence of causality. One example of this is the drop in heart disease death and lung cancer risk after smoking cessation.

4.3 Hazard Identification

4.3.1 Definition of Adverse Effect

The general aim in developing health-protective levels such as RELs is to define a level at which no impairment of the health of an exposed human is anticipated. Risk assessment guidance has therefore historically focused on the identification of an adverse effect as critical in determining health-protective levels. Thus, U.S. EPA has used a general definition of 'adverse effect' as, "any effect resulting in functional impairment and/or pathological lesions that may affect the performance of the whole organism, or that reduce an organism's ability to respond to an additional challenge" (U.S. EPA, 1994a).

However, the definition of an "adverse effect" has proved to be a source of significant difficulty and controversy. Not all effects reported for a substance are necessarily considered adverse; some adaptive biochemical responses such as enzyme induction are not considered necessarily adverse, unless they are identified as precursor events consistent with the mode of action for more obviously adverse pathophysiological events (Sherwin, 1983; ATS, 2000a).

Within the health-disease spectrum, health effects could range from mild symptoms of ill health to exacerbations of terminal illnesses of diverse kinds; an inordinate depletion of cell, tissue, and organ reserves; subclinical disease; and mortality. Reserve loss involves both reversible and irreversible alterations of the cell population and includes metabolic abnormalities and alterations of the intercellular milieu. Therefore, the earliest adverse effect is an altered ecology at the cellular level. Irreversible abnormalities that appear relatively minor may have a serious impact on health by increasing susceptibility to disease in general, or by exacerbating other disease processes (Sherwin, 1983).

NRC (2007) has pointed out that this continuum of responses presents a basic difficulty in defining any particular effect as adverse or otherwise:

"Dividing effects into dichotomous categories of 'adverse' and 'non-adverse' is problematic. Adverse effects usually develop along a continuum, starting with the uptake of a toxicant, distribution and metabolism, contact with a target organ, biologic change, physiologic response and repair, and clinical disease. Thus with some doses and hosts, biologic changes occur, but the body has sufficient defense mechanisms for detoxification or adaptation, and there is little or no adverse cumulative effect, particularly at low doses. In other situations, biologic changes are measurable and are precursors of an adverse clinical change, so an adverse effect, or the precursor of an adverse effect, could be defined in terms of a chemical metabolite or biologic change that is an indicator of both exposure and effect. The same biologic change could have little impact at a small dose (and so be termed 'non-adverse') but produce a much larger impact at a greater dose or in a more vulnerable person (and thus be termed 'adverse')."

The U.S. EPA considers both the biological and statistical significance of effects when determining if the observed effect can be defined as adverse. Their determination also takes into account what is known about the underlying mode of action (U.S. EPA, 2002). Biological significance is the determination that the observed effect (eg., a biochemical change, a functional impairment, or a pathological lesion) is likely to impair the performance or reduce the ability of an individual to function or to respond to additional challenges from the agent. For some quantal endpoints (e.g., birth defects, tumors, or some discrete pathological changes), criteria are already established to decide the type and incidence of effects, which may be considered adverse, and statistical tools to support the decision. However, changes in continuous measures such as body weight, enzyme changes, and physiological measures, are more difficult to use as endpoints because the amount of change considered to be biologically significant has not been well defined (U.S. EPA, 2002).

In particular, relatively subtle alterations in such continuous measures such as cellular proliferation, maturation, gene activation or suppression, and altered signal transduction, can lead to serious outcomes in developing humans. Thus it can be difficult, but important to the protection of developing infants and children, to determine the biological significance of seemingly minor alterations in an enzyme. Some changes in enzyme activity or levels can produce severe effects in a developing organism if they produce a change in signal transduction. For example, fetal exposure to chlorpyrifos alters receptor numbers and activity in serotonergic neurons in adults (Aldridge et al., 2004). Also, brief inhibition of cyclooxygenase-2 during the perinatal period alters neurodevelopment and severely inhibits reproductive behavior in the adult male rat (Amateau and McCarthy, 2004).

OEHHA therefore follows NRC (2007) in recommending a cautious and health-protective approach to the consideration of whether a given biological endpoint is appropriate to consider frankly "adverse," or is a biologically significant precursor lesion, in which case it would be a suitable endpoint for consideration in a risk assessment, or is rather a non-adverse adaptive or incidental change. An example of the necessary decision process is shown in the determination of a Public Health Goal for perchlorate in drinking water (Ting et al., 2006). Here the decision was made to use the precursor biochemical alteration of impaired iodine uptake, a known effect of the perchlorate ion resulting from inhibition of the active transport protein responsible for

iodide translocation. This is clearly shown at higher doses to impact thyroid hormone synthesis and this in turn is known to have severe impacts on central nervous system development in the human fetus and infant. It was concluded that even modest impacts on the iodine uptake process had the potential to impact sensitive targets, such as the fetus of a woman with suboptimal iodine intake.

4.3.2 Nature of Adverse Effects

The toxic effects of chemicals are of varying types and degrees of severity. Following an acute (one-hour) exposure to a substance released into the atmosphere, effects on the upper and lower respiratory tract may be observed as so-called "portal of entry" effects. Toxic effects from airborne substances may also be due to exposure via the skin and eyes. Systemic effects, such as hemolysis or central nervous system injury, may result from absorption of material through the lungs, and, to a lesser extent, through the skin.

Toxic effects do not have to be observed immediately to be considered due to an acute exposure, but may instead appear hours to days after that exposure. For example, a brief exposure to phosgene may result in pulmonary edema several hours later. In the case of benzene, death may result from leucopenia days following high-level acute exposure.

Certain chemicals, after a single exposure, have the potential to produce delayed adverse effects. Often acute toxicity tests do not have a sufficient follow-up period to allow thorough assessment of the potential for delayed health effects from single exposures. With respect to two kinds of delayed effects, cancer and reproductive or developmental harm, there is more information available. Carcinogenicity is treated separately in risk assessment and cancer potency factors for carcinogens are described in the *Air Toxics Hot Spots Program Risk Assessment Guidelines Part II: Technical Support Document for Describing Available Cancer Potency Factors* (OEHHA, 2005a). Reproductive and developmental toxicants are considered here because substantial research effort has been devoted toward specifically identifying such delayed effects.

Some substances exert their toxic effects through their metabolites. For example, methylene chloride's acute toxicity is mediated through its metabolite, carbon monoxide. Whenever possible, information on toxic metabolites is provided in the toxicity summaries. When detailed information is available on the relationship of dose of the parent chemical to level of metabolite and the metabolite level to degree of toxic response, this is taken into account in developing the RELs. However, RELs are always expressed in terms of the concentration of the parent compound, not the metabolite.

4.3.3 Severity of Effects

Adverse effects may occur with a range of severity from mild (sensory or subjective effects, or statistically significant incidence of precursor changes, which are reversible) to severe (clinically significant pathological changes, disabling or strongly objectionable sensory effects, persistent or irreversible histological or functional damage), or even to life-threatening. These effect levels have been used in a variety of ways including in models of progressive dose-response such as that used in U.S. EPA's categorical regression methodology. However, it is generally expected that the endpoint of choice for determination of a REL, which is intended to protect the health of

the community at large, will be a mild effect. More severe effects may be used if these are in fact the most sensitive endpoint (for example irreversible developmental effects), or if no data on mild effects are available. Under such circumstances, additional models or UFs may be used as described in the following section in order to provide adequate health protection for the majority of the exposed population. Specific applications of the severity of effect concept are described for acute (Section 5), eight-hour (Section 6) and chronic (Section 7) RELs in this document.

4.3.4 Target Organs

The nature of the target organs or systems involved in a given toxicological response is important since this is considered for hazard index (HI) calculations (Section 2.2). Consideration of the cumulative impact of exposure to multiple chemicals is one of the requirements of SB25, and a key objective for environmental justice considerations. The target organs or systems are described by general categories that may include varied effects (Table 4.3.1). For example, the target system, "respiratory system," includes upper airway irritation as well as lower airway effects, such as bronchoconstriction. For simplicity, this approach to HI calculations assumes additivity when multiple toxicants impact the same organ system or biochemical target. Other possible modes include independent/non-additive, synergistic or antagonistic. The description of synergism or antagonism is difficult, and probably requires determination of joint dose-response functions on a compound-specific basis. The number of compounds for which synergism or antagonism have been documented is fairly small. It is unclear whether this is because such interactions are genuinely infrequent, or because the standard toxicological screens are not designed to identify these effects.

In using the additive HI approach it is necessary to define what constitutes the "same" toxicological endpoint, which when impacted by multiple toxicants results in effectively cumulative damage (Salmon, 2007). This might be the same molecular target, the same physiological process, or perhaps the same anatomical unit. The traditional basis has usually been the anatomical unit by default, since actual mechanisms and physiological interactions between organs are frequently unknown. More recently, the availability of information on toxicological mechanisms has prompted discussion of both broader and narrower frames of reference. The concept of a single molecular target is attractively simple, but may be too narrow when multiple control or functional systems give input to a single critical system or process downstream from the molecular targets of various toxicants. Because the precise relative contributions of exposure to multiple substances that principally affect different areas of the same physiologic system are unknown, the assumption of additivity across a single major organ system may either under- or over-estimate the effects of chemical interactions in certain cases. However, in most cases this approach provides an appropriate health protective assumption. We have indicated in Appendix B, Table B-1 which toxicological endpoints are relevant to the specific REL for each chemical. While the REL is based on the most sensitive endpoint, other toxicological endpoints are manifested at exposures close to that which induces the toxicological endpoint that serves as the basis for the REL. Therefore, some chemicals should be evaluated for impacts on multiple target organs or systems. In addition, predisposing conditions are known to increase susceptibility to some chemicals. The target organs for those predisposing conditions should also be included in the HI approach. The target organs to be evaluated for HI are presented in Table 4.3.1, and in each chemical summary in Appendix D.

In order for the acute and chronic REL HI target organs to be consistent, developmental and reproductive, which were previously combined, have been separated into two categories. New target organ categories may need to be added, based on the toxicological data used to develop additional RELs.

TABLE 4.3.1. TARGET ORGANS OR SYSTEMS USED IN ACUTE, 8-HOUR AND CHRONIC HAZARD INDEX CALCULATIONS, WITH SAMPLE HEALTH EFFECTS FOR CURRENT ACUTE AND CHRONIC RELS

Hazard Index target organ catergories	Health effects currently included within target organ categories for acute RELs	Health effects currently included within target organ categories for chronic RELs
Hematological System	Hemolysis; anemia; platelet abnormalities; effects on hematopoietic stem cells	Lowered red and white blood cell counts
Cardiovascular System	Aggravation of angina	Carboxyhemoglobin
Nervous System	Electroencephalograph (EEG) results; performance on neurobehavioral or neuropsychological tests; lightheadedness; clinical neurological exam; headache	EEG results; astrogliosis; performance on neurobehavioral tests; tremor; lightheadedness; memory disturbances; headache
Eyes	Irritation; histological changes to eye tissue	Irritation of eyes
Alimentary Tract	Hepatotoxicity; nausea; vomiting	Hepatotoxicity; kidney lesions; urinary porphyrins; liver enzymes
Immune System	Lymphocyte proliferation; host resistance to infection	Macrophage hyperplasia
Reproductive	Anovulation; decreased ovulation, preimplantation loss; altered copulatory behavior; azoospermia; oligospermia; spontaneous abortion	Testicular degeneration

TABLE 4.3.1. TARGET ORGANS OR SYSTEMS USED IN ACUTE, 8-HOUR AND CHRONIC HAZARD INDEX CALCULATIONS, WITH SAMPLE HEALTH EFFECTS FOR CURRENT ACUTE AND CHRONIC RELS

Hazard Index target organ catergories	Health effects currently included within target organ categories for acute RELs	Health effects currently included within target organ categories for chronic RELs
Developmental	Fetotoxicity; teratogenicity, intrauterine growth retardation; altered behavior in offspring	Fetotoxicity; teratogenicity; developmental anomalies
Respiratory System	Irritation of nose and throat; increased mucus production; histological changes in nasal epithelium; histological changes in lung tissue; lung function following inhalation challenge	Irritation of nose and throat; hyperplasia of epithelium or nasal mucosa; histological changes in lung tissue; bronchiolar fibrosis; decreased pulmonary function
Skin	Irritation of skin	Potential use in eight-hour and chronic RELs, but no current examples.
Physiological response to odors	Headache; nausea	Potential use in eight-hour and chronic RELs, but no current examples
Endocrine System	Potential use in acute and eight-hour RELs, but no current examples	Thyroid enlargement
General Toxicity (e.g., failure to gain weight; weight loss)	Potential use in acute RELs, but no current examples	Potential use in eight-hour and chronic RELs, but no current examples

4.4 Dose-Response Assessment

4.4.1 Estimation of Threshold or Low Response Concentrations

Noncancer health effects assessment has been based on the concept that a threshold concentration or dose exists below which no adverse effects would occur. While such thresholds are observed among individuals, the existence and magnitude of a population threshold below which no members of the population would experience adverse effects cannot be demonstrated. In any study, the entire population of concern is not examined; rather a sample of the population from which inferences are drawn is studied. Therefore, it is not possible to distinguish whether a concentration is truly below a population threshold level for an adverse effect or is rather a level associated with a relatively low incidence of adverse effects, which cannot be distinguished from background rates in the population.

There may also be cases where no threshold exists in the general population for a particular effect. This situation may occur for responses for which there is no theoretical threshold due to the mechanism of toxicity. The most accepted example of this is chemical carcinogenesis, particularly for genotoxic carcinogens. However, there may, at least in principle, be other types of toxicity which do not show a threshold at any dose level.

Even where a true threshold exists in the dose response of a particular individual to a chemical exposure, there may in fact be no identifiable threshold in the response of the general population. This may occur in the case where some individuals in a diverse population show a threshold whereas others do not, which is at least theoretically possible if genetic polymorphisms exist which inactivate a protective mechanism. However, the most likely case is where a true threshold in the response occurs in all individuals at low doses, but the background rate or extent of that toxic response in the population is already above zero due to population-wide exposure to that pollutant or another causative factor which produces the same end-point or disease. In this case, any increment in exposure to the pollutant of concern will cause an increase in the prevalence or severity of the disease, in spite of the existence of a threshold in the individual dose-response relationship. A probable example of this is seen in the neurodevelopmental effects of lead exposure in children, which recent risk assessments have described using linear or other continuous dose-response functions (Carlisle et al., 2006). The data available for criteria pollutants such as ozone or particulate matter are consistent with linear no-threshold dose-response curves for cardiovascular mortality (Schwela, 2000; Vedal et al., 2003).

Where these special cases are demonstrated to exist on the basis of population health data, or appear likely based on mechanistic studies, it will be appropriate to use these data to develop risk-based or continuous-response models to describe the population impacts of exposure to these pollutants, rather than relying on the threshold dose-response description to identify a "safe" exposure level. It should be noted that lack of a true threshold does not necessarily imply linearity of response at all doses (although at sufficiently low dose levels any continuous non-threshold dose-response curve will necessarily approach linearity). Conversely, the observation of a non-linear dose-response curve does not necessarily imply the existence of a threshold. However, in the majority of cases for noncancer effects the existence of a threshold in the dose response is both plausible, and often, within the acknowledged limitations, demonstrable.

Therefore, the threshold assumption is regarded as the default for noncancer risk assessment, and is most often used.

Two major strategies are used for dose-response assessment methods to estimate "thresholds" of responses from study data. These are the benchmark dose (BMD) or benchmark concentration (BMC) approach and the no-observed-adverse-effect-level (NOAEL) approach.

Of the methods presented, the BMC approach is preferred. Quantal or continuous dose-response data for a toxicant (measured for at least two dose levels and a control) are required to estimate levels using the BMC method. Supporting toxicological data will not, however, always be sufficient to permit this level of quantification. In most cases, the method will allow determination of a benchmark concentration even with relatively sparse data; however, obviously the confidence in the result will be lower in this case. The alternative NOAEL method may give the appearance of providing a result more easily with poor data, but in fact the uncertainty in such a result can be extremely large, and the situation is not improved by the inability to quantify this uncertainty. OEHHA has used the BMC approach to develop two acute RELs (OEHHA, 1999a). More recently a number of chronic RELs have been developed using this approach (Collins et al., 2004). Based on recent experience with the benchmark method, new REL values will be developed using the BMC approach whenever data of sufficient quality to support this methodology are available.

4.4.1.1 Benchmark Concentration

The importance of a dose-response relationship in the evaluation of effects of chemical exposure is well-established. The NOAEL approach, which is explained below in Section 4.4.1.2, does not explicitly incorporate information on the shape of the dose-response curve and is highly dependent on the test doses chosen by the original investigators. This led to explorations of the concept that a concentration estimated to be associated with a predefined low risk could provide an alternative to the NOAEL (Mantel and Bryan, 1961; Mantel et al., 1975; Crump, 1984; Dourson, 1986; Hartung, 1987; Gaylor, 1988; Gaylor et al., 1998). Crump (1984) proposed the term "benchmark dose" and extensively evaluated this concept. In this document, the term benchmark concentration (BMC) is used since inhalation toxicology data are usually described in terms of air concentrations.

The BMC method allows a mathematical and statistical approach to the calculation of RELs (Crump, 1984; Lewis and Alexeeff, 1989; Alexeeff et al., 1992; Alexeeff et al., 1993; Barnes et al., 1995; Collins et al., 2005; Starr et al., 2005). In this document, the BMC is defined as the 95% lower confidence limit of the concentration expected to produce toxic responses in a chosen percentage of subjects (the benchmark response rate) exposed at this dose. A suitable mathematical function is fitted to the concentration versus response relationship using likelihood methodology. The function used is selected according to defined quality of fit criteria. The concentration expected to produce the benchmark response rate, and the lower confidence bound on that concentration are identified from the fitted curve. In the case of quantal data in an animal toxicity experiment, the benchmark response rate is usually selected at 5% (see Section 4.4.1.1.1 below). Other types of data, including continuous measures of toxic response, and data from epidemiological studies, require an appropriate benchmark response rate to be identified on a

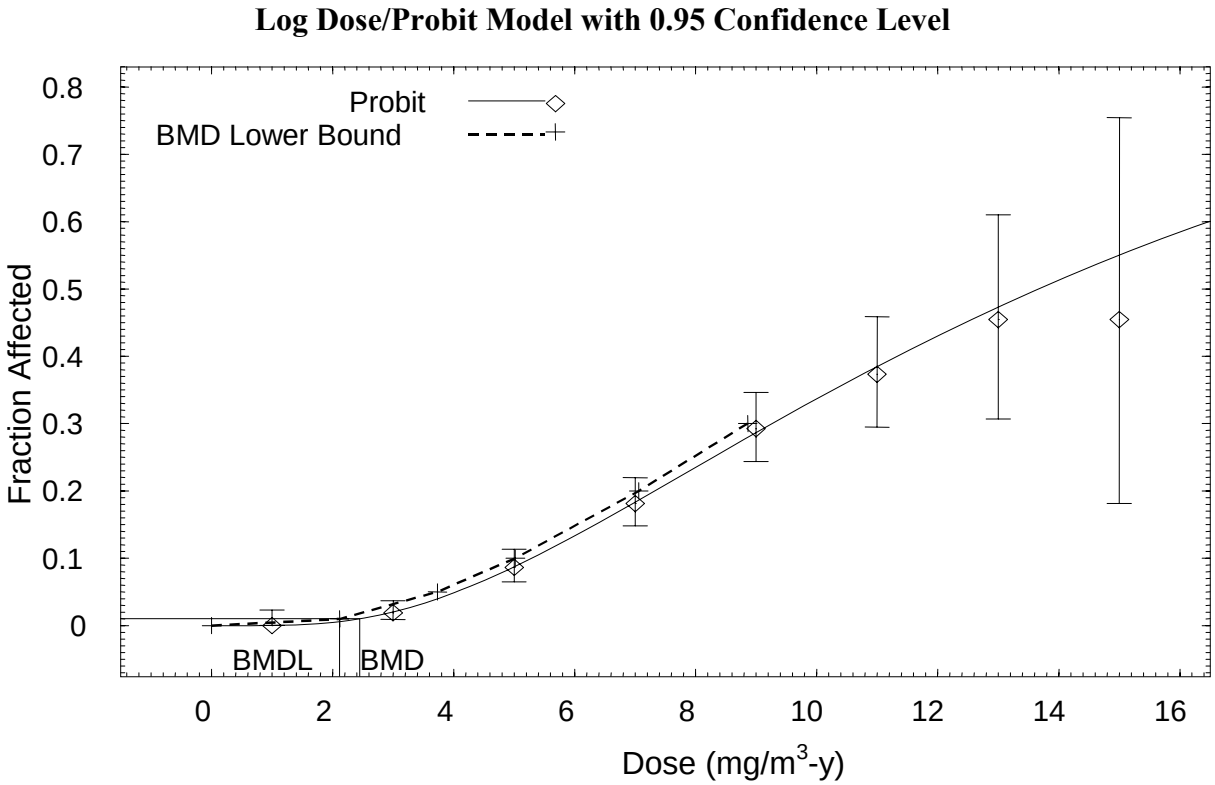
case-by-case basis. An example of the benchmark dose methodology is graphically depicted in Figure 4-1.

In spite of its advantages, there are sources of uncertainty in the experimentally derived BMC value. For example, the studies used to estimate the BMC have usually been performed with animals rather than humans e.g., (Kuwabara et al., 2006). Also, the experimental duration of exposure may differ from that which is of interest for the establishment of RELs. Additionally, the dose of toxicant delivered to the target tissue may differ between species and among humans and may depend on the type of activity in which the individual is engaged. Another area of uncertainty is that there can be a large degree of variability in the number of people who respond at any exposure. For example, there may be over a 10-fold variability in the irritation threshold (the concentration of a substance at which irritation of the eyes, nose and/or throat is first detectable) for chlorine (Anglen, 1981). In order to estimate a health protective level such as a REL for the population of concern, the BMC is therefore modified by UFs, except where explicit extrapolation models are available to allow for these differences.

$$BMC / UF = REL$$

Most frequently, the characteristics of the BMC are chosen so that its properties are similar to that of the NOAEL described below. Thus, similar UFs (Table 4.1.1.) are applied with both approaches. Specific data sets may however require or encourage the use of UFs different from what would be used with a standard NOAEL (described below), and these should be determined on a case-by-case basis.

FIGURE 4-1 LOG-PROBIT MODELING OF DOSE-RESPONSE DATA FOR SILICA[1]



[1]Data from (Hnizdo et al., 1993; Collins et al., 2005).

4.4.1.1.1 Selection of Appropriate Benchmark Concentration Criteria

Suggested response levels for the BMC based on quantal data have ranged from one in one million (Mantel and Bryan, 1961) to 10% (Dourson et al., 1985). The 1 to 5% response range approximates the lower limit of adverse effect detection likely to occur in typical human epidemiological and laboratory animal studies (Gaylor, 1992). In 1995, using developmental toxicity data, the U.S. EPA concluded that a 1% response rate was likely to be too low, while either 5% (BMC_{05}) or 10% (BMC_{10}) response rates were adequate for the purposes of estimating a benchmark concentration (Barnes et al., 1995). One reason for this conclusion was the large difference (29-fold) between observed NOAELs and the 1% benchmark using developmental toxicity data. Subsequently, the U.S. EPA (U.S. EPA, 2007a) used a 10% response rate for benchmark concentrations when deriving chronic inhalation reference concentrations (RfCs). More recently, RfC determinations for various endpoints by the U.S. EPA have used either 5% or 10% as the benchmark response rate (U.S. EPA, 2002; 2004). OEHHA has used the 5% response rate in several chronic RELs, and showed that the lower 95% confidence bound on the BMC_{05} typically appears equivalent for risk assessment purposes to a NOAEL in well designed and conducted animal studies where a quantal measure of toxic response is reported (Lewis and Alexeeff, 1989; Alexeeff et al., 1992; Alexeeff et al., 1993; Barnes et al., 1995; Collins et al., 2004; Collins et al., 2005; Starr et al., 2005; Alexeeff et al., 2006; Brown et al., 2006).

Therefore, OEHHA typically uses a 5% response rate as the default for determination of the BMC from quantal animal data (Fowles et al., 1999).

Other response rates may be selected if the data indicate that this is appropriate; for instance, in BMC determinations using human data, a case-by-case analysis is required. Thus, large epidemiological studies examining a relatively severe endpoint such as clinical disease may support the use of a 1% response criterion, as in the case of the chronic REL recently developed for respirable crystalline silica (Collins et al., 2005). In the case of a steep dose-response relationship, the selection of response rate is less influential on the final value. For acute lethality studies, 1 and 5% response rate benchmark concentrations differed, on average, by less than 2-fold from the respective NOAEL (Fowles et al., 1999).

Various criteria have been proposed for selecting an appropriate benchmark response rate for continuous data such as body weight, blood cell numbers, and levels of enzyme activity (U.S. EPA, 1995; Gaylor et al., 1998; Crump, 2002; Sand et al., 2003). One criterion is statistical confidence, e.g., criteria based on some multiple (1.0 - 3.0) of the standard deviation of the reported measurements, either above or below the mean, particularly in controls or low-dose groups. A standard deviation of 2.33 from the mean identifies values at the first and 99th percentiles, extreme values even if not adverse. If values greater than the 98th to 99th percentile are abnormal, then a concentration that changes the mean by one standard deviation yields roughly 10% excess risk in subjects in the abnormal range (Crump, 1995). A second criterion is scientific judgment as to what constitutes a biologically relevant perturbation in a measured parameter, such as one that exceeds the likely range of physiological compensation. Some clinical guidelines are generally accepted as cutoff points although they are not necessarily thresholds. These might include:

(1) reduction in lung function (>20% of expected forced expiratory volume (FEV)) as clinically significant

(2) a carboxyhemoglobin level of 1.1 to 1.3%, and

(3) a pesticide worker's blood cholinesterase level less than 80% of the individual's baseline level.

The choice of an appropriate benchmark criterion for continuous data is currently based on the particular nature of those data, including supporting information on severity of the effect and possible mechanisms of repair or compensation, rather than on any overall policy-based guidance. In the development of the chronic REL for carbon disulfide, OEHHA used as the benchmark response rate a five percent reduction in peroneal motor conduction velocity (BMC_{05}), a mild effect and definitely within the range of normal variation. In some cases, population shifts in a continuous variable such as FEV_1, blood pressure, birth weight, thyroid hormone levels (Ting et al., 2006) or IQ (*e.g.* effects of lead as reported by Lanphear et al. (2005) may result in pushing more individuals into a high-risk category, and thus small shifts can be considered adverse.

4.4.1.1.2 Selection of Confidence Limits

The benchmark dose or concentration is selected by fitting an assumed dose-response curve to the observed response data. Mathematical curve fitting of this type necessarily involves recognition of uncertainty and variability in the input data. Fitted curves or interpolated values are generally described in terms of both maximum likelihood estimates (MLE) and confidence bounds on these estimates. Variation around the predicted values is generally assumed to follow a χ^2 (chi-squared) distribution, and the χ^2 statistic is used as a criterion of fit quality and in deriving "p" values and confidence limits on estimates. The 95% lower confidence limit (LCL) of the concentration at the chosen benchmark response rate or level is generally used as the BMC, rather than the MLE. This is preferred since it takes into account sources of uncertainty intrinsic to the source data, including the variability of the test population and the number of subjects in the study. This provides an incentive for the generation and use of higher quality data, unlike the NOAEL/LOAEL methodology, which makes no explicit quantitative allowance for uncertainty in the underlying data. Use of the 95% LCL in a benchmark calculation also takes into account the quality of fit for the dose-response curve. The Benchmark Dose Workshop (Barnes et al., 1995) recommended using the 95% LCL in benchmark dose calculations. With robust data sets the 90, 95, and 99% LCLs are close to each other and to the MLE (Sand et al., 2002).

4.4.1.1.3 Selection of Models to fit the Dose-Response Curve

It is important to select an appropriate mathematical model for the type of data used for benchmark concentration calculations (Filipsson et al., 2003). The U.S. EPA's Benchmark Dose Software (BMDS) contains a variety of models (U.S. EPA, 2006a).

For dichotomous data, the models include the following:

(1) gamma distribution,
(2) logistic,
(3) multistage,
(4) probit,
(5) quantal linear,
(6) quantal quadratic, and
(7) Weibull models.

The quantal linear and the quantal quadratic are special cases of the Weibull model in which the exponents are one and two, respectively. The probit and logistic models can be run using either the dose or the logarithm of the dose. These models are useful for data where the subjects at each level of exposure did or did not experience a specific adverse effect such as eye irritation, liver enlargement, or an impaired nervous system (based on passing or failing a specific test). For nested dichotomous data, such as found in animal developmental data in which individual offspring are nested in litters, the models available are:

1) NLogistic (logistic nested),
(2) NCTR (National Center for Toxicological Research), and
(3) Rai & Van Ryzin (after the authors who described the model).

For continuous data such as body weight, enzyme activity level, blood cell counts, IQ, and nerve conduction velocity, the models available are the:

(1) linear, (3) power, and

(2) polynomial, (4) Hill.

To date the models most used by OEHHA are those for dichotomous data. Usually each model is fit to a dose-response data set of the most sensitive endpoint available, and both the MLE and the lower 95% confidence bound benchmark confidence level ($BMCL_{05}$) of the effective dose (ED_{05}) are derived from each model. When the number of subjects is very large as in the case for some occupational epidemiological exposures such as respirable, crystalline silica, the MLE_{01} and $BMCL_{01}$ (a one percent benchmark) can be determined (Collins et al., 2005). The models that give an acceptable fit ($p \geq 0.10$ by χ^2) are further examined. Some models may fit the entire range of the data equally well by the χ^2 test, but one may be better than another in describing the shape of the dose-response curve at the lower end of the dose range, which is critical in defining a benchmark such as $BMCL_{05}$. If more than one model gives an acceptable fit to the data, then some judgment is used in balancing a model's goodness of fit (as possibly indicated by a much higher p value or as determined visually from the plotted curve) versus the level of health protection provided by the $BMCL_{05}$ derived using that model. From the perspective of protecting public health, the lowest value of the $BMCL_{05}$ from a model having an acceptable fit might be taken. However, with certain data sets, some models (including the often used log-probit model) may indicate an MLE which is very far from the BMCL value (Murrell et al., 1998). For well-fitting models, the BMCL is seldom less than one third of the corresponding MLE, unless the overall precision of the data is poor. The analyst should also beware of attempts to fit complex models to data sets with insufficient precision to specify all the model parameters accurately (U.S. EPA, 2006a). Thus there must be allowance for professional judgment by toxicologists and statisticians. These considerations are discussed in the summary for each REL derived by the benchmark method.

4.4.1.2 Use of No-Observed-Adverse-Effect-Levels (NOAEL)

A NOAEL in a human or animal study may be defined as an exposure level with no biologically or statistically significant increase in the frequency or severity of adverse effects among the exposed group relative to a control group. The NOAEL must be tempered by appropriate statistical interpretation. A NOAEL is sometimes incorrectly viewed as an estimate of a threshold level for adverse effects. However, a NOAEL could be associated with a substantial (1-20%) but undetected incidence of adverse effects among the exposed experimental group or population. This is so because only a subset of individuals from the population has been observed, and because the experiment may not have been designed to observe all adverse effects associated with the substance. Therefore, one may not safely conclude that the study concentration or dose is not associated with any adverse effects (U.S. EPA, 1994a). Alternatively, a NOAEL could be many-fold lower than a true population threshold due to study design and dose spacing (Gaylor, 1992; Leisenring and Ryan, 1992).

The U.S. EPA (1994a) determined that a NOAEL not associated with any biological effect (a "no-observed-effect-level" or NOEL) identified from a study with only one dose level is unsuitable for derivation of an RfC for chronic exposure. Because there is a limited availability of multi-dose studies for the variety of chemicals considered, OEHHA may use a NOAEL

without an associated LOAEL (lowest-observed-adverse-effect-level) identified in the same study (termed a free-standing NOAEL) in deriving an acute REL, but only if there are no other suitable studies, and so long as the overall health hazard data (including any case reports or studies with shorter durations) for that substance are consistent with the NOAEL study.

4.4.1.2.1 Derivation of Reference Exposure Levels (RELs) Using NOAELs

Reference Exposure Levels are derived by the application of UFs to the NOAEL for a critical endpoint. The application and values of UFs, which are similar for the NOAEL and BMC approaches, are described below.

$$NOAEL / UF = REL$$

Prior to the determination of a NOAEL, the literature is examined to identify the relevant endpoints. Toxicological endpoints are evaluated to determine the most sensitive effect (occurring at the lowest exposure level), and a dose-response relationship is determined. The most sensitive adverse effect of relevance to human health (termed the "critical effect") is used as the basis of the REL, and as noted above this is usually a mild adverse effect.

4.4.1.2.2 Use of Lowest-Observed-Adverse-Effect-Levels (LOAEL)

A Lowest-Observed-Adverse-Effect-Level (LOAEL) may be defined as the lowest exposure level with a biologically and/or statistically significant increase in the frequency or severity of adverse effects among an exposed population relative to a control group. The highest exposure concentration which results in biological effects that are not considered adverse may be termed the lowest-observed-effect-level (LOEL); this is identical to the NOAEL (U.S. EPA, 1994a). If a NOAEL is not identifiable from the literature, it must be estimated from the lowest exposure concentration reported to produce the adverse effect; this is the lowest observed adverse effect level (LOAEL). An UF is applied to the LOAEL to estimate the NOAEL. Use of a LOAEL should be a last resort; use of the BMC methodology is preferable whenever possible. Where experimental data showing intermediate response rates are very limited, this may place constraints on the benchmark response rate and curve-fitting model used. However, even in these cases the overall uncertainty is likely to be both smaller and better quantified by the BMC methodology than by a LOAEL-based derivation.

If there exist multiple, non-identical NOAELs and LOAELs for the same compound and critical effect, the study of the best quality reporting the highest value for a NOAEL (preferred) or the lowest value for the LOAEL is used for the development of RELs.

4.4.2 Extrapolation and Uncertainties in the Database

A BMC or observed NOAEL may be a concentration where adverse effects are observable rarely, or not at all, in a specific study, but this level may not be without effect among the general human population, which includes individuals who are more sensitive than average, or who may receive repeated or extended exposures. In development of a REL, systematic extrapolation methods must be used to relate the dose-response characteristics observed in the experimental (or epidemiological) data to those expected for the general human population in a community

exposure situation. The REL must also address, and where possible quantify, uncertainties in the available data and variability in the target population. These issues are accounted for by means of explicit extrapolation models where these are available and appropriate input data can be obtained. Where these explicit models are unavailable, UFs have been used extensively with human or animal toxicity data to estimate "safe" or "acceptable" exposure levels for humans.

Extrapolation methods are used by OEHHA in deriving RELs to account for exposure duration adjustments and discontinuity, interspecies differences in exposure and pharmacokinetics, and expected differences among members of the target human population (e.g., differences between adults and children). Extrapolation methods are based on identification of measurable attributes that are judged to be relevant to addressing an area of concern, and incorporation of these data into, ideally, a mechanistic model, or (failing an established mechanistic model) an empirical mathematical model of the exposure and toxicological response.

4.4.3 Types of Uncertainty and Variability

Model-based extrapolation procedures or, where these are unavailable, UFs are used by OEHHA in deriving RELs to account for:

(1) the magnitude of effect observed at a LOAEL compared with a NOAEL (Dourson and Stara, 1983; Mitchell et al., 1993);

(2) for chronic RELs, the potentially greater effects from a continuous lifetime exposure compared to a subchronic exposure (Lehman and Fitzhugh, 1954; Bigwood, 1973; Dourson and Stara, 1983).

(3) the potentially greater sensitivity of humans relative to experimental animals not accounted for by differences in relative inhalation exposure (Vettorazzi, 1977; Dourson and Stara, 1983);

(4) the potentially increased susceptibility of sensitive individuals, for example due to inter-individual variability in response (Vettorazzi, 1977; Hattis, 1996a; Ginsberg et al., 2002; Miller et al., 2002; Dorne and Renwick, 2005a) and

(5) other deficiencies in the study design (Lehman and Fitzhugh, 1954; Bigwood, 1973; Dourson and Stara, 1983; NRC, 1993; U.S. EPA, 1993).

The use of UFs for determining "safe" or "acceptable" levels has been discussed extensively in the toxicological literature (Vettorazzi, 1977; NRC, 1977-1987; Dourson and Stara, 1983; Alexeeff et al., 1989a; Alexeeff and Lewis, 1989b; U.S. EPA, 1994a; Dourson et al., 1996).

As noted above, UFs are used when insufficient data are available to support the use of chemical-specific and species-specific extrapolation factors. In this document five UFs will be described:

(1) LOAEL uncertainty factor – UF_L;

(2) subchronic uncertainty factor – UF_S;

(3) interspecies uncertainty factor – UF_A;

(4) intraspecies uncertainty factor – UF_H, and

(5) database deficiency factor - UF_D.

Historically, UFs have most often been order-of-magnitude factors, indicating the broad level of uncertainty in addressing the area of concern. More recently, OEHHA and the U.S. EPA have used intermediate UFs, usually having a value of 3 (the rounded square root of 10) in areas estimated to have less residual uncertainty (U.S. EPA, 1994a). In special cases, other UF values may be considered appropriate. While the actual value of √10 is 3.16, in practice, a single intermediate UF is calculated as 3 rather than 3.16, while two such intermediate UFs cumulate to 10. Thus, cumulative UFs could equal 1, 3, 10, 30, 100, 300, 1000, or 3000.

4.4.4 Application of Mechanistic Data in Interspecies and Intraspecies Extrapolation

It is necessary to determine what (if anything) is known of the mechanism of action of the toxic agent as a first step in evaluating which extrapolation methodologies or UFs should be applied to the point of departure (BMC, NOAEL or LOAEL) for the extrapolation to estimate a safe level for human exposure. This will determine whether there are data to support a mechanistic model, or if a more generic model would be applicable. If the information necessary to construct a model is lacking, then the UF approach is necessary. The size of the UFs used is based on information about variability in response to broad classes of toxic agents, tests systems and target populations, and is necessarily a policy choice. It may nevertheless be possible to narrow the bounds of the uncertainty if specific features such as the site of action (either the respiratory system or other point of first contact, as used in the HEC approach, or a systemic target), and the general type of toxic response can be identified.

Extrapolation generally will be necessary to cover two basic areas of difference between the test system (e.g., animals in a toxicological experiment) and the target human population:

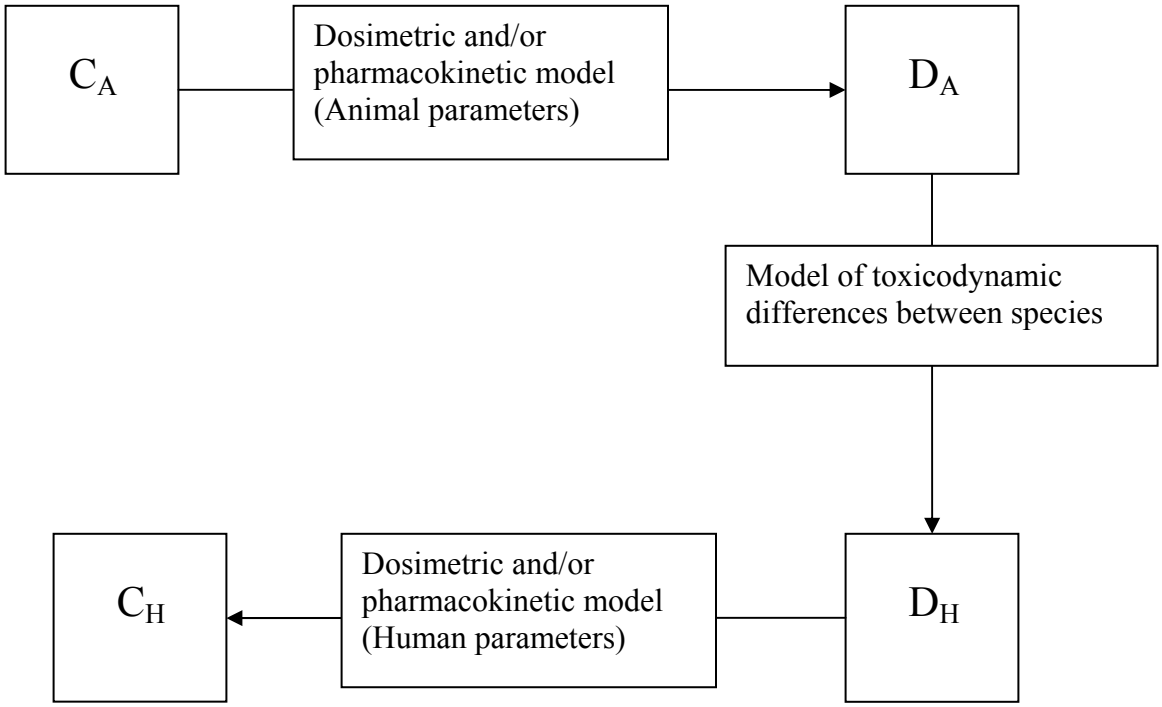
a) differences in absorption, distribution, metabolism and excretion (dosimetric and toxicokinetic adjustments), and

b) differences between species or individuals in their sensitivity to the toxic material (either the original substance or a metabolite) at the site of its action (toxicodynamic adjustments).

As will be described in greater detail below, both these types of difference need to be considered either by means of a model, or by an UF, both for extrapolation from the test species (usually a rodent) to the human, and to allow for the likely range of inter-individual variation among members of a human population which is diverse in age, sex, genetic background, health status, diet, and lifestyle.

A general scheme for extrapolation between test and target species is shown Figure 4.2 below.

FIGURE 4-2. INTERSPECIES EXTRAPOLATION

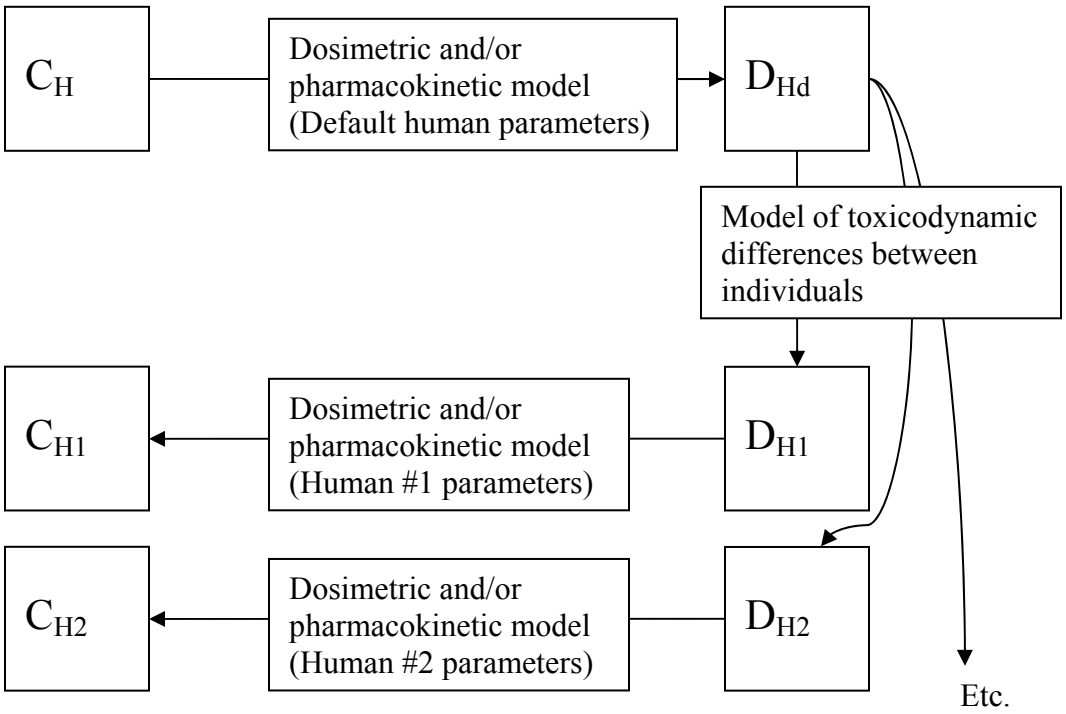


C_A = Applied concentration (e.g., BMC, LOAEL or NOAEL) in an animal experiment.
D_A = Dose of compound or active metabolite at site of action in animal.
D_H = Similarly effective dose of compound or active metabolite at site of action in a human.
C_H = Human equivalent applied concentration.

In this diagram and that which follows, the term "model" is used in the formal sense rather than implying that a detailed quantitative model of the transition is actually available. In practice such a quantitative model is usually not available, or may be incomplete, in which case the uncertainty caused by this deficiency needs to be recognized by inclusion of an UF. As will be described in Sections 4.4.7.2.1 and 4.4.8.2.1 below, detailed models are sometimes available to describe interspecies and intraspecies differences in pharmacokinetics. Unfortunately at this time there are few cases where quantitative pharmacodynamic models are available, so these extrapolations almost always utilize UFs to account for pharmacodynamic differences within humans and between species. Model parameters may be defined as single values appropriate to the test species and the default human, or as distributions representing uncertainty in the values of these parameters. In principle, variability in the values of key parameters in the animal models could also be represented by distributions, although in practice such variation is usually small due to the standardized genotype and environment of laboratory animals.

A similar scheme (Figure 4-3) may be applied in considering extrapolation from the default adult specified in the interspecies extrapolation to other specific individuals, or (when a quantitative model is available, by replacing defined single parameter values with distributions) to a range of such individuals encompassing the expected extent of variation in the target population (intraspecies extrapolation).

FIGURE 4-3 INTRASPECIES EXTRAPOLATION



C_H = Human equivalent applied concentration (default human adult).
D_{Hd} = Dose of compound or active metabolite at site of action in a default human.
D_{H1} = Similarly effective dose at site of action in human #1.
D_{H2} = Similarly effective dose at site of action in human #2.
C_{H1} = Equivalent applied concentration in human #1
C_{H2} = Equivalent applied concentration in human #2

If C_H is the human equivalent concentration of an effect threshold such as the NOAEL or $BMCL_{05}$ (adjusted for duration and for any other uncertainties), and a sufficient number of human cases (*i*), or an appropriate range of a distribution, is considered so that all but rare hypersensitive individuals are represented, then the REL is set at the level of the lowest individual equivalent concentration, or at an appropriate lower bound on the distribution of C_{Hi} values. In order to provide a REL, which is protective of children's health, it is necessary that at

least some of the cases considered, or distribution values included in the models, represent children.

A selection of useful model types and extrapolation procedures is given below. It should be noted that this selection is exemplary rather than prescriptive, and that the models used in any particular case will be determined by the availability of data and mechanistic information for that toxic agent and type of effect.

4.4.5 Extrapolating from LOAELs to NOAELs

The use of the BMC methodology allows derivation of a point of departure suitable for REL determination even when an actual NOAEL has not been observed in the experiment. Since this approach uses an empirical model fit to the actual experimental data over the range of doses examined, it is the preferred way to address the uncertainty inherent in deriving a REL from such an experiment. When this model-based extrapolation is not possible due to limitations of data quality or reporting, an observed LOAEL may be used as the basis of the REL. The UF approach is then used to estimate a health-protective level. This is a last resort, when data are entirely unsuitable for a benchmark dose analysis (e.g., all dose groups except control show 100% response rate). It should be recognized that use of the LOAEL methodology fails to reveal or quantify the actual uncertainty and variability contained in the source data, and can be influenced by the study design. A one-to-ten-fold uncertainty factor (UF_L) has been proposed to account for the higher health risk potentially associated with a LOAEL compared with use of a NOAEL (U.S. EPA, 1994a). Historically, a factor of 10 has been used in U.S. EPA and OEHHA assessments. This UF_L is applied to estimate a threshold level (NOAEL) from the LOAEL:

$$LOAEL/UF_L = NOAEL$$

The relationship between LOAELs and NOAELs for acute, and some chronic, exposures has been examined by various authors. The effectiveness of a 10-fold LOAEL to NOAEL UF was confirmed for several data sets with inhalation exposure (Gift et al., 1993; Kadry et al., 1995; Alexeeff et al., 1997; Alexeeff et al., 2002) and oral exposure (Dourson and Stara, 1983). Mitchell et al. (1993) evaluated the LOAEL to NOAEL ratio for 107 subchronic and chronic inhalation studies. They reported that 15 of the 107 studies had LOAEL to NOAEL ratios of 10 or greater. Alexeeff et al. (2002) evaluated 215 acute inhalation studies for 36 chemicals and reported that the range of LOAEL to NOAEL ratios for mild effects had 90th and 95th percentiles of 5.0 and 6.3, respectively. In contrast, the ratio of the LOAEL for serious effects to the NOAEL for all effects had 90th and 95th percentiles of 12 and 40, respectively (Alexeeff et al., 1997). Kadry et al. (1995) showed that among a small data set (four chemicals) LOAEL to NOAEL ratios were less than 5. However, where only a LOAEL has been observed, the magnitude of the difference between the observed LOAEL and the hypothetical NOAEL is uncertain.

On the basis of these data and following earlier precedents, OEHHA considers a 10-fold UF_L for extrapolation from a LOAEL to a NOAEL to be protective when applied to all types of studies. However, OEHHA has also attempted to delineate situations where UFs less than 10 could be used in the REL development process. The use of an UF less than 10 may be appropriate under certain circumstances, but application of UFs less than 10 has sometimes been somewhat

subjective, and guidance as to when it is appropriate is lacking. Consequently, OEHHA has developed criteria for use of an intermediate UF. These criteria are based primarily on data from acute exposures; the data available on chronic exposures are limited, but do not imply that the relationship between chronic LOAELS and NOAELS is substantially different from that for acute levels. When the effect is of low severity, the exposure is likely to be relatively nearer to the NOAEL. Conversely, more severe effects indicate the likelihood of a higher LOAEL to NOAEL ratio.

Following exposure, health effects of varying severity may be observed, depending on the extent of exposure, or dose, and the toxic properties of the compound. Although the relationship between exposure and health outcome is a continuous one, effects may be categorized into discrete severity levels. The purpose of acute RELs for the preparation of risk assessments under the Hot Spots Program is to evaluate impacts of short-term exposure from non-emergency releases. Thus the RELs are generally protective against mild adverse effects, although in a few cases the most sensitive endpoint, which was used in development of the REL, is severe (e.g., a reproductive/developmental endpoint).

Mild effects are defined as those with severity of grade 5 or below, as described in Table 4.4.1. This was originally developed to describe effects from acute exposures, but there is no obvious impediment to applying this approach to effect of chronic exposures also. A reasonable analysis would certainly treat irreversible effects, or those involving significant cumulative tissue damage (e.g., necrosis) as severe. Based on an analysis of LOAELs and NOAELs reported in various toxicological studies, we found that when extrapolating from a LOAEL to a NOAEL for mild (sensory irritation) effects, UFs less than 10 are justified (Alexeeff et al., 1997; Alexeeff et al., 2002). In the case of the mild adverse effect, an analysis by Alexeeff et al. (1997) of LOAEL to NOAEL ratios for over 100 datasets indicated that the 95th percentile of that ratio is 6.2. The distribution was skewed to the right; for some chemicals, a UF of 10 may not be adequate. OEHHA has chosen a UF of 6 to extrapolate from the LOAEL to the NOAEL where the effect is mild, based on this analysis. Recommended default values of UF_L for acute, eight-hour and chronic REL derivations are therefore as follows:

(1) Where the observed effect level used as the basis of the REL is a NOAEL or equivalent benchmark, the value of UF_L is 1.

(2) For a LOAEL where the observed effect is mild (for acute exposures, U.S. EPA grade 5 or below), the value of UF_L is 6.

(3) For a LOAEL where the observed effect is moderate to severe, the value of UF_L is 10.

These default values may be replaced by more specific values where appropriate data are available (e.g., for specific toxicological endpoints or chemical classes). However, the use of a LOAEL as the basis of a REL is to be avoided wherever possible, by using data sets in which a NOAEL is also observed or, preferably, by applying the BMC methodology to a study where a range of response levels with increasing dose is measured.

TABLE 4.4.1. RELATIONSHIP BETWEEN EFFECT CATEGORIES AND SEVERITY.

Severity Level	Effect Category	Effect
0	NOEL	No observed effects.
1	NOAEL	Enzyme induction or other biochemical change (excluding signal transduction effects), consistent with possible mechanism of action, with no pathologic changes, no change in organ weights, and no downstream adverse developmental effects.
2	NOAEL/LOAEL	Enzyme induction and subcellular proliferation or other changes in organelles, consistent with possible mechanism of action, but no other apparent effects.
3	NOAEL/NOAEL	Hyperplasia, hypertrophy, or atrophy, but without changes in organ weight.
4	NOAEL/LOAEL	Hyperplasia, hypertrophy, or atrophy, with changes in organ weight.
5	LOAEL	Reversible cellular changes including cloudy swelling, hydropic change, or fatty changes.
6	(LO)AEL	Degenerative or necrotic tissue changes with no apparent decrement in organ function.
7	(LO)AEL/FEL	Reversible slight changes in organ function.
8	FEL	Pathological changes with definite organ dysfunction which are unlikely to be fully reversible.
9	FEL	Pronounced pathological change with severe organ dysfunction and long-term sequelae; developmental dysfunction including biochemical changes affecting signal transduction that result in developmental defects or dysfunction.
10	FEL	Life-shortening or death.

(Adapted and expanded from U.S. EPA, 1994a)

NOEL – no-observed-effect-level; NOAEL – no-observed-adverse-effect-level; LOAEL – lowest-observed-adverse-effect-level; AEL – adverse-effect-level; FEL – frank-effect-level.

4.4.6 Extrapolating from Study Duration to REL Reference Period

The target reference period for development of a REL is one hour (single or infrequent exposure) for acute RELs, eight hours with potential for repeat exposures for the eight-hour RELs, and lifetime/annual average for the chronic RELs. Acute RELs are typically based on data from short-term exposures of a few minutes to a few hours, and eight-hour or chronic RELs typically involve data from extended repeat-dosing studies. However, the experimental duration, or exposure period in a human study, is not generally the same as the REL reference period. Scaling procedures may therefore be required to extrapolate from the specific duration (and extent of repetition) of the studies to the REL reference period. Since these are specific to the type of REL being developed they are described in the subsequent sections dealing with procedures specific to the individual REL types.

The dose-response for most toxicological processes is assumed to follow some form of dose-time integral over moderate periods of exposure. For medium-term adjustments in repeat-exposure animal studies such as six to eight hours to twenty-four, or five days a week to seven, a simple concentration multiplied by time (C x T) dependence, often referred to as Haber's Law, is assumed, so these results are adjusted by simple proportion, as described in Section 7.2.1.

The default approach to extrapolating from subchronic to chronic exposures used by OEHHA (Section 7.2.2) for development of chronic RELs is to use a 1 to 10-fold subchronic uncertainty factor (UF_S) for subchronic exposures (Table 4.4.1). Chronic studies are those where the exposure duration is 12% or more of the expected lifetime of the test species, while subchronic studies are repeat-dosing studies shorter than this but longer than standard sub-acute protocols. The same adjustment is used for human studies where the average exposure duration is less than 12% of lifetime (70 years). For exposures less than 8% of expected lifetime a 10-fold UF_S is applied, while for exposures from 8 to 12% of expected lifetime a 3-fold UF_S is applied. Where exposures are longer than 12% of expected lifetime there is no adjustment, i.e., $UF_S = 1$.

For shorter study periods such as those of a few minutes to hours that are considered in development of acute RELs and some eight-hour RELs, the basic C x T dependence is modified by means of exponents. Most commonly, an expression of the form C^n x T is used to reflect acute toxic responses where concentration is a more important determinant of response than duration over the time period of the observation. Application of this modified Haber's Law procedure in development of acute RELs is described in Section 5.6.1.

4.4.6.1 Exposure Duration REL Adjustments for Developmental Toxicants

Historically, duration adjustment of inhalation exposures for developmental toxicity studies has not been done (U.S. EPA, 2002). Unlike subchronic and chronic toxicity studies, in which months or even years of exposure may be needed before tissue damage becomes evident, developmental toxicity is frequently the result of exposure during a small window of time during gestation in which exposure may only be on the order of hours during a critical stage of development. Because the timing and duration of the sensitive period of gestation is usually unknown, the standard experimental protocol is to expose pregnant animals for several hours per day over several days during gestation in order to increase the power of the study to detect an effect. As a result, time extrapolation to the REL must take into account two principal

toxicokinetic issues to prevent, in particular, underestimation of developmental toxicity - peak tissue concentration and total tissue dose (e.g., area under the concentration-time curve (AUC)). Instances of developmental toxicants that operate predominantly by one or the other toxicokinetic factors have been observed. For example, prenatal exposure of mice to short, high exposures of ethylene oxide on day 7 of gestation was found to cause more adverse developmental effects than mice exposed to the same C x T multiple but at longer, lower exposures (Weller et al., 1999). Alternatively, pregnant rats administered all-trans-retinoic acid indicated that AUC, and not maximum plasma concentrations, was the most appropriate pharmacokinetic marker of developmental toxicity (Tzimas et al., 1997). The following procedures are designed to be health-protective even in the case where a developmental effect is the result of a possibly very brief sensitive period during a single day of exposure to the toxicant (U.S. EPA, 2004).`

4.4.6.1.1 Developmental REL Duration Adjustment from Shorter to Longer Exposures

When the principal toxicokinetic process involved in the developmental toxicity of a non-accumulating chemical is unknown, the U.S. EPA Technical Panel recommends that duration adjustment procedures from discontinuous to continuous exposures be based on equivalent multiples of concentration (C) and duration (T) for inhalation developmental toxicity studies as it is used for other health effects from inhalation exposure (U.S. EPA, 2002). This C x T approach favors a health protective overestimation of risk when adjusting the exposure duration from a shorter period to a longer period of exposure, as has been shown experimentally in dose-rate studies of developmental toxicants (Weller et al., 1999). The pharmacokinetic basis for this duration adjustment assumes that the total tissue dose during a single-day exposure period is the critical quantity in determining the level of response and ensures that the AUC, as well as the peak tissue level, will not be increased in the duration adjustment. Correspondingly, OEHHA will use this time adjustment procedure when estimating a chronic REL based on a developmental study. The default approach for duration adjustment of a developmental endpoint from discontinuous exposure to chronic continuous exposure is the same as that used for a chronic toxicity duration adjustment, and can be summarized as:

C_{AVG} = (C_{OBS}) x (H hours / 24 hours) x (D days per 7 days);

where C_{AVG} is the time-weighted average concentration, and C_{OBS} is the observed concentration.

Time extrapolation to an eight-hour REL must also take into account pharmacokinetic processes affecting a developmental endpoint, from either a single eight-hour exposure or multiple daily eight-hour exposures during gestation. Thus, estimation of the eight-hour REL will also use the daily average C x T time adjustment when extrapolating from a shorter exposure time to an eight-hour REL. The same daily average C x T adjustment should also be used when an acute REL is based on a developmental study involving exposure of animals for less than an hour for one or more days during gestation.

As more information becomes available on PBPK modeling of developmental toxicants for interspecies extrapolation from the exposed animal species to humans, modeling of blood and tissue levels may confirm the C x T adjustments on the REL exposure durations to ensure they do not exceed the peak tissue concentration or total tissue dose at the NOAEL.

4.4.6.1.2 Developmental REL Duration Adjustment from Longer to Shorter Exposures

For acute REL development, time duration adjustment will often require extrapolation from multi-hour exposure to the 1-hour exposure duration of the acute REL. Dose-rate exposure studies have shown that a C x T approach from a long exposure duration to a shorter exposure duration could underestimate the response of developmental toxicants (Weller et al., 1999). To avoid underestimation of risk when the pharmacokinetic nature of the developmental toxicant is unknown, OEHHA recommends no duration adjustment on the exposure concentration when extrapolating down to a one-hour exposure. This procedure primarily protects against higher peak tissue concentrations that would occur if a C x T time adjustment was applied. Preferably, the acute studies used as the basis of an acute REL would be those with exposure duration nearest one hour, in order to reduce the uncertainty of this approach. This approach would also apply to eight-hour RELs in which the primary study used daily exposures greater than eight hours (i.e., no time extrapolation would be applied).

4.4.6.1.3 Duration Adjustment for Bioaccumulating Developmental Toxicants

An additional pharmacokinetic issue to consider involves chemicals in which discontinuous daily exposures may take one to two weeks of gestational exposure before tissue saturation occurs. Many developmental studies begin exposures following conception. Conceivably, the critical point of gestation for developmental effects may have passed before maximal fetal/maternal blood levels were attained during the exposure period. For example, the aromatic hydrocarbon 1,2,4-trimethylbenzene shows a gradual increase in prior-to-shift blood levels (and the AUC) in humans over a 5-day period with daily eight-hour exposures (Jarnberg and Johanson, 1999). Ideally, maternal exposures would occur prior to the beginning of gestation so tissue saturation at a given exposure concentration is already present when development begins. Multi-generation studies often expose animals for at least several weeks prior to mating and could resolve this concern. In lieu of multi-generation studies and studies that started exposure prior to gestation, a modifying UF may be considered for those chemicals that slowly accumulate to maximal tissue levels during gestational exposure. Alternatively, a fractional adjustment of the exposure level can be made if sufficient pharmacokinetic data are available to identify the time to tissue saturation and tissue saturation levels. This pharmacokinetic adjustment would prevent exceedance of peak tissue levels or total tissue dose at critical time points in fetal development to account for bioaccumulation deficiencies in short-term developmental exposure studies.

For major bioaccumulators such as dioxins and some metals, developmental exposure studies in which exposure occurred only during gestation is not sufficient for establishing eight-hour or chronic RELs based on developmental toxicity. These types of toxicants can accumulate in body tissues over extended periods of time prior to gestation, leading to very high maternal body burdens that may be detrimental to the fetus during gestation. Lack of sufficient chronic exposure and multi-generation studies and lack of adequate pharmacokinetic modeling information that can predict body tissue burdens may require application of a modifying UF for pharmacokinetic deficiencies in calculating the REL.

4.4.7 Accounting for Potentially Greater Human Susceptibility

4.4.7.1 Introduction

Greater sensitivity of humans compared to animal test species for a variety of toxicological endpoints have been shown (Dourson and Stara, 1983). A well-known example is teratogenesis by various agents including thalidomide (Brown and Fabro, 1983). In general, interspecies UFs are applied to the animal study results to account for potentially greater human susceptibility (see Section 4.4.7.3). However, a preferred approach to interspecies extrapolation is to employ chemical-specific kinetic models to assess species differences in relevant tissue dosimetry. If chemical specific models are not available, generic approaches such as the human equivalent concentration (HEC; the air concentration of an agent that induces the same magnitude of toxic effect in humans as that seen in experimental animals) and/or an animal to human uncertainty factor (UF_A) may be applied. As described above (Section 4.4.4), this factor may be regarded as consisting of toxicokinetic and toxicodynamic factors, which may be considered separately where explicit models are available to describe some aspects of the extrapolation, especially toxicokinetics. Differences in acute behavioral toxicity of toluene in rats and humans are partly described by a toxicokinetic model: there are residual differences in sensitivity between species based on the tissue dose levels which might relate to actual sensitivity differences at the cellular level, or to differences in the sensitivity and comparability of the tests used in the two species (Benignus et al., 1998; Bushnell et al., 2006).

4.4.7.2 Kinetic Modeling in Interspecies Extrapolation

As part of the scientific basis for this update of the risk assessment guidelines, OEHHA conducted a pilot investigation of the application of physiologically based pharmacokinetic (PBPK) modeling to dosimetric adjustments in noncancer risk assessment. The aim was to derive alternate dosimetric adjustment factors (DAFs) or human equivalent concentration (HEC) factors based on metrics of internal dosimetry such as peak concentrations (Cmaxs) and areas under the blood or tissue concentration x time curves (AUCs). The chemicals selected for this pilot study were: ethylbenzene, vinyl chloride, toluene, styrene/styrene oxide, naphthalene/naphthalene oxides, and formaldehyde. All of these chemicals occur in outdoor and indoor air and have some prior PBPK model availability for rat or human. Initial comparisons were limited to rat/human conversions for adults and immature animals/children. In addition, since the overall objective is to improve the scientific basis for predictive toxicological criteria for air pollutants the investigation also included a series of straight chain aliphatic aldehydes: $CH_3(CH_2)_nCHO$ (n = 0 to 8). Several aliphatic aldehydes have been observed in outdoor (Uebori and Imamura, 2004) and indoor (Arcus et al., 1995) air sampling or are known to originate in building materials or furnishings.

4.4.7.2.1 PBPK Models

The type of PBPK model used by OEHHA is dependent on the physicochemical characteristics and toxicokinetic properties of the agent in question (See Appendix E for more detail; see U.S. EPA (2006b) 2 for a general description of PBPK modeling). Broadly speaking, gaseous agents fall into one of three categories.

- Category 1 gases interact mainly at the site of contact; either the nasal or respiratory tracts (RT) as portals of entry.
    - For agents in Category 1, OEHHA used either a 4- compartment RT model of the type described by Sarangapani et al. (2004) that is similar to a 3-compartment default model of the RT recommended by Hanna et al. (2001), with uptake defined by regional mass transfer coefficients. Depending on the agent being studied, for some Category 1 gases, OEHHA used nasal models as described by Frederick et al. (1998).
- Category 2 gases have effects both locally, on the RT, and systemically.
    - For Category 2 gases, OEHHA used RT-PBPK models of the type described by Sarangapani et al. (Sarangapani et al., 2004). These models included both RT compartments and body compartments for remote distribution and metabolism as recommended by Hanna (Hanna et al., 2001).
- Category 3 gases mainly have remote systemic effects.
    - For Category 3 gases, with mainly remote effects, OEHHA used either a one-compartment or, alternatively, a two-compartment lung model as described by Evelo et al. (1993), consisting of a high-perfusion alveolar exchange compartment and a low-perfusion bronchial compartment. In some instances flow-limited model components may be augmented or replaced with diffusion-limited components based on physicochemical/kinetic properties and improved model performance (e.g., dioxin).

Particle exposures are defined mainly by air concentration ($\mu g/m^3$), size distribution including mean mass aerodynamic diameter (MMAD, μm) and geometric standard deviation (σg), breathing rate, nose versus mouth contributions, and particle solubility. The benchmark human model is the Human Respiratory Tract Model for Radiological Protection from the International Commission on Radiological Protection (ICRP, 1994). This model provides tables of deposition fractions by RT region, age, sex, breathing rate and particle size. Computer models are available to predict RT clearance for a given exposure, and particle deposition and ICRP clearance parameters, e.g., Humorap 2 (Sanchez, 2002). A more complete deposition and clearance model for humans and rats is the multiple path particle deposition (MPPD) model of The Chemical Industry Institute of Toxicology (CIIT) and the Netherlands National Institute for Public Health and the Environment (RIVM) (Anjilvel and Asgharian, 1995; Brown et al., 2005; Jarabek et al., 2005). This model provides several particle number and mass-based dose metrics, although mass/surface area metrics need to be derived from graphic outputs of deposition and user supplied regional RT surface areas (Sarangapani et al., 2003). Another advantage of this model is a number of built in human child parameters for different ages. However, this model is very complex and longer-term simulations may not run successfully. Additional particle deposition and clearance models may be much simpler and adequate in many instances (Snipes, 1989a; Snipes et al., 1989b). The main dose metric of the Snipes model is mg/lung or lung burden. Also Yu and Xu (1987) provide a deposition model description for humans, rats, hamsters and guinea pigs that may be useful in many cases.

4.4.7.2.2 HEC Adjustment

The development of reference exposure concentrations (RfCs) by the U.S. EPA (1994a) requires the conversion by dosimetric adjustment of the NOAELs and LOAELs observed in laboratory animal experiments to human equivalent concentrations (HECs) for ambient exposure conditions (U.S. EPA, 1994a). The HEC procedure estimates the concentration for human exposure, which would be equivalent to the animal exposure, by adjusting for differences in minute volume and surface area of various regions of the respiratory tract between the experimental species and humans. The conversion of animal exposures to HECs is described in detail in Appendix F and involves the use of regional deposited dose ratios (RDDRs) for particles or regional gas dose ratios (RGDRs) for gases. Category 1 gases are highly reactive and/or soluble, and they do not accumulate in the blood. For these compounds, the conversion factor usually reduces to a ratio of alveolar ventilation (AV_A) to regional surface area (RSA_A) for the animal test species, divided by the same ratio for the human (AV_H / RSA_H). Adjustments for extrathoracic (ET), tracheobronchial (TB) and pulmonary (PU) regions or the total lung can be calculated (U.S. EPA, 1994a). For pulmonary exposures to a category 1 gas from rat data, adult and child specific dosimetric adjustment factors (DAFs) may be derived as follows:

DAF $= (AV_A / RSA_A) / (AV_H / RSA_H)$

DAF (Adult) $= (120\ cm^3/min/3400\ cm^2) / (7000\ cm^3/min/633{,}000\ cm^2) = 3.19$

DAF (Child) $= (120\ cm^3/min/3400\ cm^2) / (914\ cm^3/min/21{,}500\ cm^2) = 0.83$

At the other extreme of reactivity and solubility are Category 3 gases that have predominantly systemic effects. In the default methodology, the average exposure concentration is adjusted with a RGDR that represents the ratio of the blood:air partition coefficient in experimental animals to that in humans [RGDR = $(H_{b/g})_A/(H_{b/g})_H$] (see Appendix F.1.2). Category 2 gases fall somewhere between categories 1 and 3 on the continuums of reactivity and solubility. They are moderately soluble and/or reactive and may have both local (respiratory tract) and systemic effects. In practice, in the absence of data sufficient to perform more sophisticated modeling, these compounds are treated as either Category 1 or Category 3 gases depending on their physicochemical properties and the data available for the specified toxicological endpoint.

Thus, a given rat NOAEL/LOAEL concentration would be multiplied by these factors to give human equivalent concentrations (HECs) for adults and children, respectively. The U.S. EPA derives RfCs by dividing the HECs by appropriate UFs. While this is a standard methodology, it is obvious that no chemical-specific information, other than a broad characterization of gas category, is involved. The method essentially adjusts for a potential difference in absorption based on physiological and anatomical differences between species. This methodology is described in greater detail and reviewed in Appendix F which also considers extensions necessary to allow for human intraspecies variability, including age differences.

4.4.7.3 Uncertainty Factor for Animal to Human Extrapolation (UF_A)

Where data are insufficient to allow development of an extrapolation model, the default approach has been to apply a 10-fold uncertainty factor (UF_A) to animal data based on an assumption that an average human is likely to be at most 10-fold more susceptible to the effects of the substance than experimental animals (Table 4.1.1). This is truly an "uncertainty" factor since we are unsure how humans would respond, in contrast to the animals tested, to the specific chemical. However, the UF is based on the potential for greater sensitivity of humans and the larger surface area of humans compared with experimental animals (Rall, 1969; Weil, 1972; Krasovskii, 1976; Lewis and Alexeeff, 1989). This UF methodology is in contrast to the practice used in cancer risk assessment where an allometric surface area correction and a 95% confidence interval of the slope of the dose response are used. The UF approach was used by the U.S. EPA (1994a) and recommended by NRC (1977-1987) for drinking water standards. Dourson and Stara (1983) provided limited support for the concept of a ten-fold UF. Khodair et al. (1995) showed that among a small data set (six chemicals) animal NOAEL to human NOAEL ratios were less than four. Schmidt et al. (1997) evaluated interspecies variation between human and five other animal species. Sixty compounds had human data that could be matched to one or more animal species. The animal to human ratio of 10 represented approximately the 85th percentile.

The U.S. EPA has used human equivalent concentration (HEC) extrapolation and a 3-fold UF_A for RfC derivation (U.S. EPA, 1994a). In the U.S. EPA method, this intermediate value is chosen since the HEC derivation is assumed to have accounted for the toxicokinetic part of the difference between the species. However, this HEC extrapolation addresses only some of the differences; in particular, only respiratory regional exposure and deposition of the parent compound is considered; any differences in absorption, metabolism, and elimination are ignored. The remaining 3-fold UF is to account for pharmacodynamic or response differences between the species. This modified approach was also previously used by OEHHA for derivation of chronic RELs where sufficient data were available. OEHHA continues to recommend the HEC methodology where data are insufficient to support a full PBPK model. However, it is recommended that the toxicokinetic part of the UF_A be reduced to 2, rather than 1 to reflect the presence of remaining uncertainties in toxicokinetics. In some instances, it may be appropriate to retain a larger UF_A, for example if differences in deposition between the test species and humans are known to be large. OEHHA has also examined the effect of child-specific parameters on the HEC calculation.

Where both chemical- and species-specific data are unavailable, and therefore a HEC cannot be estimated, a 10-fold UF_A is normally used. The 10-fold default UF_A would only be applied after consideration of other factors that potentially affect the validity of the default assumption. Such factors include differences between humans and the test species in absorption, distribution, and metabolism, which would serve as a basis for predicting interspecies differences in susceptibility. In some cases, data may indicate that a larger UF_A is appropriate. An exception is made for data from studies of non-human primates, where a default UF_A of $\sqrt{10}$ is used because of their similarities to humans (See Table 4.4.1).

4.4.8 Increased Susceptibility of Sensitive Individuals

4.4.8.1 Introduction

RELs are intended to protect identifiable sensitive individuals from harm due to chemical exposure. Susceptibility to harm from chemical exposure may vary among individuals due to genetic variability within the population, resulting in lower levels of protective biological mechanisms. Predisposition to increased metabolic activation or to decreased detoxification are just two examples of how genetic variability influences response to toxicants (Hattis et al., 1987; Eichelbaum et al., 1992; Grandjean, 1992; U.S. EPA, 1994a; Autrup, 2000). Additionally, susceptibility to chemical-related health effects may vary over time for the same individual due to changing factors such as age, health status, and activity level. It should be recognized that RELs may not necessarily protect individuals who may develop an idiosyncratic response, such as allergic hypersensitivity, that cannot be predicted from scientific investigation of the chemical.

Thus, sensitive individuals may include infants, children, pregnant women and their fetuses, elderly persons, those with existing diseases such as lung, heart or liver disease, and persons engaging in physical activity (U.S. EPA, 1994a). Other factors, such as acute illness or immunosuppression, may cause short-term variations in individual susceptibility. Seasonal changes in absorption and toxicity have also been noted in laboratory animals (Barton and Huster, 1987).

Healthy workers, the subject of most epidemiological studies, are often found to have lower rates of morbidity and mortality than the general population (Wen et al., 1983; Monson, 1986; Rothman and Greenland, 1998); (Rothman and Greenland, 1998) (p 119)). In studies of experimental animals, highly homogeneous (inbred), healthy strains are generally used. Such strains are likely to have much less variability in response than the heterogeneous human population. Chizhikov (1973) found that animals in poor health were more likely to experience adverse effects from chronic oral exposure to chemicals than were healthy animals.

Finally, OEHHA is required to protect infants and children in developing Reference Exposure Levels. There are a number of differences in response to toxicant by age, which in some cases, increase the susceptibility of infants and children. These are described more fully elsewhere in Section 3.1.1 and Miller (2002) and OEHHA (2001).

4.4.8.2 Pharmacokinetic Factors in Inter-individual Variability

4.4.8.2.1 Physiologically Based Pharmacokinetic (PBPK) Models of Inter-individual Variability

PBPK models can give useful predictions of how the body handles a particular chemical and its metabolites. The models address issues of internal body or tissue dosimetry, route-to-route extrapolation and, in some cases, interspecies extrapolation. To date, relatively few published models for various environmental pollutants address infant and child exposure and pharmacokinetics in a systematic fashion. This is parallel to the bulk of toxicity testing in animals, which is usually initiated in young adult animals.

However, this issue has received more attention in recent years than previously. Several authors have undertaken systematic modeling studies using child-specific physiological, biochemical and

exposure parameters for various toxicants of interest (Pelekis et al., 2001; Pelekis et al., 2003; Price et al., 2003; Clewell et al., 2004; Ginsberg et al., 2004b). These studies are summarized and evaluated in Appendix E. OEHHA has used these published results and also undertaken a series of original investigations (also described in detail in Appendix E) to explore both the feasibility of using child-specific PBPK models when the necessary supporting data are available, and the appropriate values for UFs or other limited analyses where the data required for a full chemical-specific model are not available.

OEHHA's approach to applying PBPK modeling to assess children's environmental health risks has been similar to that of Pelekis et al. (2001). We have used a case study approach using published PBPK models of selected environmental toxicants and adjusting anatomical and physiological parameters to simulate infant and child ages from newborn to 18 years. Results are then compared to those using adult models. In these models, we have scaled metabolic parameters as a function of body weight. Where possible we have focused on dose metrics involving toxicologically relevant metabolites. Initial findings by this approach were presented at the 2001 Children's Environmental Health Symposium (Brown, 2001). Of the seven chemicals studied with oral and inhalation exposures (vinyl chloride, DCM, TCE, chloroform, arsenic, butadiene, and naphthalene), three chemicals showed greater internal doses in children compared to adults: DCM, TCE, and butadiene, all via the inhalation route.

The published studies and the OEHHA case studies of PBPK modeling show clearly that infants in the first year of life are likely to show increased internal dosages via the inhalation route for a variety of agents and their metabolites. It is also apparent that the current default intraspecies UF ($UF_{A\text{-}k}$) for kinetic effects of $\sqrt{10}$ is inadequate to protect neonates and young infants from some chemicals, as further discussed below.

It is worth noting that the large majority of studies and PBPK modeling exercises involve relatively short-term exposures that represent environmental, occupational, or therapeutic scenarios. Extreme situations of short-term high exposures or very long-term low exposures were not simulated. Despite this limitation, the results are considered indicative of the unique toxicokinetics of infants and children for some environmental pollutants. As such, a revised PK UF should be broadly applicable to acute (one-hour), eight-hour, and chronic RELs.

4.4.8.2.2 Uncertainty Factor for Variability within the Human Population (UF_H)

Where data are insufficient to permit development of a reliable model, an intraspecies uncertainty factor (UF_H) has traditionally been used to account for variability within the human population. This factor is intended to account for the greater susceptibility to chemical toxicity of various sensitive subpopulations, including infants and children. Previously, OEHHA has, like the U.S. EPA generally applied a 10-fold UF_H to address variability in response among individual members of the general population (U.S. EPA, 1994a).

4.4.8.2.2.1 Contribution of Kinetic and Dynamic Factors to UF_H

The variability in human response to toxicants may result from differences in toxicokinetics and toxicodynamics. The UF_H typically used in OEHHA's risk assessment methodology is thus

considered to be composed of two sub-factors to allow for both toxicokinetic (UF_{H-k}) and toxicodynamic (UF_{H-d}) differences (Table 4.1.1).

Some studies suggested that the overall 10-fold factor was reasonable. Gillis et al. (1997) suggested, based on modeled intraspecies variability, that for chronic exposures, a 10-fold factor will protect the 85th percentile. On the other hand, more recent studies have indicated that a value higher than √10 should be considered for the pharmacokinetic component of the intraspecies uncertainty factor (UF_{H-k}), especially for substances that are bioactivated, since the enzymes involved in Phase I and Phase II reactions have shown pronounced polymorphism in many cases (Renwick and Lazarus, 1998; Hattis et al., 1999).

4.4.8.2.2.2 Infants and Children

The difference in toxicokinetics is even more pressing when considering infants and children as part of the affected population. As discussed in Section 3.1, it has been suggested that children may be both more sensitive, and more diverse, than adults, as a result of both pharmacodynamic and pharmacokinetic factors affecting toxicity. Several revisions in this version of OEHHA's risk assessment methodology are designed to address this concern. An additional 10-fold UF (presumably to account for both toxicokinetic and toxicodynamic factors) has been proposed to specifically protect children in assessments conducted for pesticides in accordance with the Federal Food Quality Protection Act (FQPA) assuming infants and children are more sensitive than adults unless data to the contrary exist. In the following discussion, the approach will be to determine an appropriate value to substitute for the default value for the two separate components of UF_H, rather than to specify additional overall UFs.

In Appendix E we have summarized the more relevant data and studies bearing on the size of the default UF to protect infants and children adequately from the adverse effects of toxic air contaminants. Obviously, these studies and data are not ideal since they rely heavily on the pharmacology literature where most drugs are administered orally and not by inhalation. In addition, drug literature frequently focuses on the parent compound rather than downstream metabolites, which are often of interest to environmental toxicologists due to their frequent involvement in toxic modes of action. Modeling of environmental toxicants also presents difficulties, the foremost being a lack of relevant metabolic parameters at various stages of human development. Infant and child metabolism of environmental agents is usually estimated by scaling from adult human or animal values, a limitation when there are qualitative as well as quantitative differences in infant vs. adult metabolism (e.g., theophylline). Table 4.4.2 summarizes the PK UF values indicated by the PBPK modeling of various test chemicals. The details of these modeling exercises are given in Appendix E (text and tables; model parameters; and model equations).

4.4.8.2.2.3 Value of UF_{H-k} to Account for Toxicokinetic Differences by Age

Based on the limited information presently available, OEHHA thinks it is appropriate to increase the default UF_{H-k} from its previous value of √10 = 3.16 in order to protect neonates and young infants from potential adverse effects of airborne toxicants. OEHHA will apply a UF_{H-k} value of 10 as a default for gases acting systemically, and for particles that involve systemic exposure via dissolution and absorption in the lung or via the gastro-intestinal tract. Gases that act solely at the

portal of entry (i.e., lung or upper respiratory tract for inhaled toxicants) without involvement of metabolic activation or other complex kinetic processes would use a UF_{H-k} of √10. These are default values applicable to acute, eight-hour and chronic RELs derived from animal studies or epidemiological studies of healthy adult populations (e.g., workers). An exception to this procedure is when an exposure level is estimated from a study that includes the assessment of a sensitive human subpopulation, where a default UF_{H-k} of 1 may be appropriate.

Because the true extent of variability is frequently unknown, there may be a portion of the population for whom the chronic RELs will not be protective. When information defining susceptible individuals is available, such data will be incorporated by means of pharmacokinetic models or adjustment of UFs as necessary to protect those individuals. Ideally, more chemical-specific data in sensitive subgroups would obviate the need for the use of a default $UF_{H.}$ Unfortunately, such data are rarely available for children (or even immature animals) with environmentally relevant toxicants.

TABLE 4.4.2. CHEMICALS STUDIED BY PBPK, GROUPED BY MODELED PK UF FOR INFANTS AND CHILDREN

PK UF ≤ √10	PK UF > √10 to 9.9	PK UF ≥ 10
Furan	MTBE	Butadiene/Butadiene
Perchloroethylene	Styrene/Styrene oxide	monoxide/Diepoxybutane
Naphthalene/Naphthalene oxides	Ethylene/Ethylene oxide	Dichloromethane
Carbon tetrachloride	Vinyl chloride	TCE and metabolites
Chloroform	Toluene	Benzo[a]pyrene
Arsenic and metabolites	m-Xylene	
Ethylbenzene*	Toluene/Xylene mixtures	
1,1-Dichloroethylene	Isopropanol	
Benzene*		
Bromochloromethane*		
Methyl chloroform*		
Diethyl ether*		

*Note that simulation results for these chemicals are not shown in the text but are based on parameters in Haddad et al. (2001) and Gargas et al. (1986) using the same approach as for toluene and xylene.

4.4.9 Uncertainty Associated with Deficiencies in the Data

In some cases, the database on an environmental chemical may be insufficient to be confident that the REL will be protective. Since this type of deficiency necessarily implies a lack of adequate data to construct an appropriate extrapolation model, it is accommodated by application of a database deficiency uncertainty factor (UF_D) (Table 4.1.1). The use of additional UFs in order to compensate for deficiencies in the chemical database is similar to the U.S. EPA modifying factor of 1 to 10 to account for data uncertainties in their procedures for calculating RfDs (U.S. EPA, 1993).

4.4.9.1 Database Deficiency Factor for Lack of Developmental Toxicity Data

Under SB 25, OEHHA is mandated to ensure that our health standards take into account the potential greater vulnerability of infants and children to chemical exposure and toxicity. Some chemicals can affect the developing fetus or development in infants and children. If studies in immature animals are lacking, it may be impossible to predict effects on developing organs and tissues. OEHHA will use a database deficiency factor (UF_D), with a default value of $\sqrt{10}$, when animal developmental studies are not available for a chemical in order to help ensure that RELs protect infants and children.

4.4.9.2 Estimation of Inhalation Effects from Oral Exposure Data

Strong weight is given to inhalation exposure-based health effects data. If adequate inhalation data are not available, oral exposure data are also considered. Both the U.S. EPA (1994a) and the NRC (1986b) support route-to-route extrapolation under certain circumstances. Route-to-route extrapolation may sometimes be inappropriate (e.g., where chemicals act at the portal of entry).

Use of oral exposure studies to develop RELs requires consideration of kinetic differences between routes, including differences in absorption across the lung versus the gastrointestinal tract. Wherever possible, such extrapolations should be undertaken using PBPK models which allow for the route-specific features of uptake and distribution of the specific chemical.

Where data are unavailable to support this approach it may be possible to use default assumptions or limited data to allow for route-to-route differences, at least in simple and straightforward cases. While route-specific differences in absorption and potency may occur, no additional UF is generally applied for non-inhalation data. Instead, attempts should be made to adjust for absorption and other kinetic differences (e.g., first pass metabolism following oral exposure) when possible. Owen (1990) found that the median inhalation/oral absorption coefficient ratio was 1.0 for 34 substances. For 32 of the substances (94%), inhalation absorption coefficients were at most 10-fold higher than oral absorption coefficients for the same substance. The two exceptions (6%) with much greater absorption by inhalation were metals with very low oral absorption (<1%): inhalation absorption of beryllium and elemental mercury was estimated to be 500-fold and 7,500-fold higher, respectively, than corresponding oral absorption. Fifteen substances (44%) were predicted to have greater inhalation than oral absorption, and 7 substances (21%) were predicted to have at least 2-fold greater inhalation than oral absorption. Pepelko (1987; 1991) provided additional evidence that differences between toxic effects following oral and inhalation exposures are generally within a 10-fold dose range. Inhalation and oral doses associated with a 25% additional risk of cancer (RRD(25)) were estimated for various chemicals. Carcinogens were more potent via oral exposure compared with inhalation exposure in 15 of 23 rodent data sets, and 20 oral exposure data sets (87%) predicted inhalation results within a 10-fold factor. Greater than 10-fold differences in potency were found in rats exposed to asbestos, hexavalent chromium (Cr^{VI}), hydrazine, or vinyl chloride.

4.4.10 Summary of Uncertainty Factors

A summary of UFs used for acute, eight-hour and chronic REL development is given in Table 4.1.1..

4.4.11 Other Extrapolation Methods

4.4.11.1 Effects of Exposure Continuity and Duration

Acute, eight-hour and chronic RELs are intended to protect members of the general population from the types of exposures resulting from facility emissions or ambient levels of air pollutants. Modeling and interpretation of such exposure patterns are covered in the Exposure Assessment section of the Hot Spots Technical Support Documents (OEHHA, 2000b). It may also be necessary to apply models or adjustments to the exposures received by the subjects (animal or human) of studies used as the basis for derivation of RELs, to determine reference levels appropriate to the specified 1-hour, eight-hour or continuous exposure durations. Since animal experiments often involve durations other than those specified for the RELs, and possibly discontinuous or repeated exposure patterns, specific adjustment procedures are prescribed for derivation of RELs. Since these are different for the three types of REL, they are described separately in the following sections covering issues specific to each type of REL.

4.5 Supporting Data

Summaries describing the development of the acute, eight-hour and chronic RELs for each chemical are found in Appendix D. In addition, a list of acronyms is provided in Appendix A. All toxicity summaries for the newest RELs include a discussion of the information upon which the calculations are based. This discussion includes the following key elements:

1. Physical and chemical properties: Descriptions include information on volatility, reactivity, stability, toxic secondary compounds, flammability, density, water solubility, color, odor, and some additional properties.

2. Occurrence and use: The typical major uses of the chemical are described as well as where it is likely to be found. If available, measured ambient air levels are provided.

3. Routes of exposure: The routes of exposure that may lead to toxic effects are mentioned for each substance. Since the intent of this document is to provide information on airborne toxicants, the data presented focuses on inhalation exposure studies and may be supplemented by relevant non-inhalation toxicology studies. If inhalation data are unavailable or are of poor quality for a particular chemical, other routes of exposure may be considered for the development of RELs. For extrapolation from oral to inhalation exposures, ideally a PBPK model dealing with both routes is used. Failing that, methodology presented by U.S. EPA (1994a) should be used.

4. Summary of toxic effects: Toxic effects are described for relevant endpoints. Where possible, all of the following attributes are mentioned: endpoints, test species, concentration or dose, duration and frequency of exposure, type of effect level (such as benchmark dose or

NOAEL), reversibility of findings, UFs applied, and RELs derived. Note: while an overview of the toxicity of the chemical is provided in the summary, only the papers deemed key to the REL are described in detail.

5. Pharmacokinetics and metabolism: A discussion of pharmacokinetics is included if information is available. This may include information on absorption, distribution, metabolism, and excretion. The inhalation route of exposure is examined preferentially. Metabolites of the parent compound are also identified when known. Where data are available to support it, a pharmacokinetic model may be derived; if used in the derivation it is described in the summary.

6. Children's sensitivity to the chemical relative to adults: A discussion of the potential for infants' and children's differential sensitivity to the chemical is provided, and any adjustments to the REL to protect children's health are described. Effects on other potentially sensitive subpopulations are also considered.

7. Quality assurance measures: Weak or conflicting data are reviewed. Studies are evaluated for any recognized violations of sound laboratory or statistical practices.

8. Sources of data: In the absence of well-documented experimental dose-response studies in humans, reliance on toxicological data from animal studies and human data from workplace and other exposures is appropriate. In addition, in vitro toxicity studies are sometimes reviewed, particularly for information on mechanism of action.

9. Oral RELs: Substances emitted to the air may deposit on soil, water or plants with subsequent human exposure via non-inhalation routes. Since oral exposure is the predominant non-inhalation pathway, non-inhalation RELs are referred to as oral RELs. Where appropriate, oral RELs are included to capture the contribution of this pathway, for example, for the nonvolatile compounds anticipated to be present in the air adsorbed to particulate matter. In the absence of adequate inhalation data, oral REL data may be used in the development of inhalation RELs.

5 Acute Reference Exposure Levels

This section presents methods for deriving acute (one-hour) inhalation Reference Exposure Levels (RELs) for toxic air contaminants (TACs). The acute REL is an exposure that is not likely to cause adverse effects in a human population, including sensitive subgroups (such as infants and children), exposed to that concentration for one hour on an intermittent basis.

5.1 Time Frame of Interest

In the Air Toxics Hot Spots Program, routine industrial emissions are evaluated for potential public health impacts. Facility emissions may fluctuate considerably, with daily and hourly maximum and minimum concentrations. The commonly used air dispersion models can be used to model concentrations hour by hour throughout a year, giving an indication of the one-hour maximum exposure concentrations. The hourly fluctuations are a reflection of the changing meteorological conditions that are included in the model. Section 5.4.1 provides more description of the underlying assumptions and applicability of the acute REL.

In general, the one-hour modeled maximum concentrations in the Air Toxics Hot Spots Program are used in a HI approach in order to evaluate "acute" exposures and potential public health impacts from such exposures. The HI is the ratio of the one-hour maximum modeled ground level concentration (GLC) to the acute reference exposure level (REL). If the ratio exceeds one, then the risk manager needs to consider whether risk reduction is appropriate. An exceedance of one does not mean adverse effects will occur. Rather, it is an indication of the erosion of the margin of safety for exposure to that chemical.

5.2 Acute RELs and Severity of Effect

The toxic effects that are observed for a chemical may be regarded as mild adverse effects, severe adverse effects, and life-threatening effects (Section 4.3.3). Except for death, these graded effect levels are not sharply demarcated but each merges into adjacent categories. In general, as dose increases, so does the severity of the response. As for other types of REL, the critical effect chosen for development of an acute REL is generally either a mild adverse effect, or a precursor effect with known relation to the mechanism of toxicity. In view of the importance of respiratory irritation as an endpoint for consideration in acute toxicity, some specific considerations in relation to this endpoint are given below.

5.2.1.1.1 Lower Airway Responsiveness

Lower airway effects are often the most sensitive physiologic response following inhalation exposure to certain chemicals. Because these endpoints have been used for development of certain acute RELs, we have defined specific criteria for tests of lower airway function to be considered in categorizing effect severity. In some individuals, the lower airways respond acutely to various stimuli by decreasing airway diameter (Woolcock, 1994). Although various methods are used to measure pulmonary function, airway responsiveness is commonly and easily assessed and is a sensitive indicator of airway narrowing in response to external agents (Gold, 1994; Woolcock, 1994). While varying spirometric criteria have been used for the determination

of airway hyper-responsiveness .(Wiebicke et al., 1990; Bernstein et al., 1992; Eschenbacher et al., 1992; Jubber et al., 1993; Cherniack, 1995), the standards used in this document are consistent with previously specified guidelines and research protocols (Eiser et al., 1983; Sterk et al., 1993; Aris et al., 1995; ATS, 2000b).

Following inhalation exposure to the chemical, the following spirometric changes (compared to pre-exposure findings) are criteria for inclusion as a mild effect:

(1) statistically significant but clinically insignificant changes in forced expiratory volume in 1 second (FEV_1) (i.e., <10% decrement in FEV_1 compared to pre-exposure baseline); or

(2) statistically or clinically significant changes in specific airway resistance (SR_{aw}) or airway conductance (SG_{aw}) following inhalation challenge with the chemical of interest (clinical significance is a 100% increase in SR_{aw} or a 50% decrease in SG_{aw}) without a 20% drop in FEV_1 or symptoms consistent with bronchoconstriction, such as chest tightness, wheezing and shortness of breath (Table 5.2.1).

TABLE 5.2.1. SYSTEM FOR CATEGORIZATION OF PULMONARY FUNCTION INTO EFFECT SEVERITY LEVELS

Endpoint[1]	Mild	Severe
Spirometry Test Result (compared to baseline)	Statistically significant but < 20% decrement in FEV_1[1]	≥ 20% decrement in FEV_1
Methacholine Challenge Test Result	≥ 100% increase in specific airway resistance (SR_{aw}) or ≥ 50% decrease in airway conductance (SG_{aw}) no symptoms of bronchoconstriction < 20% decrement in FEV_1	100% increase in specific airway resistance (SR_{aw}) or 50% decrease in airway conductance (SG_{aw}) accompanied by (1) symptoms of bronchoconstriction or (2) ≥ 20% decrement in FEV_1
Clinical Findings	None anticipated	Chest tightness, shortness of breath, wheezing Wheeze detected by examination Hypoxia or decreased oxygen saturation

[1] Forced expiratory volume in one second (FEV_1).

5.2.1.1.2 Definition of Severe Adverse Effects for Acute RELs

The hallmark of a severe adverse outcome is a change in organ function and/or tissue damage that may be detectable by clinical examination (effect severity levels 5-9 in Table 4.4.1). For example, loss of balance, asthma exacerbation, hemolysis, cardiac ischemia and adverse

outcomes of a pregnancy are all clinically significant findings. With some exceptions (such as adverse outcome of a pregnancy), effects may be reversible, although prolonged exposure may result in irreversible effects.

Following the criteria for pulmonary function testing presented in Table 5.2.1, and in contrast to the mild effect level, pulmonary function test results considered indicative of a severe effect are a 20% or greater decrement in FEV_1 compared to baseline, with or without symptoms of bronchoconstriction such as wheezing, chest tightness and shortness of breath. Alternatively, a 100% increase in SR_{aw} or a 50% decrease in SG_{aw} compared to baseline, accompanied by symptoms of bronchoconstriction, is also consistent with a severe adverse effect. Also consistent with a severe adverse effect is forced expiratory volume in 1 second to forced vital capacity ratio less than 70% ($FEV_1/FVC < 70\%$), which suggests obstructive airway disease. Other measures of the integrity of the lung, such as the diffusing capacity (D_L), a measure of the efficiency of gas exchange across the air-blood barrier, may be taken into consideration, but would not be the sole determinant of the severity of the effect.

As with all health effects, certain individuals may be more susceptible to adverse health consequences following exposure above the acute REL. These sensitive individuals may suffer health effects that may require assistance at a lower level of exposure than the general population. For example, individuals with asthma, who following exposure to sulfur dioxide are likely to exhibit bronchoconstriction at a lower concentration than the general population, may require greater protection from this substance than non-asthmatic persons. Acute RELs are designed to be protective for the range of susceptible persons in the general population including infants and children.

In addition to the histochemical and pathological effects in Table 4.4.1 OEHHA's system for categorizing clinical symptoms and signs into severity levels is presented in Table 5.2.2, which is intended to provide examples of health effects commonly considered for each severity level, but is not meant to be a comprehensive list of all possible health effects. Please refer to the individual toxicological summaries in Appendix D for chemical-specific information.

TABLE 5.2.2. OEHHA CATEGORIZATION OF SEVERITY OF ADVERSE HEALTH EFFECTS FOR ACUTE RELS

Acute Exposure Level	Symptoms	Signs/Laboratory Findings
Mild Adverse	Mild subjective complaints with few to no objective findings: • Mild mucous membrane (eye, nose, throat) irritation • Urge to cough • Mild skin irritation • Mild headache, dizziness, nausea • Behavioral changes	Statistically significant findings of pre-clinical significance: • Mild conjunctivitis • Mild lung function changes[1] • Abnormal immunotoxicity test results • Mild decreases in hemoglobin concentration or hematocrit • Cardiovascular system: ectopic heart beats • Serum enzyme changes • Body weight changes • Mild, reversible histological changes e.g. liver
Severe Adverse	Potentially disabling effects that affect one's judgment and ability to take protective actions; prolonged exposure may result in irreversible effects: • Severe mucous membrane irritation • Blurry vision • Shortness of breath, wheezing • Severe nausea • Severe headache • Incoordination • Drowsiness • Panic, confusion	Clinically significant findings: • Findings consistent with central or peripheral nervous system toxicity • Loss of consciousness • Hemolysis • Asthma exacerbation • "Mild" pulmonary edema • Clinically significant lung function changes[1] • Cardiac ischemia • Some cardiac arrhythmias, e.g., atrial fibrillation • Renal insufficiency • Hepatitis • Reproductive/developmental endpoints, e.g., infertility, spontaneous abortion, congenital anomalies, other adverse birth outcomes

[1] Refer to Table 5.2.1 for detailed categorization of lung function tests.

5.3 Exposure Duration and Patterns

As indicated in Section 5.1, the focus of acute RELs is on short-term exposures. A one-hour exposure is used as the timescale for which toxicity is assessed, which is consistent with the hour-by-hour monitoring or modeling that is generally conducted for facilities under the Hot Spots Program. Sometimes it is necessary to extrapolate from other durations of experimental exposure, or reports of human exposure situations, to a one-hour exposure duration. This is described in Section 5.6, and is also discussed on a chemical-by-chemical basis in the toxicity reviews for many compounds.

5.3.1 Exposure Concentration Averaging Period

The acute REL is a concentration that is not likely to cause adverse effects in a human population, including sensitive subgroups, exposed on an intermittent basis to that concentration for one hour. Intermittent exposure is difficult to define. The U.S. EPA views intermittent exposure as that lasting less than 24 hours and occurring no more frequently than monthly. This is, in part, based on an assumption that an acute exposure concentration is at least 10-fold higher than the monthly average, and the presumption that individual exposures are independent of one another. They point out that very few chemicals will have sufficient data to determine the safe "periodicity" of an acute exposure. Thus, U.S. EPA (1994b) has identified three issues to be addressed: length of acute exposure, periodicity of exposures, and the relationship between the acute exposure and the chronic background (U.S. EPA, 1994b). These will be discussed below.

In acute toxicology experiments, the study design usually involves exposures of short duration to an otherwise unexposed animal. However, real world "acute" exposures occur intermittently, rather than as rare events in a lifetime. Thus, the typical ambient exposure scenario is not reflected in the standard acute toxicology experimental design. The possibility of cumulative effects from intermittent ambient exposure cannot be addressed in acute REL development. Hence, acute environmental exposures are considered by the U.S. EPA to occur no more frequently than monthly. The U.S. EPA also recommends that longer inter-exposure periods be established for chemicals with long clearance times or for those with evidence of cumulative or sensitizing effects.

A related exposure issue is the fact that peak exposures are superimposed on lower long-term exposures to the same compound. This is also not reflected in the standard acute toxicology design. For some compounds this will result in an increased body burden relative to the typical toxicology experimental design and in a potential lowering of the acute exposure needed to produce an adverse effect. The U.S. EPA's approach is to assume that the peak exposures are at least 10 times the monthly average so that the acute exposure can be considered to be relatively independent of the longer-term chronic exposure to the same substance (U.S. EPA, 1994b).

Despite these limitations, it is imperative to examine whether short-term exposures to peak concentrations might result in adverse public health impacts. OEHHA's RELs should be compared to the modeled one-hour maximum (or multi-hour as noted for specific reproductive/developmental toxicants) concentrations used in the HI approach to risk assessment. OEHHA recommends that these acute RELs be used to evaluate exposures that occur no more frequently than every two weeks in a given year. The two-week interval was

chosen because in most acute toxicology experiments two weeks is the duration of time an animal is observed for signs of adverse outcomes following exposure.

An assumption in making this recommendation is that the REL is protective of adverse health effects that are not cumulative. Thus, the effects of each peak exposure are independent of previous or subsequent peak exposures that occur as often as every two weeks. This recommendation is only valid for substances that do not bioaccumulate. When bioaccumulation is known to occur and body burden is associated with an adverse effect, or where cumulative tissue damage occurs with repeated exposures, longer inter-exposure periods should be specified.

The modeled one-hour peak concentrations are typically much greater than the maximum average annualized concentrations used for determining chronic exposure and risk. Thus, it is assumed that acute exposures are independent of the long-term average exposure based on the modeled annualized maximum average concentration. However, under certain meteorological conditions (poor mixing, persistent calm winds), it is conceivable that there are many hours in a day or within a few days where exposures are close to the peak one-hour in any given year. Concentrations close to the maximum one-hour exposure may occur many times during the year including on consecutive days. In addition, it is conceivable that exposure concentrations close to the maximum may occur in consecutive hours. Currently, the local air districts, Air Board, and facilities do not ascertain how often exposures close to the one-hour maximum occur in a given day, week, month or year. This contributes to the uncertainty in evaluating the adverse health effects of peak one-hour exposures.

In evaluating chemicals with developmental toxicity, we found that the standard experimental paradigm of repeated exposure over several days did not lend itself easily to extrapolation to a one-hour acute REL. Since developmental endpoints are frequently manifested in a small window of time during gestation, the standard protocol is to expose pregnant animals for several hours per day over several days during gestation in order to increase the power of the study to detect an effect. Issues that affect the extrapolation to one hour include not only when the sensitive gestational period is, but also toxicokinetic issues. Whether or not a single one-hour exposure could produce a reproductive or developmental adverse outcome depends on the toxicokinetics governing the concentration of the chemical in maternal and fetal tissues, timing of exposure, mechanism of action, and other factors. These issues are not easily taken into account in extrapolating to a one-hour acute REL. Thus, for those acute RELs addressing a developmental endpoint determined under our previous methodology, the REL was for the exposure duration chosen for a single day in the experimental protocol. In this revised methodology, OEHHA proposes to use the exposure concentration in a developmental toxicity study as the basis of the one-hour REL, regardless of the daily exposure duration in the study. Given the seriousness of developmental endpoints and our mandate to ensure our risk assessment methods adequately protect infants and children, this is justified. It is rarely clear in a developmental toxicity study if the toxicity depends on tissue concentration during a discrete time interval or on total dose over the course of exposure. This may be particularly important for developmental endpoints where short time periods of extreme vulnerability to toxicants may be accompanied by uncertainties in toxicological mechanisms. The duration of the period of vulnerability may itself be highly uncertain.

5.4 Pre-Existing Acute Exposure Guidelines

Acute exposure standards have been developed by several different organizations. However, there are no inhalation exposure values that were derived using a consistent basis to protect the public from planned industrial emissions. Values designed for protection of the general public exist, but they are intended to address accidental releases and use methodologies that are not well documented. Occupational exposure guidelines are available for hundreds of substances, but have an inconsistent basis, often have not incorporated recently available data, and are not designed to protect sensitive subpopulations. The existing exposure guidelines considered for possible relevance to OEHHA's acute RELs are described below.

5.4.1 The California Ambient Air Quality Standards (CAAQS)

CAAQSs are promulgated by the California Air Resources Board (CARB) based on recommendations from OEHHA, and are specified concentrations and durations of exposure to air pollutants which reflect the relationship between the intensity and composition of air pollution to undesirable effects. The CAAQS for a criteria air pollutant has in the past been adopted as the acute REL. If necessary, a one-hour value was derived using time extrapolation (described below). The CARB on April 28, 2005 reviewed the current one-hour ozone standard and left it unchanged, but promulgated a new eight-hour ozone standard. The two together are meant to provide adequate protection of sensitive populations including children.

5.4.1.1 Ambient Air Quality Standards as Acute RELs

Almost all acute RELs were developed *de novo*. However, the California Ambient Air Quality Standards for Criteria Air Pollutants were reviewed. If they were found to be appropriate, they were adopted as the relevant acute toxicity RELs. For the six criteria air pollutants carbon monoxide, nitrogen dioxide, sulfates, ozone, hydrogen sulfide, and sulfur dioxide, the CAAQS for short-term (one-hour) exposure is used as the REL, or one-hour values were derived by extrapolation from the 24-hour standard.

5.4.2 The Threshold Limit Value-Time Weighted Average (TLV-TWA) and Short-Term Exposure Limit (STEL)

The TLV-TWAs and STELs are developed by the American Conference of Governmental Industrial Hygienists (ACGIH) and updated annually (ACGIH, 2006); similarly, the National Institute for Occupational Safety and Health (NIOSH) recommended exposure limits also exist (NIOSH, 2005). The TLV-TWA is defined as the time-weighted average concentration for a normal eight-hour workday and a 40-hour workweek to which nearly all workers may be repeatedly exposed, day after day, without adverse effect. The STEL is defined as a 15-minute TWA exposure which should not be exceeded at any time during the workday.

Occupational exposure limits have sometimes been used to derive chemical exposure guidelines for the general public (NATICH and McCullough, 1991; Robinson and Paxman, 1992; U.S. EPA, 1994a). More than 600 ACGIH TLVs and NIOSH RELs are available. These values have been attractive because of the large number of accessible values and the concept that they are intended to protect a human population from inhalation exposures. However, these values are

not designed for or recommended for protection of the general public, and in many cases may not prevent adverse health effects among workers (Roach and Rappaport, 1990). OEHHA has therefore not taken the TLV-TWAs and STELs directly into account in developing acute RELs, but has taken advantage of the data identified and evaluations offered by ACGIH when relevant.

5.4.3 Various Emergency Exposure Guidance Levels

A variety of guidance levels have been developed to assist in dealing with accidental chemical releases. As such, these values focus on emergency planning and response, not on the routine emissions and exposure which are the focus of this document. Thus NRC (2001) described the objective of U.S. EPA's AEGL program (see below) as *"to develop guideline levels for once-in-a-lifetime, short-term exposures to airborne concentrations of acutely toxic, high-priority chemicals."* Emergency guidelines are typically defined as predicted thresholds above which some level of adverse health effect is anticipated: standard margins of safety are not incorporated. Also, in many cases these guidelines are designed to identify tolerable conditions for emergency first responders such as firefighters or military personnel, rather than to protect the general population. Such guidance values are seldom comparable to the acute RELs, and are not suitable for protecting the health of the general public from routine emissions. However they may incorporate relevant information as to the type of effects to be expected and the dose response for exposure to compounds of interest.

Emergency Exposure Guidance Levels (EEGLs) are designed to provide guidelines for military personnel operating under emergency conditions that are peculiar to military operations and for which regulatory agencies have not set standards, and are defined by the NAS as the ceiling concentrations of substances in air that may be judged by the Department of Defense to be acceptable for the performance of specific tasks during rare emergency conditions lasting for periods of 1 to 24 hours (NRC, 1986a). "Emergency" connotes an unexpected situation with potential for loss of life. The Short-term Public Emergency Guidance Level (SPEGL) is defined by the NAS as a suitable concentration for unpredicted, single, short-term, emergency exposure of the general public (NRC, 1986a). In contrast to the EEGL, the SPEGL takes into account the wide range of susceptibility of the general public, but it is not designed for repeated or multiple exposures.

The American Industrial Hygiene Association (AIHA) has defined Emergency Response Planning Guidelines (ERPGs) as concentration ranges where adverse health effects could be observed (AIHA, 2006). ERPGs have a specific emphasis on responding to accidental releases.

The U.S. EPA has developed Acute Emergency Guidance Levels (AEGLs) to provide information to incident commanders in an emergency. The NRC has published a methodology for developing AEGLs (NRC, 2001). As of April 2007, AEGLs for 31 chemicals have been finalized (U.S. EPA, 2007).

5.5 Human Equivalent Concentration (HEC) Procedure for Acute RELs

When animal studies are used for acute REL development, the U.S. EPA HEC procedure (described in Appendix F) may be used as a partial adjustment for interspecies toxicokinetic differences, in which case the 10-fold interspecies UF is reduced to 6 ($UF_{H\text{-}k} = 2$, $UF_{H\text{-}d} = \sqrt{10}$).

The modifications of the HEC procedure to account for children may also be used. These procedures will be used where applicable as the acute RELs are updated to reflect additional available research and to fulfill the mandates of SB 25 to account for potentially greater vulnerability of children when setting health standards.

5.6 Effects of Exposure Duration – Special Considerations for Acute Effects

Studies of adverse health effects associated with exposures in humans or experimental animals are generally conducted for time periods different from that which is of interest in the acute exposure scenario. Typical exposure scenarios involve several hours for human exposures and several daily exposures for two weeks in animals. OEHHA acute RELs, on the other hand, are designed to be protective for one-hour exposures (with the exception of some developmental toxicants where the REL is for several hour exposures).

Acute inhalation toxicology studies (exposure duration of 8 hours or less) are preferred over other exposure routes. In their absence, studies using exposures of longer durations may be employed if appropriate (e.g., symptoms noted after short period of time; developmental endpoints). If inhalation toxicity data are unavailable, studies on other exposure routes may be used. Studies that include an adequate follow-up period (hours to days, depending on the chemical and endpoint) to account for delayed health effects are preferred to those that terminate observation immediately following exposure. In order to adjust experimental exposure durations to one-hour, OEHHA uses a method termed time extrapolation.

5.6.1 Concentration and Time Extrapolation using Haber's Law

"Haber's Law" states that the product of the concentration (C) and time of exposure (T) required to produce a specific physiologic effect is equal to a constant level or severity of response (K), or C * T = K (Rinehart and Hatch, 1964). When the duration of experimental exposure differs from the desired exposure duration for which an acute exposure level is being calculated (in this case 1 hour), a modification of Haber's Law is used to adjust the experimental exposure duration to the desired duration of the acute exposure level:

$$C^n * T = K$$

where *n* is a chemical-specific parameter greater than zero (ten Berge et al., 1986). When n is equal to one ($n = 1$), the toxicity of a chemical is equally dependent on changes in concentration and duration of exposure; when n is less than one ($n < 1$), the duration of exposure is a greater determinant of toxicity than the concentration; finally, when n is greater than one ($n > 1$), the toxicity of a chemical is determined to a greater extent by exposure concentration than by duration.

5.6.1.1 Value of the concentration exponent, *n*

Ideally, the magnitude of *n* should be determined for all chemicals by evaluating the concentration versus response relationships for several different exposure durations. However, this information is available for only a limited number of substances. Empirically-derived values of the exponent *n* range from 0.8-3.5 (ten Berge et al., 1986). The time-concentration-response

relationship depends on the time-frame considered and the endpoint measured. There are usually multiple "n" values for a single chemical that are applicable to different response endpoints. For example, the "n" for irritation of ammonia is 4.6, while the "n" for lethality of ammonia is 2. (See Appendix G.). As concentration becomes the more important factor, the value of *n* will increase. Values of *n* greater than three suggest that concentration has a strong predominance over time.

The value for the exponent *n* used by OEHHA in acute toxicity summaries is chosen as follows. First, when an empirically derived value for the exponent is available from the open literature, this is adopted for time extrapolation, using the modification of Haber's Law as described above. Appendix G shows published or OEHHA derived values for *n* which were used in acute RELs previously developed by OEHHA (1999a).

When a derived value is not available and there are insufficient data from which to determine a value *de novo*, a default value for *n* must be used. The published or OEHHA derived values for *n* shown in Appendix G range from 0.8 to 4.6. The mean value in this range rounds to 2; the interquartile range (25%-75%) is from 1 to 2.2. Previously, the mean value of $n = 2$ was used by OEHHA (1999a).when extrapolating from an exposure duration that is greater than one hour to a 1-hour level. However, when this issue was considered by (NRC, 2001) they concluded that it would be more appropriate to use the value $n = 3$, which approximates the 90th percentile of the range of values reported by ten Berge (1986). OEHHA now therefore recommends the use of $n = 3$ when extrapolating from experimental exposures greater than one hour to the 1-hour period of concern for the acute RELs.

When extrapolating from an experimental exposure duration of less than one hour to a 1-hour level, the value of $n = 1$ was used. Using a value of $n = 1$ is more health-protective than a value of $n = 3$. A value of $n = 1$ results in a relatively rapid decrease in the derived REL when extrapolations are made from shorter to longer exposures. For example, when extrapolating from a 30 minute exposure at the published NOAEL of 60 ppm (Purser et al., 1984) to a 60 minute exposure for hydrogen cyanide, using n = 1 results in an extrapolated 1-hour NOAEL of 30 ppm; when using n = 3, the extrapolated NOAEL is 48 ppm.

In summary, the default exponents used by OEHHA in the formula $C^n T = K$ for extrapolation to 1-hour acute RELs are as follows:

- From less than 1 hour $n = 1$
- From greater than 1 hour $n = 3$

5.6.1.2 Haber's Law and Irritants

The applicability or otherwise of Haber's Law to irritants has been the subject of various studies. The NAS has suggested that Haber's Law does not apply for "some irritants" (NRC, 1986a; 1986b; 1993). This statement is apparently based on the observation that for some substances, irritation appears to be solely concentration dependent. In fact, the time course of response to the small number of sensory irritants for which data are available suggests that although the response

follows a modified dose/time integral relationship (like most other toxicities) over very short time scales of a few seconds or minutes, this relationship has reached a plateau where the level of response is dependent only on concentration well within the one-hour time scale of concern for acute REL derivation (Shusterman et al., 2006).

Response to mild sensory irritants is mediated through binding to the trigeminal nerve receptors. In humans, this results in a complex response including a burning sensation of the eyes, nose, and throat. Other notable symptoms are coughing, nasal congestion, rhinorrhea, sinus and Eustachian tube dysfunction, and worsening of lower airway function in some asthmatics (the so-called "naso-bronchial reflex") (Widdicombe, 1990; Raphael et al., 1991). The response in the rodent is simpler, consisting primarily of a reflex depression of the breathing rate. When a mouse is exposed to an irritant, the decrease in respiratory rate is proportional to the concentration of the airborne chemical. Also, a minimum respiratory rate is reached and remains at a plateau, or fades in response during exposure. This has been used as the basis for a bioassay of sensory irritant properties (Alarie, 1966), in which irritant potency is expressed as the concentration producing a 50% depression in respiratory rate (RD_{50}).

Although the receptors have not been fully characterized, receptor binding has been found to follow the classic Michaelis-Menten receptor kinetics. There is evidence that many chemicals bind to a common receptor, sometimes referred to as the common “chemical sense” receptor (Cometto-Muniz et al., 1997; Bryant and Silver, 2000). There is also evidence of multiple receptor types on the trigeminal nerve (Nielsen and Vinggaard, 1988). The irritant response can be described by the Michaelis-Menten equation in an animal model (Kristiansen et al., 1986; Nielsen and Vinggaard, 1988). Competitive agonism has been demonstrated in the mouse RD_{50} bioassay (Bos et al., 1991). This is additional evidence for a receptor mediated mechanism, with a common receptor for these two chemicals.

The degree of receptor binding is mediated by the tissue concentration of the toxicant, not the duration of exposure once equilibrium is reached, which generally occurs relatively quickly. At equilibrium, at a constant exposure concentration, a constant level of receptor binding would be expected presumably leading to a constant effect level, independent of the duration (the “T” factor in the Haber’s Law equation). Michaelis-Menten kinetics also predict that receptors would be saturated at higher irritant chemical concentrations and therefore additional trigeminal mediated irritation response with increased exposure concentration would not be expected in this situation.

Thus, irritancy should be more a function of the air concentration of the irritant than of the total dose. Often these chemicals are non-reactive volatile organic compounds (VOCs), although in some cases they may be reactive. This simple picture is complicated by additional factors such as desensitization of receptors that can cause a decline in perceived irritation over time for some chemicals (Nielsen, 1991; Shusterman et al., 2006).

The trigeminal system is distinct from odor perception. Persons lacking a functional sense of smell (anosmics) still perceive chemical irritants in a similar fashion to people with a normal sense of smell. Odor perception occurs at a lower threshold than irritation, and in some cases at a much lower threshold. In both odor perception and trigeminal irritancy from a chemical, there can be wide variability in threshold air concentrations in the general population.

If the irritancy reaction is a function of the concentration, then the fact that children have higher breathing rates than adults should not influence the health impact of a particular concentration. There is no evidence that infants and children have different or more irritancy receptors than adults. Therefore, OEHHA has not assumed that children are more sensitive than adults to the sensory effects of eye, nasal or respiratory irritants. However, it must be considered that many irritants, especially those that are chemically reactive, may have the potential to exacerbate or induce asthma, which is a special concern for children's health.

OEHHA will consider trigeminally-mediated sensory irritant endpoints to be independent of the duration of exposure over the one-hour timescale, unless data indicate such time dependence. Data establishing time dependency should be in a concentration range relevant for trigeminal mediated effects and not at considerably higher concentrations. Higher concentrations may cause irritancy through tissue damage, and thus show time dependence because of accumulating tissue damage. The National Academy of Science Subcommittee on Acute Exposure Guideline Levels made the same determination for their Acute Exposure Guideline Levels (AEGLs) (NRC, 2001). Sometimes trigeminally-mediated irritancy is difficult to distinguish (based on available data for a chemical) from tissue damage mechanisms, or there may be mixed mechanisms. Empirical determination of an "n" value indicating that duration of exposure for a particular chemical influences toxicity will be accepted as an adequate basis for Haber's Law adjustments in these cases.

5.6.1.3 Strong Irritants Causing Tissue Damage and/or Hyperplasia

Some chemicals cause irritancy through tissue damage that can result in hyperplasia or other nasal, eye or respiratory tissue damage. The tissue damage resulting from exposure to these chemicals may be both time and concentration dependent and in some cases be dependent on the total cumulative dose or the concentration. For example, formaldehyde-induced hyperplasia appears to be more concentration dependent than exposure duration dependent according to an analysis of several studies cited in the development of OEHHA's chronic REL (OEHHA, 2000a). Trigeminally-mediated irritancy may occur at a lower concentration, while tissue damage or hyperplasia may occur at a higher concentration. Tissue damage or hyperplasia may also occur with longer exposure to the same concentration (Barrow et al., 1986). This is particularly evident with highly reactive chemicals such as chlorine which have both sensory irritant and direct tissue-damaging properties in the upper respiratory tract (Jiang et al., 1983; Bos et al., 1991). Chemicals which cause sensory irritation in the upper respiratory tract as a result of lower short-term exposures may also cause pathological changes in the lower respiratory tract, especially following more extended or more intense exposures (Shusterman, 1999). However, persistent histological changes may not always be seen with the isolated one-hour exposures against which acute RELs are intended to be protective. In such cases, the one-hour REL would likely be different from a chronic REL.

If such tissue damage in the nose or other parts of the respiratory tract accumulates over time, then the toxic effect would be dependent upon both time and concentration. Such damage could trigger pain receptors, and needs to be distinguished from trigeminally-mediated irritation. In contrast to the case of pure sensory irritation, the use of Haber's Law where tissue damage is a factor in the response is appropriate.

The higher breathing rates of children may need to be considered for chemicals causing cumulative damage at the point of entry, for which either total dose or AUC is the determinant of toxicity. An analysis of the mechanism of toxicity may help to determine whether children are more sensitive than adults to irritants that cause tissue damage or hyperplasia.

5.6.2 Time Extrapolation for Acute RELs Based on Developmental Studies

In the previous guidelines, OEHHA (1999a) considered that extrapolation to one hour using Haber's law was not appropriate in the case of repeated dose studies for developmental endpoints. OEHHA chose a single day's exposure for each chemical (ranged from 1 to 8 hours) as the exposure duration for which the REL is to be applied. Thus, no time extrapolation was used for developmental toxicants. Several of the acute RELs derived using these earlier guidelines based on developmental studies have averaging times longer than one hour. These averaging times include six hours for benzene, carbon disulfide, EGEE, EGEEA, and EGME, and seven hours for carbon tetrachloride and chloroform (OEHHA, 1999a).

OEHHA has developed a different underlying methodology in the present version of these guidelines, which has been described in Section 4.4.6.1. As in the cases noted above, the time extrapolation used when deriving acute RELs will most often be from a longer experimental duration to a shorter one-hour reference period for the REL. In these cases, the revised methodology treats the experimentally applied concentration as the basis for the acute REL, *i.e.* the concentration present during the experiment is not to be exceeded during any 1-hour period.. The revised methodology will, in these cases, result in an acute REL which is numerically the same as that obtained by the previous method, although the previous complication of having a non-standard averaging time is avoided.

5.7 Areas for Further Research

5.7.1 Acute Toxicity Data

Many chemicals lack adequate data on acute toxicity. There are approximately 450 chemicals on the Air Toxics Hot Spots list of substances to be quantified (Appendix C). This is the list of substances, which facilities must report in their emissions inventories. We have to date only developed acute RELs for 51 of these compounds, six of which have so far been revised according to these new guidelines. While not all of the 450 chemicals have reported emissions in California, more work needs to be done in analyzing available literature for the remaining compounds and in revising existing RELs to take explicit account of children's health issues.

5.7.2 LOAEL to NOAEL Uncertainty Factor

The application of UFs to account for extrapolation from a LOAEL to a NOAEL warrants further analysis (see Section 4.4.5). When evaluating dose-response relationships, the slope of the dose-response curve determines the distance between the LOAEL and the NOAEL from a particular study. Some endpoints tend to have steep dose-response slopes and may not warrant a 10-fold UF to extrapolate from a LOAEL to a NOAEL; other endpoints have a shallow dose-response slope and may warrant a 10-fold (or higher) UF for extrapolating from the LOAEL to

the NOAEL. An analysis of the distribution of the LOAEL to NOAEL ratios for 112 datasets (Gift et al., 1993; Kadry et al., 1995; Alexeeff et al., 1997; Almstrup et al., 2002) suggested an intermediate UF of 6 to extrapolate from the LOAEL to a NOAEL for mild effects. Further analysis of 215 data sets for 36 pollutants yielded LOAEL to NOAEL ratios of 2.0, 5.0, 6.3, and 10.0 for the 50th, 90th, 95th, and 99th percentile, respectively (Alexeeff et al., 2002). The 90% confidence interval for the 95th percentile was 5.0-7.5. Thus, the LOAEL to NOAEL UF of 6 would be protective for 95% of the responses, and a value of 10 for 99%. However, the 99th percentile value was considered unstable. For this reason the 95th percentile value is chosen for extrapolation of the LOAEL to a NOAEL for mild effects.

Little variability was noted among species, particularly at the median. This analysis is based on toxicity data from mild acute inhalation studies and may not be applicable to other exposure routes, exposure durations, or more severe toxicity. (A value of 10 should be used by default for effects considered severe.) In addition, this analysis did not focus specifically on children.

5.7.3 Interspecies Uncertainty Factor

An interspecies UF of 10 is commonly used to extrapolate from animal studies to the human response (UF_A) (Section 4.4.7.3). The available analyses supporting use of the 10-fold interspecies UF_A were conducted on studies of toxicity by the oral route of administration (Dourson and Stara, 1983). Further analysis of available data on chemicals for which there is both human and animal data for the same endpoints by the inhalation route of exposure is warranted.

In some cases, there may be a reason that a different UF could be used. Some part of the uncertainty encompassed by this factor may be replaced by species-specific models of deposition, distribution or metabolism where data exist to support these models. In certain cases there may be specific reasons for concluding that the toxicodynamic component of this variability may be smaller or larger than the $\sqrt{10}$ assumed by default. For example, in lethality studies, the exposure to irritant chemicals producing lung edema may have very similar dose-response slopes because the basic loss of cellular integrity at high doses may not be a phenomenon that would vary substantially from one species to another. However, in general data on the extent of toxicodynamic differences between species are limited, although the situation for acute exposures may be simpler than for the case of chronic exposures. The existing analyses are limited in terms of toxicological endpoints examined. Interspecies variability may differ significantly for different toxicological endpoints. This is another area where more research is warranted.

5.7.4 Uncertainty Factor for Database Deficiencies

An additional UF may be used in cases where there are identifiable deficiencies in the data (Section 4.4.9). For example, a database deficiency factor of $\sqrt{10}$ (UF_D) may be applied to protect developing infants and children if no developmental data are available. Judgment is still needed when some developmental data are available. The ideal dataset for evaluating developmental endpoints would include studies in two species in which exposure occurs during gestation and a two-generation reproductive study in each of two species. In practice such a

large database is unusual. Available mechanistic data will be considered when deciding when to apply the data base deficiency factor and what its value should be. The more robust the database, the less likely that the factor will be needed. Other types of data deficiency besides developmental toxicity may also be addressed with this factor.

5.7.5 Time Extrapolation for Acute RELs

We have used time extrapolation with a modified Haber's Law to extrapolate from the experimental duration in the acute study to an equivalent concentration for a one-hour exposure, for endpoints other than sensory irritation. There are empirical data for the value of n in Haber's equation for some chemicals. More data would be valuable for additional chemicals. Further analysis of the validity of the Haber's Law application for different toxicological endpoints would be useful.

5.7.6 Additivity of Adverse Effects

We currently use an additive approach to assess the impacts of multiple chemicals on a target organ (Sections 2.2; 5.3). Some interactions may be synergistic and others antagonistic. Additivity has generally been accepted as health-protective at low environmental concentrations. However, there is a need for key studies on the additivity or synergism of chemicals at low concentrations that act on the same target organ. Further literature evaluation would also be helpful to elucidate whether the additive approach is the most valid approach for all scenarios (DeVito et al., 2000; Crofton et al., 2005; U.S. EPA, 2007a).

6 Eight-Hour Reference Exposure Levels

This section presents additional information for deriving eight-hour inhalation Reference Exposure Levels (RELs) for toxic air contaminants (TACs). Eight-hour RELs are concentrations at or below which adverse health effects are not likely to occur in the general human population with intermittent exposures of eight hours per day, 5 days per week.

6.1 Populations at Risk

The Air Toxics Hot Spots Program Guidance Manual for Preparation of Health Risk Assessments (OEHHA, 2003) calls for evaluation of sensitive receptors such as daycare centers and schools as well as offsite workers. Onsite workers are under the jurisdiction of the California Occupational Safety and Health Administration. Current occupational standards such as threshold level values (TLVs) and permissible exposure levels (PELs) are usually expressed as a time weighted average (TWA) over an eight-hour shift. Noncancer health impacts for children at schools have been evaluated using either the chronic or acute RELs. Acute RELs are only useful for evaluating impacts of estimated maximum one-hour air concentrations when such exposures occur infrequently. Exposure duration for children and offsite workers will vary, but an eight-hour exposure duration assumption would be reasonable, particularly if children and offsite workers are exposed to facility emissions at their school or place of work and not at their residential locations. Chronic RELs are designed to be protective against long term 24-hour a day exposure and thus may overestimate some noncancer chemical risks associated with shorter, daily exposure (e.g., eight-hour exposures).

Alternatively, chronic RELs may underestimate the noncancer risk where facility operation and emissions occur only 8 hours per day but coincide with the presence of nearby offsite workers and attendance at daycares and schools. Many facilities operate five days a week, eight hours per day. Such facilities have been modeled as if the total emissions were occurring twenty-four hours a day, seven days a week, three hundred sixty five days a year. The annual average ground level concentration (GLC) could then be compared to the chronic REL to determine noncancer chronic health risk. This approach has the advantage of simplicity but is a less accurate modeling approach because of diurnal meteorological variation and non-continuous facility emissions. This approach assumes that a higher eight-hour pollutant concentration followed by a sixteen-hour period of no exposure is toxicologically equivalent to the average concentration over twenty-four hours. Development of eight-hour RELs would allow more accurate evaluation of the impacts of exposure to modeled eight-hour ground level concentrations by comparison with noncancer health standards specifically tailored to actual exposure duration.

6.2 Characterization of 8-Hour Exposures

An eight-hour REL, designed to protect against periodic exposure that could occur as often as daily, may share characteristics of both acute and chronic exposure. Frequent eight-hour exposures to a chemical with a short half-life in the body that does not cause tissue damage or accumulate may resemble a series of acute exposures. The previous exposures may have little or no impact on the current-day exposure. In these cases, acute exposure methodology would be employed for derivation of the eight-hour REL. Frequent eight-hour exposures to a chemical that accumulates in the body, or causes cumulative tissue damage, and/or activating or

deactivating enzyme induction is considered a chronic exposure, requiring chronic exposure methodology for derivation of the eight-hour REL. The REL for an eight-hour period would need to be adjusted in such cases to reflect cumulative dose from previous eight-hour exposures. Pharmacokinetic modeling may be appropriate to determine the cumulative dose from serial eight-hour exposures.

6.2.1 Eight-Hour Averaging Period for Chronic Toxicity

In cases where the evidence shows that an eight-hour REL should be derived based on chronic exposure, a modification of the default approach adopted for the chronic RELs is used (see Section 7.2.1). The default approach to estimating an equivalent time-weighted average concentration (C_{AVG}) from the observed concentration (C_{OBS}) in non-occupational studies may be summarized as:

$$C_{AVG} = C_{OBS} \text{ x (H hours per 8 hours) x (D days per 5 days/week)}$$

The default approach to estimating an equivalent inhalation-weighted average concentration (C_{AVG}) from the observed concentration (C_{OBS}) for occupationally exposed humans is:

$$C_{AVG} = C_{OBS} \text{ x (10 m}^3\text{/day occupational exposure / 20 m}^3\text{/day total exposure)}$$

$$\text{x (D days per 5 days/week)}$$

Commonly encountered exposure scenarios in both worker studies and experimental animal toxicology studies involve exposures of 6 to 8 hours per day for 5 days per week. Considerably less time adjustment, and associated uncertainty, occurs applying an eight-hour REL under these exposure scenarios relative to applying a chronic REL.

For simplicity, it may be desirable in some cases to use the chronic REL as a health guidance value for repeated eight-hour exposures. This might be appropriate for substances where the response is concentration rather than time dependent, and for substances that accumulate in the body and have long internal half-lives, such as dioxins and some metals, or substances that demonstrate cumulative toxicity, where large pharmacokinetic uncertainties exist. Thus, in these cases the chronic and eight-hour REL may be the same.

6.2.2 Eight-Hour Averaging Period for Acute Recurrent Toxicity

There are some acute RELs that have used single health studies with six- or eight-hour human exposures to derive NOAELs or LOAELs. It may be appropriate to use such studies to derive eight-hour RELs using acute REL methodology if the data show that the chemical is quickly eliminated and does not cause cumulative tissue damage. Similarly, intermittent exposure studies in experimental animals, often with daily exposures at or near six hours, may exhibit toxicity that reflects a daily recurrent acute effect rather than a chronic cumulative-type injury. In some cases, that daily recurrent acute effect may consist of sensory irritation, in which case no concentration adjustment is applied to extrapolate to an eight-hour REL (see Section 5.8.1). A cautious interpretation of such situations is necessary, however, since a number of agents shown to cause sensory irritation during a single one-hour (acute) exposure have also been shown to

cause persistent (and therefore to some degree cumulative) histological damage in various parts of the respiratory tract following repeated (chronic) exposures.

In cases where daily intermittent exposure shows a recurrent acute effect other than sensory irritation, acute REL methodology is applied for time extrapolation to an eight-hour exposure employing a modification of Haber's Law.

$$C^n * T = K,$$

In this equation, (C) is concentration, (T) is time of exposure, and *n* is a chemical-specific parameter greater than zero. When the value of n is unknown, default exponents are used by OEHHA for extrapolation to 8 hours. When extrapolating from an experimental exposure duration of less than 8 hours to an eight-hour level, the value of n=1 was used. A value of n = 1 results in a relatively rapid decrease in the derived REL when extrapolations are made from shorter to longer exposures and is considered an appropriate health-protective approach. Most human worker and experimental animal studies with daily intermittent exposures have time durations at or near 6-8 hours per day, which are well-suited for extrapolating to an eight-hour REL. Data on experimental or workplace exposures longer than 8 hours are less likely to be encountered, but if this were the case a value of *n* = 3 would be used as for the acute RELs. Daily exposures considerably less than 6 hours are not as preferable and may be more practical for acute REL derivation.

6.2.3 Eight-Hour REL Exposure Duration Adjustments for Developmental Toxicants

Because the timing and duration of the sensitive period of gestation is usually unknown, time extrapolation to an eight-hour REL must take into account two principal toxicokinetic issues to prevent, in particular, underestimation of developmental toxicity - peak tissue concentration and total tissue dose (e.g., area under the concentration-time curve, or AUC). Additionally, for those developmental toxicants where there is a suspicion that the chemical or its metabolites may accumulate with daily eight-hour exposures, a duration adjustment from discontinuous to continuous exposures based on equivalent multiples of concentration (C) and duration (T) is recommended (See Section 4.4.6.1). This C x T approach avoids possible underestimation of risk when adjusting the exposure duration from a shorter period to a longer period of exposure.

As more information becomes available on PBPK modeling of developmental toxicants for interspecies extrapolation from the exposed animal species to humans, modeling of blood and tissue levels may confirm the C x T adjustments on the REL exposure durations to ensure they do not exceed the peak tissue concentration or total tissue dose at the NOAEL.

For developmental studies in which the daily exposures are greater than eight-hours, a "not to be exceeded" health guidance is recommended in which no adjustment is applied to the duration with extrapolation down to 8 hours. This procedure avoids underestimation of risk when the pharmacokinetic nature of the developmental toxicant is unknown.

For bioaccumulating toxicants such as dioxins and some metals, developmental exposure studies in which exposure occurred only during gestation is not sufficient for establishing an eight-hour REL based on developmental toxicity. These types of toxicants can accumulate in body tissues

over extended periods of time prior to gestation, leading to maternal body burdens that may be detrimental to the fetus during gestation. Lack of sufficient chronic-exposure and multi-generation studies, and adequate pharmacokinetic modeling information that can predict body tissue burdens, may require application of a modifying UF for pharmacokinetic deficiencies in calculating the REL.

6.3 Human Equivalent Concentration (HEC) and Uncertainty Factor Applications

Application of HEC adjustments and UFs for eight-hour REL derivation uses the same formulae as are used for the acute and chronic RELs. For eight-hour RELs based on chronic effects, the UFs used for chronic exposure are applied; for eight-hour RELs based on acute recurrent effects, the UFs used for acute exposures are applied.

6.4 Hazard Index Calculation

In calculating the HI, the same standardized target organ categories are used for the eight-hour RELs as for acute and chronic RELs (see Section 4.3.4). Calculation of the HI is described in Section 2.2.

7 Chronic Reference Exposure Levels

This section presents additional information for deriving chronic inhalation Reference Exposure Levels (RELs) for hazardous airborne substances. Chronic exposure is evaluated using ambient air concentrations of emitted chemicals averaged over a year. The annualized average air concentration forms the basis for both chronic noncancer and cancer risk evaluation. In reality, exposure over a 24-hour period does not occur at a continuous level. Chronic RELs are concentrations at or below which adverse health effects are not likely to occur in the general human population exposed continuously over a lifetime.

7.1 Priority for Evaluation of Chemicals

Chronic noncancer RELs have been developed for 80 substances as of May, 2005; these are described in Appendix B of this Technical Support Document and in OEHHA (OEHHA, 2000a). Substances were selected for chronic REL development primarily based on (1) the magnitude of current known emissions in California, (2) the availability of a strong scientific database on which to estimate a chronic REL, and (3) toxicity. We include impacts on children's health or other sensitive subpopulations in prioritizing chemicals for chronic REL development.

The amount of data and the quality of the information will ultimately determine whether a chronic REL can be derived for a specific chemical. Margins of safety or UFs can be used to address the common data gaps encountered in risk assessment, but in some cases, chronic RELs cannot be developed because the data are not relevant to inhalation exposure, or because too much uncertainty exists in the database and subsequent derivations. As more data become available over time, chronic RELs may be added or re-evaluated.

Exposure above a particular chronic REL may or may not lead to the development of adverse health effects. Conversely, there may be individuals exhibiting idiosyncratic responses (unpredictable health effects) at concentrations below the chronic RELs. Health effects associated with individual chemicals are presented in Appendix D individual summaries of acute, eight-hour, and chronic RELs.

7.2 Exposure Concentration Averaging Period

The exposure period of concern in the development of chronic RELs is a full lifetime, which encompasses periods of potentially increased susceptibility to adverse health effects from chemical exposure, particularly during childhood and the later years of life. The chronic REL is intended to be protective for individuals exposed continuously over their lifetime. Scientific data available to assess these effects generally consist of discontinuous exposures over a shorter interval. In such cases, default or chemical-specific assumptions are required to estimate concentrations causing comparable effects if exposures were to be continued over the entire lifetime.

7.2.1 Effects of Exposure Continuity and Duration

Studies of adverse health effects associated with long-term exposures of humans or experimental animals generally involve discontinuous exposures. Commonly encountered exposure scenarios involve exposures of six to eight hours per day for five days per week. OEHHA's chronic RELs, however, are intended to protect the general public who could be exposed continuously. In practice, discontinuous facility emissions are generally adjusted to a continuous daily or annual average.

The default approach adopted for the chronic RELs presented in this document to account for differences in effects associated with discontinuous and continuous inhalation exposures to substances is an equivalent time-weighted average approach. This is the same approach used in the derivation of U.S. EPA RfCs (U.S. EPA, 1994a). It is similar to modified Haber's law approach used for acute and 8-hour RELs in the special case where $n = 1$. Values of n greater than 1 have not been shown to be applicable in chronic exposure situations, although toxicokinetic effects such as extensive bioaccumulation may require other types of special treatment.

The default approach for estimating an equivalent time-weighted average concentration (C_{AVG}) from the observed concentration (C_{OBS}) in non-occupational studies may be summarized as:

$$C_{AVG} = C_{OBS} \text{ x (H hours per 24 hours) x (D days per 7 days)}$$

The default approach to estimating an equivalent inhalation-weighted average concentration (C_{AVG}) from the observed concentration (C_{OBS}) in studies of occupationally exposed humans is:

$$C_{AVG} = C_{OBS} \text{ x (10 m}^3\text{/day occupational exposure / 20 m}^3\text{/day total exposure)}$$

$$\text{x (D days per 7 days)}$$

7.2.2 Differences between Lifetime and Less-than-Lifetime Exposures

Studies of adverse health effects associated with exposures of humans or experimental animals generally involve less-than-lifetime exposures. The OEHHA chronic RELs, however, are intended to protect the general public who could be exposed over their entire lifetime. Studies that expose experimental animals for at least 12% of the expected lifetime for the test species are considered chronic exposure studies. RELs based on such chronic animal studies are not adjusted for less-than-lifetime exposures. Similarly using this convention, chronic exposure for humans is considered to be greater than 12% of a lifetime of 70 years. Thus, human exposures of greater than 8 years are considered chronic exposures and are not adjusted either in their calculation or application. Although a potential source of uncertainty, this approximation appears reasonable for the majority of chemicals.

There are certain situations, such as in cancer risk assessment, where dependence on cumulative dose over long periods up to and including a lifetime (subject to weighting during critical periods early in life) may reasonably be assumed. Models of dose-time cumulation over relatively short timescales have been explored for various acute toxicity endpoints, and are described elsewhere

in this document. However, for most situations involving chronic noncancer toxicity an explicit description of the time/dose relationship over longer intervals (including several weeks or months to a full lifetime) is not available. Toxicity studies tend to be conducted for specific periods representing subchronic, chronic and lifetime exposures, but these are seldom directly related to one another, and frequently report different endpoints. Subchronic exposures are those with duration less than 12% of expected lifetime for the test species, except in the case of mice and rats where the U.S. EPA has considered 13 weeks subchronic. Therefore, the default approach to extrapolating from subchronic to chronic exposures used by OEHHA and the U.S. EPA is to use a 1 to 10-fold uncertainty factor, UF_S for subchronic exposures.

The UF_S to extrapolate from subchronic to chronic exposures is determined as follows:

(1) exposures less than 8% of expected lifetime were given a 10-fold UF
(2) exposures from 8 to <12% of expected lifetime were given a 3-fold UF, and
(3) exposures ≥12% of expected lifetime were given a 1-fold UF.

Average life spans assumed for humans and experimental animals are presented in Table 7.2.1.

TABLE 7.2.1. AVERAGE LIFE-SPAN FOR HUMANS VS. EXPERIMENTAL ANIMALS

Species	Approximate average Life-span (years)[1]	Subchronic exposure duration (weeks)[2]
Human	**70**	**≤ 364**
Baboon	55	≤ 286
Cat	15	≤ 78
Dog	15	≤ 78
Guinea pig	6	≤ 31
Hamster	2.5	≤ 13[3]
Mouse	2	≤ 13[3]
Rabbit	6	≤ 31
Rat	2	≤ 13
Rhesus monkey	35	≤ 182

[1] U.S. EPA (1988).
[2] Subchronic exposures are usually defined as those over less than 12% of average lifetime (U.S. EPA, 1994a).
[3] Special rule adopted by U.S. EPA that exposures of 13 weeks or less are subchronic regardless of the species involved (U.S. EPA, 1994a).

Unlike the extensive exposure concentration-duration-effect analyses that have been conducted for acute lethality data in experimental animals, only limited work has been done to compare the differences between acute, sub-chronic, chronic and lifetime exposure scenarios.

Kadry and associates (1995) showed that among a small data set (6 chlorinated chemicals) subchronic NOAEL to chronic NOAEL ratios were less than 10. Nessel et al. (1995) reported that for 9 inhalation studies the mean and median subchronic NOAEL to chronic NOAEL ratios

were 4.5 and 4.0 respectively (range = 1 to 8). However, in a study of published animal NOAELs for a larger group of pesticides, Nair and associates (1995) found that 19 of 148 (13%) of the subchronic to chronic NOAEL ratios differed by more than 10-fold. The U.S. EPA reported that, based on an analysis of responses to 100 substances, the subchronic to chronic ratios formed a distribution with a median value of 2 and an upper 95th percentile of 15; the value of 10 represents the 90th percentile (Swartout, 1997). This supports the selection of a default maximum value of 10 for the UF_S.

7.3 Pre-Existing Chronic Exposure Guidelines

Chronic exposure levels have been derived using several different approaches, but inhalation exposure values estimated using a consistent basis to protect the general public are only available for certain chemicals. The U.S. EPA RfCs, now published for 73 chemicals, are a notable example. Other values designed for the protection of the general public, such as the U.S. EPA reference doses (RfDs), are available for more chemicals but are intended primarily to deal with non-inhalation exposures to chemicals and are usually based on toxicity data obtained following oral exposure. It is likely that the oral and dermal routes would underestimate the health effects of inhalation exposure, unless the health effect is an identifiable systemic effect and not affected by first-pass metabolism. If the effect is systemic, then appropriate adjustments for absorption can be made.

7.3.1 U.S. EPA Reference Concentrations

The U.S. EPA developed an inhalation reference concentration (RfC) method (Jarabek et al., 1989; U.S. EPA, 1994a; 2002). The inhalation RfC considers toxic effects for both the respiratory system (portal-of-entry) and for effects peripheral to the respiratory system (extra-respiratory effects). The RfC is comparable to earlier Acceptable Daily Intake (ADI) and RfD methods but addresses inhalation specific issues such as respiratory dynamics and delivered doses by inhalation. Dosimetry models are used to extrapolate the internal dose metric across species and to estimate the human equivalent concentration (HEC), as described in Appendix F.

7.3.2 U.S. EPA Reference Doses

The U.S. EPA developed an oral reference dose (RfD) concept in 1987 (Barnes and Dourson, 1988). This provides a protocol for study selection, identifying NOAELs, applying UFs, and assessing the weight of evidence. As of September 2005, U.S. EPA RfDs were available for more than 350 substances (U.S. EPA, 2007b). The major limitation of these values for application to inhalation REL development is that they are almost entirely based on studies of exposures by routes other than inhalation. However, they have utility for substances treated as multi-pathway chemicals in the Air Toxics Hot Spots Program's risk assessments. Additionally, route-to-route extrapolation is sometimes possible, depending on the compound's toxicity and pharmacokinetics.

7.3.3 Occupational Threshold Limit Values

Occupational exposure limits have been used to derive chemical exposure guidelines for the general public (NATICH and McCullough, 1991; Robinson and Paxman, 1992). As of May,

2005, more than 600 American Conference of Governmental Industrial Hygienists (ACGIH) Threshold Limit Values (TLVs) (ACGIH, 2005) and National Institute for Occupational Safety and Health (NIOSH) Permissible Exposure Limits (PELs) (NIOSH, 2005) were available. The California Occupational Safety and Health Administration also has approximately 600 occupational PELs. However, these values lack a consistent basis, are not designed for or recommended for protection of the general public, and in many cases may not prevent adverse health effects among workers (Roach and Rappaport, 1990). Occupational exposure guidelines, which are available for hundreds of substances, have been used in many states to derive inhalation exposure guidelines for the general public. These values, however, have an inconsistent basis, which often included risk management and feasibility considerations specific to industrial facilities, in addition to health-based criteria, and have not always incorporated recently available data. Most importantly, occupational exposure guidelines are designed to protect healthy adult workers, and do not allow for possibly more sensitive members of the general population such as children and the elderly, or those with genetically predetermined sensitivities. Thus, OEHHA does not use occupational guidelines for chronic RELs, which are intended to protect the general public.

7.3.4 California Ambient Air Quality Standards

California Ambient Air Quality Standards (CAAQS) are available for criteria air pollutants (CAPCOA, 1993). Where defined according to a basis appropriate to lifetime exposures, the CAAQS was adopted as the chronic inhalation REL.

7.4 Severity of Chronic Effects

The relationship between effect categories and severity levels is shown in Table 4.4.1 above for acute exposure. While these definitions of severity have only been developed for acute exposures, it is anticipated that they are generally applicable to chronic exposures as well. As with the acute effects, effects that are irreversible or that involve significant cumulative tissue damage (e.g., necrosis) are considered severe.

7.5 Hyperplasia

The use of hyperplasia as a toxicological endpoint for setting chronic RELs should consider whether the hyperplasia may progress to dysplasia and neoplasia. In a chronic study, if hyperplasia was the most sensitive endpoint for that chemical, it was used as an endpoint for REL development. Hyperplasia can be seen as a normal response (e.g., to hormones), and is also seen in response to a number of sensory irritants which are not carcinogens. When hyperplasia was used as the toxicological endpoint and as a mild effect, the histological grade was low (e.g., one on a scale of one to five) and, in accordance with Table 4.4.1 there was no increase in organ weight noted. Increasing histological grade, changes in organ weight and other morphological changes would be cause to view the hyperplasia as a severe effect.

References

ACGIH. (2005). *Documentation of the Threshold Limit Values and Biological Exposure Indices Annual Reports of the Committees on TLVs® and BEIs® for Year 2005 Publication #0106A*

Cincinnati (OH): American Conference of Governmental Industrial Hygienists, Inc.

ACGIH. (2006). *Documentation of the Threshold Limit Values and Biological Exposure Indices Annual Reports of the Committees on TLVs® and BEIs® for Year 2006 Publication #0106*. Cincinnati (OH): American Conference of Governmental Industrial Hygienists, Inc.

Agunod M, Yamaguchi N, Lopez R, Luhby AL and Glass GB (1969). Correlative study of hydrochloric acid, pepsin, and intrinsic factor secretion in newborns and infants. Am J Dig Dis 14(6): 400-414.

AIHA. (1991). *Emergency Response Planning Guidelines*. Akron (OH): American Industrial Hygiene Association.

AIHA. (2006). *Public Exposure Guidelines*. American Industrial Hygiene Association. http://archive.orr.noaa.gov/cameo/locs/expguide.html.

Alarie Y (1966). Irritating properties of airborne materials to the upper respiratory tract. Arch Environ Health 13(4): 433-49.

Alcorn J and McNamara PJ (2003). Pharmacokinetics in the newborn. Adv Drug Deliv Rev 55(5): 667-686.

Aldridge JE, Seidler FJ and Slotkin TA (2004). Developmental exposure to chlorpyrifos elicits sex-selective alterations of serotonergic synaptic function in adulthood: critical periods and regional selectivity for effects on the serotonin transporter, receptor subtypes, and cell signaling. Environ Health Perspect 112(2): 148-55.

Alexeeff GV, Broadwin R, Liaw J and Dawson SV (2002). Characterization of the LOAEL-to-NOAEL uncertainty factor for mild adverse effects from acute inhalation exposures. Regul Toxicol Pharmacol 36(1): 96-105.

Alexeeff GV, Deng KK, Broadwin RL and Salmon AG (2006). Benchmark dose evaluation for human irritation [Abstract # 901]. The Toxicologist 90(1): 183.

Alexeeff GV, Fowles JR, Hill M and Dodge D (1997). Stochastic evaluation of acute inhalation thresholds from reported LOAELs [Abstract #851]. The Toxicologist 36(1 Part 2): 167.

Alexeeff GV and Lewis DC. (1989b). *Factors influencing quantitative risk analysis of noncancer health effects from air pollutants*. 89-91.3. Air & Waste Management Assoc.

Alexeeff GV, Lewis DC and Lipsett MJ (1992). Use of toxicity information in risk assessment for accidental release of toxic gases. J Hazard Mater 29(3): 387-403.

Alexeeff GV, Lewis DC and Ragle NL (1993). Estimation of potential health effects from acute exposure to hydrogen fluoride using a "benchmark dose" approach. Risk Anal 13(1): 63-69.

Alexeeff GV, Lipsett MJ and Kizer KW (1989a). Problems associated with the use of immediately dangerous to life and health (IDLH) values for estimating the hazard of accidental chemical releases. Am Ind Hyg Assoc J 50(11): 598-605.

Almstrup K, Fernandez MF, Petersen JH, Olea N, Skakkebaek NE and Leffers H (2002). Dual effects of phytoestrogens result in u-shaped dose-response curves. Environ Health Perspect 110(8): 743-8.

Amateau SK and McCarthy MM (2004). Induction of PGE2 by estradiol mediates developmental masculinization of sex behavior. Nat Neurosci 7(6): 643-50.

Anglen DM. (1981). Sensory response of human subjects to chlorine in air. dissertation. Ann Arbor (MI): University of Michigan.

Anjilvel S and Asgharian B (1995). A multiple-path model of particle deposition in the rat lung. Fundam Appl Toxicol 28(1): 41-50.

Arant Jr. BS (1978). Developmental patterns of renal functional maturation compared in the human neonate. J Pediatr 92(5): 705-712.

Arcus A, Broadwin R, Lam R and Vork K. (1995). *A Database of Indoor Air Volatile Organic Compounds and Selected Combustion Products: Construction and Development (draft). Report to Indoor Air Quality and Personal Exposure Assessment Section*. Sacramento (CA): Air Resources Board.

Aris RM, Tager I, Christian D, Kelly T and Balmes JR (1995). Methacholine responsiveness is not associated with O_3-induced decreases in FEV_1. Chest 107(3): 621-628.

ATS (2000a). American Thoracic Society. What constitutes an adverse health effect of air pollution? . Am J Respir Crit Care Med 161(2 Pt 1): 665-673.

ATS (2000b). American Thoracic Society. Guidelines for methacholine and exercise challenge testing - 1999. Am J Respir Crit Care Med 161(1): 309-329.

Augustinsson KB and Barr M (1963). Age variation In plasma arylesterase activity in children. Clin Chim Acta 8: 568-573.

Autrup H (2000). Genetic polymorphisms in human xenobiotica metabolizing enzymes as susceptibility factors in toxic response. Mutat Res 464(1): 65-76.

Avery GB, Randolph JG and Weaver T (1966). Gastric acidity in the first day of life. Pediatrics 37(6): 1005-1007.

Ballatori N and Clarkson TW (1982). Developmental changes in the biliary excretion of methylmercury and glutathione. Science 216(4541): 61-63.

Barnes DG, Daston GP, Evans JS, Jarabek AM, Kavlock RJ, Kimmel CA, Park C and Spitzer HL (1995). Benchmark Dose Workshop: Criteria for use of a benchmark dose to estimate a reference dose. Regul Toxicol Pharmacol 21(2): 296-306.

Barnes DG and Dourson M (1988). Reference dose (RfD): Description and use in health risk assessments. Regul Toxicol Pharmacol 8(4): 471-486.

Barrow C, Buckley L, James R, Steinhagen W and Chang J (1986). Sensory Irritation: Studies on correlation to pathology, structure-activity, tolerance-development, and prediction of species differences to nasal injury. Chapter 7. In: Toxicology of the Nasal Passages. Barrow C. Hemisphere. Washington (DC) & New York (NY): 101-122.

Barton JC and Huster WJ (1987). Seasonal changes in lead absorption in laboratory rats. Environ Health Perspect 73: 209-214.

Belay ED, Bresee JS, Holman RC, Khan AS, Shahriari A and Schonberger LB (1999). Reye's syndrome in the United States from 1981 through 1997. N Engl J Med 340(18): 1377-82.

Benignus VA, Boyes WK and Bushnell PJ (1998). A dosimetric analysis of behavioral effects of acute toluene exposure in rats and humans. Toxicol Sci 43(2): 186-95.

Bernstein DI, Ploysongsang Y, Mittman RJ, Piyamahunt A and Bernstein IL (1992). The relationship between airway responsiveness measured before and after the allergen-induced late asthmatic response. Chest 101(2): 437-441.

Besunder JB, Reed MD and Blumer JL (1988). Principles of drug biodisposition in the neonate. A critical evaluation of the pharmacokinetic-pharmacodynamic interface (Part I). Clin Pharmacokinet 14(4): 189-216.

Bigwood EJ (1973). The acceptable daily intake of food additives. CRC Crit Rev Toxicol 2(1): 41-93.

Bos PM, Zwart A, Reuzel PG and Bragt PC (1991). Evaluation of the sensory irritation test for the assessment of occupational health risk. Crit Rev Toxicol 21(6): 423-450.

Brodersen R, Friis-Hansen B and Stern L (1983). Drug-induced displacement of bilirubin from albumin in the newborn. Dev Pharmacol Ther 6(4): 217-229.

Brown JP (2001). Physiological and anatomical parameters for infants and children's PBPK modeling. Children's Environmental Health--Developing a Risk Assessment Framework.Office of Environmental Health Hazard Assessment, California Environmental Protection Agency. Monterey (CA), April.

Brown JP, Collins JF, Salmon AG, Marty MA and Alexeeff GV (2006). Use of benchmark dose methodology on human non-cancer data to develop protective criteria for child exposures to arsenic [Abstract # 2185]. The Toxicologist 90(1): 448.

Brown JS, Wilson WE and Grant LD (2005). Dosimetric comparisons of particle deposition and retention in rats and humans. Inhal Toxicol 17(7-8): 355-385.

Brown NA and Fabro S (1983). The value of animal teratogenicity testing for predicting human risk. Clin Obstet Gynecol 26(2): 467-77.

Bryant B and Silver W (2000). Chemesthesis: The common chemical sense. In: The Neurobiology of Taste and Smell (2nd Edition). Finger T., Silver W. and Restrepo D. Wiley-Liss. NY: 73-100.

Buehler BA, Rao V and Finnell RH (1994). Biochemical and molecular teratology of fetal hydantoin syndrome. Neurol Clin 12(4): 741-748.

Bushnell PJ, Boyes WK, Shafer TJ, Bale AS and Benignus VA (2006). Approaches to extrapolating animal toxicity data on organic solvents to public health. Neurotoxicology.

Calabrese EJ (1983). Principles of Animal Extrapolation. Environmental Science and Technology. New York: Wiley.

Canalle R, Burim RV, Tone LG and Takahashi CS (2004). Genetic polymorphisms and susceptibility to childhood acute lymphoblastic leukemia. Environ Mol Mutagen 43(2): 100-109.

CAPCOA. (1993). *The Air Toxics Hot Spots Program Revised 1992 Risk Assessment Guidelines*. Cameron Park (CA): California Air Pollution Control Officers' Association Toxics Committee

CARB. (1990). *Proposed Identification of Vinyl Chloride as a Toxic Air Contaminant. Technical Support Document. Part C*. California Air Resources Board.

Carlisle J, Kaley K and Dowling K. (2006). *Development of health criteria for school site risk assessment pursuant to health and safety code section 901(g); Proposed child-specific benchmark change in blood lead concentration for school site risk assessment*. California Environmental Protection Agency. http://www.oehha.ca.gov/public_info/public/kids/pdf/Lead031606.pdf.

Cherniack RM (1995). Physiologic diagnosis and function in asthma. Clin Chest Med 16(4): 567-581.

Chhabra RS, Huff JE, Schwetz BS and Selkirk J (1990). An overview of prechronic and chronic toxicity/carcinogenicity experimental study designs and criteria used by the National Toxicology Program. Environ Health Perspect 86: 313-321.

Chiron C, Raynaud C, Maziere B, Zilbovicius M, Laflamme L, Masure MC, Dulac O, Bourguignon M and Syrota A (1992). Changes in regional cerebral blood flow during brain maturation in children and adolescents. J Nucl Med 33(5): 696-703.

Chizhikov VA (1973). [Effect of harmful substances on animals with weakened health under chronic feeding conditions]. Gig Sanit 38(7): 7-12.

Clewell HJ, Gentry PR, Covington TR, Sarangapani R and Teeguarden JG (2004). Evaluation of the potential impact of age- and gender-specific pharmacokinetic differences on tissue dosimetry. Toxicol Sci 79(2): 381-393.

Cole TB, Jampsa RL, Walter BJ, Arndt TL, Richter RJ, Shih DM, Tward A, Lusis AJ, Jack RM, Costa LG and Furlong CE (2003). Expression of human paraoxonase (PON1) during development. Pharmacogenetics 13(6): 357-364.

Collins JF, Alexeeff GV, Lewis DC, Dodge DE, Marty MA, Parker TR, Budroe JD, Lam RH, Lipsett MJ, Fowles JR and Das R (2004). Development of acute inhalation reference exposure levels (RELs) to protect the public from predictable excursions of airborne toxicants. J Appl Toxicol 24(2): 155-166.

Collins JF, Salmon AG, Brown JP, Marty MA and Alexeeff GV (2005). Development of a chronic inhalation reference level for respirable crystalline silica. Regul Toxicol Pharmacol 43(3): 292-300.

Cometto-Muniz JE, Cain WS and Hudnell HK (1997). Agonistic sensory effects of airborne chemicals in mixtures: Odor, nasal pungency, and eye irritation. Percept Psychophys 59(5): 665-674.

Conceicao GM, Miraglia SG, Kishi HS, Saldiva PH and Singer JM (2001). Air pollution and child mortality: A time-series study in Sao Paulo, Brazil. Environ Health Perspect 109 (Suppl 3): 347-350.

Costa LG, Richter RJ, Li WF, Cole T, Guizzetti M and Furlong CE (2003). Paraoxonase (PON 1) as a biomarker of susceptibility for organophosphate toxicity. Biomarkers 8(1): 1-12.

Costa LG, Vitalone A, Cole TB and Furlong CE (2005). Modulation of paraoxonase (PON1) activity. Biochem Pharmacol 69(4): 541-550.

Cresteil T (1998). Onset of xenobiotic metabolism in children: Toxicological implications. Food Addit Contam 15 (Suppl): 45-51.

Crofton KM, Craft ES, Hedge JM, Gennings C, Simmons JE, Carchman RA, Carter Jr. WH and DeVito MJ (2005). Thyroid-hormone-disrupting chemicals: Evidence for dose-dependent additivity or synergism. Environ Health Perspect 113(11): 1549-1554.

Crump KS (1984). A new method for determining allowable daily intakes. Fundam Appl Toxicol 4(5): 854-871.

Crump KS (1995). Calculation of benchmark doses from continuous data. Risk Anal 15: 78-89.

Crump KS (2002). Critical issues in benchmark calculations from continuous data. Crit Rev Toxicol 32(3): 133-153.

DeVito MJ, Menache MG, Diliberto JJ, Ross DG and Birnbaum LS (2000). Dose-response relationships for induction of CYP1A1 and CYP1A2 enzyme activity in liver, lung, and skin in

female mice following subchronic exposure to polychlorinated biphenyls. Toxicol Appl Pharmacol 167(3): 157-172.

Dorne JL and Renwick AG (2005a). The refinement of uncertainty/safety factors in risk assessment by the incorporation of data on toxicokinetic variability in humans. Toxicol Sci 86(1): 20-26.

Dourson ML (1986). New approaches in the derivation of acceptable daily intake (ADI). Comm Toxicol 1: 35-48.

Dourson ML, Felter SP and Robinson D (1996). Evolution of science-based uncertainty factors in noncancer risk assessment. Regul Toxicol Pharmacol 24(2 Pt 1): 108-120.

Dourson ML, Hertzberg RC, Hartung R and Blackburn K (1985). Novel methods for the estimation of acceptable daily intake. Toxicol Ind Health 1(4): 23-33.

Dourson ML and Stara JF (1983). Regulatory history and experimental support of uncertainty (safety) factors. Regul Toxicol Pharmacol 3(3): 224-238.

Ecobichon DJ and Stephens DS (1973). Perinatal development of human blood esterases. Clin Pharmacol Ther 14(1): 41-47.

Eichelbaum M, Kroemer HK and Mikus G (1992). Genetically determined differences in drug metabolism as a risk factor in drug toxicity. Toxicol Lett 64-65 (Spec No): 115-122.

Eiser NM, Kerrebijn KF and Quanjer PH (1983). Guidelines for standardization of bronchial challenges with (nonspecific) bronchoconstricting agents European Society for Clinical Respiratory Physiology Working Group "Bronchial hyperreactivity".Bull Eur Physiopathol Resp. 19: 495-514.

Eluma FO, Sucheston ME, Hayes TG and Paulson RB (1984). Teratogenic effects of dosage levels and time of administration of carbamazepine, sodium valproate, and diphenylhydantoin on craniofacial development in the CD-1 mouse fetus. J Craniofac Genet Dev Biol 4(3): 191-210.

Eschenbacher WL, Moore TB, Lorenzen TJ, Weg JG and Gross KB (1992). Pulmonary responses of asthmatic and normal subjects to different temperature and humidity conditions in an environmental chamber. Lung 170(1): 51-62.

Evelo CT, Oostendorp JG, ten Berge WF and Borm PJ (1993). Physiologically based toxicokinetic modeling of 1,3-butadiene lung metabolism in mice becomes more important at low doses. Environ Health Perspect 101(6): 496-502.

Fanucchi MV and Plopper CG (1997). Pulmonary developmental responses to toxicants. In: Comprehensive Toxicology Roth R. Pergamon. New York: Vol. 8: 203-220.

Fanucchi MV, Plopper CG, Evans MJ, Hyde DM, Van Winkle LS, Gershwin LJ and Schelegle ES (2006). Cyclic Exposure to Ozone Alters Distal Airway Development in Infant Rhesus Monkeys. Am J Physiol Lung Cell Mol Physiol 291(4): L644-650.

Filipsson AF, Sand S, Nilsson J and Victorin K (2003). The benchmark dose method--review of available models, and recommendations for application in health risk assessment. Crit Rev Toxicol 33(5): 505-542.

Foradori AC, Bertinchamps A, Gulibon JM and Cotzias GC (1967). The discrimination between magnesium and manganese by serum proteins. J Gen Physiol 50(9): 2255-66.

Fowles JR, Alexeeff GV and Dodge D (1999). The use of benchmark dose methodology with acute inhalation lethality data. Regul Toxicol Pharmacol 29(3): 262-278.

Frederick CB, Bush ML, Lomax LG, Black KA, Finch L, Kimbell JS, Morgan KT, Subramaniam RP, Morris JB and Ultman JS (1998). Application of a hybrid computational fluid dynamics and physiologically based inhalation model for interspecies dosimetry extrapolation of acidic vapors in the upper airways. Toxicol Appl Pharmacol 152(1): 211-231.

Friis-Hansen B (1961). Body water compartments in children: Changes during growth and related changes in body composition. Pediatrics 28: 169-181.

Friis-Hansen B (1971). Body composition during growth. In vivo measurements and biochemical data correlated to differential anatomical growth. Pediatrics 47(1 Suppl 2): 264-274.

Galizia A and Kinney PL (1999). Long-term residence in areas of high ozone: Associations with respiratory health in a nationwide sample of nonsmoking young adults. Environ Health Perspect 107(8): 675-679.

Gargas ML, Andersen ME and Clewell 3rd. HJ (1986). A physiologically based simulation approach for determining metabolic constants from gas uptake data. Toxicol Appl Pharmacol 86(3): 341-352.

Gauderman WJ, Avol E, Gilliland F, Vora H, Thomas D, Berhane K, McConnell R, Kuenzli N, Lurmann F, Rappaport E, Margolis H, Bates D and Peters J (2004). The effect of air pollution on lung development from 10 to 18 years of age. N Engl J Med 351(11): 1057-1067.

Gauderman WJ, Avol E, Lurmann F, Kuenzli N, Gilliland F, Peters J and McConnell R (2005). Childhood asthma and exposure to traffic and nitrogen dioxide. Epidemiology 16(6): 737-743.

Gaylor D, Ryan L, Krewski D and Zhu Y (1998). Procedures for calculating benchmark doses for health risk assessment. Regul Toxicol Pharmacol 28(2): 150-164.

Gaylor DW (1988). Applicability of cancer risk assessment techniques to other toxic effects. Toxicol Ind Health 4(4): 453-459.

Gaylor DW (1992). Incidence of developmental defects at the no observed adverse effect level (NOAEL). Regul Toxicol Pharmacol 15(2 Pt 1): 151-160.

Gebremichael A, Chang AM, Buckpitt AR, Plopper CG and Pinkerton KE (1995). Postnatal development of cytochrome P4501A1 and 2B1 in rat lung and liver: Effect of aged and diluted sidestream cigarette smoke. Toxicol Appl Pharmacol 135(2): 246-253.

Gibbons RA, Dixon SN, Hallis K, Russell AM, Sansom BF and Symonds HW (1976). Manganese metabolism in cows and goats. Biochim Biophys Acta 444(1): 1-10.

Gift JS, Webb CK and Jarabek AM (1993). Suitability of LOAEL-to-NOAEL 10-fold uncertainty factor for health assessments of inhaled toxicants [Abstract # 475]. The Toxicologist 13: 140.

Gillis CA, Keenan RE, Carlson-Lynch HL and Price PS (1997). Characterization of the inter-individual (UFH) factors: Alternative models and approaches [Abstract #1053]. The Toxicologist 36: 207.

Ginsberg G (2000). Xenobiotic metabolism and chemical fate from early childhood onward. Children's Environmental Health Symposium. Oakland (CA).

Ginsberg G, Hattis D, Russ A and Sonawane B (2004b). Physiologically based pharmacokinetic (PBPK) modeling of caffeine and theophylline in neonates and adults: Implications for assessing children's risks from environmental agents. J Toxicol Environ Health A 67(4): 297-329.

Ginsberg G, Hattis D, Sonawane B, Russ A, Banati P, Kozlak M, Smolenski S and Goble R (2002). Evaluation of child/adult pharmacokinetic differences from a database derived from the therapeutic drug literature. Toxicol Sci 66(2): 185-200.

Ginsberg G, Slikker Jr. W, Bruckner J and Sonawane B (2004c). Incorporating children's toxicokinetics into a risk framework. Environ Health Perspect 112(2): 272-283.

Gold WG (1994). Pulmonary function testing. In: Respiratory Medicine. 2nd ed. Murray J. F. and Nadel J. A. W.B. Saunders. Philadelphia: 815-817.

Goldstein BD (1983). Toxic substances in the atmospheric environment. A critical review. J Air Pollut Control Assoc 33(5): 454-467.

Grandjean P (1992). Individual susceptibility to toxicity. Toxicol Lett 64-65 (Spec No): 43-51.

Guberman AH, Besag FM, Brodie MJ, Dooley JM, Duchowny MS, Pellock JM, Richens A, Stern RS and Trevathan E (1999). Lamotrigine-associated rash: risk/benefit considerations in adults and children. Epilepsia 40(7): 985-91.

Guignard JP, Torrado A, Da Cunha O and Gautier E (1975). Glomerular filtration rate in the first three weeks of life. J Pediatr 87(2): 268-272.

Ha EH, Hong YC, Lee BE, Woo BH, Schwartz J and Christiani DC (2001). Is air pollution a risk factor for low birth weight in Seoul? Epidemiology 12(6): 643-648.

Hackney JD and Linn WS (1983). Controlled clinical studies of air pollutant exposure: Evaluating scientific information in relation to air quality standards. Environ Health Perspect 52: 187-191.

Haddad S, Beliveau M, Tardif R and Krishnan K (2001). A PBPK modeling-based approach to account for interactions in the health risk assessment of chemical mixtures. Toxicol Sci 63(1): 125-131.

Hakkola J, Tanaka E and Pelkonen O (1998). Developmental expression of cytochrome P450 enzymes in human liver. Pharmacol Toxicol 82(5): 209-217.

Hanna LM, Lou SR, Su S and Jarabek AM (2001). Mass transport analysis: Inhalation RfC methods framework for interspecies dosimetric adjustment. Inhal Toxicol 13(5): 437-463.

Harada M (1995). Minamata disease: Methylmercury poisoning in Japan caused by environmental pollution. Crit Rev Toxicol 25(1): 1-24.

Harding R, Pinkerton K and Plopper CG (2004). The Lung: Development, Aging and the Environment. London (UK); San Diego (CA): Elsevier Academic Press.

Hartung R (1987). Dose-response relationships. In: Toxic Substances and Human Risk. Tardiff R. G. and Rodricks J. V. Plenum Press. New York: 29-46.

Hattis D (1996a). Variability in susceptibility--how big, how often, for what responses to what agents? Environ Toxicol Pharmacol 2: 135-145.

Hattis D (1996b). Human interindividual variability in susceptibility to toxic effects: From annoying detail to a central determinant of risk. Toxicology 111(1-3): 5-14.

Hattis D, Banati P and Goble R (1999). Distributions of individual susceptibility among humans for toxic effects. How much protection does the traditional tenfold factor provide for what fraction of which kinds of chemicals and effects? Ann N Y Acad Sci 895: 286-316.

Hattis D, Erdreich L and Ballew M (1987). Human variability in susceptibility to toxic chemicals--a preliminary analysis of pharmacokinetic data from normal volunteers. Risk Anal 7(4): 415-426.

Hattis D, Ginsberg G, Sonawane B, Smolenski S, Russ A, Kozlak M and Goble R (2003). Differences in pharmacokinetics between children and adults--II. Children's variability in drug elimination half-lives and in some parameters needed for physiologically-based pharmacokinetic modeling. Risk Anal 23(1): 117-142.

Heath LM, Soole KL, McLaughlin ML, McEwan GT and Edwards JW (2003). Toxicity of environmental lead and the influence of intestinal absorption in children. Rev Environ Health 18(4): 231-250.

Herngren L, Ehrnebo M and Boreus LO (1983). Drug binding to plasma proteins during human pregnancy and in the perinatal period. Studies on cloxacillin and alprenolol. Dev Pharmacol Ther 6(2): 110-124.

Hill AB (1971). Chapter XXIV: Statistical Evidence and Inference. In: Principles of Medical Statistics. Oxford University Press. Oxford (UK).

Hines RN and McCarver DG (2002). The ontogeny of human drug-metabolizing enzymes: Phase I oxidative enzymes. J Pharmacol Exp Ther 300(2): 355-360.

Hnizdo E, Murray J, Sluis-Cremer GK and Thomas RG (1993). Correlation between radiological and pathological diagnosis of silicosis: An autopsy population based study. Am J Ind Med 24(4): 427-445.

Hook JB and Hewitt WR (1977). Development of mechanisms for drug excretion. Am J Med 62(4): 497-506.

Horstman D, Roger LJ, Kehrl H and Hazucha M (1986). Airway sensitivity of asthmatics to sulfur dioxide. Toxicol Ind Health 2(3): 289-298.

Howdeshell KL, Furr J, Lambright CR, Rider CV, Wilson VS and Gray LE, Jr. (2007). Cumulative effects of dibutyl phthalate and diethylhexyl phthalate on male rat reproductive tract development: altered fetal steroid hormones and genes. Toxicol Sci 99(1): 190-202.

IARC. (2006). *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*. Preamble. International Agency for Research on Cancer http://monographs.iarc.fr/ENG/Preamble/index.php.

ICRP. (1994). Human Respiratory Tract Model for Radiological Protection. Elsevier Science Inc. Tarrytown (NY)

Ikeda M (1988). Multiple exposure to chemicals. Regul Toxicol Pharmacol 8(4): 414-421.

Islam MS and Schlipkoter HW (1989). Reversible fraction of airway resistance in healthy children of areas with different levels of atmospheric pollutants. Exp Pathol 37(1-4): 23-26.

Jarabek AM, Asgharian B and Miller FJ (2005). Dosimetric adjustments for interspecies extrapolation of inhaled poorly soluble particles (PSP). Inhal Toxicol 17(7-8): 317-334.

Jarabek AM, Menache MG, Overton Jr. JH, Dourson ML and Miller FJ (1989). Inhalation reference dose (RfDi): An application of interspecies dosimetry modeling for risk assessment of insoluble particles. Health Phys 57 (Suppl 1): 177-183.

Jarnberg J and Johanson G (1999). Physiologically based modeling of 1,2,4-trimethylbenzene inhalation toxicokinetics. Toxicol Appl Pharmacol 155(3): 203-214.

Jiang X, Buckley L and Morgan K (1983). Pathology of toxic responses to the RD50 concentration of chlorine gas in the nasal passages of rats and mice. Toxicol Appl Pharmacol 71(2): 225-236.

Jonker D, Woutersen RA, van Bladeren PJ, Til HP and Feron VJ (1990). 4-Week oral toxicity study of a combination of eight chemicals in rats: Comparison with the toxicity of the individual compounds. Food Chem Toxicol 28(9): 623-631.

Jubber AS, Foster RW, Hassan NA, Carpenter JR and Small RC (1993). Airway response to inhaled methacholine in normal human subjects. Pulm Pharmacol 6(3): 177-184.

Kadry AM, Khodair AJ, Skowronski GA and Abdel-Rahman MS (1995). Evaluation of the current method for extrapolating subchronic and chronic test results for estimation of lifetime exposure levels [Abstract #180]. The Toxicologist 15(1): 33.

Kearns GL, Abdel-Rahman SM, Alander SW, Blowey DL, Leeder JS and Kauffman RE (2003). Developmental pharmacology--Drug disposition, action, and therapy in infants and children. N Engl J Med 349(12): 1157-1167.

Khodair AJ, Kadry AM, Skowronski GA and Abdel-Rahman MS (1995). Comparison of animal and human data to estimate the no adverse effect level (NOAEL) in humans [Abstract #179]. The Toxicologist 15(1): 33.

Klaassen CD (1972). Immaturity of the newborn rat's hepatic excretory function for ouabain. J Pharmacol Exp Ther 183(3): 520-526.

Klaassen CD (1973). Comparison of the toxicity of chemicals in newborn rats to bile duct-ligated and sham-operated rats and mice. Toxicol Appl Pharmacol 24(1): 37-44.

Krasovskii GN (1976). Extrapolation of experimental data from animals to man. Environ Health Perspect 13: 51-58.

Kristiansen U, Hansen L, Nielsen GD and Holst E (1986). Sensory irritation and pulmonary irritation of cumene and n-propanol: Mechanisms of receptor activation and desensitization. Acta Pharmacol Toxicol (Copenh) 59(1): 60-72.

Kunzli N, Lurmann F, Segal M, Ngo L, Balmes J and Tager IB (1997). Association between lifetime ambient ozone exposure and pulmonary function in college freshmen--results of a pilot study. Environ Res 72(1): 8-23.

Kuwabara Y, Alexeeff G, Broadwin R and Salmon A (2006). Evaluation of the RD_{50} for determining acceptable levels of exposure to airborne sensory irritants. [Abstract #882]. The Toxicologist 90(1): 179.

Lanphear BP, Hornung R, Khoury J, Yolton K, Baghurst P, Bellinger DC, Canfield RL, Dietrich KN, Bornschein R, Greene T, Rothenberg SJ, Needleman HL, Schnaas L, Wasserman G, Graziano J and Roberts R (2005). Low-level environmental lead exposure and children's intellectual function: an international pooled analysis. Environ Health Perspect 113(7): 894-9.

Lehman AJ and Fitzhugh OG (1954). 100-Fold margin of safety. Assoc Food Drug Off USQ Bull 18: 33-35.

Lehmann KP, Phillips S, Sar M, Foster PM and Gaido KW (2004). Dose-dependent alterations in gene expression and testosterone synthesis in the fetal testes of male rats exposed to di (n-butyl) phthalate. Toxicol Sci 81(1): 60-8.

Leisenring W and Ryan L (1992). Statistical properties of the NOAEL. Regul Toxicol Pharmacol 15(2 Pt 1): 161-171.

Levy G, Khanna NN, Soda DM, Tsuzuki O and Stern L (1975). Pharmacokinetics of acetaminophen in the human neonate: Formation of acetaminophen glucuronide and sulfate in relation to plasma bilirubin concentration and D-glucaric acid excretion. Pediatrics 55(6): 818-825.

Lewis DC and Alexeeff GV (1989). Quantitative risk assessment of noncancer health effects for acute exposure to air pollutants. In: 82nd Annual Meeting of the Air and Waste Management Association. Association A. a. W. M.Anaheim (CA): p. 89-91.

Lilienfeld AM and Lilienfeld DE (1980). Foundations of Epidemiology, 2nd Edition Oxford University Press.

Loomis D, Castillejos M, Gold DR, McDonnell W and Borja-Aburto VH (1999). Air pollution and infant mortality in Mexico City. Epidemiology 10(2): 118-123.

Mantel N, Bohidar NR, Brown CC, Ciminera JL and Tukey JW (1975). An improved Mantel-Bryan procedure for "safety" testing of carcinogens. Cancer Res 35(4): 865-872.

Mantel N and Bryan WR (1961). "Safety" testing of carcinogenic agents. J Natl Cancer Inst 27: 455-470.

Maughan B, Taylor C, Taylor A, Butler N and Bynner J (2001). Pregnancy smoking and childhood conduct problems: A causal association? J Child Psychol Psychiatry 42(8): 1021-1028.

McCarver DG and Hines RN (2002). The ontogeny of human drug-metabolizing enzymes: Phase II conjugation enzymes and regulatory mechanisms. J Pharmacol Exp Ther 300(2): 361-366.

McConnell R, Berhane K, Yao L, Jerrett M, Lurmann F, Gilliland F, Kunzli N, Gauderman J, Avol E, Thomas D and Peters J (2006). Traffic, susceptibility, and childhood asthma. Environ Health Perspect 114(5): 766-772.

McFadden SW and Haddow JE (1969). Coma produced by topical application of isopropanol. Pediatrics 43(4): 622-623.

Miles M (1983). Pediatric Pharmacokinetics. In: Applied Clinical Pharmacokinetics. Mungall D. McGraw-Hill. New York: 367-388.

Miller MD, Marty MA, Arcus A, Brown J, Morry D and Sandy M (2002). Differences between children and adults: Implications for risk assessment at California EPA. Int J Toxicol 21(5): 403-418.

Miller RA (1941). Observations on the gastric acidity during the first month of life. Arch Dis Child 16: 22-30.

Milsap RL and Jusko WJ (1994). Pharmacokinetics in the infant. Environ Health Perspect 102 (Suppl 11): 107-110.

Mitchell WA, Gift JS and Jarabek AM (1993). Suitability of LOAEL to NOAEL 10-fold uncertainty factor for health assessments of inhaled toxicants [Abstract #475]. The Toxicologist 13: 140.

Monson RR (1986). Observations on the healthy worker effect. J Occup Med 28(6): 425-433.

Morselli PL (1976). Clincial pharmacokinetics in neonates. Clin Pharmacokinet 1(2): 81-98.

Morselli PL (1989). Clinical pharmacology of the perinatal period and early infancy. Clin Pharmacokinet 17 (Suppl 1): 13-28.

Morselli PL, Franco-Morselli R and Bossi L (1980). Clinical pharmacokinetics in newborns and infants. Age-related differences and therapeutic implications. Clin Pharmacokinet 5(6): 485-527.

Muller G, Spassovski M and Henschler D (1972). Trichloroethylene exposure and trichloroethylene metabolites in urine and blood. Arch Toxikol 29(4): 335-40.

Murrell JA, Portier CJ and Morris RW (1998). Characterizing dose-response I: Critical assessment of the benchmark dose concept. Risk Anal 18(1): 13-26.

Nair RS, Sherman JH, Stevens MW and Johannsen FR (1995). Selecting a More Realistic Uncertainty Factor: Reducing Compounding Effects of Multiple Uncertainties. Monsanto Company, St. Louis (MO).

NAS. (1977-1987). *Drinking Water and Health*. 1-7. Washington (DC): National Academy of Sciences. http://darwin.nap.edu/openbook.php?record_id=1780&page=905.

NATICH and McCullough M. (1991). *National Air Toxics Information Clearinghouse Database Report on State, Local and EPA Air Toxics Activities: Final*. Research Triangle Park (NC): National Air Toxics Information Clearinghouse

Needleman HL, Schell A, Bellinger D, Leviton A and Allred EN (1990). The long-term effects of exposure to low doses of lead in childhood. An 11-year follow-up report. N Engl J Med 322(2): 83-88.

Nessel CS, Lewis SC, Stauber KL and Adgate JL (1995). Subchronic to chronic extrapolation: Toxicologic evidence for a reduced uncertainty factor. Hum Ecol Risk Assess 1(5): 516-526.

Nielsen GD (1991). Mechanisms of activation of the sensory irritant receptor by airborne chemicals. Crit Rev Toxicol 21(3): 183-208.

Nielsen GD and Vinggaard AM (1988). Sensory irritation and pulmonary irritation of C3-C7 n-alkylamines: Mechanisms of receptor activation. Pharmacol Toxicol 63(4): 293-304.

NIOSH. (2005). *NIOSH Pocket Guide to Chemical Hazards*. Washington (DC) National Institute for Occupational Safety and Health. http://www.cdc.gov/niosh/npg/.

NRC. (1977-1987). *Drinking Water and Health*. 1-7. Washington (DC): National Research Council, National Academy Press. http://darwin.nap.edu/openbook.php?record_id=1780&page=905.

NRC. (1986a). *Criteria and Methods for Preparing Emergency Exposure Guidance Level (EEGL), Short Term Public Emergency Guidance Level (SPEGL) and Continuous Exposure Guidance Level (CEGL) Documents*. Washington (DC): National Academy Press.

NRC (1986b). Dose-Route Extrapolations: Using Inhalation Toxicity Data to Set Drinking Water Limits. In: Drinking Water and Health. Council N. R. National Academy Press. Washington (DC): 6.

NRC. (1993). *Guidelines for Developing Community Emergency Exposure Levels for Hazardous Substances.* . Washington (DC), National Academy Press.

NRC. (1994). *Science and Judgement in Risk Assessment*. Washington D.C., National Academy Press. http://www.nap.edu/books/030904894X/html/index.html.

NRC. (2001). *Standing Operating Procedures for Developing Acute Exposure Guideline Levels for Hazardous Chemicals*. Washington (DC): Subcommittee on Acute Exposure Guideline Levels, National Research Council, National Academy Press.

NRC. (2007). *Scientific Review of the Proposed Risk Assessment Bulletin from the Office of Management and Budget*. Washington (DC): National Academy Press.

OEHHA. (1999a). *The Air Toxics Hot Spots Program Risk Assessment Guidelines. Part I: Technical Support Document for Determination of Acute Reference Exposure Levels for Airborne Toxicants*. Available online at http://www.oehha.ca.gov/air/acute_rels/acuterel.html#download. http://www.oehha.ca.gov/air/acute_rels/acuterel.html#download.

OEHHA. (2000a). *The Air Toxics Hot Spots Program Risk Assessment Guidelines Part III: Technical Support Document for the Determination of Noncancer Chronic Reference Exposure Levels*. Available online at http://www.oehha.ca.gov/air/chronic_rels/pdf/relsP32k.pdf. http://www.oehha.ca.gov/air/chronic_rels/pdf/relsP32k.pdf.

OEHHA. (2000b). *The Air Toxics Hot Spots Program Risk Assessment Guidelines. Part IV: Technical Support Document for Exposure Assessment and Stochastic Analysis*. Air Toxicology and Epidemiology Section, Office of Environmental Health Hazard Assessment, California Environmental Protection Agency. http://www.oehha.ca.gov/air/hot_spots/finalStoc.html#download.

OEHHA. (2001). *Prioritization of Toxic Air Contaminants Under the Children's Environmental Health Protection Act*. Available online at

http://www.oehha.ca.gov/air/toxic_contaminants/pdf_zip/SB25%20TAC%20prioritization.pdf.
http://www.oehha.ca.gov/air/toxic_contaminants/pdf_zip/SB25%20TAC%20prioritization.pdf.

OEHHA. (2003). *The Air Toxics Hot Spots Program Guidance Manual for Preparation of Health Risk Assessments*. August. Available online at http://www.oehha.ca.gov/air/hot_spots/pdf/HRAguidefinal.pdf.
http://www.oehha.ca.gov/air/hot_spots/pdf/HRAguidefinal.pdf.

OEHHA. (2005a). *The Air Toxics Hot Spots Program Risk Assessment Guidelines. Part III: Technical Support Document for Describing Available Cancer Potency Factors*. Available online at http://www.oehha.ca.gov/air/hot_spots/pdf/May2005Hotspots.pdf.
http://www.oehha.ca.gov/air/hot_spots/pdf/May2005Hotspots.pdf.

OEHHA. (2005b). *Proposed Identification of Environmental Tobacco Smoke as a Toxic Air Contaminant*. Part B: Health Effects. California Environmental Protection Agency. http://www.oehha.ca.gov/air/environmental_tobacco/2005etsfinal.html.

Orup Jr. HI, Holmes LB, Keith DA and Coull BA (2003). Craniofacial skeletal deviations following in utero exposure to the anticonvulsant phenytoin: Monotherapy and polytherapy. Orthod Craniofac Res 6(1): 2-19.

Owen BA (1990). Literature-derived absorption coefficients for 39 chemicals via oral and inhalation routes of exposure. Regul Toxicol Pharmacol 11(3): 237-252.

Pelekis M, Gephart LA and Lerman SE (2001). Physiological-model-based derivation of the adult and child pharmacokinetic intraspecies uncertainty factors for volatile organic compounds. Regul Toxicol Pharmacol 33(1): 12-20.

Pelekis M, Nicolich MJ and Gauthier JS (2003). Probabilistic framework for the estimation of the adult and child toxicokinetic intraspecies uncertainty factors. Risk Anal 23(6): 1239-1255.

Pepelko WE (1987). Feasibility of route extrapolation in risk assessment. Br J Ind Med 44(10): 649-651.

Pepelko WE (1991). Effect of exposure route on potency of carcinogens. Regul Toxicol Pharmacol 13(1): 3-17.

Perera FP, Jedrychowski W, Rauh V and Whyatt RM (1999). Molecular epidemiologic research on the effects of environmental pollutants on the fetus. Environ Health Perspect 107 (Suppl 3): 451-460.

Pineiro-Carrero VM and Pineiro EO (2004). Liver. Pediatrics 113(4 Suppl): 1097-106.

Plopper CG and Fanucchi MV (2004). Development of Airway Epithelium. In: The Lung : Development, Aging and The Environment Harding R., Pinkerton K. and Plopper C. G. Elsevier Academic Press. San Diego.

Plopper CG, Mariassy AT, Wilson DW, Alley JL, Nishio SJ and Nettesheim P (1983). Comparison of nonciliated tracheal epithelial cells in six mammalian species: Ultrastructure and population densities. Exp Lung Res 5(4): 281-294.

Plunkett LM, Turnbull D and Rodericks JV (1992). Differences between adults and children affecting exposure assessment. In: Similarities and Differences Between Children and Adults. Implications for Risk Assessment. Guzelian P. S., Henry C. J. and Olin S. S. ILSI Press. Washington (DC): 79-94.

Price K, Haddad S and Krishnan K (2003). Physiological modeling of age-specific changes in the pharmacokinetics of organic chemicals in children. J Toxicol Environ Health A 66(5): 417-433.

Purser DA, Grimshaw P and Berrill KR (1984). Intoxication by cyanide in fires: a study in monkeys using polyacrylonitrile. Arch Environ Health 39(6): 394-400.

Rall DP (1969). Difficulties in extrapolating the results of toxicity studies in laboratory animals to man. Environ Res 2(5): 360-367.

Rane A and Wilson JT (1976). Clinical pharmacokinetics in infants and children. Clin Pharmacokinet 1(1): 2-24.

Raphael G, Meredith S, Baraniuk J and Kaliner M (1991). Nasal reflexes. In: Rhinitis (2nd Edition). Settipane G. OceanSide Publications. Providence (RI): 135-142.

Renwick AG, Dorne JL and Walton K (2000). An analysis of the need for an additional uncertainty factor for infants and children. Regul Toxicol Pharmacol 31(3): 286-296.

Renwick AG and Lazarus NR (1998). Human variability and noncancer risk assessment--An analysis of the default uncertainty factor. Regul Toxicol Pharmacol 27(1): 3-20.

Rice D and Barone Jr. S (2000). Critical periods of vulnerability for the developing nervous system: Evidence from humans and animal models. Environ Health Perspect 108(Suppl 3): 511-533.

Rice DC (1996). Evidence for delayed neurotoxicity produced by methylmercury. Neurotoxicology 17(3-4): 583-596.

Rinehart WE and Hatch T (1964). Concentration-tme product (Ct) as an expression of dose in sublethal exposures to phosgene. Am Ind Hyg Assoc J 25: 545-553.

Ritz B and Yu F (1999). The effect of ambient carbon monoxide on low birth weight among children born in southern California between 1989 and 1993. Environ Health Perspect 107(1): 17-25.

Roach SA and Rappaport SM (1990). But they are not thresholds: A critical analysis of the documentation of Threshold Limit Values. Am J Ind Med 17(6): 727-753.

Robillard J, Guillery E and Petershack J (1999). Renal function during fetal life. In: Pediatric Nephrology. Barratt T. M., Avner E. and Harmon W. Lippincott Williams & Wilkins. Baltimore: 21-37.

Robinson JC and Paxman DG (1992). The role of threshold limit values in U.S. air pollution policy. Am J Ind Med 21(3): 383-396.

Roels H, Meiers G, Delos M, Ortega I, Lauwerys R, Buchet JP and Lison D (1997). Influence of the route of administration and the chemical form (MnCl2, MnO2) on the absorption and cerebral distribution of manganese in rats. Arch Toxicol 71(4): 223-30.

Rothman KJ and Greenland S (1998). Modern Epidemiology. Philidelphia (PA): Lippincott, Raven Publishers.

Rothman KJ and Greenland S (2005). Causation and causal inference in epidemiology. Am J Public Health 95 (Suppl 1): S144-S150.

Rozman KK and Klaassen CD (1996). Absorption, distribution, and excretion of toxicants. In: Casarett and Doull's Toxicology; the basic science of poisons. Klaassen C. D. McGraw-Hill. New York: p 104.

Sagiv SK, Mendola P, Loomis D, Herring AH, Neas LM, Savitz DA and Poole C (2005). A time-series analysis of air pollution and preterm birth in Pennsylvania, 1997-2001. Environ Health Perspect 113(5): 602-606.

Salmon A (2007). Standard risk assessment procedures: Are they adequate to account for cumulative risks? International Journal of Toxicology in press.

Sanchez G. (2002). Humorap: A Mathematica package for solving the ICRP 66 and 78 compartmental models and more. http://web.usal.es/~guillerm/

Sand S, Filipsson AF and Victorin K (2002). Evaluation of the benchmark dose method for dichotomous data: Model dependence and model selection. Regul Toxicol Pharmacol 36(2): 184-197.

Sand SJ, von Rosen D and Filipsson AF (2003). Benchmark calculations in risk assessment using continuous dose-response information: The influence of variance and the determination of a cut-off value. Risk Anal 23(5): 1059-1068.

Sarangapani R, Gentry PR, Covington TR, Teeguarden JG and Clewell 3rd. HJ (2003). Evaluation of the potential impact of age- and gender-specific lung morphology and ventilation rate on the dosimetry of vapors. Inhal Toxicol 15(10): 987-1016.

Sarangapani R, Teeguarden JG, Gentry PR, Clewell 3rd. HJ, Barton HA and Bogdanffy MS (2004). Interspecies dose extrapolation for inhaled dimethyl sulfate: A PBPK model-based analysis using nasal cavity N7-methylguanine adducts. Inhal Toxicol 16(9): 593-605.

Schmidt CW, Gillis CA, Keenan RE and Price PS (1997). Characterizing inter-chemical variation in the interspecies uncertainty factor (UFA) [Abstract #1057]. The Toxicologist 36(1 Pt 2): 208.

Schwela D (2000). Air pollution and health in urban areas. Rev Environ Health 15(1-2): 13-42.

Sherwin RP (1983). What is an adverse health effect? Environ Health Perspect 52: 177-182.

Shusterman D (1999). Upper and lower airway sequelae of irritant inhalations. Clin Pulm Med 6(1): 18-31.

Shusterman D, Matovinovic E and Salmon A (2006). Does Haber's Law apply to human sensory irritation? Inhal Toxicol 18(7): 457-471.

Siegel M, Lebenthal E and Krantz B (1984). Effect of caloric density on gastric emptying in premature infants. J Pediatr 104(1): 118-122.

Signer E (1975). Gastric emptying in newborns and young infants. Measurement of the rate of emptying using indium-113m-microcolloid. Acta Paediatr Scand 64(3): 525-530.

Smiley-Jewell SM, Liu FJ, Weir AJ and Plopper CG (2000). Acute injury to differentiating Clara cells in neonatal rabbits results in age-related failure of bronchiolar epithelial repair. Toxicol Pathol 28(2): 267-276.

Snipes MB (1989a). Long-term retention and clearance of particles inhaled by mammalian species. Crit Rev Toxicol 20(3): 175-211.

Snipes MB, McClellan RO, Mauderly JL and Wolff RK (1989b). Retention patterns for inhaled particles in the lung: Comparisons between laboratory animals and humans for chronic exposures. Health Phys 57 (Suppl 1): 69-77; discussion 77-78.

Snodgrass WR (1992). Physiological and biochemical differences between children and adults as determinants of toxic response to environmental pollutants. In: Similarities and Differences Between Children and Adults. Implications For Risk Assessment. Guzelian P. S., Henry C. J. and Olin S. S. ILSI Press. Washington (DC): p. 35-42.

Starr TB, Goodman JI and Hoel DG (2005). Uses of benchmark dose methodology in quantitative risk assessment. Regul Toxicol Pharmacol 42(1): 1-2.

Sterk PJ, Fabbri LM, Quanjer PH, Cockroft DW, O'Byrne PM, Anderson SD, Juniper EF and Malo JL (1993). Airway responsiveness: Standardized challenge testing with pharmacological, physical and sensitizing stimuli in adults. Eur Resp J 6(Suppl 16): 53-83.

Streissguth AP, Aase JM, Clarren SK, Randels SP, LaDue RA and Smith DF (1991). Fetal alcohol syndrome in adolescents and adults. Jama 265(15): 1961-1967.

Stromland K, Nordin V, Miller M, Akerstrom B and Gillberg C (1994). Autism in thalidomide embryopathy: A population study. Dev Med Child Neurol 36(4): 351-356.

Swartout JC (1997). Exposure-duration uncertainty factor for the RfD [Abstract #1060]. The Toxicologist 36: 209.

ten Berge WF, Zwart A and Appelman LM (1986). Concentration-time mortality response relationship of irritant and systemically acting vapours and gases. J Hazard Mater 13: 301-309.

Ting D, Howd RA, Fan AM and Alexeeff GV (2006). Development of a health-protective drinking water level for perchlorate. Environ Health Perspect 114(6): 881-6.

Treluyer JM, Gueret G, Cheron G, Sonnier M and Cresteil T (1997). Developmental expression of CYP2C and CYP2C-dependent activities in the human liver: In-vivo/in-vitro correlation and inducibility. Pharmacogenetics 7(6): 441-452.

Treluyer JM, Jacqz-Aigrain E, Alvarez F and Cresteil T (1991). Expression of CYP2D6 in developing human liver. Eur J Biochem 202(2): 583-588.

Tyrala EE, Hillman LS, Hillman RE and Dodson WE (1977). Clinical pharmacology of hexachlorophene in newborn infants. J Pediatr 91(3): 481-486.

Tzimas G, Thiel R, Chahoud I and Nau H (1997). The area under the concentration-time curve of all-trans-retinoic acid is the most suitable pharmacokinetic correlate to the embryotoxicity of this retinoid in the rat. Toxicol Appl Pharmacol 143(2): 436-444.

U.S. DHHS. (2004). *The Health Consequences of Smoking; A Report of the Surgeon General*. Washington (D.C.) U.S. Department of Health and Human Services. http://www.cdc.gov/tobacco/sgr/sgr_2004.

U.S. EPA. (1988). *Recommendations for and Documentation of Biological Values for Use in Risk Assessment*. EPA/600/6-87-008. Washington (DC), United States Environmental Protection Agency.

U.S. EPA. (1993). Reference Dose (RfD): Description and Use in Health Risk Assessments. Background Document1A. March 15. Retrieved Sept. 21, 2005, from http://www.epa.gov/iris/rfd.htm

U.S. EPA. (1994a). *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* EPA/600/8-90/066F. Washington (DC) , United States Environmental Protection Agency. http://www.epa.gov/iris/subst/0364.htm

U.S. EPA. (1994b). *Methods for Exposure-Response Analysis and Health Assessment for Acute Inhalation Exposure to Chemicals. Development of the Acute Reference Exposure (Draft).* Research Triangle Park (NC), United States Environmental Protection Agency.

U.S. EPA. (1995). *The Use of the Benchmark Dose Approach in Health Risk Assessment*. EPA/630/R-94/007. Washington (DC), United States Environmental Protection Agency file:///W:/OEHHA/ATES/children's%20health%20TACs/SB25%20Risk%20Assess%20gdlnes/Noncancer%20TSD/Papers/USEPA1995.pdf.

U.S. EPA. (2000). *Strategy for Research on Environmental Risks to Children*. EPA/600/R-00/068. Washington (DC), United States Environmental Protection Agency.

U.S. EPA. (2002). *A Review of the Reference Dose and Reference Concentration Process*. EPA/630/P-02/002F. Washington (DC), United States Environmental Protection Agency. http://www.epa.gov/iris/RFD_FINAL%5B1%5D.pdf.

U.S. EPA. (2004). *An Examination of EPA Risk Assessment Principles and Practices*. EPA/100/B-04/001. Washington (DC), United States Environmental Protection Agency. http://www.epa.gov/OSA/pdfs/ratf-final.pdf.

U.S. EPA (2006a). Benchmark Dose Software (BMDS) Tutorial. United States Environmental Protection Agency. http://www.epa.gov/ncea/bmds_training/methodology/model.htm. June 29, 2006.

U.S. EPA. (2006b). *Approaches for the application of physiologically based pharmacokinetic (PBPK) models and supporting data in risk assessment*. EPA/600/R-05/043F. U.S.EPA.

U.S. EPA. (2006c). *A Framework for Assessing Health Risks of Environmental Exposures to Children*

EPA/600/R-05/093A External Review Draft

Washington (DC), United States Environmental Protection Agency. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=150263.

U.S. EPA (2007a). Acute Exposure Guideline Levels. United States Environmental Protection Agency. http://www.epa.gov/oppt/aegl/pubs/compiled.pdf. May 14, 2007.

U.S. EPA (2007b). Integrated Risk Information System (IRIS) Database. United States Environmental Protection Agency. http://www.epa.gov/IRIS/.

U.S. FDA (2006). Redbook 2000 Toxicological Principles for the Safety Assessment of Food Ingredients. Office of Food Additive Safety, U.S. Food and Drug Administration. http://www.cfsan.fda.gov/~redbook/red-toca.html.

Uebori M and Imamura K (2004). Analysis of aliphatic and aromatic carbonyl compounds in ambient air by LC/MS/MS. Anal Sci 20(10): 1459-1462.

Vedal S, Brauer M, White R and Petkau J (2003). Air pollution and daily mortality in a city with low levels of pollution. Environ Health Perspect 111(1): 45-52.

Vettorazzi G (1977). Safety factors and their application in the toxicological evaluation. In: The Evaluation of Toxicological Data for the Protection of Public Health : Proceedings of the International Colloquium, Luxembourg, December 1976. for the Commission of the European Communities by Pergamon Press. Oxford, [Eng.] ; New York: 207-223.

Watzka M, Bidlingmaier F, Schramm J, Klingmuller D and Stoffel-Wagner B (1999). Sex- and age-specific differences in human brain CYP11A1 mRNA expression. J Neuroendocrinol 11(12): 901-905.

Weil CS (1972). Statistics vs safety factors and scientific judgment in the evaluation of safety for man. Toxicol Appl Pharmacol 21(4): 454-463.

Weller E, Long N, Smith A, Williams P, Ravi S, Gill J, Henessey R, Skornik W, Brain J, Kimmel C, Kimmel G, Holmes L and Ryan L (1999). Dose-rate effects of ethylene oxide exposure on developmental toxicity. Toxicol Sci 50(2): 259-270.

Wen CP, Tsai SP and Gibson RL (1983). Anatomy of the healthy worker effect: A critical review. J Occup Med 25(4): 283-289.

Widdicombe JG (1990). Nasal pathophysiology. Respir Med 84 Suppl A: 3-9; discussion 9-10.

Wiebicke W, Jorres R and Magnussen H (1990). Comparison of the effects of inhaled corticosteroids on the airway response to histamine, methacholine, hyperventilation, and sulfur dioxide in subjects with asthma. J Allergy Clin Immunol 86(6 Pt 1): 915-923.

Wilhelm M and Ritz B (2005). Local variations in CO and particulate air pollution and adverse birth outcomes in Los Angeles County, California, USA. Environ Health Perspect 113(9): 1212-1221.

Williams GM, O'Callaghan M, Najman JM, Bor W, Andersen MJ, Richards D and U C (1998). Maternal cigarette smoking and child psychiatric morbidity: A longitudinal study. Pediatrics 102(1): e11.

Woodruff TJ, Parker JD and Schoendorf KC (2006). Fine particulate matter (PM2.5) air pollution and selected causes of postneonatal infant mortality in California. Environ Health Perspect 114(5): 786-790.

Woolcock AJ (1994). Asthma. In: Respiratory Medicine. Murray J. F. and Nadel J. A. W. B. Saunders. Philadelphia (PA): 1288-1330.

Yolton K, Dietrich K, Auinger P, Lanphear BP and Hornung R (2005). Exposure to environmental tobacco smoke and cognitive abilities among U.S. children and adolescents. Environ Health Perspect 113(1): 98-103.

Yu CP and Xu GB (1987). Predictive models for deposition of inhaled diesel exhaust particles in humans and laboratory species. Res Rep Health Eff Inst(10): 3-22.

Zeltner TB and Burri PH (1987). The postnatal development and growth of the human lung. II. Morphology. Respir Physiol 67(3): 269-282.

Appendix A. List of Acronyms

A.1 List of Acronyms

σg	geometric standard deviation
AAQS	Ambient Air Quality Standards
ACGIH	American Conference of Governmental Industrial Hygienists
ADI	acceptable daily intake
AEGL	Acute Emergency Guidance Level
AEL	adverse effect level
AHH	aryl hydrocarbon hydroxylase
AICE	American Institute of Chemical Engineers
AIHA	American Industrial Hygiene Association
ALL	acute lymphoblastic leukemia
ARB	Air Resources Board
ATS	American Thoracic Society
AUC	area under the (blood or tissue concentration x time) curve
AV_A	alveolar ventilation (animal)
AV_H	alveolar ventilation (human)
BMC	benchmark concentration
$BMCL_{05}$	lower 95% confidence interval of the benchmark concentration
BMD	benchmark dose
BW	body weight
C x T	concentration multiplied by time
C_A	applied concentration in an animal experiment.
CAAQS	California Ambient Air Quality Standards
CalEPA	California Environmental Protection Agency
CalOSHA	California Occupational Safety and Health Administration
CAPCOA	California Air Pollution Control Officer's Association
C_{AVG}	average concentration
CFT	computational fluid dynamics
CIIT	Chemical Industry Institute of Toxicology
Cmaxs	peak concentrations
C_{OBS}	observed concentration
Cr^{VI}	hexavalent chromium
CYP	cytochrome P450
DAF	dosimetric adjustment factor
DCM	dichloromethane
DES	diethylstilbestrol

D_{H-d}	dose of compound or active metabolite at site of action in default human
D_L	diffusing capacity
DOD	Department of Defense
DPR	Department of Pesticide Regulation (State of California, CalEPA)
DPX	DNA-protein cross-links
EC_{05}	5% effective concentration (expected to produce a 5% toxic response rate)
EEGL	Emergency Exposure Guidance Level
ERPG	Emergency Response Planning Guidelines
ET	extrathoracic
ETS	environmental tobacco smoke
FAS	Fetal Alcohol Syndrome
FDA	Food and Drug Administration
FEL	frank effect level
FEV_1	forced expiratory volume in one second
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act
FQPA	Federal Food Quality Protection Act
FVC	forced vital capacity
GFR	glomerular filtration rate
GLC	ground level concentration
GLPs	Good Laboratory Practices
GSH	glutathione
GSTs	Glutathione sulfotransferases
H&SC	Health & Safety Code
HARP	Air Resources Board's Hot Spots Analysis and Reporting Program
HEC	Human Equivalent Concentration
Hg	mercury
HI	hazard index
IARC	International Agency for Research on Cancer
IBT	Industrial Bio-Test Laboratories Inc.
ICRP	International Commission on Radiological Protection
IRDC	International Research and Development Corporation
LCL	lower confidence limit
LD_{50}	lethal dose to 50% of test animals in a given experiment
LOAEL	lowest-observed-adverse-effect-level
LOEL	lowest-observed-effect-level
MLE	maximum likelihood estimate
MMAD	mean mass aerodynamic diameter
MPPD	multiple path particle deposition model

MV	minute volume
NAC	National Advisory Committee
NAS	National Academy of Sciences
NATICH	National Air Toxics Information Clearinghouse
NIOSH	National Institute for Occupational Safety and Health
NOAEL	no-observed-adverse-effect-level
NOEL	no observed effect level
NRC	National Research Council
NTP	National Toxicology Program
OEHHA	Office of Environmental Health Hazard Assessment
OP	organophosphate
OR	odds ratio
PAH	polycyclic aromatic hydrocarbon
Pb	lead
PBPK	physiologically based pharmacokinetic modeling
PCE	perchloroethylene
PEL	Permissible Exposure Limits
PK UF	pharmacokinetic uncertainty factors
PU	pulmonary region
RD50	respiratory dose 50 (dose of gas causing 50% decrease in respiration rate)
RDDRs	regional deposited dose ratios
REL	Reference Exposure Level
RfC	Reference Concentration
RfD	Reference Dose
RFLP	restriction fragment length polymorphism
RGDR	regional gas dose ratio
RIVM	The Netherlands National Institute for Public Health and the Environment
RR	relative risk
RSA_A	regional surface area (animal)
RSA_H	regional surface area (human)
RT	respiratory tracts
SA	surface area
SB	Senate Bill
SG_{aw}	specific airway conductance
SMR	standardized mortality ratios
SPEGL	Short-term Public Emergency Guidance Level
SR_{AW}	specific airway resistance
SRP	Scientific Review Panel

STEL	Short-term Exposure Limit
TACs	Toxic Air Contaminants
TB	tracheobronchial region
TCA	trichloroacetic acid
TCE	trichloroethylene
TD	toxicodynamic
TK	toxicokinetic
TLV-TWA	Threshold Limit Value-Time Weighted Average
TSDs	Technical Support Documents
UCL	upper confidence limit
UF_A	interspecies uncertainty factor
UF_{A-d}	interspecies uncertainty factor toxicodynamic component
UF_{A-k}	interspecies uncertainty factor toxicokinetic component
UF_D	database deficiency uncertainty factor
UF_H	intraspecies uncertainty factor
UF_{H-d}	intraspecies uncertainty factor toxicodynamic component
UF_{H-k}	intraspecies uncertainty factor toxicokinetic component
UF_L	LOAEL uncertainty factor
UF_S	subchronic uncertainty factor
UFs	uncertainty factors
U.S. DHHS	United States Department of Health and Human Services
U.S. EPA	United States Environmental Protection Agency
Val	valine
Vd	volume of distribution
VOCs	volatile organic compounds
WHO	World Health Organization
χ^2	chi-squared

Appendix B. Acute, 8-Hour, and Chronic Reference Exposure Levels (RELs) Summary Table

B.1 Introduction to Reference Exposure Levels Summary Table

The acute noncancer Reference Exposure Levels (RELs) originally appeared in the "*Air Toxics Hot Spots Program Risk Assessment Guidelines Part I: The Determination of Acute Reference Exposure Levels for Airborne Toxicants*" March 1999 (OEHHA 1999a). In a subsequent publication, "*Air Toxics Hot Spots Program Risk Assessment Guidelines Part III: Technical Support Document for the Determination of Noncancer Chronic Reference Exposure Levels*" (OEHHA 2000), chronic noncancer RELs were presented. Existing acute and chronic RELs have undergone public review and peer review by the State's Scientific Review Panel (SRP) on Toxic Air Contaminants (TACs). The 8-hour RELs are presented for the first time in this document. Seven chemicals are being re-evaluated using updated methods put forth in this guidelines document including benchmark dose (BMC) analysis and PBPK modeling. The acute, 8-hour, and chronic REL summaries for these seven chemicals are in Appendix D of this document.

OEHHA developed chronic inhalation RELs for chemicals on the list of substances for which emissions need to be quantified (see Appendix C) under the Air Toxics Hot Spots Program. These substances were selected primarily based on:

(1) the magnitude of current known emissions in California and

(2) the availability of a scientific database on which to estimate a chronic REL. The California Air Resources Board (ARB) previously adopted chronic RELs for acetaldehyde, diesel exhaust particulates, and perchloroethylene during the identification of these substances as TACs.

This public review draft lists only new RELs developed using the methodology presented in the revised technical support document. The final version will also list RELs developed using the original (OEHHA1999a and 2000) methodologies, until such time as those values are replaced by newly developed values. Prioritization for updating these original values will follow the scheme presented in the *Prioritization of Toxic Air contaminants under the Children's environmental Health Protection Act* (OEHHA, 2001).

TABLE B.1.1. OEHHA ACUTE (A), 8-HOUR (8) AND CHRONIC (C) REFERENCE EXPOSURE LEVEL (REL) SUMMARY[1]

Substance		Inhalation REL† (μg/m^3)	Hazard Index Target Organs	Human Data	Oral REL (μg/kg BW)
Acetaldehyde (75-07-0)	**A**	750	Sensory irritation; eyes	X	
	8	568	Respiratory system	X	
	C	43†	Respiratory system		
Acrolein (107-02-8)	**A**	2.3	Sensory irritation; eyes	X	
	8	1.6	Respiratory system		
	C	0.1	Respiratory system		
Inorganic Arsenic (7440-38-2) **& arsenic compounds (including arsine)**	**A**	0.20	Developmental; nervous, & cardiovascular systems		
	8	0.015	Development; nervous system	X	
	C	0.015	Development; nervous system	X	3.5
Formaldehyde (50-00-0)	**A**	55	Sensory irritation; eyes	X	
	8	9	Respiratory system	X	
	C	9	Respiratory system	X	
Manganese & compounds	**A**	--			
	8	0.05	Nervous system	X	
	C	0.03	Nervous system	X	
Mercury (inorganic) (7439-97-6)	**A**	0.6	Nervous system, development		
	8	0.06	Nervous system	X	
	C	0.03	Nervous system	X	0.16

[1] New acute, 8-hour, and chronic RELs, whose summaries appear in this document (See Appendix D).

Appendix C. Substances for which Emissions Must Be Quantified

SUBSTANCES FOR WHICH EMISSIONS MUST BE QUANTIFIED

75070	Acetaldehyde
60355	Acetamide
75058	Acetonitrile
98862	Acetophenone
53963	2-Acetylaminofluorene [PAH-Derivative, POM]
107028	Acrolein
79061	Acrylamide
79107	Acrylic acid
107131	Acrylonitrile
107051	Allyl chloride
7429905	Aluminum
1344281	Aluminum oxide (fibrous forms)
117793	2-Aminoanthraquinone [PAH-Derivative, POM]
92671	4-Aminobiphenyl [POM]
61825	Amitrole
7664417	Ammonia
6484522	Ammonium nitrate
7783202	Ammonium sulfate
62533	Aniline
90040	o-Anisidine
-	Anthracene [PAH, POM], (see PAH)
7440360	Antimony
*	Antimony compounds including but not limited to:
1309644	Antimony trioxide
7440382	Arsenic
1016	Arsenic compounds (inorganic) including but not limited to:
7784421	Arsine
1017	Arsenic compounds (other than inorganic)
-	Asbestos (see Mineral fibers)
7440393	Barium
*	Barium Compounds
-	Benz[a]anthracene [PAH, POM], (see PAH)
71432	Benzene
92875	Benzidine (and its salts) [POM]
1020	Benzidine-based dyes [POM] including but not limited to:
1937377	Direct Black 38 [PAH-Derivative, POM]
2602462	Direct Blue 6 [PAH-Derivative, POM]
16071866	Direct Brown 95 (technical grade) [POM]
-	Benzo[a]pyrene [PAH, POM], (see PAH)
-	Benzo[b]fluoranthene [PAH, POM], (see PAH)
271896	Benzofuran
98077	Benzoic trichloride {Benzotrichloride}
-	Benzo[j]fluoranthene [PAH, POM] (see PAH)
-	Benzo[k]fluoranthene [PAH, POM] (see PAH)
98884	Benzoyl chloride
94360	Benzoyl peroxide
100447	Benzyl chloride
7440417	Beryllium

*	Beryllium compounds
92524	Biphenyl [POM]
111444	Bis(2-chloroethyl) ether {DCEE}
542881	Bis(chloromethyl) ether
103231	Bis(2-ethylhexyl) adipate
7726956	Bromine
*	Bromine compounds (inorganic) including but not limited to:
7789302	Bromine pentafluoride
10035106	Hydrogen bromide
7758012	Potassium bromate
75252	Bromoform
106990	1,3-Butadiene
540885	t-Butyl acetate
141322	Butyl acrylate
71363	n-Butyl alcohol
78922	sec-Butyl alcohol
75650	tert-Butyl alcohol
85687	Butyl benzyl phthalate
7440439	Cadmium
*	Cadmium compounds
156627	Calcium cyanamide
105602	Caprolactam
2425061	Captafol
133062	Captan
63252	Carbaryl [PAH-Derivative, POM]
1050	Carbon black extracts
75150	Carbon disulfide
56235	Carbon tetrachloride
463581	Carbonyl sulfide
1055	Carrageenan (degraded)
120809	Catechol
133904	Chloramben
57749	Chlordane
108171262	Chlorinated paraffins (average chain length, C12; approximately 60% Chlorine by weight)
7782505	Chlorine
10049044	Chlorine dioxide
79118	Chloroacetic acid
532274	2-Chloroacetophenone
106478	p-Chloroaniline
1058	Chlorobenzenes including but not limited to:
108907	Chlorobenzene
25321226	Dichlorobenzenes (mixed isomers) including:
95501	1,2-Dichlorobenzene
541731	1,3-Dichlorobenzene
106467	p-Dichlorobenzene {1,4-Dichlorobenzene}
120821	1,2,4-Trichlorobenzene
510156	Chlorobenzilate [POM] {Ethyl-4,4'-dichlorobenzilate}
67663	Chloroform
107302	Chloromethyl methyl ether (technical grade)
1060	Chlorophenols including but not limited to:

95578	2-Chlorophenol
120832	2,4-Dichlorophenol
87865	Pentachlorophenol
25167833	Tetrachlorophenols including but not limited to:
58902	2,3,4,6-Tetrachlorophenol
95954	2,4,5-Trichlorophenol
88062	2,4,6-Trichlorophenol
95830	4-Chloro-o-phenylenediamine
76062	Chloropicrin
126998	Chloroprene
95692	p-Chloro-o-toluidine
7440473	Chromium
*	Chromium compounds (other than hexavalent)
18540299	Chromium, hexavalent (and compounds) including but not limited to:
10294403	Barium chromate
13765190	Calcium chromate
1333820	Chromium trioxide
7758976	Lead chromate
10588019	Sodium dichromate
7789062	Strontium chromate
-	Chrysene [PAH, POM], (see PAH)
7440484	Cobalt
*	Cobalt compounds
1066	Coke oven emissions
7440508	Copper
*	Copper compounds
1070	Creosotes
120718	p-Cresidine
1319773	Cresols (mixtures of) {Cresylic acid} including:
108394	m-Cresol
95487	o-Cresol
106445	p-Cresol
4170303	Crotonaldehyde
98828	Cumene
80159	Cumene hydroperoxide
135206	Cupferron
1073	Cyanide compounds (inorganic) including but not limited to:
74908	Hydrocyanic acid
110827	Cyclohexane
108930	Cyclohexanol
66819	Cycloheximide
	Decabromodiphenyl oxide [POM] (see Polybrominated diphenyl ethers)
1075	Dialkylnitrosamines including but not limited to:
924163	N-Nitrosodi-n-butylamine
1116547	N-Nitrosodiethanolamine
55185	N-Nitrosodiethylamine
62759	N-Nitrosodimethylamine
621647	N-Nitrosodi-n-propylamine
10595956	N-Nitrosomethylethylamine
615054	2,4-Diaminoanisole
1078	Diaminotoluenes (mixed isomers) including but not limited to:

95807	2,4-Diaminotoluene {2,4-Toluene diamine}
334883	Diazomethane
226368	Dibenz[a,h]acridine [POM]
224420	Dibenz[a,j]acridine [POM]
-	Dibenz[a,h]anthracene [PAH, POM], (see PAH)
194592	7H-Dibenzo[c,g]carbazole
-	Dibenzo[a,e]pyrene [PAH, POM], (see PAH)
-	Dibenzo[a,h]pyrene [PAH, POM], (see PAH)
-	Dibenzo[a,i]pyrene [PAH, POM], (see PAH)
-	Dibenzo[a,l]pyrene [PAH, POM], (see PAH)
132649	Dibenzofuran [POM]
96128	1,2-Dibromo-3-chloropropane {DBCP}
96139	2,3-Dibromo-1-propanol
84742	Dibutyl phthalate
-	p-Dichlorobenzene (1,4-Dichlorobenzene) (see Chlorobenzenes)
91941	3,3'-Dichlorobenzidine [POM]
72559	Dichlorodiphenyldichloroethylene {DDE} [POM]
75343	1,1-Dichloroethane {Ethylidene dichloride}
94757	Dichlorophenoxyacetic acid, salts and esters {2,4-D}
78875	1,2-Dichloropropane {Propylene dichloride}
542756	1,3-Dichloropropene
62737	Dichlorovos {DDVP}
115322	Dicofol [POM]
--	Diesel engine exhaust
9901	Diesel engine exhaust, particulate matter **{**Diesel PM**}**
9902	Diesel engine exhaust, total organic gas
#	Diesel fuel (marine)
111422	Diethanolamine
117817	Di(2-ethylhexyl) phthalate {DEHP}
64675	Diethyl sulfate
119904	3,3'-Dimethoxybenzidine [POM]
60117	4-Dimethylaminoazobenzene [POM]
121697	N,N-Dimethylaniline
57976	7,12-Dimethylbenz[a]anthracene [PAH-Derivative, POM]
119937	3,3'-Dimethylbenzidine {o-Tolidine} [POM]
79447	Dimethyl carbamoyl chloride
68122	Dimethyl formamide
57147	1,1-Dimethylhydrazine
131113	Dimethyl phthalate
77781	Dimethyl sulfate
534521	4,6-Dinitro-o-cresol (and salts)
51285	2,4-Dinitrophenol
42397648	1,6-Dinitropyrene [PAH-Derivative, POM]
42397659	1,8-Dinitropyrene [PAH-Derivative, POM]
25321146	Dinitrotoluenes (mixed isomers) including but not limited to:
121142	2,4-Dinitrotoluene
606202	2,6-Dinitrotoluene
123911	1,4-Dioxane
-	Dioxins (Chlorinated dibenzodioxins) (see Polychlorinated dibenzo-p-dioxins) [POM]
630933	Diphenylhydantoin [POM]

122667	1,2-Diphenylhydrazine {Hydrazobenzene} [POM]
1090	Environmental Tobacco Smoke
106898	Epichlorohydrin
106887	1,2-Epoxybutane
1091	Epoxy resins
140885	Ethyl acrylate
100414	Ethyl benzene
75003	Ethyl chloride {Chloroethane}
-	Ethyl-4,4'-dichlorobenzilate (see Chlorobenzilate)
74851	Ethylene
106934	Ethylene dibromide {EDB, 1,2-Dibromoethane}
107062	Ethylene dichloride {EDC, 1,2-Dichloroethane}
107211	Ethylene glycol
151564	Ethyleneimine {Aziridine}
75218	Ethylene oxide
96457	Ethylene thiourea
1101	Fluorides and compounds including but not limited to:
7664393	Hydrogen fluoride
1103	Fluorocarbons (brominated)
1104	Fluorocarbons (chlorinated) including but not limited to:
76131	Chlorinated fluorocarbon {CFC-113} {1,1,2-Trichloro-1,2,2-trifluoroethane}
75456	Chlorodifluoromethane {Freon 22}
75718	Dichlorodifluoromethane {Freon 12}
75434	Dichlorofluoromethane {Freon 21}
75694	Trichlorofluoromethane {Freon 11}
50000	Formaldehyde
110009	Furan
--	Gasoline engine exhaust including but not limited to:
--	Gasoline engine exhaust (condensates & extracts)
9910	Gasoline engine exhaust, particulate matter
9911	Gasoline engine exhaust, total organic gas
1110	Gasoline vapors
111308	Glutaraldehyde
1115	Glycol ethers and their acetates including but not limited to:
111466	Diethylene glycol
111966	Diethylene glycol dimethyl ether
112345	Diethylene glycol monobutyl ether
111900	Diethylene glycol monoethyl ether
111773	Diethylene glycol monomethyl ether
25265718	Dipropylene glycol
34590948	Dipropylene glycol monomethyl ether
629141	Ethylene glycol diethyl ether
110714	Ethylene glycol dimethyl ether
111762	Ethylene glycol monobutyl ether
110805	Ethylene glycol monoethyl ether
111159	Ethylene glycol monoethyl ether acetate
109864	Ethylene glycol monomethyl ether
110496	Ethylene glycol monomethyl ether acetate
2807309	Ethylene glycol monopropyl ether
107982	Propylene glycol monomethyl ether

108656	Propylene glycol monomethyl ether acetate
112492	Triethylene glycol dimethyl ether
76448	Heptachlor
118741	Hexachlorobenzene
87683	Hexachlorobutadiene
608731	Hexachlorocyclohexanes (mixed or technical grade) including but not limited to:
319846	alpha-Hexachlorocyclohexane
319857	beta-Hexachlorocyclohexane
58899	Lindane {gamma-Hexachlorocyclohexane}
77474	Hexachlorocyclopentadiene
67721	Hexachloroethane
680319	Hexamethylphosphoramide
110543	Hexane
302012	Hydrazine
7647010	Hydrochloric acid
-	Hydrocyanic acid (see Cyanide compounds)
7783064	Hydrogen sulfide
123319	Hydroquinone
-	Indeno[1,2,3-cd]pyrene [PAH, POM], (see PAH)
13463406	Iron pentacarbonyl
1125	Isocyanates including but not limited to:
822060	Hexamethylene-1,6-diisocyanate
101688	Methylene diphenyl diisocyanate {MDI} [POM]
624839	Methyl isocyanate
-	Toluene-2,4-diisocyanate (see Toluene diisocyanates)
-	Toluene-2,6-diisocyanate (see Toluene diisocyanates)
78591	Isophorone
78795	Isoprene, except from vegetative emission sources
67630	Isopropyl alcohol
80057	4,4'-Isopropylidenediphenol [POM]
7439921	Lead
1128	Lead compounds (inorganic) including but not limited to:
301042	Lead acetate
-	Lead chromate (see Chromium, hexalent)
7446277	Lead phosphate
1335326	Lead subacetate
1129	Lead compounds (other than inorganic)
108316	Maleic anhydride
7439965	Manganese
*	Manganese compounds
7439976	Mercury
*	Mercury compounds including but not limited to:
7487947	Mercuric chloride
593748	Methyl mercury {Dimethylmercury}
67561	Methanol
72435	Methoxychlor [POM]
75558	2-Methylaziridine {1,2-Propyleneimine}
74839	Methyl bromide {Bromomethane}
74873	Methyl chloride {Chloromethane}
71556	Methyl chloroform {1,1,1-Trichloroethane}

56495	3-Methylcholanthrene [PAH-Derivative, POM]
3697243	5-Methylchrysene [PAH-Derivative, POM]
101144	4,4'-Methylene bis(2-chloroaniline) {MOCA} [POM]
75092	Methylene chloride {Dichloromethane}
101779	4,4'-Methylenedianiline (and its dichloride) [POM]
78933	Methyl ethyl ketone {2-Butanone}
60344	Methyl hydrazine
74884	Methyl iodide {Iodomethane}
108101	Methyl isobutyl ketone {Hexone}
75865	2-Methyllactonitrile {Acetone cyanohydrin}
80626	Methyl methacrylate
109068	2-Methylpyridine
1634044	Methyl tert-butyl ether
90948	Michler's ketone [POM]
1136	Mineral fibers (fine mineral fibers which are man-made, and are airborne particles of a respirable size greater than 5 microns in length, less than or equal to 3.5 microns in diameter, with a length to diameter ratio of 3:1) including but not limited to:
1056	Ceramic fibers
1111	Glasswool fibers
1168	Rockwool
1181	Slagwool
1135	Mineral fibers (other than man-made) including but not limited to:
1332214	Asbestos
12510428	Erionite
1190	Talc containing asbestiform fibers
1313275	Molybdenum trioxide
-	Naphhthalene [PAH, POM], (see PAH)
7440020	Nickel
*	Nickel compounds including but not limited to:
373024	Nickel acetate
3333673	Nickel carbonate
13463393	Nickel carbonyl
12054487	Nickel hydroxide
1271289	Nickelocene
1313991	Nickel oxide
12035722	Nickel subsulfide
1146	Nickel refinery dust from the pyrometallurgical process
7697372	Nitric acid
139139	Nitrilotriacetic acid
602879	5-Nitroacenaphthene [PAH-Derivative, POM]
98953	Nitrobenzene
92933	4-Nitrobiphenyl [POM]
7496028	6-Nitrochrysene [PAH-Derivative, POM]
607578	2-Nitrofluorene [PAH-Derivative, POM]
302705	Nitrogen mustard N-oxide
100027	4-Nitrophenol
79469	2-Nitropropane
5522430	1-Nitropyrene [PAH-Derivative, POM]
57835924	4-Nitropyrene [PAH-Derivative, POM]
86306	N-Nitrosodiphenylamine

156105	p-Nitrosodiphenylamine [POM]
684935	N-Nitroso-N-methylurea
59892	N-Nitrosomorpholine
100754	N-Nitrosopiperidine
930552	N-Nitrosopyrrolidine
*	Oleum (see Sulfuric acid and oleum)
--	PAHs (Polycyclic aromatic hydrocarbons) [POM] including but not limited to:
1151	PAHs, total, w/o individ. components reported [PAH, POM]
1150	PAHs, total, with individ. components also reported [PAH, POM]
83329	Acenaphthene [PAH, POM]
208968	Acenaphthylene [PAH, POM]
120127	Anthracene [PAH, POM]
56553	Benz[a]anthracene [PAH, POM]
50328	Benzo[a]pyrene [PAH, POM]
205992	Benzo[b]fluoranthene
192972	Benzo[e]pyrene [PAH, POM]
191242	Benzo[g,h,i]perylene [PAH, POM]
205823	Benzo[j]fluoranthene [PAH, POM]
207089	Benzo[k]fluoranthene [PAH, POM]
218019	Chrysene [PAH, POM]
53703	Dibenz[a,h]anthracene [PAH, POM]
192654	Dibenzo[a,e]pyrene [PAH, POM]
189640	Dibenzo[a,h]pyrene [PAH, POM]
189559	Dibenzo[a,i]pyrene [PAH, POM]
191300	Dibenzo[a,l]pyrene [PAH, POM]
206440	Fluoranthene [PAH, POM]
86737	Fluorene [PAH, POM]
193395	Indeno[1,2,3-cd]pyrene [PAH, POM]
91576	2-Methyl naphthalene [PAH, POM]
91203	Naphthalene [PAH, POM]
198550	Perylene [PAH, POM]
85018	Phenanthrene [PAH, POM]
129000	Pyrene [PAH, POM]
#	PAH-Derivatives (Polycyclic aromatic hydrocarbon derivatives) [POM] (including but not limited to those substances listed in Appendix A with the bracketed designation [PAH-Derivative, POM])
56382	Parathion
1336363	PCBs (Polychlorinated biphenyls), total [POM] including but not limited to:
32598133	3,3',4,4'-Tetrachlorobiphenyl (PCB 77)
70362504	3,4,4',5-Tetrachlorobiphenyl (PCB 81)
32598144	2,3,3',4,4'-Pentachlorobiphenyl (PCB 105)
74472370	2,3,4,4',5-Pentachlorobiphenyl (PCB 114)
31508006	2,3',4,4',5-Pentachlorobiphenyl (PCB 118)
65510443	2,3',4,4',5'-Pentachlorobiphenyl (PCB 123)
57465288	3,3',4,4',5-Pentachlorobiphenyl (PCB 126)
38380084	2,3,3',4,4',5-Hexachlorobiphenyl (PCB 156)
69782907	2,3,3',4,4',5'-Hexachlorobiphenyl (PCB 157)
52663726	2,3',4,4',5,5'-Hexachlorobiphenyl (PCB 167)
32774166	3,3',4,4',5,5'-Hexachlorobiphenyl (PCB 169)
39635319	2,3,3',4,4',5,5'-Heptachlorobiphenyl (PCB 189)

82688	Pentachloronitrobenzene {Quintobenzene}
79210	Peracetic acid
127184	Perchloroethylene {Tetrachloroethene}
2795393	Perfluorooctanoic acid {PFOA} and its salts, esters, and sulfonates
108952	Phenol
106503	p-Phenylenediamine
90437	2-Phenylphenol [POM]
75445	Phosgene
7723140	Phosphorus
--	Phosphorus compounds:
7803512	Phosphine
7664382	Phosphoric acid
10025873	Phosphorus oxychloride
10026138	Phosphorus pentachloride
1314563	Phosphorus pentoxide
7719122	Phosphorus trichloride
126738	Tributyl phosphate
78400	Triethyl phosphine
512561	Trimethyl phosphate
78308	Triorthocresyl phosphate [POM]
115866	Triphenyl phosphate [POM]
101020	Triphenyl phosphite [POM]
85449	Phthalic anhydride
2222	Polybrominated diphenyl ethers {PBDEs}, including but not limited to:
1163195	Decabromodiphenyl oxide [POM]
--	Polychlorinated dibenzo-p-dioxins {PCDDs or Dioxins} [POM] including but not limited to:
1086	Dioxins, total, w/o individ. isomers reported {PCDDs} [POM]
1085	Dioxins, total, with individ. isomers also reported {PCDDs} [POM]
1746016	2,3,7,8-Tetrachlorodibenzo-p-dioxin {TCDD} [POM]
40321764	1,2,3,7,8-Pentachlorodibenzo-p-dioxin [POM]
39227286	1,2,3,4,7,8-Hexachlorodibenzo-p-dioxin [POM]
57653857	1,2,3,6,7,8-Hexachlorodibenzo-p-dioxin [POM]
19408743	1,2,3,7,8,9-Hexachlorodibenzo-p-dioxin [POM]
35822469	1,2,3,4,6,7,8-Heptachlorodibenzo-p-dioxin [POM]
3268879	1,2,3,4,6,7,8,9-Octachlorodibenzo-p-dioxin [POM]
41903575	Total Tetrachlorodibenzo-p-dioxin [POM]
36088229	Total Pentachlorodibenzo-p-dioxin [POM]
34465468	Total Hexachlorodibenzo-p-dioxin [POM]
37871004	Total Heptachlorodibenzo-p-dioxin [POM]
--	Polychlorinated dibenzofurans {PCDFs or Dibenzofurans} [POM] including but not limited to:
1080	Dibenzofurans (Polychlorinated dibenzofurans) {PCDFs} [POM]
51207319	2,3,7,8-Tetrachlorodibenzofuran [POM]
57117416	1,2,3,7,8-Pentachlorodibenzofuran [POM]
57117314	2,3,4,7,8-Pentachlorodibenzofuran [POM]
70648269	1,2,3,4,7,8-Hexachlorodibenzofuran [POM]
57117449	1,2,3,6,7,8-Hexachlorodibenzofuran [POM]
72918219	1,2,3,7,8,9-Hexachlorodibenzofuran [POM]
60851345	2,3,4,6,7,8-Hexachlorodibenzofuran [POM]
67562394	1,2,3,4,6,7,8-Heptachlorodibenzofuran [POM]

55673897	1,2,3,4,7,8,9-Heptachlorodibenzofuran [POM]
39001020	1,2,3,4,6,7,8,9-Octachlorodibenzofuran [POM]
55722275	Total Tetrachlorodibenzofuran [POM]
30402154	Total Pentachlorodibenzofuran [POM]
55684941	Total Hexachlorodibenzofuran [POM]
38998753	Total Heptachlorodibenzofuran [POM]
#	POM (Polycyclic organic matter) (including but not limited to those substances listed in Appendix A with the bracketed designation of [POM], [PAH, POM], or [PAH-Derivative, POM])
1120714	1,3-Propane sultone
57578	beta-Propiolactone
123386	Propionaldehyde
114261	Propoxur {Baygon}
115071	Propylene
75569	Propylene oxide
-	1,2-Propyleneimine (see 2-Methylaziridine)
110861	Pyridine
91225	Quinoline
106514	Quinone
1165	Radionuclides including but not limited to:
24267569	Iodine-131
1166	Radon and its decay products
50555	Reserpine [POM]
#	Residual (heavy) fuel oils
7782492	Selenium
*	Selenium compounds including but not limited to:
7783075	Hydrogen selenide
7446346	Selenium sulfide
1175	Silica, crystalline (respirable)
7440224	Silver
*	Silver compounds
1310732	Sodium hydroxide
100425	Styrene
96093	Styrene oxide
*	Sulfuric acid and oleum
8014957	Oleum
7446719	Sulfur trioxide
7664939	Sulfuric acid
100210	Terephthalic acid
79345	1,1,2,2-Tetrachloroethane
-	Tetrachlorophenols (see Chlorophenols)
7440280	Thallium
*	Thallium compounds
62555	Thioacetamide
62566	Thiourea
7550450	Titanium tetrachloride
108883	Toluene
-	2,4-Toluenediamine (see 2,4-Diaminotoluene)
26471625	Toluene diisocyanates including but not limited to:
584849	Toluene-2,4-diisocyanate
91087	Toluene-2,6-diisocyanate

95534	o-Toluidine
8001352	Toxaphene {Polychlorinated camphenes}
-	1,1,1-Trchloroethane (see Methyl chloroform)
79005	1,1,2-Trichloroethane {Vinyl trichloride}
79016	Trichloroethylene
-	2,4,6-Trichlorophenol (see Chlorophenols)
96184	1,2,3-Trichloropropane
121448	Triethylamine
1582098	Trifluralin
25551137	Trimethylbenzenes including but not limited to:
95636	1,2,4-Trimethylbenzene
540841	2,2,4-Trimethylpentane
51796	Urethane {Ethyl carbamate}
7440622	Vanadium (fume or dust)
1314621	Vanadium pentoxide
108054	Vinyl acetate
593602	Vinyl bromide
75014	Vinyl chloride
100403	4-Vinylcyclohexene
75025	Vinyl fluoride
75354	Vinylidene chloride
1206	Wood preservatives (containing arsenic and chromate)
1330207	Xylenes (mixed**)** including:
108383	m-Xylene
95476	o-Xylene
106423	p-Xylene
7440666	Zinc
*	Zinc compounds including but not limited to:
1314132	Zinc oxide

Appendix D. Individual Acute, 8-Hour, and Chronic Reference Exposure Level Summaries

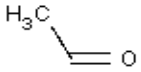
D.1 Summaries using this version of the Hot Spots Risk Assessment guidelines

Acetaldehyde
Acrolein
Arsenic
Formaldehyde
Manganese
Mercury

Acetaldehyde Reference Exposure Levels

(ethanal; acetic aldehyde; acetylaldehyde; ethylaldehyde; diethylacetyl)

CAS: 75-07-0



1. Summary

Acetaldehyde has been prioritized as a Toxic Air Contaminant (TAC) with the potential to differentially impact infants and children. Acetaldehyde was chosen for a focused literature review due to its effects as a respiratory irritant and possible exacerbation of asthma. In addition, acetaldehyde has high California Hot Spots and mobile source emissions, and secondary formation in the atmosphere (OEHHA 2001). Based on acute and chronic inhalation studies conducted mostly in experimental animals, the target tissue for acetaldehyde has consistently been at the portal of entry with effects occurring primarily in the upper respiratory tract at lowest concentrations.

The major noncancer health effects of acute exposure in humans to acetaldehyde vapors consist of irritation to the eyes, skin, and respiratory tract. Low to moderate air concentrations (25 ppm to 200 ppm) cause eye and upper respiratory tract irritation. OEHHA used the critical effect of eye irritation as the basis for determination of the acute Reference Exposure Level (REL). The 8-hour Reference Exposure Level (REL) also accounted for sensory irritation based on an early human exposure study. Signs of acute toxicity in animals at high concentrations (~10,000 ppm) include labored respiration, mouth breathing, weight loss, and liver damage.

Subchronic and chronic exposure to acetaldehyde causes inflammation and injury to the respiratory tract (e.g. lesions including hyperplasia and metaplasia of the olfactory mucosa). Exposure to acetaldehyde, as seen in experimental animal studies, causes histopathological changes in the nose, larynx, and trachea including degeneration, hyperplasia, and metaplasia. Chronic toxicity to rats and hamsters following inhalation exposure to acetaldehyde includes increased mortality and growth retardation. OEHHA used degenerative, inflammatory and hyperplastic changes of the nasal mucosa in rats as the basis for the 8-hour and chronic REL.

Children, especially those with diagnosed asthma, may be more likely to show impaired pulmonary function and symptoms of asthma than are adults following exposure to acetaldehyde.

1.1 Acetaldehyde Acute REL

Reference Exposure Level	**750 μg/m^3 (420 ppb)**
Critical effect(s)	Sensory irritation, eye redness and swelling
Hazard index target(s)	Eyes, nose, throat

1.2 Acetaldehyde 8-Hour REL

Reference Exposure Level	**568 μg/m^3 (316 ppb)**
Critical effect(s)	Degeneration of olfactory nasal epithelium
Hazard index target(s)	Respiratory system

1.3 Acetaldehyde Chronic REL

Reference Exposure Level	**43 μg/m^3 (24 ppb)**
Critical effect(s)	Degenerative, inflammatory and hyperplastic changes of the nasal mucosa in animals
Hazard index target(s)	Respiratory system

2. Physical & Chemical Properties

Description	Colorless liquid or gas (above 21°C)
Molecular formula	C_2H_4O
Molecular weight	44.05 g/mol
Density	0.79 g/cm^3
Boiling point	21 °C
Melting point	-123.5 °C
Vapor pressure	755 mm Hg @ 20°C
Odor threshold	0.09 mg/m^3
Solubility	Miscible in all proportions with water and the most common organic solvents.
Conversion factor	1.8 mg/m^3 = 1 ppm @ 25°C

3. Occurrence and Major Uses

Acetaldehyde is used as an intermediate for the manufacture of a number of other chemicals, including acetic acid, acetic anhydride, ethyl acetate, peracetic acid, pentaerythritol, chloral, glyococal, alkylamines, and pyridines (HSDB, 2004). Sources of acetaldehyde emissions include interior finish materials such as sheet vinyl flooring and carpets, and wood-based building products such as fiberboard and particleboard. Some consumer products also emit acetaldehyde, including adhesives and glues, coatings, lubricants, inks, nail polish removers, liquid wax for wood preservation, detergent and cleansers, deodorants, fuels, and mold inhibitors (Beall and Ulsamer, 1981; CARB, 1993). Emissions of acetaldehyde also occur during combustion processes such as cigarette smoking and use of fireplaces and woodstoves, although long-term indoor concentrations tend to be dominated by non-combustion sources.

An emissions study of new building materials found that samples of carpet, fiberboard, particleboard, and non-rubber resilient flooring emitted acetaldehyde (Burt et al., 1996; IWMB, 2003). Air concentrations based on the acetaldehyde emission rates from these various building products, when modeled to standard State office and classroom dimensions, ranged from 4.6 to 26 $\mu g/m^3$ (2.6 to 14 ppb).

Indoor concentrations of acetaldehyde often greatly exceed outdoor levels and appear to dictate personal exposures, which is consistent with the more significant and widespread indoor sources of this aldehyde. In 2002, the annual average outdoor concentration of acetaldehyde in the South Coast Air Basin was 2.5 $\mu g/m^3$ (1.4 ppb). In Brazil, which has a high usage of ethanol as a transportation fuel, outdoor acetaldehyde concentrations have been measured as high as 63 $\mu g/m^3$ (35 ppb) while a highway tunnel had 3.43 times higher levels of acetaldehyde (430 $\mu g/m^3$ (240 ppb)). The mean acetaldehyde concentrations in U.S. homes range from 15 to 36 $\mu g/m^3$,(8.3 to 20 ppb) but reached as high as 103 $\mu g/m^3$ (57.2 ppb) in newly manufactured homes (Zweidinger *et al.,* 1990; Lindstrom *et al.,* 1995; Hodgson *et al.,* 2002; Kinney *et al.,* 2002). Acetaldehyde concentrations measured in Southern California portable classrooms ranged from 5.7 to 12.8 $\mu g/m^3$ (3.2 to 7.1 ppb) with a mean of 9.8 $\mu g/m^3$ (5.4 ppb) (Shendell *et al.,* 2004). Similar concentrations were found in classrooms of the main buildings. Measured concentrations of acetaldehyde in public/office buildings range from 3 to 16 $\mu g/m^3$(1.7 to 8.9 ppb).

Environmental tobacco smoke (ETS) has been found to be a dominant source of environmental acetaldehyde. Although long-term acetaldehyde levels in smoking and non-smoking homes tend to be similar, acetaldehyde concentrations in homes as a result of exposure from ETS for nonsmoking Californians has been estimated at 11-15 $\mu g/m^3$ (6.1 to 8.3 ppb) (Miller *et al.,* 1998). Fifty-seven homes using a 72-hour exposure period were studied to assess the concentrations of acetaldehyde in the air, which ranged from 3 to 23 $\mu g/m^3$ (1.7 to 12.8 ppb). However, no significant difference was observed between the homes of smokers and nonsmokers (Brown *et al.,* 1994). A 48-hour integrated measurement of breathing-zone concentrations revealed that people who work in garages (9 smokers and 13 nonsmokers) had significantly higher levels of breath acetaldehyde

than controls (4 smokers and 11 nonsmokers) and the smokers had significantly higher levels of breath acetaldehyde than the nonsmokers.

The concentration of breath acetaldehyde (endogenous level) in non-alcoholic, non-smokers range from 0.7 to 11.0 μg/m^3(0.4 to 6.1 ppb), but can be somewhat higher in smokers (16 ± 3 μg/m^3 = 8.9ppb). The higher concentrations are seen in the breath of smokers after they ingest alcohol. With alcohol consumption, the concentrations of acetaldehyde produced vastly exceed the trace amounts generated from microorganisms or other possible endogenous substrates. When subjects with normal aldehyde dehydrogenase (ALDH) activity drink small amounts of alcohol (0.4-0.8 g/kg), the concentrations of breath acetaldehyde may reach between 200 and 2200 μg/m^3 (111 to 1222 ppb) (Shaskan and Dolinsky, 1985; Jones, 1995).

In a controlled human study, five healthy nonsmoking adults inhaled low doses of ethanol (ETOH) and concentrations of ETOH and acetaldehyde were measured in the alveolar air using only the last portion of air in the sampling bag after forced expiration through a three-way valve (Tardif et al., 2004). Exposures were for six consecutive hours to 25, 100, or 1000 ppm ETOH. After two hours of exposure at 25 ppm, acetaldehyde and ETOH were measured in the alveolar air at 0.06 and 7.5 ppm, respectively.

In Asian subjects with genetically deficient ALDH the concentration of acetaldehyde in the breath after drinking can reach 8.8-22 mg/m^3 (4.9 to 12.2 ppm) Higher concentrations of acetaldehyde have been shown to activate mast cells, which then induce histamine release. In one case study, a patient had a severe bronchial asthma attack after ingesting food containing small amounts of alcohol, and was found to be homozygous for the ALDH-2 mutant genotype. Both acetaldehyde and ethanol inhalation tests were performed. The ethanol inhalation test was negative, but the acetaldehyde inhalation test (5, 10, 20, or 40 mg/ml) was positive because $FEV_{1.0}$ was decreased by 33.5% at the 20 mg/ml concentration of acetaldehyde (Saito et al., 2001).

4. Disposition

Acetaldehyde is readily absorbed through the lungs into the blood following inhalation exposure. Acetaldehyde is rapidly exchanged and equilibrated between blood entering the lungs and alveolar air. Male Sprague-Dawley rats exposed to acetaldehyde vapor concentrations in air ranging from 9 to 1000 mg/l (0.009 to 1 mg/m^3 or 500 to 555 ppb) for one hour had higher levels of acetaldehyde in the blood than liver (Watanabe *et al.,* 1986). Levels in the arterial blood were also higher than in peripheral venous blood.

Two studies were performed using humans and dogs to determine the percent retention of inhaled acetaldehyde in the respiratory tract (Egle, 1970; Egle Jr., 1972a; 1972b). In humans, the total respiratory tract retention of acetaldehyde was 45-70% when inhaled either orally or nasally (Egle, 1970). Physiological respiratory total retention in multiple breath experiments was independent of tidal volume, and uptake was controlled by frequency and duration of ventilation. Total respiratory tract retention of acetaldehyde in

dogs was found to be very close to human retention values and inversely related to ventilatory rate in the same manner as humans (Egle Jr., 1972b). Uptake was also found to be higher in the upper than the lower respiratory tract and unrelated to changes in concentration inhaled or tidal volume (Egle Jr., 1972b; Morris, 1997b).

Acetaldehyde deposition efficiency is strongly dependent on the inspired concentration, with deposition being less efficient at high compared to low concentrations. Species differences have been observed in uptake efficiency with uptake being significantly higher in the mouse, rat, and hamster compared to the guinea pig at 100 ppm, but at 10 ppm the rat had the lowest uptake (Morris, 1997b).

Following oral administration, acetaldehyde is readily absorbed from the gastrointestinal tract and undergoes extensive first pass metabolism in the liver; only 5% remains unchanged (Morris, 1997b).

Acetaldehyde rapidly diffuses through cellular membranes and is distributed to various organs for metabolism. The half-life in rats was 10 minutes, and the time to total body clearance was 40 minutes (Shiohara et al., 1984). Inhaled acetaldehyde does not undergo a first pass effect and is distributed to all tissues including the liver. Inhaled acetaldehyde undergoes extrahepatic metabolism and is metabolized by aldehyde dehydrogenase in the lungs to acetate. Aldehyde dehydrogenase is found in both the cytosol and the mitochondria. Inhaled acetaldehyde undergoes extrahepatic metabolism by the respiratory-olfactory epithelium, kidneys, blood, brain, and spleen, and only small amounts reach the liver. Acetaldehyde also crosses the blood-brain barrier.

Various isoenzymes of alcohol dehydrogenase transform ethanol into acetaldehyde, which in turn is rapidly oxidized by aldehyde dehydrogenase (ALDH) into nontoxic acetate. Functional genetic polymorphisms and ethnic variation exist at various genes encoding these enzyme proteins which all act to alter the rate of synthesis of the toxic metabolite acetaldehyde, or decrease its further oxidation. About 50% of the Asian population are alcohol-sensitive and have a deficiency or low activity in ethanol-metabolizing enzymes that can result in high blood and breath acetaldehyde levels following alcohol consumption.

A small amount of acetaldehyde is produced in the body during normal intermediary metabolism and is also a product of microbial fermentation of sugars in the gut. However, based on studies in animals, the critical effects of exposure to exogenous acetaldehyde occur at the site of initial contact (i.e., the respiratory tract following inhalation).

At least two isozymes of aldehyde dehydrogenase were found in the rodent nasal mucosa, differing with respect to their apparent Vmax and Km values (Morris, 1997b). Male F344 rats were exposed to 1500 ppm acetaldehyde for 6 hours/day for 5 days. Oxidation of acetaldehyde occurred more rapidly in the homogenates of the respiratory than the olfactory mucosa (Morris, 1997b). The nasal tissue is the first to contact acetaldehyde vapors upon inhalation. The aldehyde dehydrogenase acts as a defense mechanism

helping to minimize or prevent toxic injury to nasal tissues exposed to airborne compounds. Pretreatment with an ALDH inhibitor reduced nasal acetaldehyde deposition rates (Morris, 1997b).

Acetaldehyde can be eliminated unchanged in urine, expired air, and skin (Baselt and Cravey, 1989). Acetaldehyde is highly reactive and can bind to amino acids and blood and membrane proteins (Mohammad et al., 1949; Eriksson et al., 1977; Gaines et al., 1977; Donohue Jr. et al., 1983; Tuma and Sorrell, 1985; Dellarco, 1988; Hoffmann et al., 1993; Wickramasinghe et al., 1994; Tyulina et al., 2006). Acetaldehyde causes lipid peroxidation, which can lead to adduct formation and free radical-induced cell injury. Acetaldehyde that is metabolized by aldehyde dehydrogenase to acetate is readily excreted in the urine. Acetaldehyde can act as a hapten; and antibodies against acetaldehyde conjugates have been detected in human and rabbit serum (Gaines et al., 1977).

5. Acute Toxicity of Acetaldehyde

5.1 Acute Toxicity to Adult Humans

The major acute effects of human exposure to acetaldehyde vapors consist of irritation to the eyes, skin and respiratory tract, and bronchoconstriction in asthmatics. The key study used to determine the acute Reference Exposure Level (REL) was a study performed in human volunteers investigating sensory response to solvent vapors (Silverman et al., 1946). The purpose of the study was to determine the sensory response limit for solvent concentrations when estimating ventilation requirements for comfortable working conditions. The sensory limits were reported and compared to the maximum allowable concentration, which was stated as 200 ppm for acetaldehyde at the time of the study. Twelve volunteer human subjects of both sexes were used for each solvent exposure. The time of exposure was fifteen minutes. During the 15 minute exposure period, motion pictures were shown to occupy the subjects' attention and divert their thoughts from the atmospheric exposure in the chamber.

The results, though described in a limited way, are useful because the analysis was performed in human subjects and the concentrations tested were as low as 25 ppm. At 50 ppm, the majority of subjects experienced eye irritation. The subjects that did not report eye irritation had reddened eyelids and bloodshot eyes after exposure at 200 ppm. Several subjects reported unspecified irritation at 25 ppm and "objected strenuously." Finally, nose and throat irritation were reported as occurring at concentrations greater than 200 ppm (Silverman et al., 1946).

A second acute human study was found in the historical literature, where fourteen male subjects were exposed to 134 ppm acetaldehyde in a chamber for 30 minutes (Sim and Pattle, 1957). Subjects reported mild upper respiratory tract irritation (Sim and Pattle, 1957). However, a major confounder with this study appears in the methods section,

which stated that subjects were permitted to smoke inside the "chamber" during the 30 minutes.

Acetaldehyde provocation tests have been conducted with asthmatic and non-asthmatic human subjects using aerosolized acetaldehyde solutions. Nine male asthmatic patients with nine age- and sex-matched healthy volunteers inhaled ascending doses of 5, 10, 20, or 40 mg/ml of acetaldehyde and bronchial responsiveness was measured (Myou et al., 1993). The acetaldehyde solutions were in saline and were inhaled from a nebulizer for 2 minutes by mouth tidal breathing wearing a noseclip. Forced expiratory volume (FEV_1) was measured. A greater than 20% decrease in FEV_1 was caused by inhalation of acetaldehyde in a dose-dependent manner in asthmatics that received a placebo and did not receive a histamine H_1 blocker (terfenadine). Percent decrease in FEV_1 in asthmatics receiving the placebo after inhalation of each concentration of acetaldehyde was larger than that in asthmatics treated with terfenadine and in healthy subjects (Myou et al., 1993). These findings support the hypothesis that bronchial hyper-responsiveness is a precondition of acetaldehyde induced bronchoconstriction, which is caused indirectly via histamine release in asthmatics.

In another acute human study, nine asthmatic subjects were used to determine whether bronchial responsiveness to inhaled methacholine (a stardard test used to identify agents that potentially exacerbate asthma) was altered when asthmatic subjects inhaled a sub threshold concentration of aerosolized acetaldehyde which did not cause bronchoconstriction, and whether any increase in bronchial hyper-responsiveness after acetaldehyde was mediated by histamine release. For each subject, the concentration of acetaldehyde producing a 20% fall in FEV_1 was determined (PC_{20}) using ascending doses (5, 10, 20, or 40 mg/ml) of acetaldehyde. The mean concentration of PC_{20} for the nine subjects was 23.3 mg/ml of acetaldehyde. A sub threshold concentration of 0.8 mg/ml acetaldehyde was chosen and the nine subjects inhaled that or saline for four minutes (Myou et al., 1994). After each inhalation, a methacholine provocation test was performed. Acetaldehyde potentiated bronchial hyper-responsiveness to provocation by methacholine (Myou et al., 1994). In addition, the investigators determined that histamine was not responsible for acetaldehyde induced bronchial hyper-responsiveness at sub threshold concentrations (Myou et al., 1994).

The response to methacholine and acetaldehyde challenges have also been measured in 81 non-smoking adults (61 asthmatics and 20 normal controls) to determine differences in airway responsiveness between asthmatics and healthy subjects and to examine the relationship between acetaldehyde responsiveness and the variability of peak expiratory flow (PEF) (Prieto et al., 2000). The results of this study indicate that airway hyper-responsiveness to acetaldehyde is a sensitive and specific indicator for separating normal and asthmatic subjects. Five to 40 mg/mL acetaldehyde solutions were inhaled and FEV_1 was measured 60 to 90 seconds after inhalation of each concentration until FEV_1 dropped by more than 20% (Prieto et al., 2000). In this study, the PC_{20} values for acetaldehyde ranged from 1.96 to 40 mg/mL with a geometric mean value of 17.55 mg/mL. However, in this study, inhaled acetaldehyde was much less potent as a bronchoconstrictor than

methacholine in asthmatic patients. Peak expiratory flow variation was significantly but weakly related to acetaldehyde responsiveness ($p = 0.004$) (Prieto et al., 2000).

Bronchial responsiveness to acetaldehyde has been studied in sixteen asthmatics subjects without alcohol sensitivity and ten subjects with alcohol-induced bronchoconstriction (Fujimura et al., 1999). In this acute human study, subjects inhaled acetaldehyde or methacholine (0.04, 0.08, 0.16, 0.31, 0.63, 1.25, 2.5, 5, 10, and 20 mg/ml) in saline administered by nebulizer. The mean provocative concentration of acetaldehyde (PC_{20} in the alcohol and non-alcohol-senstive groups were 21.0 mg/mL and 31.7 mg/mL acetaldehyde, respectively. Bronchial responsiveness of acetaldehyde relative to methacholine was augmented in asthmatic subjects with alcohol-induced bronchoconstriction when compared to asthmatic subjects who were tolerant to alcohol (Myou et al., 1993; Fujimura et al., 1999; Prieto et al., 2000)

Another acute human study showed increased sensitivity to acetaldehyde by alcohol-sensitive adults (Myou et al., 1995). Seven male and three female asthmatics with alcohol-induced bronchoconstriction and sixteen asthmatics without alcohol-induced sensitivity were exposed to 0.04, 0.08, 0.16, 0.31, 0.63, 1.25, 2.5, 5, 10, 20, 40, and 80 mg/mL acetaldehyde or methacholine solution dissolved in saline for two minutes, administered with a nebulizer, and FEV_1 was recorded (Myou et al., 1995). Bronchial responsiveness was greater in the alcohol-sensitive asthmatic group compared to the alcohol-non-sensitive asthmatic subjects.

As indicated above, the provocation tests involved acetaldehyde solutions that were aerosolized, and then inhaled by mouth. Aerosolized acetaldehyde solutions have been shown to be about 265 times less potent than methacholine in constricting the airways of asthmatic subjects, with aerosolized acetaldehyde solutions of 80 mg/ml resulting in cough, dyspnea, and throat irritation in the asthmatic subjects (Myou et al., 1993). Studies involving aerosolized solutions of acetaldehyde were not used by OEHHA as the basis for the REL derivation.

In summary, exposure to acetaldehyde at concentrations as low as 25 ppm result in sensory irritation in human volunteers. Adult asthmatics that inhaled aerosolized solutions of acetaldehyde showed increased irritation and bronchoconstriction.

5.2 Acute Toxicity to Infants and Children

No studies on the effects of acute exposure to acetaldehyde in non-adult humans were located. However, as noted above for adults, there is some evidence that following acute exposure to acetaldehyde, asthmatics are more sensitive to acetaldehyde exposure and are likely to show asthma-like symptoms such as wheezing, shortness of breath, bronchoconstriction, and/or decrements in pulmonary function consistent with immediate and/or delayed bronchoconstriction. Furthermore, some asthmatics may respond with significant reductions in lung function due to the irritant effects on asthma, sensitized or not. The potential association between acetaldehyde exposure and asthma is of special concern for children because they have higher prevalence rates of asthma than adults, and

their asthma episodes can be more severe due to their smaller airways, which may result in more hospitalizations of children, especially for the first four years of life (Mannino et al., 1998). In addition, infants and children may have qualitatively different responses due to different target tissue sensitivities during windows of susceptibility in the developmental process.

Findings also support the view that toxic air contaminants, such as acetaldehyde, in communities in proximity to major emission sources, including both industrial and traffic sources, have adverse effects on asthma in children (Delfino et al., 2003) . The average daily residential exposure to acetaldehyde in high school students living in inner-city neighborhoods of New York City and Los Angeles and living with a smoker was evaluated. The exposure concentration range measured in juveniles living with smokers was 6.3 to 14 $\mu g/m^3$ (Nazaroff, 2004). This study estimated that approximately 16 million juveniles are exposed to environmental tobacco smoke, and hence acetaldehyde by living with smokers.

5.3 Acute Toxicity to Experimental Animals

Acetaldehyde causes sensory irritation in experimental animals. Male B6C3F1 or Swiss-Webster mice were exposed to acetaldehyde in a head-only exposure chamber for 10 minutes and sensory irritation was quantified by measuring respiratory rate depression during the exposures (Steinhagen and Barrow, 1984). The respiratory rates were recorded with a plethysmograph and the average maximum decrease in respiratory rate for one minute was computed from the response of each group of animals. Five concentrations (750 to 4200 ppm) were used to construct a concentration-response curve and the RD_{50} was calculated (the concentration eliciting a 50% decrease in respiratory rate). RD_{50} values were 2932 and 2845 ppm for B6C3F1 and Swiss-Webster mice, respectively (Steinhagen and Barrow, 1984).

In a study using young adult albino male Wistar rats, acetaldehyde (nose-only) exposure resulted in an initial rapid decrease in breathing frequency during the first minutes of exposure (Cassee et al., 1996a). The mimimum decrease in respiratory rate considered significant was 12%. The animals were exposed to acetaldehyde vapors for thirty minutes. The exposure concentrations were reported as 2800, 4600, and 6500 ppm for acetaldehyde. The RD_{50} for acetaldehyde in the single-compound study was calculated to be 3046 ppm (Cassee et al., 1996a).

Similarly, male F-344 rats were exposed in a head-only inhalation chamber to acetaldehyde (approximately 800 to 10,000 ppm though exact concentrations from the graph were not provided in the paper) for 10 minutes and experienced sensory irritation as measured by reduction in respiratory rate (Babiuk et al., 1985). The RD_{50} (the level inducing a 50% reduction in respiratory breathing rate) was 2991 ppm (95% CI 2411-3825) for this study (Babiuk et al., 1985).

In addition to sensory irritation, histopathological effects have been observed after exposure to acetaldehyde. Albino, male Wistar rats, 8 weeks old, were exposed for 6 hours a day, either on one or three day exposures on consecutive days, in a nose-only inhalation chamber to acetaldehyde (750 or 1500 ppm) (Cassee et al., 1996b). Acetaldehyde exposure resulted in histopathological nasal changes with the three-day exposure group consisting of increased incidence and severity of "single-cell necrosis" in olfactory epithelium with increasing concentration. Biochemical changes consisted of concentration-dependent increases of nonprotein sulfhydryl groups in nasal respiratory epithelium with one- and three-day exposure, which was statistically significant with exposure to 1500 ppm. Activities of biotransformation enzymes (glutathione peroxidase, glutathione S-transferase, glutathione reductase, formaldehyde dehydrogenase, and nonspecific aldehyde dehydrogenase) were not affected by any of the exposures (Cassee et al., 1996b).

Acute lethality studies have also been performed with acetaldehyde. In an historical acute inhalation study in rats, groups of eight per dose were exposed to acetaldehyde vapors 14,000 to 17,600 ppm (30,600 to 31,680 mg/m^3)) for thirty minutes (Skog, 1950). The acute LD_{50} value for acetaldehyde inhalation was 20,600 ppm (37,080 mg/m^3) (Skog, 1950).

Appelman et al. (1982) determined the LC_{50} for acetaldehyde after acute exposure in rats. Twenty male and twenty female albino Wistar rats were used for the acute study. The animals were exposed in horizontally placed glass exposure cylinders with a total airflow through the cylinder of 8 l/min. Concentrations were given as the mean of 10 to 15 determinations and were as follows: 10,436, 12,673, 15,683, and 16,801 ppm. Rats were exposed to acetaldehyde once for four hours. Within the first half-hour of the four-hour LC_{50} study, rats exhibited restlessness, closed eyes and labored breathing to acetaldehyde concentrations as low as 10,436 ppm. In the subacute portion of the study, rats exhibited severe dyspnoea and excitation within the first half-hour of exposure to 5000 ppm. The behavior of animals exposed to 2200 ppm or lower for six hours was unremarkable. The four-hour LC_{50} and the 95% confidence limits were calculated to be 13,300 ppm (95% CL: 11,200, 15,400) (Appelman et al., 1982)

Syrian Golden hamsters were exposed acutely to acetaldehyde vapors for 4 hours at doses ranging from 14,450 to 17,600 ppm (26,010 to 31, 680 mg/m^3) (Kruysse, 1970). After one to two hours of exposure at all concentrations, the animals showed severe lacrimation, salivation, and nasal discharges. The 4-hour LC_{50} was determined to be 17,000 ppm (30,600 mg/m^3) for this study. In all exposure groups, the animals that died during exposure had convulsions. However at all concentrations, some animals survived, but only after a deep narcosis and apnea (Kruysse, 1970).

Aldehyde dehydrogenase 2 (ALDH2) is an important enzyme that oxidizes acetaldehyde. Isse et al. (2005) compared the acute acetaldehyde toxicity between wild-type (Aldh2+/+) and Aldh2-inactive transgenic (Aldh2-/-) mice after inhalation. The null aldehyde dehydrogenase 2 (ALDH2) transgenic mice (-/-) or wildtype (+/+) mice were exposed by inhalation to 5000 ppm acetaldehyde for four hours. Mice were observed at 0, 2, 20, 40,

60, 120, and 240 minutes after administration. Within the first twenty minutes, hypoactivity, crouching, bradypnea, closed eyes, and piloerection were observed in both the wildtype and the knockout mice. By one hour, the ADLH (-/-) mice were showing a staggering gait (Isse et al., 2005). This study concluded that acute acetaldehyde toxicity after inhalation is higher in aldehyde dehydrogenase 2 knockout than in wild-type mice (Isse et al., 2005).

Female CD1 mice were exposed in inhalation chambers to a target acetaldehyde exposure of 200 ppm (actual mean of 5 exposures was 180 ± 35 ppm), twice the threshold limit value, for single and multiple three-hour exposures, which were evaluated for changes in their susceptibility to experimentally induced *Streptococcus* aerosol infection and pulmonary bactericidal activity to inhaled *Klebsiella* pneumoniae after one or five days (Aranyi et al., 1986). The results showed increased pulmonary bactericidal activity in response to 200 ppm of acetaldehyde possibly by a pollutant-induced recruitment of unexposed alveolar macrophages. This study suggests that inhaled toxicants such as acetaldehyde may alter susceptibility to or severity of respiratory infection (Aranyi et al., 1986).

Table 5.3.1 summarizes the acute animal data for acetaldehyde inhalation. The data indicate that humans are more sensitive to the acute effects of acetaldehyde than animals. For the endpoint of sensory irritation, measured as reduction in respiratory rate, the lowest RD_{50} for mice and rats were 2845 and 2991 ppm, respectively. With respect to histopathological changes, effects were observed at 1500 ppm. In the acute lethality studies, the lowest LC_{50} was 13,300 ppm in rats. In contrast, the LOAEL for humans was reported to be 25 ppm in one historical study (Silverman et al., 1946). Thus, humans appear to be at least 30 times more sensitive than animals to the acute effects of acetaldehyde.

Table 5.3.1 Summary of Acute Studies in Experimental Animals

Endpoint	Strain/Species	Exposure	Response	Reference
Sensory irritation	B6C3F1 mice	750 to 4200 ppm for10 min	RD_{50} = 2932 ppm	(Steinhagen and Barrow, 1984)
	Swiss Webster mice	750 to 4200 ppm for10 min	RD_{50} = 2845 ppm	(Steinhagen and Barrow, 1984)
	F-344 rats	~800 to 10,000 ppm for 10 min	RD_{50} = 2991 ppm	Babiuk et al., 1985
	Wistar rats	2800, 4600, or 6500 ppm for 30 min	RD_{50} = 3046 ppm	Cassee et al., 1996a
Histopathological	Wister rats	750 or 1500 ppm for 1 and 3 days	Olfactory epithelial lesions at 1500 ppm	Cassee et al., 1996b
Lethality	Rats	14,000 to 17,600 ppm for 30 min	LD_{50} = 20,600 ppm	Skog, 1950
	Wistar rats	10,436 to16,801 ppm for 4 hours	LC_{50} = 13,300 ppm	Appelman et al., 1982
	Syrian Golden hamsters	14,450 to 17,600 ppm for 4 hours	LC_{50} = 17,000 ppm	Kruysse, 1970
Behavioral/Other effects	Syrian Golden hamsters	14,450 for one to 2 hours	lacrimation, salivation, and nasal discharges	Kruysse, 1970
	Wistar rats	10,436 ppm within first 30 min	restlessness, closed eyes and labored breathing	Appelman et al., 1982
	Wistar rats	5000 ppm for 30 min	severe dyspnoea and excitation	Appelman et al., 1982
		5000 ppm for 20 minutes	crouching, bradypnea, closed eyes, and piloerection	Isse et al., 2005a
	CD1 mice	200 ppm for 3 hours	increased pulmonary bactericidal activity	Aranyi et al., 1986

6. Chronic Toxicity of Acetaldehyde

6.1 Chronic Toxicity to Adult Humans

No studies were found for human chronic exposures. Therefore the chronic REL was based on an animal study. However, as mentioned previously, it is important to note that acetaldehyde can be produced endogenously after food intake and ethanol consumption. Therefore, certain segments of the population may be at higher risk for chronic exposure due to alcoholism or frequent drinking or smoking. Those members of the population who smoke or are consistently exposed to ETS may be at increased risk of problems related to chronic toxicity of acetaldehyde.

6.2 Chronic Toxicity to Infants and Children

No studies were found on chronic exposure of infants and children to acetaldehyde. However, we anticipate that chronic exposure to acetaldehyde may exacerbate breathing problems in infants and children with asthma.

6.3 Chronic Toxicity to Experimental Animals

Exposure to inhaled acetaldehyde produces non-carcinogenic injury including degeneration and hyperplasia in the rat respiratory tract. The nasal cavity is the primary target with nasal olfactory mucosa being more sensitive than respiratory mucosa to the effects of acetaldehyde (Morris, 1997a; b). Deposition efficiency of inhaled acetaldehyde is highly dependent on airflow rate and on the inspired concentration in rodents (Morris, 1997a; b). Pretreatment with an ALDH inhibitor reduces nasal acetaldehyde deposition rates in anesthetized rodents (Morris and Blanchard, 1992).

In a subchronic study, male and female rats were exposed to acetaldehyde (6 hr/day, 5 days/week) for four weeks to concentrations of 400, 1000, 2200, or 5000 ppm, which resulted in degeneration of olfactory nasal tissues at all concentrations. Therefore a lowest observable adverse effect level (LOAEL) for this study was 400 ppm (Table 6.3.1) (Appelman et al., 1982). Nasal respiratory tissue lesions were seen at the three highest concentrations, tracheal and laryngeal lesions were observed only at the two highest concentrations, and mild injury to the lower respiratory tract was observed only at the highest concentration. Respiratory distress (dyspnea) was noted at 5000 ppm. Subsequent 4-week exposure studies in the same rat species, but males only, at 150 and 500 ppm, resulted in observed degeneration of olfactory nasal tissues at 500 ppm, but not in the 150 ppm exposure group (Appelman et al., 1986). Therefore, 150 ppm was designated the no observable adverse effect level (NOAEL).

Table 6.3.1: Incidence of Nasal Olfactory Tissue Effects in Rats

Degeneration of nasal olfactory epithelium	Exposure Group (ppm)						
	0	150	400	500	1000	2200	5000
Number examined	40	10	20	10	20	20	20
Number affected	2	0	16	10	20	19	20

(Appelman et al., 1982)

Exposure of rats to 243 ppm (442 mg/m^3) acetaldehyde for 8 hr/day, 5 days/week for 5 weeks resulted in an "intense" nasal inflammatory reaction with olfactory epithelium hyperplasia and polymorphonuclear and mononuclear infiltration of the submucosa (Saldiva et al., 1985). Changes in pulmonary mechanics, including increased functional residual capacity, residual volume, total lung capacity, and respiratory frequency was observed, but may have been the result of mechanical damage during pulmonary function testing.

In a subchronic exposure of hamsters to 0, 390, 1340, or 4560 ppm acetaldehyde 6 hr/day, 5 days/week for 90 days resulted in growth retardation, and ocular and nasal irritation in the high dose group. Histopathological changes were observed only in the respiratory tract and consisted of necrosis and inflammatory changes of the epithelium in the nasal cavity, larynx, bronchi and lungs in the high dose animals, and mild trachea epithelial lesions in the mid-dose group. No adverse effects were observed at 390 ppm (Kruysse et al., 1975).

In a subsequent study, 36 hamsters per dose group were chronically exposed in a whole body inhalation chamber to 0, 1500, or 2500 ppm acetaldehyde for 7 hr/day, 5 days/week for 52 weeks resulting in growth retardation and hyperplasia and metaplasia of the nasal and tracheal epithelium in exposed animals (Feron et al., 1982). Rhinitis and epithelial lesions of the larynx were also noted at the highest exposure. The average concentration in the high exposure group (2500 ppm) was lowered several times during the study due to severe growth retardation to a final concentration of 1650 ppm. The authors noted that the nasal lesions were very similar to those previously seen in hamsters repeatedly exposed to 4560 ppm in the 13-week study Kruysse et al. (1975) study. Following a 26-week recovery period, the upper respiratory tract lesions were still present in high exposure animals, but were nearly or completely absent at the low exposure animals (Feron et al., 1982). However, the authors note that the acetaldehyde-induced hyperplasia and metaplasia of the nasal and laryngeal epithelium persisted and was irreversible (Feron et al., 1982).

In chronic inhalation studies, rats were exposed to 0, 750, 1500, or 3000 ppm acetaldehyde for 6 hr/day, 5 days/week for up to 28 months (Woutersen et al., 1984; Woutersen et al., 1986; Woutersen and Feron, 1987). The concentration in the high-dose group was gradually lowered over 15 months to 1000 ppm due to early mortality, respiratory distress (dyspnea) and severe growth retardation. Nasal olfactory tissue degeneration, hyperplasia, and metaplasia were seen at all exposure levels including the LOAEL of 750 ppm. A NOAEL was not determined for this study. Larynx and nasal

respiratory epithelium lesions were observed at the two highest concentrations (1500 and 3000 ppm), and slight to severe rhinitis and sinusitis was observed at the highest concentration (3000 ppm). Growth retardation occurred in males of each test group and in females of the two highest concentration groups.

In a pulmonary immune response study, groups of non-sensitized and ovalbumin (OA)-sensitized guinea pigs were exposed to 0 or 200 ppb (360 $\mu g/m^3$) acetaldehyde for 6 hr/day, 5 days/week for four weeks (Lacroix et al., 2002). In sensitized, acetaldehyde-exposed animals, subsequent challenge with OA aerosol did not modify the inflammatory and allergic responses induced by sensitization alone. In nonsensitized guinea pigs, acetaldehyde exposure resulted in slight irritation (metaplasia/hyperplasia) of the lung, trachea and nasal respiratory epithelium, and induced a significant increase in the number of alveolar macrophages and total number of cells in bronchoalveolar lavage fluid (Lacroix et al., 2002). The respiratory lesions in guinea pigs at 200 ppb acetaldehyde occurred at roughly 2000-fold less than the NOAEL established for respiratory lesions in rats and hamsters under similar exposure duration protocols. These data suggest that guinea pigs are considerably less sensitive to acetaldehyde exposure than rats or hamsters.

Inhaled acetaldehyde is genotoxic and is a clastogen, and inducer of sister chromatid exchanges (Dellarco, 1988). *In vivo* and *in vitro* studies have shown that acetaldehyde can form DNA-DNA and DNA-protein crosslinks (Morris, 1997a). Acetaldehyde vapor causes chronic tissue injury and tumor formation in nasal tissues at exposure concentrations of 750 ppm or higher (Feron et al., 1982; Woutersen et al., 1986).

7.0 Developmental and Reproductive Toxicity

Both clinical and experimental studies have shown that ethyl alcohol causes developmental and reproductive toxicity. Acetaldehyde, the primary metabolite of ethyl alcohol, has been suggested as a possible etiologic agent in fetal alcohol syndrome (FAS) (Pratt, 1980; West, 1994; Eriksson, 2001). Current studies suggest that ethyl alcohol and acetaldehyde work through different mechanisms, but it is still unknown if one or both are the basis for FAS. Acetaldehyde has been shown to cross the placenta in mice and was distributed to embryos (Blakley and Scott Jr., 1984). Placental transfer occurred when acetaldehyde was administered via i.p. injection to pregnant CD-1 mice at 200 mg/kg on day 10 of gestation, and acetaldehyde was detected within the embryo within 5 minutes (Silverman et al., 1946). Maximal concentrations of acetaldehyde were also reached in the maternal blood, liver, and yolk sac in the first five minutes.

Acetaldehyde also freely crosses the placenta of Wistar rats (Zorzano and Herrera, 1989). Following i.v. injection of acetaldehyde (10 mg/kg) to pregnant rats on gestation day 21, acetaldehyde concentrations reached peak levels within five minutes in the maternal blood, fetal blood, and amniotic fluid. After just two minutes of maternal intravenous administration of acetaldehyde at high concentrations, it freely crosses the placenta.

Acetaldehyde is a small liposoluble molecule and is able to cross membranes by simple diffusion (Zorzano and Herrera, 1989).

Acetaldehyde has been shown to cause adverse developmental effects in some rodent species when administered in high doses via i.p. or i.v. injection. Rats were exposed 50, 75, or 100 mg/kg acetaldehyde by i.p. on gestation day 10, 11, or 12 and then sacrificed on day 21. Significant fetal resorptions and malformations were observed including: edema, microcephaly, micrognathia, micromelia, hydrocephaly, exencephaly, and hemorrhages. Somatometric measurements of fetus, crown rump length, transumbilical distance, and tail length notes severe growth retardation (Sreenathan et al., 1982). In another study in rats, after a single i.p. injection of 50, 75, or 100 mg/kg, teratogenicity, embryolethality, and growth retardation were observed (Blakley and Scott Jr., 1984).

In vitro models have found that acetaldehyde was teratogenic to C_3H mouse embryos between 8 and 10 days of gestation after 28 hours of exposure (Thompson and Folb, 1982). Morphological parameters and DNA synthesis were measured and correlated. Eight and nine-day embryos were exposed to doses of 7.4, 19.7, or 39.4 mg/l acetaldehyde. The 39.4 mg/l dose group at eight days showed a significant effect on somite count, neural tube fusion, CNS development (size and symmetry), and significant reduction in DNA synthesis. The nine-day embryos at 39.4 mg/l had increased somite count, absent heart beat, and a significant increase in limb development, while the 19.7 mg/l group had significant abnormalities in development of visceral arches, CNS development, and reduction in DNA synthesis.

Acetaldehyde significantly induced cytotoxicity *in vitro* in cultured rat embryonic midbrain cells. The levels of p53, bcl-2, and 8-OHdG were also changed by acetaldehyde treatment (Lee et al., 2005). The purpose of this study was to elucidate the molecular mechanisms involved in alcohol-induced Fetal Alcohol Syndrome (FAS) during embryo and fetal development. It is not clear whether the observed toxicity associated with FAS is due to direct exposure to ethanol, to its metabolite(s) (e.g. acetaldehyde) or to both.

Both acetaldehyde and ethanol significantly inhibited the gonadotropin-stimulated biosynthesis of testosterone, and acetaldehyde was 4,000 times more potent than ethanol *in vitro* in enzymatically dispersed cells. Testicular steroidogenesis was blocked by acetaldehyde selectively, specifically inhibiting the conversion of androstenedione to testosterone (Cicero and Bell, 1980; Cicero et al., 1980a; Cicero et al., 1980b). As little as 50 μM acetaldehyde was effective in suppressing testicular steroidogenesis; however cell viability was unaffected.

Currently, acetaldehyde is on the Proposition 65 list of carcinogens (listed April 1, 1988); however, it is not currently under consideration as a developmental and reproductive toxicant (DART).(OEHHA, 2007).

8.0 Derivation of Reference Exposure Levels

8.1 Acetaldehyde Acute Reference Exposure Level

Study	Silverman et al., 1946
Study population	24 adult human volunteers
Exposure method	Whole body inhalation
Exposure continuity	
Exposure duration	15 minutes
Critical effects	Eye and upper respiratory tract irritation
LOAEL	45 mg/m^3 (25 ppm)
NOAEL	not observed
Benchmark concentration	not derived
Time-adjusted exposure	not applied (sensory irritation, no Haber's Law adjustment)
Human Equivalent Concentration	not applied
LOAEL uncertainty factor (UF_L)	6 (default: mild effect, no NOAEL)
Subchronic uncertainty factor (UFs)	not applied
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	1 (default, human study)
Toxicodynamic (UF_{A-d})	1 (default, human study)
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	1 (site of contact; no systemic effects)
Toxicodynamic (UF_{H-d})	10 (asthma exacerbation in children)
Cumulative uncertainty factor	60
Reference Exposure Level	**750 μg/m^3 (420 ppb)**

Acute Reference Exposure Levels are levels at which intermittent one-hour exposures are not expected to result in adverse health effects (see Section 5 in the Technical Support Document).

The study by Silverman *et al.,* (1946) was selected for development of the acute REL as it investigated short-term exposure of human volunteers to acetaldehyde. Eye irritation was the most noticeable endpoint for non-asthmatic adults. Upper respiratory tract, nose, throat, and bronchial irritation typically followed that effect closely. Exposure to 50 ppm for 15 minutes caused moderate eye irritation in all subjects, whereas 25 ppm caused complaints of slight eye irritation in an unspecified number of volunteers. Nose and throat irritation and transient conjunctivitis were seen at concentrations of 200 ppm or greater. From the key study it was possible to determine a LOAEL of 25 ppm for slight eye irritation. However, a NOAEL was not determined for this study.

OEHHA was unable to use pulmonary response as an endpoint because the studies done to date in humans have all used aerosolized acetaldehyde vs. inhalation of acetaldehyde vapor. However, in numerous studies on adult humans with and without asthma,

provocation with acetaldehyde resulted in significant pulmonary decrements and more so in asthmatics.

The trigeminal nerve, which gathers sensory signals from the nasal mucosa amongst several other places, appears to be the only sensory nerve pathway directly involved with the respiratory response to inhaled irritants. In rodents, a reflex decrease in respiratory rate is observed after the initial sensory irritation (Bos et al., 2002); the human response is more complex in its expression although similar in neurological mechanism.

In this key study, the output (acetaldehyde vapor) is sent generally into an environmental chamber in an effort to mimic real-life exposures and the subject's nose, respiratory tract, eyes, and uncovered skin are concomitantly exposed to the chemical stimulus (Silverman et al., 1946). Generally speaking, the lowest concentration of an irritant that can be discerned by sniffing or by ocular exposure is considered to be the threshold for irritation (Doty et al., 2004). As a general rule, most volatile chemicals that are capable of eliciting irritative sensations (e.g., via the trigeminal nerve) can also elicit an odor (via CN I); furthermore, the odor is often evoked at concentrations one or more orders of magnitude below those that evoke irritation. For most volatile chemicals, ocular irritation is equivalent in sensitivity to nasal irritation in humans with thresholds of equivalent magnitude (Cometto-Muniz and Cain, 1995; 1998; Cometto-Muniz et al., 1999; 2001; 2002; Doty et al., 2004).

A default uncertainty factor of six is associated with the use of a LOAEL for mild effects in the absence of a NOAEL (see Section 4.4.5 of the TSD). The key study used to determine the acute REL was a human study, therefore the interspecies uncertainty factor, toxicokinetic (UF_{A-k}) and toxicodynamic (UF_{A-d}) components were each assigned the default value of one. Eye irritancy appears to be more a function of concentration rather than duration of exposure (Yang et al., 2001), so no time correction factor was applied.

For the toxicokinetic component of the intraspecies uncertainty factor (UF_{H-k}) a value of one was used since sensory irritation is not expected to involve large toxicokinetic differences among individuals, and the effects are largely confined to the site of contact, in this case, the eyes, nose, and upper respiratory tract, with negligible or no systemic effects. The deposition kinetics of reactive gases is generally thought not to be greatly different between adults and children. Because of this, a value of one is used for the kinetic component of the intraspecies uncertainty factor (UF_{H-k}), rather than a more extended values of $\sqrt{10}$ or ten used where metabolic processes also contribute to inter-individual variability.

A toxicodynamic uncertainty factor (UF_{H-d}) of ten was used to account for the potential greater susceptibility of children. While ocular irritation is not expected to be substantially different between children and adults, the respiratory irritant effect, with documented potential to exacerbate asthma, is clearly an effect with the potential to differentially impact infants and children. The toxicodynamic component of the intraspecies uncertainty factor UF_{H-d} is therefore assigned an increased value of ten to account for potential asthma exacerbation. As mentioned earlier, asthmatics are more

sensitive to the irritative properties of inhaled aerosolized acetaldehyde solutions, which significantly decreased forced expiratory volume in one second (FEV_1) by more than 20% in asthmatics. And, alcohol sensitive asthmatics had a selective hyper-responsiveness to acetaldehyde (Myou et al., 1993; Fujimura et al., 1999; Prieto et al., 2000). These considerations are applied equally to the acute, 8-hour and chronic REL.

Limitations with the Silverman et al. (1946) key study include: small sample size, subjective and non-quantitative measure of irritation, absence of a clear description of exposure method and experimental procedure, which was further unsubstantiated by lack of a clear experimental procedure that was referenced as Cook et al. (1945).

In conclusion, using the LOAEL of 45 mg/m^3 (25 ppm) from Silverman et al. (1946) divided by the cumulative uncertainty factor of 60, an acute reference exposure level (REL) for acetaldehyde, with the endpoint of eye irritation, was determined to be 750 ug/m^3 or 420 ppb, which is the level considered safe for infants and children during an acute exposure period.

8.2 Acetaldehyde 8-Hour Reference Exposure Level

The 8-hour Reference Exposure Level is a concentration at or below which adverse noncancer health effects would not be anticipated for repeated 8-hour exposures (see Section 6 in the Technical Support Document).

Both eye irritation and nasal mucosal histopathology are legitimate concerns for the 8-hour REL and occur in a broadly similar concentration range over the relevant time scale. The human study by Silverman et al. (1946) provided limited information on the experimental procedure, used a small sample size, and used a subjective non-quantitative form of measure as the endpoint (self-reported irritancy). Further, the repeated nature of an 8-hour REL makes use of the acute study inappropriate. Therefore, the 8-hour REL was derived using the subchronic animal study (Appelman et al., 1982; 1986) in rats exposed to acetaldehyde six hours per day, five days per week for four weeks. Incidence of degeneration of nasal olfactory epithelium was the most sensitive end-point.

Study	Appelman *et al.*, 1982; 1986
Study population	Wistar rats (10-40 animals/group)
Exposure method	Discontinuous whole-body inhalation exposure to 0, 273, 728, 910, 1820, 4004, 9100 mg/m^3 (0, 150, 400, 500, 1000, 2200, or 5000 ppm)
Exposure continuity	6 hours per day, 5 days/week
Exposure duration	4 weeks
Critical effects	Nasal degeneration of olfactory epithelium
LOAEL	720 mg/m^3 (400 ppm)
NOAEL	270 mg/m^3 (150 ppm)
Benchmark Concentration (BMC_{05}) (using continuous model)	178 mg/m^3 (99 ppm)
Time-adjusted exposure	133.5 mg/m^3 (74.25 ppm) = (178*6/8*5/5)
Human DAF concentration	113.5 mg/m^3 (63.11 ppm) = 133.5*0.85 (DAF)
LOAEL uncertainty factor (UF_L)	1
Subchronic uncertainty factor (UFs)	1
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	2 (with DAF adjustment)
Toxicodynamic (UF_{A-d})	√10 (default: no interspecies toxicodynamic data)
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	√10 (inter-individual variation)
Toxicodynamic (UF_{H-d})	10 (asthma exacerbation in children)
Cumulative uncertainty factor	200
Reference Exposure Level	**568 μg/m^3 (316 ppb)**

The animal studies by Appelman et al. (1982; 1986) used subchronic exposure of Wistar rats to acetaldehyde for six hours per day, 5 days per week, for four weeks. Incidence of degeneration of nasal olfactory epithelium was the most sensitive end-point. The animal

study has a histopathological endpoint for which there is a presumption of Haber's law (C x t) cumulation, at least over moderate timeframes. Therefore, the average experimental exposure was adjusted from six to eight hours per day.

The 8-hour REL was determined using the Benchmark Dose (BMC) program (Crump and Howe, 1983; Crump, 1984) and procedures developed by the U.S. EPA (2003). The BC_{05} is defined as the 95% lower confidence limit of the concentration expected to produce a response rate of 5%. The animal data from the Appelman et al. (1982; 1986) studies were used to develop a BC_{05} for acetaldehyde.

The male and female data were analyzed both together and separately (Table 8.2.1). The study with exposure concentrations of 150 and 500 ppm used only males. Data on incidence of degeneration of olfactory epithelium were converted to a continuous data set ranked by severity of effect (Table 8.2.1). The means and standard deviations at each dose-group are shown, which were calculated from the severity grading of individual animals in each dose group. Each severity category had a name and a corresponding value assigned: no effect = zero, minimal = one, slight = two, moderate = three, marked = 4, moderate with hyperplasia = 5, severe with hyperplasia = 6, and very severe with hyperplasia =7. The means and standard deviations for each dose group were entered into the BMC program using continuous modeling. The Hill and Polynomial models in the BMC program gave the best fit to the data (Table 8.2.2). The mean of the three models that best fit the data was calculated to be 99 ± 1.20 ppm and used as the BC_{05}.

Table 8.2.1. Incidence of Degeneration of Olfactory Epithelium using Weighted Means by Severity[1] .

	Males			**Females[2]**		
Dose (ppm)	Number	Mean	Stdev	Number	Mean	Stdev
0	30	0.07	0.25	10	0	0
150	10	0	0			
400	10	2.6	1.17	10	0.9	0.74
500	10	2.5	0.97			
1000	10	2.8	0.63	10	3.6	0.70
2200	10	5.3	2.21	10	5.1	1.91
5000	10	6.7	0.67	10	6.9	0.32

[1]Severity categories: no effect=0; minimal=1; slight=2; moderate=3; marked=4; moderate w/ hyperplasia=5; severe w/ hyperplasia=6; and very severe w/ hyperplasia=7.
[2] In the 150 and 500 ppm dose groups, only male animals were used.

Table 8.2.2. BMC Results Modeling Incidence of Degeneration of Nasal Olfactory Epithelium Using Weighted Means by Severity in Rats Using a Continuous Model.

Method	BMCL*	BMC*	P-value	AIC
Hill Model	100	205	0.07	55.96
Polynomial (2°)	101	126	0.02	56.18
Polynomial (3°)	97	165	0.03	55.95

* BMCL and BMC are in units of ppm.

The standard Human Equivalent Concentration (HEC) adjustment was not used for the dosimetric interspecies extrapolation. Instead, species information based on pharmacokinetic modeling for toxicants that result in specific nasal olfactory tissue damage was applied for interspecies extrapolation of acetaldehyde toxicity. Dosimetry data for the nasal olfactory epithelium shows that the rat is more efficient in scrubbing organic vapors in this region of the nasal cavity than humans (Frederick et al., 1998; Frederick et al., 2001). Consequently, rats receive a similar, or greater, tissue dose of inhaled organic vapors than humans in the olfactory epithelium. This interspecies adjustment also takes into account differences in the deposition of inhaled vapors and breathing rates. While rodents are obligate nose breathers, humans are not, which has implications for exposure of nasal tissues. Other factors when extrapolating toxicity findings from rodents to humans include dosimetry, nasal anatomy and airflow dynamics, target tissue metabolism, species differences in gross anatomy, distribution of nasal airway epithelia, and distribution and composition of mucous secretory products (Feron et al., 2001).

The dosimetric adjustment factor (DAF) is a factor derived by OEHHA based on the modeled comparative flux of formaldehyde in the upper respiratory tracts of rats, rhesus monkeys and humans (Kimbell et al., 2001) (see Section 4.4.7.2.2 of the TSD). In this model, a three-dimensional, anatomically realistic, computational flow dynamic model was used to estimate mass flux across 20 consecutive bins representing the nasal passages. The mean flux at each bin was weighted by the percent of non-squamous epithelium in that bin to derive a weighted average flux for each bin. Averaging across all 20 bins provides an overall estimate of the flux for comparison between species (rat 13.63 pmol/mm^2; human 30.80 pmol/mm^2). Peak flux values were also estimated for the rat (2620 pmol/mm^2) and human (2082 pmol/mm^2), and averaged with the mean flux values to estimate the DAF. The DAF is the ratio of this value for the rat to that for humans. The reactivity of acetaldehyde is similar to that of formaldehyde. Therefore, application of the DAF to acetaldehyde assumes that it deposits similarly to formaldehyde in the nasal passages. The uncertainty associated with this assumption is reflected in the use of the toxicokinetic component of the interspecies uncertainty factor $UF_{A\text{-}k}$ equaling two. Sensitivity to acetaldehyde of the rat olfactory epithelium is a major factor for olfactory tissue damage, even though the specific activity of aldehyde dehydrogenase is greater in the respiratory epithelium (Bogdanffy et al., 1998; Stanek and Morris, 1999).

The LOAEL uncertainty factor (UF_L) of one was chosen, since both a LOAEL and NOAEL were determined in the key studies (Appelman et al., 1982; Appelman et al., 1986), and the benchmark approach was used to determine the chronic REL. In addition, the subchronic uncertainty factor (UFs) was assigned a value of one since this was a subchronic study.

The value of two was chosen for the toxicokinetic component of the interspecies uncertainty factor (UF_{A-k}) to account for additional differences between humans and rodents. For example, it is unknown if humans have nasal ALDH2. In addition, previous studies have shown marked differences between animal species (hamster, rat, mice, and guinea pig) in the uptake of acetaldehyde vapor (Morris, 1997b). However, since acetaldehyde exerts mainly a localized effect on nasal olfactory epithelium, toxicokinetics including distribution and metabolism play less of a key role.

The toxicodynamic portion of the interspecies uncertainty factor (UF_{A-d}) is 3.16 because the key studies are in non-primates and data on toxicodynamic interspecies differences are not available.

An uncertainty factor (UF_{H-k}) of 3.16 was used to account for intra-individual toxicokinetic variation. The intraspecies uncertainy factor was selected because acetaldehyde is a reactive substance that produces lesions at the point of contact with the tissue, therefore there would be less variability to take into account for children versus adults. The toxicodynamic uncertainty factor (UF_{H-d}) of 10 was used to account for the potentially greater susceptibility of children and asthmatics. The resulting cumulative uncertainty factor was calculated as 200 and used to determine the acute REL of the experimental animal study.

The BC_{05} of 99 ± 1.20 ppm from the Benchmark Dose analysis was time-adjusted from six to eight hours for five days per week. Then, the resulting value was multiplied by the dosimetric adjustment factor (DAF) based on PBPK modeling. Finally, the resulting value was divided by the cumulative uncertainty factor of 200. This produced an 8-hour REL with the endpoint of degeneration of olfactory epithelium in rats of 568 $\mu g/m^3$ (316 ppb) (Appelman et al., 1982; Appelman et al., 1986)

Eye irritation and nasal mucosal histopathology are both legitimate concerns for the 8-hour REL for acetaldehyde and occur in a broadly similar concentration range over the relevant time scale. However, repeated 8-hour exposures could result in tissue damage, therefore the REL using the animal study with a histopathological endpoint of 568 $\mu g/m^3$ (316 ppb) was used. The experimental animal study used as the basis for the 8-hour REL with an endpoint of degeneration of nasal olfactory epithelium would also be protective of the human sensory response too, since the acute REL derived from the Silverman et al. (1946) human study is higher. The animal study was chosen because it was a well-conducted study with adequate dose groups and a time-period relevant for the 8-hour REL. In addition, using benchmark dose and PBPK modeling decreased the uncertainty associated with the REL derivation compared with using the traditional NOAEL/LOAEL and HEC procedures.

8.3 Acetaldehyde Chronic Reference Exposure Level

Study	Appelman *et al.,* 1982; 1986
Study population	Wistar rats (10-40 animals/group)
Exposure method	Discontinuous whole-body inhalation exposure to 0, 273, 728, 910, 1820, 4004, 9100 mg/m^3 (0, 150, 400, 500, 1000, 2200, or 5000 ppm)
Exposure continuity	6 hours per day, 5 days/week
Exposure duration	4 weeks
Critical effects	Nasal degeneration of olfactory epithelium
LOAEL	720 mg/m^3 (400 ppm)
NOAEL	270 mg/m^3 (150 ppm)
Benchmark Concentration (BMC_{05}) (using continuous model)	178 mg/m^3 (99 ppm)
Time-adjusted exposure	31.79 mg/m^3 (17.68 ppm) = (178*6/24*5/7)
Human DAF concentration	27.02 mg/m^3 (15.03 ppm) = 31.79*0.85 (DAF)
LOAEL uncertainty factor (UF_L)	1
Subchronic uncertainty factor (UFs)	√10 (exposure 8-12% of lifetime)
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	2 (with DAF adjustment)
Toxicodynamic (UF_{A-d})	√10 (default: no interspecies toxicodynamic data)
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	√10 (inter-individual variation)
Toxicodynamic (UF_{H-d})	10 (asthma exacerbation in children)
Cumulative uncertainty factor	632
Reference Exposure Level	**43 μg/m^3 (24 ppb)**

The chronic Reference Exposure Level is a concentration at which adverse noncancer health effects would not be expected from chronic exposures (see Section 7 in the Technical Support Document).

The chronic REL was based on four-week exposure data in rats from Appelman *et al.,* (1982, 1986), and supported by Saldiva *et al.*, (1985); Woutersen *et al*., (1986, 1984); and (Woutersen and Feron, 1987), which included a 28-month chronic study in rats. Incidence of degeneration of nasal olfactory epithelium was the most sensitive end-point. The proposed chronic REL was estimated by a benchmark concentration (BMC) modeling approach using the continuous polynomial and Hill models of analysis (Crump and Howe, 1983; Crump, 1984) as previously described in detail in Section 8.2. The average experimental exposure data were adjusted to reflect chronic exposure. Table 8.2.1 shows the data expressed as the mean and standard deviation of the degeneration of nasal olfactory epithelium by severity for each dose group, which were the data used for the BMC model. As shown in Table 8.2.2, three models were selected that best fit the data and their mean and standard deviation was 99 ± 1.20 ppm and therefore used as the BC_{05}.

As described in detail in Section 8.2, OEHHA derived a dosimetric adjustment factor (DAF) for acetaldehyde rather than using the traditional Human Equivalent Concentration (HEC) value. The application of the DAF was based on a model developed with formaldehyde and assumes that acetaldehyde deposits similarly to formaldehyde in the nasal passages. The uncertainty associated with this assumption is reflected in the use of the toxicokinetic component of the interspecies uncertainty factor UF_{A-k} equaling two.

The LOAEL uncertainty factor (UF_L) of one was chosen, since both a LOAEL and NOAEL were determined in the key studies (Appelman et al., 1982; Appelman et al., 1986) , and the benchmark approach was used to determine the chronic REL.

The subchronic uncertainty factor (UFs) was assigned a value of three since the chronic REL is representative of exposures over a lifetime, and because the supporting chronic study (Woutersen et al., 1986) didn't give a dramatic increase in injury compared to the four-week studies by Appelman *et al.*, (1982; 1986). In addition, Saldiva *et al.,* (1985) observed "intense" nasal lesions in rats exposed to 442 mg/m^3 (243 ppm) for slightly longer exposure durations than that used by Appelman *et al.,* (1982; 1986).

The value of two was also chosen for the toxicokinetic component of the interspecies uncertainty factor (UF_{A-k}) to account for additional differences between humans and rodents. For example, it is unknown if humans have nasal ALDH2. In addition, previous studies have shown marked differences between animal species (hamster, rat, mice, and guinea pig) in the uptake of acetaldehyde vapor (Morris, 1997b). However, since acetaldehyde exerts mainly a localized effect on nasal olfactory epithelium, toxicokinetics including distribution and metabolism play less of a key role.

The toxicodynamic portion of the interspecies uncertainty factor (UF_{A-d}) is 3.16 because the key studies are in non-primates and data on toxicodynamic interspecies differences are not available.

Intraspecies variability can be as much as a factor of 1,000-fold for VOCs measured in human subjects (Fenske and Paulson, 1999). An uncertainty factor (UF_{H-k}) of 3.16 was used to account for intra-individual toxicokinetic variation. The intraspecies uncertainy factor was selected because acetaldehyde is a reactive substance that produces lesions at the point of contact with the tissue, therefore there would be less kinetic variability to take into account for children versus adults. The toxicodynamic uncertainty factor (UF_{H-d}) of 10 was used to account for the potentially greater susceptibility of children and asthmatics.

The current chronic RfC for acetaldehyde determined by the U.S. EPA and based on Appelman *et al.,* (1982; 1986) is 9 µg/m^3 (5 ppb) and is within the range of normal human breath acetaldehyde concentrations of 0.7 to 11.0 µg/m^3 (0.4 to 6.1 ppb). OEHHA's proposed chronic REL of 43 µg/m^3 (24 ppb) is above the range of human breath concentrations of acetaldehyde, but is mostly exceeded when humans consume significant amounts of alcohol, resulting in human breath concentrations ranging from

200 to 2200 μg/m^3. Thus, frequent alcohol use and abuse by humans is a major source of acetaldehyde exposure to the airway tissue that can exceed the chronic REL.

Standard BMC methodology was not in place when U.S. EPA developed its RfC for acetaldehyde in 1991. The U.S. EPA (1991) determined an acetaldehyde RfC of 9 μg/m^3 (5 ppb) based on the Appelman *et al.,* (1982, 1986) studies using NOAEL/LOAEL methodology. The principal study established a NOAEL of 273 mg/m^3. Adjustments for duration of exposure and corrections for relative areas of human and animal extrathoracic region of the respiratory tract were made resulting in a human equivalent concentration of 8.7 mg/m^3. A total UF of 1000 (10 for intraspecies, 10 for extrapolation to chronic exposure, and 10 for interspecies and incompleteness of the database) was then applied, resulting in the RfC of 9 μg/m^3.

The LOAEL of 750 ppm from the chronic exposure data by Woutersen *et al.,* (1984, 1986) and Woutersen and Feron (1987) produced similar injuries and was confined to the nasal olfactory epithelium as the LOAEL of 400 ppm from the 4-week Appelman studies. Thus, the subchronic UF was reduced from 10 to 3.16, to account for similar findings from the chronic studies. Analyses were also performed on the incidence of respiratory epithelial changes using the LOAEL from the chronic rat studies, although it was a less sensitive end-point (Woutersen *et al.,* 1984, 1986; Woutersen and Feron 1987). The 100% response rate at the LOAEL combined with the lack of a NOAEL prevented the chronic studies from becoming the basis of the REL.

Significant strengths for the chronic REL include (1) the use of a well conducted repeated exposure study with histopathological analysis and (2) independent studies demonstrating comparable key effects (nasal lesions) in experimental animals. However, major areas of uncertainty are the lack of adequate human chronic inhalation dose-response data in adults and children, and inadequate long-term inhalation animal data, therefore a subchronic animal study was used.

8.4 Acetaldehyde as a Toxic Air Contaminant

In view of the potential of acetaldehyde to exacerbate asthma (Section 5.1, 5.2), and the differential impacts of asthma on children including higher prevalence rates, coupled with widespread exposure (e.g., indoors from exposure to environmental tobacco smoke, and outdoors due to numerous emissions sources), OEHHA recommends that acetaldehyde be identified as a toxic air contaminant (TAC) that may disproportionately impact children pursuant to Health and Safety Code, Section 39669.5(c).

9. References

Appelman LM, Woutersen RA and Feron VJ (1982). Inhalation toxicity of acetaldehyde in rats. I. Acute and subacute studies. Toxicology 23(4): 293-307.

Appelman LM, Woutersen RA, Feron VJ, Hooftman RN and Notten WR (1986). Effect of variable versus fixed exposure levels on the toxicity of acetaldehyde in rats. J Appl Toxicol 6(5): 331-336.

Aranyi C, O'Shea WJ, Graham JA and Miller FJ (1986). The effects of inhalation of organic chemical air contaminants on murine lung host defenses. Fundam Appl Toxicol 6(4): 713-720.

Babiuk C, Steinhagen WH and Barrow CS (1985). Sensory irritation response to inhaled aldehydes after formaldehyde pretreatment. Toxicol Appl Pharm 79(1): 143-149.

Baselt RC and Cravey RH (1989). Disposition of Toxic Drugs and Chemicals in Man. 3rd. Edition. Chicago (IL): Year Book Medical Publishers Inc.

Beall JR and Ulsamer AG (1981). Toxicity of volatile organic compounds present indoors. Bull N Y Acad Med 57(10): 978-96.

Blakley PM and Scott Jr. WJ (1984). Determination of the proximate teratogen of the mouse fetal alcohol syndrome. 1. Teratogenicity of ethanol and acetaldehyde. Toxicol Appl Pharm 72(2): 355-363.

Bogdanffy MS, Sarangapani R, Kimbell JS, Frame SR and Plowchalk DR (1998). Analysis of vinyl acetate metabolism in rat and human nasal tissues by an in vitro gas uptake technique. Toxicol Sci 46(2): 235-246.

Bos PMJ, Busschers M and Arts JHE (2002). Evaluation of the sensory irritation test (Alarie test) for the assessment of respiratory tract irritation. J Occup Environ Med 44(10): 968-976.

Brown V, Crump D, Gardiner D and Gavin M (1994). Assessment of a passive sampler for the determination of aldehydes and ketones in indoor air. Environmental Technology 15: 679-685.

Burt J, Nelson N and Kaufman J (1996). Effects of ventilation flushout on indoor air quality in a newly constructed office building. Appl. Occup. Environ. Hyg. 11(6): 546-552.

CARB (1993). Acetaldehyde as a Toxic Air Contaminant. Part B, Health Assessment. Sacramento CA: California Air Resources Board.

Cassee FR, Arts JH, Groten JP and Feron VJ (1996a). Sensory irritation to mixtures of formaldehyde, acrolein, and acetaldehyde in rats. Arch Toxicol 70(6): 329-337.

Cassee FR, Groten JP and Feron VJ (1996b). Changes in the nasal epithelium of rats exposed by inhalation to mixtures of formaldehyde, acetaldehyde, and acrolein. Fundam Appl Toxicol 29(2): 208-218.

Cicero TJ and Bell RD (1980). Effects of ethanol and acetaldehyde on the biosynthesis of testosterone in the rodent testes. Biochem Biophys Res Commun 94(3): 814-819.

Cicero TJ, Bell RD and Badger TM (1980a). Acetaldehyde directly inhibits the conversion of androstenedione to testosterone in the testes. Adv Exp Med Biol 132: 211-217.

Cicero TJ, Bell RD, Meyer ER and Badger TM (1980b). Ethanol and acetaldehyde directly inhibit testicular steroidogenesis. J Pharmacol Exp Ther 213(2): 228-233.

Cometto-Muniz JE and Cain WS (1995). Relative sensitivity of the ocular trigeminal, nasal trigeminal and olfactory systems to airborne chemicals. Chem Senses 20(2): 191-198.

Cometto-Muniz JE and Cain WS (1998). Trigeminal and olfactory sensitivity: Comparison of modalities and methods of measurement. Int Arch Occup Environ Health 71(2): 105-110.

Cometto-Muniz JE, Cain WS, Abraham MH and Gola JM (1999). Chemosensory detectability of 1-butanol and 2-heptanone singly and in binary mixtures. Physiol Behav 67(2): 269-276.

Cometto-Muniz JE, Cain WS, Abraham MH and Gola JM (2001). Ocular and nasal trigeminal detection of butyl acetate and toluene presented singly and in mixtures. Toxicol Sci 63(2): 233-244.

Cometto-Muniz JE, Cain WS, Abraham MH and Gola JM (2002). Psychometric functions for the olfactory and trigeminal detectability of butyl acetate and toluene. J Appl Toxicol 22(1): 25-30.

Crump KS (1984). A new method for determining allowable daily intakes. Fundam Appl Toxicol 4(5): 854-871.

Crump KS and Howe R. (1983). Probit-A: computer program to extrapolate quantile animal toxicological data to low doses. Crump KS & Company, Inc.

Delfino RJ, Gong H, Jr., Linn WS, Pellizzari ED and Hu Y (2003). Asthma symptoms in hispanic children and daily ambient exposures to toxic and criteria air pollutants. Environ Health Perspect 111(4): 647-656.

Dellarco VL (1988). A mutagenicity assessment of acetaldehyde. Mutat Res 195(1): 1-20.

Donohue Jr. TM, Tuma DJ and Sorrell MF (1983). Acetaldehyde adducts with proteins: Binding of [^{14}C]acetaldehyde to serum albumin. Arch Biochem Biophys 220(1): 239-246.

Doty RL, Cometto-Muniz JE, Jalowayski AA, Dalton P, Kendal-Reed M and Hodgson M (2004). Assessment of upper respiratory tract and ocular irritative effects of volatile chemicals in humans. Crit Rev Toxicol 34(2): 85-142.

Egle JL, Jr. (1970). Retention of inhaled acetaldehyde in man. J Pharmacol Exp Ther 174(1): 14-9.

Egle Jr. JL (1972a). Effects of inhaled acetaldehyde and propionaldehyde on blood pressure and heart rate. Toxicol Appl Pharm 23(1): 131-135.

Egle Jr. JL (1972b). Retention of inhaled acetaldehyde in the dog. Arch Environ Health 24(5): 354-357.

Eriksson CJ (2001). The role of acetaldehyde in the actions of alcohol (update 2000). Alcohol Clin Exp Res 25(5 Suppl ISBRA): 15S-32S.

Eriksson CJ, Sippel HW and Forsander OA (1977). The occurrence of acetaldehyde binding in rat blood but not in human blood. FEBS Lett 75(1): 205-208.

Fenske JD and Paulson SE (1999). Human breath emissions of VOCs. J Air Waste Manag Assoc 49(5): 594-598.

Feron VJ, Arts JH, Kuper CF, Slootweg PJ and Woutersen RA (2001). Health risks associated with inhaled nasal toxicants. Crit Rev Toxicol 31(3): 313-347.

Feron VJ, Kruysse A and Woutersen RA (1982). Respiratory tract tumours in hamsters exposed to acetaldehyde vapour alone or simultaneously to benzo(a)pyrene or diethylnitrosamine. Eur J Cancer Clin Oncol 18(1): 13-31.

Frederick CB, Bush ML, Lomax LG, Black KA, Finch L, Kimbell JS, Morgan KT, Subramaniam RP, Morris JB and Ultman JS (1998). Application of a hybrid computational fluid dynamics and physiologically based inhalation model for interspecies dosimetry extrapolation of acidic vapors in the upper airways. Toxicol Appl Pharm 152(1): 211-231.

Frederick CB, Gentry PR, Bush ML, Lomax LG, Black KA, Finch L, Kimbell JS, Morgan KT, Subramaniam RP, Morris JB and Ultman JS (2001). A hybrid computational fluid dynamics and physiologically based pharmacokinetic model for comparison of predicted tissue concentrations of acrylic acid and other vapors in the rat and human nasal cavities following inhalation exposure. Inhal Toxicol 13(5): 359-376.

Fujimura M, Myou S, Kamio Y, Ishiura Y, Iwasa K, Hashimoto T and Matsuda T (1999). Increased airway responsiveness to acetaldehyde in asthmatic subjects with alcohol-induced bronchoconstriction. Eur Respir J 14(1): 19-22.

Gaines KC, Salhany JM, Tuma DJ and Sorrell MF (1977). Reaction of acetaldehyde with human erythrocyte membrane proteins. FEBS Lett 75(1): 115-119.

Hodgson AT, Beal D and McIlvaine JE (2002). Sources of formaldehyde, other aldehydes and terpenes in a new manufactured house. Indoor Air 12(4): 235-42.

Hoffmann T, Meyer RJ, Sorrell MF and Tuma DJ (1993). Reaction of acetaldehyde with proteins: Formation of stable fluorescent adducts. Alcohol Clin Exp Res 17(1): 69-74.

HSDB. (2004). Hazardous Substances Data Bank. from http://toxnet.nlm.nih.gov.

Isse T, Oyama T, Matsuno K, Ogawa M, Narai-Suzuki R, Yamaguchi T, Murakami T, Kinaga T, Uchiyama I and Kawamoto T (2005). Paired acute inhalation test reveals that acetaldehyde toxicity is higher in aldehyde dehydrogenase 2 knockout mice than in wild-type mice. J Toxicol Sci 30(4): 329-337.

IWMB. (2003). Building Material Emissions Study. from www.ciwmb.ca.gov/Publications.

Jones AW (1995). Measuring and reporting the concentration of acetaldehyde in human breath. Alcohol Alcohol 30(3): 271-285.

Kimbell JS, Subramaniam RP, Gross EA, Schlosser PM and Morgan KT (2001). Dosimetry modeling of inhaled formaldehyde: Comparisons of local flux predictions in the rat, monkey, and human nasal passages. Toxicol Sci 64(1): 100-110.

Kinney PL, Chillrud SN, Ramstrom S, Ross J and Spengler JD (2002). Exposures to multiple air toxics in New York City. Environ Health Perspect 110 Suppl 4: 539-46.

Kruysse A. (1970). Acute inhalation toxicity of acetaldehyde in hamsters. Centraal Instituut Voor Voedingsonderzoek.

Kruysse A, Feron VJ and Til HP (1975). Repeated exposure to acetaldehyde vapor. Studies in Syrian golden hamsters. Arch Environ Health 30(9): 449-452.

Lacroix G, Tissot S, Rogerieux F, Beaulieu R, Cornu L, Gillet C, Robidel F, Lefevre JP and Bois FY (2002). Decrease in ovalbumin-induced pulmonary allergic response by benzaldehyde but not acetaldehyde exposure in a guinea pig model. J Toxicol Env Heal A 65(14): 995-1012.

Lee RD, An SM, Kim SS, Rhee GS, Kwack SJ, Seok JH, Chae SY, Park CH, Choi YW, Kim HS, Cho HY, Lee BM and Park KL (2005). Neurotoxic effects of alcohol and acetaldehyde during embryonic development. J Toxicol Env Heal A 68(23-24): 2147-2162.

Lindstrom A, Proffitt D and Fortune C (1995). Effects of modified residential construction on indoor air quality. Indoor Air 5: 258-269.

Mannino DM, Homa DM, Pertowski CA, Ashizawa A, Nixon LL, Johnson CA, Ball LB, Jack E and Kang DS (1998). Surveillance for asthma--United States, 1960-1995. MMWR CDC Surveill Summ 47(1): 1-27.

Miller SL, Branoff S and Nazaroff WW (1998). Exposure to toxic air contaminants in environmental tobacco smoke: an assessment for California based on personal monitoring data. J Expo Anal Environ Epidemiol 8(3): 287-311.

Mohammad A, Olcott HS and Fraenkel-Conrat H (1949). The reaction of proteins with acetaldehyde. Arch Biochem 24(2): 270-280.

Morris JB (1997a). Dosimetry, toxicity and carcinogenicity of inspired acetaldehyde in the rat. Mutat Res 380(1-2): 113-124.

Morris JB (1997b). Uptake of acetaldehyde vapor and aldehyde dehydrogenase levels in the upper respiratory tracts of the mouse, rat, hamster and guinea pig. Fundam Appl Toxicol 35(1): 91-100.

Morris JB and Blanchard KT (1992). Upper respiratory tract deposition of inspired acetaldehyde. Toxicol Appl Pharm 114(1): 140-146.

Myou S, Fujimura M, Kamio Y, Bando T, Nakatsumi Y and Matsuda T (1995). Repeated inhalation challenge with exogenous and endogenous histamine released by acetaldehyde inhalation in asthmatic patients. Am J Respir Crit Care Med 152(2): 456-460.

Myou S, Fujimura M, Nishi K, Matsuda M, Ohka T and Matsuda T (1994). Potentiating effect of inhaled acetaldehyde on bronchial responsiveness to methacholine in asthmatic subjects. Thorax 49(7): 644-648.

Myou S, Fujimura M, Nishi K, Ohka T and Matsuda T (1993). Aerosolized acetaldehyde induces histamine-mediated bronchoconstriction in asthmatics. Am Rev Respir Dis 148(4 Pt 1): 940-943.

Nazaroff WW (2004). Inhalation of hazardous air pollutants from environmental tobacco smoke in US residences. J Expo Anal Env Epid 14: S71-S77.

OEHHA. (2007). *Safe Drinking Water and Toxic Enforcement Act of 1986. Chemicals Known to the State to Cause Cancer or Reproductive Toxicity. June 1, 2007*. Office of Environmental Health Hazard Assessment, California Environmental Protection Agency. http://www.oehha.ca.gov/prop65/prop65_list/files/060107LST.pdf.

Pratt OE (1980). The fetal alcohol syndrome: Transport of nutrients and transfer of alcohol and acetaldehyde from mother to fetus. Psychopharmacol Alcohol: 229-256.

Prieto L, Sanchez-Toril F, Brotons B, Soriano S, Casan R and Belenguer JL (2000). Airway responsiveness to acetaldehyde in patients with asthma: Relationship to methacholine responsiveness and peak expiratory flow variation. Clin Exp Allergy 30(1): 71-78.

Saito Y, Sasaki F, Tanaka I, Sato M, Okazawa M, Sakakibara H and Suetsugu S (2001). Acute severe alcohol-induced bronchial asthma. Intern Med 40(7): 643-645.

Saldiva PH, do Rio Caldeira MP, Massad E, Calheiros DF, Cardoso LM, Bohm GM and Saldiva CD (1985). Effects of formaldehyde and acetaldehyde inhalation on rat pulmonary mechanics. J Appl Toxicol 5(5): 288-292.

Shaskan EG and Dolinsky ZS (1985). Elevated endogenous breath acetaldehyde levels among abusers of alcohol and cigarettes. Prog Neuro-Psychoph 9(3): 267-272.

Shendell DG, Winer AM, Stock TH, Zhang L, Zhang JJ, Maberti S and Colome SD (2004). Air concentrations of VOCs in portable and traditional classrooms: results of a pilot study in Los Angeles County. J Expo Anal Environ Epidemiol 14(1): 44-59.

Shiohara E, Tsukada M, Chiba S, Yamazaki H, Nishiguchi K, Miyamoto R and Nakanishi S (1984). Subcellular aldehyde dehydrogenase activity and acetaldehyde oxidation by isolated intact mitochondria of rat brain and liver after acetaldehyde treatment. Toxicology 30(1): 25-30.

Silverman L, Schultes HF and First MW (1946). Further studies on sensory response to certain industrial solvent vapors. J Ind Hyg Toxicol 28: 262-266.

Skog E (1950). A toxicological investigation of lower aliphatic aldehydes. I. Toxicity of formaldehyde, acetaldehyde, propionaldehyde, butyraldehyde, as well as acrolein and crotonaldehyde. Acta Pharmacol Toxicol 6: 299-318.

Sreenathan RN, Padmanabhan R and Singh S (1982). Teratogenic effects of acetaldehyde in the rat. Drug Alcohol Depend 9(4): 339-350.

Stanek JJ and Morris JB (1999). The effect of inhibition of aldehyde dehydrogenase on nasal uptake of inspired acetaldehyde. Toxicol Sci 49(2): 225-231.

Steinhagen WH and Barrow CS (1984). Sensory irritation structure-activity study of inhaled aldehydes in B6C3F1 and Swiss-Webster mice. Toxicol Appl Pharm 72(3): 495-503.

Tardif R, Liu L and Raizenne M (2004). Exhaled ethanol and acetaldehyde in human subjects exposed to low levels of ethanol. Inhal Toxicol 16(4): 203-207.

Thompson PA and Folb PI (1982). An in vitro model of alcohol and acetaldehyde teratogenicity. J Appl Toxicol 2(4): 190-195.

Tuma DJ and Sorrell MF (1985). Covalent binding of acetaldehyde to hepatic proteins: Role in alcoholic liver injury. Prog Clin Biol Res 183: 3-17.

Tyulina OV, Prokopieva VD, Boldyrev AA and Johnson P (2006). Erythrocyte and plasma protein modification in alcoholism: A possible role of acetaldehyde. Biochim Biophys Acta-Molecular Basis of Disease 1762(5): 558-563.

Watanabe A, Hobara N and Nagashima H (1986). Blood and liver acetaldehyde concentration in rats following acetaldehyde inhalation and intravenous and intragastric ethanol administration. Bull Environ Contam Toxicol 37(4): 513-6.

West JR (1994). Recent findings on the mechanisms by which alcohol damages the developing nervous system. Alcohol Clin Exp Res 18(2): 9A.

Wickramasinghe SN, Thomas S and Hasan R (1994). Reaction of ^{14}C-acetaldehyde with whole blood in vitro: Further evidence for the formation of unstable complexes with plasma proteins and red cells. Alcohol Alcohol 29(1): 51-57.

Woutersen RA, Appelman LM, Feron VJ and Van der Heijden CA (1984). Inhalation toxicity of acetaldehyde in rats. II. Carcinogenicity study: Interim results after 15 months. Toxicology 31(2): 123-133.

Woutersen RA, Appelman LM, Van Garderen-Hoetmer A and Feron VJ (1986). Inhalation toxicity of acetaldehyde in rats. III. Carcinogenicity study. Toxicology 41(2): 213-231.

Woutersen RA and Feron VJ (1987). Inhalation toxicity of acetaldehyde in rats. IV. Progression and regression of nasal lesions after discontinuation of exposure. Toxicology 47(3): 295-305.

Yang X, Zhang YP, Chen D, Chen WG and Wang R (2001). Eye irritation caused by formaldehyde as an indoor air pollution--a controlled human exposure experiment. Biomed Environ Sci 14(3): 229-36.

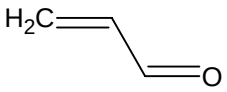
Zorzano A and Herrera E (1989). Disposition of ethanol and acetaldehyde in late pregnant rats and their fetuses. Pediatr Res 25(1): 102-106.

Zweidinger R, Hoffman A and Gage L (1990). Comparison of Indoor and Outdoor Aldehyde Concentrations During the IACP Roanoke Virginia USA Residential Study. Measurement of Toxic and Related Air Pollutants International Symposium.U.S. Environmental Protection Agency. 994-999. Raleigh, NC, May 1-4, 1990

Acrolein Reference Exposure Levels

(2-propenal, acrylic aldehyde, acryladehyde, acraldehyde)

CAS 107-02-8



1. Summary

Acrolein is a powerful irritant. Due to its highly reactive nature, the effects of acrolein are generally limited to the site of contact; skin, eyes and mucous membranes. Inhalation exposure to low levels (≤ 1 ppm) causes irritation of the eyes, nose and throat. Moderately higher exposures may result in severe lacrimation, and irritation of the mucous membranes of the respiratory tract. Death due to respiratory failure has been associated with high level exposures.

1.1 Acrolein Acute REL

Reference Exposure Level	**2.3 μg/m³ (1.0 ppb)**
Critical effect(s)	Subjective ocular irritation
Hazard Index target(s)	Eyes

1.2 Acrolein 8-Hour REL

Reference Exposure Level	**1.6 μg/m³ (0.68 ppb)**
Critical effect(s)	Lesions in respiratory epithelium
Hazard Index target(s)	Respiratory

1.3 Acrolein Chronic REL

Reference Exposure Level	**0.1 μg/m³ (0.05 ppb)**
Critical effect(s)	Lesions in respiratory epithelium
Hazard Index target(s)	Respiratory

2. Physical & Chemical Properties

Description	Colorless or yellow liquid with piercing disagreeable odor
Molecular formula	C_3H_4O
Molecular weight	56.1 g/mol
Density	0.843 g/cm^3 @ 20° C
Boiling point	53° C
Melting point	-87° C
Vapor pressure	220 mm Hg @ 20° C
Flashpoint	-26° C
Explosive limits	2.8% - 31% by volume
Solubility	soluble in ethanol, diethyl ether, and up to 20% w/v in water
Odor threshold	0.5 ppm
Metabolites	glycidaldehyde, acrylic acid
Conversion factor	1 ppm in air = 2.3 mg/m^3 @ 25° C

3. Occurrence and Major Uses

Acrolein is principally used as a chemical intermediate in the production of acrylic acid and its esters. Acrolein is used directly as an aquatic herbicide and algicide in irrigation canals, as a microbiocide in oil wells, liquid hydrocarbon fuels, cooling-water towers and water-treatment ponds, and as a slimicide in the manufacture of paper (IARC, 1995). Combustion of fossil fuels, tobacco smoke, and pyrolyzed animal and vegetable fats contribute to the environmental prevalence of acrolein. Acrolein is a byproduct of fires and is one of several acute toxicants to which firefighters are exposed. It is also formed from atmospheric reactions of 1,3-butadiene. The annual statewide emissions of acrolein from mobile, stationary and natural sources (not including atmospheric transformation) reported in the California Toxics Inventory for 2004 were preliminarily estimated to be 2,242 tons contributing to a statewide ambient level of 0.53 ppb (CARB, 2005b).

4. Metabolism

The metabolism of acrolein comprises several pathways. It rapidly reacts with sulfhydryl groups, especially protein cysteine residues and glutathione. The glutathione conjugate may be oxidized or reduced to mercapturic acids (N-acetyl –S-2-carboxyethylcysteine and N-acetyl-S-3-hydroxypropylcysteine, respectively), with the reduction pathway predominating, followed by urinary elimination. Alternatively, acrolein may be epoxidized to glycidaldehyde, which is in turn attacked by glutathione and oxidatively processed to the mercapturic acid, N-acetyl-S-2-carboxy-2-hydroxyethylcysteine. In a third pathway, the Michael addition of water to acrolein is followed by oxidation to malonic and finally oxalic acids (Parent et al., 1998). The formation of homopolymers of acrolein is thought to occur but appears to be limited to the gut. Acrolein may also be oxidized to acrylic acid, mainly in the liver. Following inhalation exposure, the predominant metabolites are the 3-hydroxypropyl and 2-carboxyethyl mercapturic acids mentioned above (Linhart et al., 1996).

5. Acute Toxicity of Acrolein

5.1 Acute Toxicity to Adult Humans

Sensory irritation is the primary adverse effect associated with acute, low level exposures to acrolein. The irritative effects of acrolein are noticeable at low levels of exposure (≤0.25 ppm) and rapidly become more pronounced with increasing concentration, while brief exposure (1.5 min) to 0.3 ppm (0.7 mg/m³) causes irritation of the eyes and nose (Weber-Tschopp et al., 1977). The powerful irritant and lacrimator properties of acrolein led to its use in gas grenades and artillery shells by the French in 1916. At a concentration of 7 mg/m³, acrolein caused severe lacrimation and irritation of the mucous membranes of the respiratory tract (Prentiss, 1937). A case report of respiratory failure and death in individuals exposed to vapors from overheated frying pans containing fat and food items implicated acrolein as the principal toxicant (Gosselin et al., 1979).

Ocular irritation is one of the most sensitive responses to acrolein. In a study by Darley et al. (1960), 36 human volunteers were exposed to 0.06, 1.3-1.6, and 2.0-2.3 ppm for 5 minutes. Acrolein was dissolved in water and delivered to the eyes in a stream of oxygen through face masks. Carbon-filter respirators were worn during exposure so that only the eyes were exposed to the test material. The subjects, who were without a history of chronic upper respiratory or eye problems, rated the degree of eye irritation every 30 seconds during exposure as none (0), medium (1), or severe (2). The individuals' maximum values were used in the analysis that revealed a concentration-dependent incidence of eye irritation (Table 5.1.1). The lowest observed adverse effect level (LOAEL) for eye irritation in human volunteers was estimated by an unspecified method to be 0.06 ppm (0.14 mg/m³) acrolein during the five minute exposures. A NOAEL was not observed in this study.

Table 5.1.1 Ocular Irritation with Acrolein (from Darley et al., 1960)

Acrolein concentration	Irritation score
Filtered air	0.283
0.06 ppm	0.471
1.3-1.6 ppm	1.182
2.0-2.3 ppm	1.476

Ocular and upper respiratory tract irritation were also examined in a chamber study by Weber-Tschopp et al. (1977) involving healthy volunteers. Thirty one men and 22 women were exposed to increasing acrolein levels (0-0.60 ppm) for 40 min, while 21 men and 25 women were exposed to a constant 0.3 ppm for 60 min. Subjective reports of irritation and annoyance, and objective measures of eye-blink and respiratory rates were taken during the exposure periods. During exposure to increasing levels of acrolein, eye irritation, as measured by subjective report and blink frequency, was a more sensitive measure of irritation than nasal irritation. By comparison, for less reactive volatile compounds in studies surveyed by Doty et al. (2004), the thresholds for ocular and

intranasal irritation were of the same magnitude. In the Weber-Tschopp study of acrolein, significantly ($p<0.01$) higher eye irritation was first observed at 0.07 ppm, and nasal irritation at 0.26 ppm compared to controls. Significant depression of respiratory rates was observed at 0.60 ppm ($p<0.05$). With continuous exposure to 0.3 ppm acrolein, subjective eye and nasal irritation increased rapidly during the first 20 minutes and tended to plateau by 40 min. After 10 min of continuous exposure, a decrease in respiratory rate of 10% was evident in 47% of the subjects, while eye blink rate doubled in 66%. The authors suggest a threshold for adverse effects in the range of 0.09-0.30 ppm.

The effects of irritants such as acrolein may be accentuated in individuals with prior sensitization. Roux et al. (1999) investigated the interaction between passive sensitization of human isolated airways and exposure to pollutants (specifically, ozone and acrolein). Lung tissue from nonatopic, nonasthmatic patients was immunologically sensitized by incubation in sera from atopic asthmatic patients. Roux et al. reported that *in vitro* passive sensitization of the isolated tissues and exposure to acrolein act in a synergistic manner on human bronchial smooth muscle reactivity in response to both specific and nonspecific agonists. In tissues sensitized by incubation in sera from asthmatic patients, preexposure to 0.3 μM acrolein for 10 or 20 minutes significantly increased the maximal contractile response to a specific antigen (*Dermatophagoides pteronyssinus*) by 20.5 ± 6.5 % and 34.9 ± 7.4%, respectively. In addition, in sensitized tissue pre-exposed to 0.3 μM acrolein for 10 minutes, contractile response was increased by 33.5 ± 6.2% and 32.5 ± 5.1% for carbachol and histamine, respectively. Thus acrolein exposure appears to exacerbate asthma.

Mucus hypersecretion is one of the hallmarks of inflammatory airway disorders, including asthma. Borchers et al. (1999b) examined the effect of 0.01-100 nM acrolein on mucus glycoprotein (mucin) gene expression in cultured human airway epithelial cells. After a 4 hour exposure to acrolein in vitro, epithelial cells were found to have elevated mucin mRNA levels. It is not clear whether acrolein acts directly on epithelial cells or indirectly through inflammatory mediators released after acrolein exposure, however, asthma exacerbation is a likely result of acrolein exposure in susceptible individuals.

Predisposing Conditions for Acrolein Toxicity

Medical: Persons with pre-existing eye, skin, respiratory, allergic, asthmatic or heart conditions might be at increased risk due to acrolein exposure. As a respiratory irritant, there is evidence that acrolein exacerbates asthma via the induction of bronchial hyper-responsiveness (Leikauf et al., 1989a; Leikauf et al., 1989b), and mucus hyper-secretion (Borchers et al., 1998; Borchers et al., 1999a; Borchers et al., 1999b). Acrolein has been listed as a TAC that may disproportionately impact children due to concerns related to asthma exacerbation.

Chemical: Cancer patients treated with cyclophosphamide could be at increased risk because acrolein is a metabolite of cyclophosphamide (NTIS, 1981).

5.2 Acute Toxicity to Infants and Children

The literature specifically examining the effects of acrolein inhalation in infants and children is limited and comprises case studies of accidental exposure, and exposures to multiple substances. The most frequent souces of acrolein in childhood exposures are environmental tobacco smoke (ETS) and acrolein formed from overheated cooking oils. Mahut et al. (1993) describe the case of a 27 month-old boy hospitalized for acute respiratory failure following exposure for about an hour to acrid smoke from vegetable oil burning on an electric hot plate. The child was reportedly cyanotic with labored, crackling breathing, and was experiencing severe respiratory acidosis. Eighteen months following exposure, X-ray and CT scans showed bronchial thickening, massive over-inflation, patchy emphysema and diffuse bronchiectasis. In this case, and in cases of exposure to ETS, infants may be more susceptible to the adverse effects of acrolein in part due to an inability to escape exposure. Children also may be more susceptible to the effects of respiratory irritants due to the immature state of their airways.

As noted in OEHHA (2001): "*OEHHA considers asthma to impact children more than adults. Children have higher prevalence rates of asthma than do adults (Mannino et al., 1998). In addition, asthma episodes can be more severe due to the smaller airways of children, and result in more hospitalizations in children, particularly from the ages of 0 to 4 years, than in adults (Mannino et al., 1998; CDHS, 2000)." "Thus, on a population-wide basis, children are more impacted by asthma than adults, and since acrolein exacerbates asthma, children may be more impacted by acrolein toxicity than adults."* Data strongly suggesting that acrolein exacerbates asthma derive from studies using human tissue *in vitro* (Roux et al., 1999; Borchers et al., 1999a) and in animals *in vivo* (Leikauf et al., 1989a; 1989b; Borchers et al., 1998; Borchers et al., 1999b).

5.3 Acute Toxicity to Experimental Animals

Experimental exposures of rodents to acrolein at and above levels that are irritating to the eyes and respiratory tract in humans provide evidence for several adverse effects and their possible mechanisms. Acrolein prompts a proliferative response in nasal epithelium as shown by increased DNA synthesis (Roemer et al., 1993) and expression of mucin genes (Borchers et al., 1998). The latter effect in turn is associated with the hyper-secretion of mucus that may contribute to chronic obstructive pulmonary disease and asthma (Borchers et al., 1998). Bronchial hyper-responsiveness, a hallmark of asthma, increases with acrolein exposure (Leikauf et al., 1989a) supporting a connection between acrolein exposure and exacerbation of asthma in humans. The dose-dependent decreases in protective epithelial enzyme activities (Cassee et al., 1996b) and levels of sulfhydryls (Lam et al., 1985; McNulty et al., 1984) are likely to be involved in the observed formation of lesions in the nasal epithelium (Cassee et al., 1996b).

Table 5.3.1 Acrolein Effects in Experimental Animals

Study	Model	Exposure	Outcome
Roemer et al. 1993	Proliferation of rat nasal and tracheal epithelium	0, 0.2, 0.6 ppm 6 h/d, 1 or 3 d	Increased DNA synthesis at 0.2 ppm (LOAEL)
Borchers et al. 1998	Mucus hyper-secretion, mucin gene expression in rat trachea and lungs	0.3, 0.75, 1.5, 3.0 ppm 6 h/d, 5 d/w	Hyper-secretion and gene expression at 0.75 ppm. (NOAEL = 0.3 ppm)
Leikauf et al. 1989a	Bronchial hyper-responsiveness and airway resistance in guinea pigs	1.3 ppm, 2 h	Resistance increased from 0.86 to 1.29 ml·cm H_2O/ml. Acetylcholine to double airway resistance dropped from 114 to 44.7 µg/kg/min
Buckley et al. 1984	Nasal histopathology at (RD_{50}) in mice;	1.7 ppm, 6 h/d, 5d	Exfoliation and squamous metaplasia of epithelium
Morris et al. 2003	Decrease in respiratory rate (RD_{50}) in mice	0.3, 1.6, 3.9 ppm, 10 min	Control RD_{50} at 1.50 ppm vs 0.82 ppm in allergic mice
Kane et al. 1979	Decrease in respiratory rate (RD_{50}) in mice	15 min	RD_{50} 1.7 ppm
Cassee et al. 1996b	Histopathology of rat nasal epithelium	0, 0.25, 0.67, 1.4 ppm, 6 h/d, 1-3 d	Dose-dependent lesions and decreased enzyme activities in nasal epithelium
Lam et al. 1985	Sulfhydryl depletion in rat respiratory mucosa	0, 0.1, 0.5, 1.0, 2.5 ppm 3 h	Dose-dependent depletion of non-protein sulfhydryls
McNulty et al. 1984	Sulfhydryl depletion in rat respiratory mucosa and liver	0.1, 0.3, 1, 2.5, 5 ppm 3 h	Dose-dependent depletion of non-protein sulfhydryls in nasal mucosa but not liver

Roemer et al. (1993) exposed Male Sprague Dawley rats by inhalation to 0, 0.2 or 0.6 ppm acrolein for 6 h per day on one or three successive days. Nasal and tracheal epithelial and free lung cells were analyzed for proliferative responses using 5-bromodeoxyuridine (BrdU) labeling to identify DNA synthesizing cells. A single exposure to acrolein increased the DNA synthesizing cells 3-fold. After three exposures the increase was distinctly lower. All sites analyzed showed approximately the same concentration/response pattern. Since significant changes in cell proliferation were detected at 0.2 ppm (0.46 mg/m^3) acrolein, it is a LOAEL for this experiment.

Enhanced mucus secretion is a normal airway response to inhaled irritants. However, mucus hypersecretion is involved in the development of chronic obstructive pulmonary diseases; as such, it is considered an adverse effect. Borchers et al. (1998) exposed male rats to 3.0 ppm acrolein for 6 h/d, 5 d/wk for up to 12 days and examined the lungs and trachea for mucin cell metaplasia and expression of the mucin genes MUC2 and MUC5ac. The effects of acrolein concentration on mucin mRNA levels were further examined in rats exposed daily to 0.3, 0.75, 1.5, 3.0 ppm. Acrolein exposure resulted in a time-dependent increase in mucous cell differentiation and mucus hypersecretion in rat lungs. These changes were accompanied by increases in lung MUC5ac mRNA to levels

3-fold higher than in controls, and readily immunohistochemically detectable levels of MUC5ac. MUC5ac mRNA was elevated by concentrations as low as 0.75 ppm while MUC2 mRNA was not affected by any of the levels tested. Thus 0.3 ppm (0.69 mg/m^3) is a NOEL for this effect. The trachea of treated animals showed sloughing of the epithelium accompanied by excessive mucus and inflammatory cells in the lumen.

Bronchial hyper-responsiveness is a hallmark of reactive airway diseases such as asthma, and may be induced by inhaled irritants. Leikauf et al. (1989a) exposed guinea pigs to 1.3 ppm acrolein for 2 h and measured the induction of bronchial hyperresponsiveness by the amount of infused acetylcholine necessary to double specific airway resistance 1, 2, 6, and 24 h after exposure compared to baseline. The dose of acetylcholine required to double airway resistance decreased from 114.0 ± 6.6 to 44.7 ± 4.2 µg/kg/min ($p < 0.001$) at 2 h following acrolein exposure and remained low for at least 24 h. Acrolein exposure was found to increase levels of the bronchoconstrictor leukotriene C_4 (LTC_4) in bronchoaveolar lavage fluids prior to the observation of bronchial hyperresponsiveness. This hyperresponsiveness was prevented by treatment with an inhibitor of LTC_4 synthesis or an LTC_4 receptor antagonist. Acrolein was thus shown to induce bronchial hyperresponsiveness, an effect apparently mediated by LTC_4.

Buckley et al. (1984) investigated whether lesions occur in the respiratory tract of Swiss-Webster mice after exposure to the RD_{50} concentrations of ten sensory irritants including acrolein. After exposure of mice for 6 hr/day for 5 days to 1.7 ppm acrolein, the respiratory tract was examined for histopathologic changes. Acrolein (and all other irritants) produced lesions in the nasal cavity with a distinct anterior-posterior severity gradient. Acrolein specifically caused severe exfoliation and squamous metaplasia of the respiratory epithelium and moderate ulceration of the olfactory epithelium. Acrolein did not induce lesions in the lower respiratory tract.

Morris et al. (2003) compared the respiratory responses to acrolein in healthy mice with those in mice previously sensitized to ovalbumin. Inhalation exposure to ovalbumin prior to acrolein exposure elicited an allergic response in the sensitized mice that was characteristic of allergic airway disease. Upon subsequent acrolein exposure, the RD_{50}, a measure of the dose required to reduce the respiratory rate by 50%, was 1.50 ppm in naïve mice and 0.82 ppm in the mouse model of allergic airway disease. Thus in sensitized animals, a lower concentration of acrolein is required to elicit the same changes in breathing rate observed in non-allergic animals. In both intact mice and in isolated mouse upper respiratory tracts, acrolein exposure caused a significant ($P < 0.05$) increase in flow resistance, an effect that was immediate and not exposure time dependent. Pretreatment with capsaicin to defunctionalize sensory neurons significantly attenuated the breathing rate and obstructive responses supporting the role of sensory neuron stimulation in the response to acrolein. For comparison, Kane et al. (1979) also used the RD_{50} as a measure of sensory irritation and estimated an RD_{50} of 1.7 ppm in mice during 15 minutes of acrolein exposure.

Cassee et al. (1996b) exposed male Wistar rats to 0, 0.25, 0.67, or 1.4 ppm acrolein for 6 h per day on one or three successive days. Immediately following the last exposure, the

rats were killed. Mucosa from the respiratory or olfactory parts of the nose were collected from 3 rats per group for biochemical analyses. The skulls of the other rats in each group were prepared for histopathology and cell proliferation measurements. Nasal epithelium, examined microscopically, showed dose-dependent evidence of disarrangement, necrosis, thickening, and desquamation of the respiratory/transitional epithelium (Table 5.3.2). Significant basal cell hyperplasia, observed at the lowest dose (0.25 ppm), increased with exposure. The activity of glutathione reductase (GR) was reduced after one-day exposure to acrolein, while the activities of GR, glutathione-S-transferase and aldehyde dehydrogenase were reduced following the three-day exposures. These results and those mentioned below suggest that acrolein interferes with enzyme systems involved in its detoxification.

Table 5.3.2 Nasal Lesions in Rats with Acrolein Exposure
(from Cassee et al., 1996b)

Site and type of lesion	Extent	Incidence	
		Low	Medium
Noses examined		5	6
Disarrangement, necrosis, desquamation of respiratory, transitional epithelium	Slight (mainly disarrangement)	4	3
	Moderate	1	3
	Severe and extensive	0	0
Basel cell hyperplasia and/or increased mitotic figures	Slight (focal)	3	2
	Moderate	0	4
	Severe (extensive)	0	0

Pronounced and possibly irreversible biochemical changes occur with acrolein levels that are extremely irritating. Acrolein depletes glutathione (GSH) and other free thiol groups both in vitro and in vivo (McNulty et al., 1984; Lam et al., 1985; Grafstrom et al., 1987; U.S.EPA, 2003; Yang et al., 2004). Inhalation exposure of rats to a concentration of 5 ppm (11.4 mg/m^3) for 3 hours caused irreversible depletion of non-protein sulfhydryls in the nasal mucosa (Lam et al., 1985). Under similar exposure conditions, 5 ppm (11.5 mg/m^3) for 3 hours, McNulty et al. (1984) reported a 63% decrease in glutathione in nasal mucosal but not in liver. In addition, ^{14}C-labeled acrolein has been shown to bind irreversibly to sulfhydryl groups on cytochrome P450 in rats (Gurtoo et al., 1981). The binding of acrolein to sulfhydryl groups is localized to the area of contact (e.g., nasal membranes or lung epithelium), and is not a systemic effect (Lam et al., 1985).

The pulmonary immunological defense against a bacterial challenge using *Staphylococcus aureus* in mice was impaired in a dose-dependent manner following a single exposure to acrolein at concentrations of 3 and 6 ppm (6.9 and 13.8 mg/m^3) for 8 hours (Astry and Jakab, 1983). In this study, the control exposure was not described.

6. Chronic Toxicity of Acrolein

6.1 Chronic Toxicity to Adult Humans

Information regarding the chronic toxicity of acrolein in humans is limited. There is inadequate direct evidence for carcinogenicity of acrolein in humans or experimental animals (IARC, 1985). However, a metabolite of acrolein, the reactive epoxide glycidaldehyde, has been shown to be mutagenic and carcinogenic in mice and rats. Therefore, acrolein has been designated a Group C substance, with possible human carcinogenic potential (U.S.EPA, 1987). In addition, acrolein-DNA adducts have been found in aortic tissue following 6 hr inhalation exposure to 1 and 10 ppm acrolein (Penn et al., 2001).

A source of chronic acrolein exposure for some individuals is tobacco smoking. Much of the pulmonary irritancy associated with tobacco smoke has been attributed to acrolein and research in this area suggests mechanisms for some of acrolein's pulmonary effects. As part of a defense response, pulmonary neutrophils release oxidants, proteases and cytokines such as IL-8, all of which may promote inflammation and potentiate tissue damage. To limit tissue damage and resolve the inflammation, neutrophils normally undergo consitutive apoptosis. Experiments with isolated human neutrophils exposed to acrolein at levels achievable during active smoking (1-50 μM) found that acrolein inhibited neutrophil apoptosis, increased IL-8 production, and activated mitogen-activated protein kinases (MAPK) (Finkelstein et al., 2001). At acrolein concentrations up to 10 μM, inhibition of apoptosis was accompanied by increased cell viability. At higher acrolein levels, cell viability decreased as necrotic cell death increased. While the mechanisms behind acrolein's concentration-dependent effects on neutrophils are not clear, the effects observed at the lower exposure levels suggest that acrolein may contribute to pulmonary inflammation and exacerbate allergic responses by prolonging the survival of neutrophils, and stimulating the production of inflammation-related cytokines and enzymes. At higher levels, frank cellular toxicity becomes more prominent.

6.2 Chronic Toxicity to Infants and Children

No data addressing the effects of chronic acrolein exposure among infants and children were located. Inasmuch as acrolein is one of the major irritants in environmental tobacco smoke (Takabe et al.) at relatively high concentrations in smokers' homes (1.6-3.6 $\mu g/m^3$; 0.70-1.57 ppm (Nazaroff and Singer, 2004)), children living with smokers may be disproportionately exposed to acrolein as they are less able to avoid exposure than are adult nonsmokers. To the extent that respiratory irritants such as acrolein elicit bronchoconstriction and excessive mucus secretion characteristic of asthma, children, with their smaller airways and greater prevalence of asthma, may experience more diminution of pulmonary function and more episodes of asthma with chronic exposure.

6.3 Chronic Toxicity to Experimental Animals

Long-term exposure to acrolein causes structural and functional changes in the respiratory tract. These effects were examined in male Fischer-344 rats exposed for 6 hours/day, 5 days/week for 62 days to acrolein at concentrations of 0, 0.4, 1.4, and 4.0 ppm (0, 0.92, 3.2, and 9.2 mg/m^3)(Kutzman, 1981; Kutzman et al., 1985). Each group of 24 animals was assessed for pulmonary function immediately prior to the end of the experiment. Pulmonary function tests included lung volumes, forced respiratory capacity, pulmonary resistance, dynamic compliance, diffusing capacity of carbon monoxide, and multi-breath nitrogen washout. At the end of the experiment, animals were killed and histopathological changes in the lungs were recorded. Eight additional rats were designated for histopathology and 8 rats were used for reproductive testing only. All analyses were performed at 6 days post-exposure to minimize the acute effects of acrolein. Mortality was high (56%) in rats exposed to 4.0 ppm (9.2 mg/m^3). The observed mortality was due to acute bronchopneumonia in these cases. The animals from this group that survived had reduced body weight. No histological changes were observed in extra-respiratory tissues in any group. There was a concentration-dependent increase in histological changes to the nasal turbinates (increased submucosal lymphoid aggregates), beginning at 0.4 ppm. Concentration-dependent damage to the peribronchiolar and bronchiolar regions included epithelial necrosis and sloughed cells lying free in the lumen. No lung lesions were observed in the 0.4 ppm group. The LOAEL for nasal lesions (squamous epithelial metaplasia and neutrophil infiltration) in this study was 0.4 ppm.

Feron et al. (1978) exposed groups of 20 Syrian golden hamsters, 12 SPF Wistar rats and 4 Dutch rabbits (of both sexes) to acrolein vapor at 0, 0.4, 1.4 and 4.9 ppm (0, 0.92, 3.2, and 11.3 mg/m^3) 6 h/d, 5 d/wk for 13 weeks. The most prominent effects at the highest level included mortality in rats (3 of each sex), and ocular and nasal irritation, growth depression, and histopathological changes of the respiratory tract in each species. The changes in the airways induced by acrolein consisted of destruction, and hyperplasia and metaplasia of the lining epithelium accompanied by inflammatory alterations. Rats were the most susceptible species examined and showed treatment-related histopathological abnormalities in the nasal cavity down to 0.4 ppm (LOAEL), whereas this level was a NOAEL in hamsters and rabbits. The results for individual rats at 0.4 ppm were not given.

Bouley et al. (1975; 1976) exposed male SPF OFA rats continuously to 0.55 ppm (1.3 mg/m^3) of acrolein for up to 63 days. This level of acrolein led to a greater susceptibility to airborne *Salmonella enteritidis* infection during the first three weeks compared to control rats but it disappeared spontaneously when exposure was continued beyond three weeks. The general toxic effect of diminished weight gain (due to reduced feeding) compared to the control group lasted as long as exposure and disappeared only after acrolein was discontinued. Sneezing, a sign of nasal irritation, was consistently observed in the exposed animals on days 7 through 21 but ceased thereafter. No histopathology of the nasal cavity of or any other tissue was reported.

In one of the few chronic studies reported, Feron and Kruysse (1977) exposed hamsters (18/gender) to 4 ppm (9.2 mg/m^3) acrolein for 7 hours/day, 5 days/week, for 52 weeks. Mild to moderate histological changes were observed in the upper and lower respiratory tract. No evidence of toxicity to other organs was apparent at necropsy, although body weight was decreased. Hematology, urinalysis, and serum enzymes were not affected by exposure. Thus 4 ppm is a chronic LOAEL for hamsters. As noted above, hamsters appear to be a less sensitive species than rats (Feron et al., 1978).

Exposures of rodents have generally formed the basis for the determination of acrolein's chronic effects. However, an interspecies comparison was conducted by Lyon and associates (Lyon et al., 1970) who investigated the effects of repeated or continuous exposures of acrolein on Sprague-Dawley rats (n = 15/exposure group), guinea pigs (n = 15), beagle dogs (n = 2), and male squirrel monkeys (n = 9). Animals were exposed to 0.7 or 3.7 ppm (1.6 or 8.5 mg/m^3) acrolein for 8 hours/day, 5 days/week, for 6 weeks, or continuously to 0.22, 1.0, or 1.8 ppm (0.5, 2.3, or 4.1 mg/m^3) for 90 days. The results below suggest that dogs and monkeys were more susceptible to acrolein's effects than were the rodents.

Two monkeys in the 3.7 ppm intermittent exposure group died within 9 days. Monkeys and dogs salivated excessively during the first week. Squamous metaplasia and basal cell hyperplasia of the trachea were observed in monkeys and dogs; 7 of the 9 monkeys repeatedly exposed to 3.7 ppm also exhibited bronchiolitis obliterans with squamous metaplasia in the lungs. Bronchopneumonia was noted in the dogs. Inflammation in the lung interstitiae was more prominent in the dogs than in the monkeys. Rats and guinea pigs did not exhibit signs of toxicity when exposed intermittently to 3.7 ppm. Continuous exposure to 1.0 and 1.8 ppm, but not 0.22 ppm acrolein, resulted in salivation and ocular discharge in the monkeys and dogs. Rats and guinea pigs appeared normal at all concentrations. Rats exhibited significant weight loss in the 1.0 and 1.8 ppm continuous exposure groups. Nonspecific inflammatory changes were observed in sections of brain, heart, lung, liver and kidney from all species exposed to 1.8 ppm. The lungs from the dogs showed confluent bronchiopneumonia. Focal histological changes in the bronchiolar region and the spleen were detected at 0.22 ppm in dogs. Nonspecific inflammatory changes at the 0.22 ppm level were apparent in liver, lung, kidney and heart from monkeys, guinea pigs and dogs. Unfortunately the nasal cavity was not examined in this study. While there were no unexposed control animals for any species, the cross-species comparison shows substantial interspecies variability in susceptibility.

7. Developmental and Reproductive Toxicity

There are no reports of reproductive or developmental toxicity following inhalation exposure to acrolein. Kutzman (1981) studied reproductive fitness in male and female rats following acrolen inhalation for 6 h/d, 5 d/wk for 62 days. Treated males were mated with untreated females, and treated females with untreated males. No treatment-related differences were found in the parameters assessed including pregnancy rate, number of corpora lutea, embryo viability, early and late deaths, and preimplantation losses.

Similarly, the morphology of sperm collected from the epididymides of treated males was examined and reportedly not affected. Bouley et al. (1975; 1976) exposed three male and 21 female SPF-OFA rats continuously to 0.55 ppm (1.26 mg/m^3) acrolein vapor for 25 days. The rats were allowed to mate on day 4 of the exposure. The number of acrolein-exposed pregnant rats and the number and mean body weight of their fetuses were similar to controls.

In rats, acrolein can induce teratogenic and embryotoxic effects when administered directly into the amniotic fluid, or when added to cultured rat embryos (Slott and Hales, 1986). Additionally, acrolein injected into chicken embryos resulted in embryotoxicity and some teratogenic effects at moderate to high doses (0.001-0.1 mg/egg) (Chhibber and Gilani, 1986). However, intravenous injection of acrolein in pregnant rabbits showed no developmental effects in the offspring (Claussen et al., 1980). Based on this latter study, the World Health Organization (1992) concluded that human exposure to acrolein was unlikely to affect the developing embryo.

8. Derivation of Reference Exposure Levels

8.1 Acrolein Acute Reference Exposure Level

Study	Darley et al., 1960
Study population	36 healthy human volunteers
Exposure method	5 min exposure: carbon-filter respirators worn
Exposure continuity	
Exposure duration	5 min
Critical effects	subjective ocular irritation
LOAEL	0.06 ppm
NOAEL	not observed
Benchmark concentration	not derived
Time-adjusted exposure	not applied
Human Equivalent Concentration	n/a
LOAEL uncertainty factor (UF_L)	6 (default: mild effect, no NOAEL)
Subchronic uncertainty factor (UFs)	not applied
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	1 (default: human study)
Toxicodynamic (UF_{A-d})	1 (default: human study)
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	1 (site of contact; no systemic effects)
Toxicodynamic (UF_{H-d})	10 (greater susceptibility of children to asthma exacerbation)
Cumulative uncertainty factor	60
Reference Exposure Level	**2.3 μg/m^3 (1.0 ppb)**

Acute Reference Exposure Levels are levels at which intermittent one-hour exposures are not expected to result in adverse health effects (see Section 5 of the Technical Support Document).

The study by Darley et al. (1960) was selected as the best available acute exposure study employing human subjects. In addition, the ocular mucosa and the nasal mucosa are both innervated by cranial nerve V (trigeminal nerve). As noted by Doty et al. (2004), numerous studies employing n-alcohols, ketones, alkylbenzenes, terpenes, butyl acetate and toluene, report thresholds for ocular and intranasal irritation to be of the same magnitude suggesting that for most volatiles, tests of ocular and nasal irritancy are of equivalent sensitivity. Thus the endpoint of ocular irritancy used in this study is expected to also reflect irritancy of the upper respiratory tract. Confidence in this REL calculation is moderate as the LOAEL used is based on an estimated LOAEL of 0.06 ppm rather than a measured level. A default uncertainty factor of 6 is associated with the use of a LOAEL for mild effects in the absence of a NOAEL (see Section 4.4.5 of the TSD). Due to its high reactivity, the effects of exposure to acrolein in the air are largely confined to the site of contact, in this case the eyes, with negligible or no systemic effects. This localization of effects to the site of contact is supported by the confinement of acrolein's effects to the upper respiratory tract in the animal studies of acute inhalation exposure. Based on modeling of adults and 3-month old children that takes into account age-related ventilation rates and respiratory tract surface area, the deposition kinetics of reactive gases are generally thought not to be greatly different between adults and children (Ginsberg et al., 2005). Because of this, a value of 1 is used for the kinetic component of the intraspecies uncertainty factor (UF_{H-k}), rather than a more extended values of $\sqrt{10}$ or 10 which are used where metabolic processes also contribute to inter-individual variability. While ocular irritation is not expected to be substantially different between children and adults, the respiratory irritant effect, with documented potential to exacerbate asthma, is clearly an effect with the potential to differentially impact infants and children. The toxicodynamic component of the intraspecies uncertainty factor UF_{H-d} is therefore assigned an increased value of 10 to account for potential asthma exacerbation. These considerations are applied equally to the acute, 8-hour and chronic REL. The acute REL for acrolein exposure is calculated to be 2.3 $\mu g/m^3$ (1.0 ppb).

The acute REL above is supported by a study in humans by Weber-Tschopp et al. (1977). During a 40 min exposure to increasing concentrations of acrolein, significant ocular irritation was first reported at 0.07 ppm. This represents the LOAEL for this effect and is similar to the LOAEL of 0.06 ppm in Darley et al. (1960). The same uncertainty and adjustment factors, and rationale apply as in Darley, giving an acute REL of 2.7 $\mu g/m^3$ (1.2 ppb).

Study	Weber-Tschopp et al. (1977)
Study population	54 healthy human volunteers
Exposure method	Exposure chamber
Exposure continuity	Increasing concentration (0-0.6 ppm)
Exposure duration	40 min
Critical effects	subjective ocular irritation
LOAEL	0.07 ppm
NOAEL	not observed
Benchmark concentration	not derived
Time-adjusted exposure	not applied
Human Equivalent Concentration	n/a
LOAEL uncertainty factor (UF_L)	6 (default: mild effect, no NOAEL)
Subchronic uncertainty factor (UFs)	not applied
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	1 (default: human study)
Toxicodynamic (UF_{A-d})	1 (default: human study)
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	1 (site of contact; no systemic effects)
Toxicodynamic (UF_{H-d})	10 (asthma exacerbation in children)
Cumulative uncertainty factor	60
Reference Exposure Level	**2.7 µg/m³ (1.2 ppb)**

A similar acute REL was calculated as shown below based on lesions in nasal epithelium in rats exposed to acrolein for 6 h/d for 3 d (Cassee et al., 1996b). There were sufficient data in this study to permit the application of the BMD method in preference to the NOAEL/LOAEL approach. A $BMC_{0.5}$ of 56 µg/m^3 was derived based on the incidence of moderate to severe lesions at each exposure level. Irritancy was not the endpoint in this study so a time adjustment was applied using $C^n*T=K$ (n=3) to adjust the 18 hours of exposure to 1 hour that gave 147 µg/m^3 (see Section 5.7.1 of the TSD). Interspecies uncertainty factors of 2 for toxicokinetic differences with use of a dosimetric adjustment factor (DAF) of 0.85 (dosimetric adjustment factor – described below and in Section 4.4.7.2.2 of the TSD), and √10 for toxicodynamic variability were combined with a combined intraspecies UF of 10 (1 for kinetic and 10 for dynamic variability, reflecting the expectation of greater toxicodynamic variability) for a cumulative UF of 60 and an acute REL of 0.91 ppb.

Study	Cassee et al., 1996b
Study population	11 rats
Exposure method	
Exposure continuity	6 hr/day
Exposure duration	3 days
Critical effects	lesions of the respiratory epithelium
LOAEL	1.73 ppm
NOAEL	not observed
Benchmark concentration (BMC_{05})	56 µg/m^3
Time-adjusted exposure	C^n*T n = 3
Extrapolated concentration	147 µg/m^3 $(56^3*6/1*3/1)^{1/3}$
Human concentration adjustment	125 µg/m^3 = 147*0.85 (DAF)
LOAEL uncertainty factor (UF_L)	not applied
Subchronic uncertainty factor (UFs)	not applied
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	2 (with DAF adjustment)
Toxicodynamic (UF_{A-d})	√10 (default: no interspecies toxicodynamic data)
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	1
Toxicodynamic (UF_{H-d})	10 (asthma exacerbation in children)
Cumulative uncertainty factor	60
Reference Exposure Level	**2.1 µg/m^3 (0.91 ppb)**

The DAF is a factor derived by OEHHA based on the modeled comparative flux of formaldehyde in the upper respiratory tracts of rats, rhesus monkeys and humans by Kimbell et al. (2001) (see Section 4.4.7.2.2 of the TSD). Kimbel et al used three-dimensional, anatomically realistic, computational flow dynamic models to estimate mass flux across 20 consecutive bins representing the nasal passages. The mean flux at each bin was weighted by the percent of non-squamous epithelium in that bin to derive a weighted average flux for each bin. Averaging across all 20 bins provides an overall estimate of the flux for comparison between species (rat 13.63 pmol/mm^2; human 30.80 pmol/mm^2). Peak flux values were also estimated for the rat (2620 pmol/mm^2) and human (2082 pmol/mm^2), and averaged with the mean flux values to estimate the DAF (0.85). The DAF is the ratio of this value for the rat to that for humans. Although acrolein is more reactive than formaldehyde, both compounds appear to have their effects primarily on the respiratory (vs. olfactory) epithelium (Cassee et al., 1996a). This supports the assumption that in applying the DAF to acrolein, acrolein and formaldehyde deposit similarly in the nasal passages. In the absence of acrolein-specific modeling data, any residual uncertainty associated with this assumption is reflected in the use of an interspecies UF_{A-k} of 2.

8.2 Acrolein 8-Hour Reference Exposure Level

Study	Kutzman et al., 1985
Study population	96 adult Fisher-344 rats
Exposure method	Discontinuous whole body 0.4 – 4.0 ppm
Exposure continuity	6 hr/day, 5 days/week
Exposure duration	62 days
Critical effects	Lesions in the respiratory epithelium
LOAEL	0.4 ppm
NOAEL	not observed
Benchmark concentration	not derived
Time-adjusted exposure	$C^n * T = K$, where n =1.2
Extrapolated 8 hour concentration	310 ppb = $(0.4^{1.2}*6/8)^{1/1.2}$
Human concentration adjustment	260 ppb = 310*0.85 (DAF)
LOAEL uncertainty factor (UF_L)	6 (default: mild effect, no NOAEL)
Subchronic uncertainty factor (UFs)	not applied
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	2 (default with DAF adjustment)
Toxicodynamic (UF_{A-d})	√10 (default: no interspecies toxicodynamic data)
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	1
Toxicodynamic (UF_{H-d})	10 (asthma exacerbation in children)
Cumulative uncertainty factor	380
Reference Exposure Level	**1.6 μg/m³ (0.68ppb)**

The 8-hour Reference Exposure Level is a concentration at or below which adverse non-cancer health effects would not be anticipated for repeated 8-hour exposures (see Section 6 in the TSD).

The 8-hour and chronic RELs are based on the observation of lesions in rat respiratory epithelium by Kutzman et al. (1985) following exposure to acrolein for 6 h/d, 5 d/wk for 62 d. The critical effect of lesion formation is not a sensory irritancy effect so a time (T) adjustment was applied using $C^n * T = K$, where n =1.2 (Table G-1, Appendix G of the TSD) to extrapolate to an 8 hour concentration of 310 ppb. Use of C^n (where C is concentration) is a modification of Haber's Law as described in Section 5.7.1 of the TSD. K is a constant. A UF of 6 was applied for the use of a LOAEL. An adjusted human concentration of 260 ppb was estimated using a DAF of 0.85 (see previous section for a discussion of the DAF). Use of the DAF is expected to correct for pharmacokinetic differences between species so an interspecies kinetic UF of 2 was used instead of √10. The default interspecies UF_{A-d} of √10 was applied to compensate for the absence of data on pharmacodynamic differences between species. An intraspecies UF_{H-k} of 1 was used since, although the data are only for adult animals, the pharmacokinetic differences between adult and young animals are not expected to be great based on the similar inhalation dosimetry associated with reactive gases in adults and infants (Ginsberg et al., 2005). The potential pharmacodynamic differences among individuals (especially those

with and without asthma) and between adults and infants (due to the immaturity of the infants respiratory tract) are expected to be greater. For example, irritant gases more readily stimulate the hyper-reactive airways of asthmatics while enhanced mucus production in response to irritant gases may more easily block the infant's narrower airways. As described in Section 5.2, exacerbation of asthma by acrolein is expected to disproportionately affect children. For these reasons, an intraspecies UF_{H-d} of 10 was employed. The UF_{H-d} of 10 is the default in the absence of human kinetic data. This resulted in a cumulative UF of 380 and an 8-hour REL of 1.6 μg/m³ (0.68 ppb).

8.3 Acrolein Chronic Reference Exposure Level

Study	Kutzman et al., 1985
Study population	96 adult Fischer-344 rats
Exposure method	Discontinuous whole body to 0 – 4.0 ppm
Exposure continuity	6 hr/day, 5 days/week
Exposure duration	62 days
Critical effects	Lesions in the respiratory epithelium
LOAEL	0.4 ppm
NOAEL	not observed
Benchmark concentration	not derived
Time adjusted exposure	0.071 ppm = 0.4*6/24*5/7
Human concentration adjustment	60 ppb = 0.071 * 0.85 (DAF)
LOAEL uncertainty factor (UF_L)	6 (default: mild effect, no NOAEL)
Subchronic uncertainty factor (UFs)	√10 (exposure 8-12% of lifetime)
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	2 (with DAF adjustment)
Toxicodynamic (UF_{A-d})	√10 (default: no interspecies toxicodynamic data)
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	1
Toxicodynamic (UF_{H-d})	10 (asthma exacerbation in children)
Cumulative uncertainty factor	1200
Reference Exposure Level	**0.12 μg/m³ (0.05 ppb)**

The chronic Reference Exposure Level is a concentration at which adverse noncancer health effects would not be expected from chronic exposures (see Section 7 in the Technical Support Document).

The chronic REL was developed based on the same study as the 8-hr REL but with a time extrapolation to continuous exposure since the endpoint was not trigeminal irritancy (see Section 1.2.3 in the TSD). In addition to the UFs applied to the 8-hr REL, a UF of √10 was applied since this was a subchronic exposure study representing 8-12% of the rat's lifetime. The cumulative UF of 1200 gives a chronic REL of 0.12 μg/m³ (0.05 ppb). For comparison with the proposed chronic REL, a chronic REL was estimated from the data of Feron et al. (1978) in rats, which found a LOAEL of 0.4 ppm after exposure for 13 weeks. Using time extrapolation and a DAF of 0.85, a human equivalent

concentration of 60 ppb (138 μg/m^3) was calculated. A UF of 6 was applied for LOAEL to NOAEL conversion, √10 for use of subchronic exposure, and √10 for interspecies toxicodynamic variability. A factor of 2 was used for interspecies toxicokinetic uncertainty associated with the use of the DAF. For intraspecies variability, while these data are from adult animals, a UF_{H-k} of 1 was used since the pharmacokinetic differences between adult and young animals are not expected to be as great as the potential pharmacodynamic differences, for which an intraspecies UF_{H-d} of 10 was employed. This gave an estimated chronic REL of 0.12 μg/m^3 (0.05 ppb). This study was considered supportive of the Kutzman results.

Study	Feron et al. (1978)
Study population	48 adult Wistar rats
Exposure method	Discontinuous whole body to 0 – 4.9 ppm
Exposure continuity	6 hr/day, 5 day/week
Exposure duration	13 weeks
Critical effects	Lesions in respiratory epithelium
LOAEL	0.4 ppm
NOAEL	not observed
Benchmark concentration	not derived
Time-adjusted exposure	0.071 ppm = 0.4*6/24*5/7
Human concentration adjustment	60 ppb = 0.071 * 0.85 (DAF)
LOAEL uncertainty factor (UF_L)	6 (default: mild effect, no NOAEL)
Subchronic uncertainty factor (UFs)	√10 (exposure 8-12% of lifetime)
Interspecies Uncertainty Factor	
Toxicokinetic (UF_{A-k})	2 (with DAF adjustment)
Toxicodynamic (UF_{A-d})	√10 (default: no interspecies toxicodynamic data)
Intraspecies Uncertainty Factor	
Toxicokinetic (UF_{H-k})	1
Toxicodynamic (UF_{H-d})	10 (asthma exacerbation in children)
Cumulative uncertainty factor	1200
Reference Exposure Level	**0.12 μg/m^3 (0.05 ppb)**

The U.S. EPA (2003) based its RfC of 0.02 μg/m^3 on the study by Feron et al. (1978) from which a HEC of 0.02 mg/m^3 was derived based on a regional gas dosimetric ratio (RGDR) of 0.14 and an adjusted LOAEL of 0.16 mg/m^3 (0.14 * 0.16 = 0.02). U.S. EPA applied a total uncertainty factor of 1,000 (3 for interspecies extrapolation from a dosimetrically adjusted dose; 10 for intra-human variability; 3 for the use of a LOAEL; 10 for subchronic to chronic extrapolation). In contrast to the RGDR of 0.14, to better account for differences in rat and human exposures to reactive gases, OEHHA used a DAF of 0.85 based on comparative modeling of gas flux in human and rat nasal passages described above instead of a RGDR. This, combined with UFs of 6 for interspecies uncertainty (2 for use of the DAF, √10 for toxicodynamic differences), √10 for the use of a subchronic study, and 6 for the use of a LOAEL (vs US EPA's 3, 3, and 10, respectively) account for the difference between the REL and the U.S. EPA RfC.

For comparison, the state of Minnesota Department of Health reports a subchronic Health Risk Value (HRV) for acrolein of 0.2 $\mu g/m^3$, a level thought to be without significant risk following inhalation exposure for 13 weeks (MDH, 2002).

8.4 Acrolein as a Toxic Air Contaminant

Acrolein was designated by the ARB as a toxic air contaminant (TAC) in accordance with section 39657(b) of the California Health and Safety Code on April 8, 1993 (Title 17, California Code of Regulations, section 93001)(CCR, 2007). In view of the differential impacts on infants and children identified in Section 6.2 (more severe effects associated with bronchoconstriction and asthma exacerbation, less ability to escape or avoid exposure), OEHHA identified acrolein as a TAC which may disproportionately impact children pursuant to Health and Safety Code, Section 39669.5(c).

9. References

Astry CL and Jakab GJ (1983). The effects of acrolein exposure on pulmonary antibacterial defenses. Toxicol Appl Pharmacol 67(1): 49-54.

Borchers MT, Carty MP and Leikauf GD (1999a). Regulation of human airway mucins by acrolein and inflammatory mediators. Am J Physiol 276(4 Pt 1): L549-55.

Borchers MT, Wert SE and Leikauf GD (1998). Acrolein-induced MUC5ac expression in rat airways. Am J Physiol 274(4 Pt 1): L573-81.

Borchers MT, Wesselkamper S, Wert SE, Shapiro SD and Leikauf GD (1999b). Monocyte inflammation augments acrolein-induced Muc5ac expression in mouse lung. Am J Physiol 277(3 Pt 1): L489-97.

Bouley G, Dubreuil A, Godin J, Boisset M and Boudene C (1976). Phenomena of adaptation in rats continuously exposed to low concentrations of acrolein. Ann Occup Hyg 19(1): 27-32.

Bouley G, Dubreuil A, Godin J and Boudene C (1975). [Effects in the rat of a weak dose of acrolein inhaled continuously]. Eur J Toxicol Environ Hyg 8(5): 291-7.

Buckley LA, Jiang XZ, James RA, Morgan KT and Barrow CS (1984). Respiratory tract lesions induced by sensory irritants at the RD50 concentration. Toxicol Appl Pharmacol 74(3): 417-29.

CARB (2005b). Annual Statewide Toxics Summary - acrolein Sacramento, CA. http://www.arb.ca.gov/adam/toxics/statepages/acrostate.html.

Cassee FR, Arts JH, Groten JP and Feron VJ (1996a). Sensory irritation to mixtures of formaldehyde, acrolein, and acetaldehyde in rats. Arch Toxicol 70(6): 329-37.

Cassee FR, Groten JP and Feron VJ (1996b). Changes in the nasal epithelium of rats exposed by inhalation to mixtures of formaldehyde, acetaldehyde, and acrolein. Fundam Appl Toxicol 29(2): 208-18.

CCR (2007). California Code of Regulations Section 93001 Hazardous Air Pollutants Identified as Toxic Air Contaminants. Sacramento, CA: California Office of Administrative Law. 8-20-07. http://ccr.oal.ca.gov/linkedslice/default.asp?SP=CCR-1000&Action=Welcome.

Chhibber G and Gilani SH (1986). Acrolein and embryogenesis: an experimental study. Environ Res 39(1): 44-9.

Claussen U, Hellmann W and Pache G (1980). The embryotoxicity of the cyclophosphamide metabolite acrolein in rabbits, tested in vivo by i.v. injection and by the yolk-sac method. Arzneimittelforschung 30(12): 2080-3.

Darley E, Middleton J and Garber M (1960). Plant damage and eye irritation from ozone-hydrocarbon reactions. Agricul Food Chem 8(6): 483-484.

Doty RL, Cometto-Muniz JE, Jalowayski AA, Dalton P, Kendal-Reed M and Hodgson M (2004). Assessment of upper respiratory tract and ocular irritative effects of volatile chemicals in humans. Crit Rev Toxicol 34(2): 85-142.

Feron VJ and Kruysse A (1977). Effects of exposure to acrolein vapor in hamsters simultaneously treated with benzo[a]pyrene or diethylnitrosamine. J Toxicol Environ Health 3(3): 379-94.

Feron VJ, Kruysse A, Til HP and Immel HR (1978). Repeated exposure to acrolein vapour: subacute studies in hamsters, rats and rabbits. Toxicology 9(1-2): 47-57.

Finkelstein EI, Nardini M and van der Vliet A (2001). Inhibition of neutrophil apoptosis by acrolein: a mechanism of tobacco-related lung disease? Am J Physiol Lung Cell Mol Physiol 281(3): L732-9.

Ginsberg GL, Foos BP and Firestone MP (2005). Review and analysis of inhalation dosimetry methods for application to children's risk assessment. J Toxicol Environ Health A 68(8): 573-615.

Gosselin B, Wattel F, Chopin C, Degand P, Fruchart JC, Van der Loo D and Crasquin O (1979). [A case of acute acrolein poisoning]. Nouv Presse Med 8(30): 2469-72.

Grafstrom RC, Sundqvist K, Dypbukt JM and Harris CC (1987). Pathobiological effects of aldehydes in cultured human bronchial cells. IARC Sci Publ(84): 443-5.

Gurtoo HL, Marinello AJ, Struck RF, Paul B and Dahms RP (1981). Studies on the mechanism of denaturation of cytochrome P-450 by cyclophosphamide and its metabolites. J Biol Chem 256(22): 11691-701.

IARC (1995). Dry Cleaning, Some Chlorinated Solvents and Other Industrial Chemicals. IARC monographs 63: 337-371.

Kane LE, Barrow CS and Alarie Y (1979). A short-term test to predict acceptable levels of exposure to airborne sensory irritants. Am Ind Hyg Assoc J 40(3): 207-29.

Kimbell JS, Overton JH, Subramaniam RP, Schlosser PM, Morgan KT, Conolly RB and Miller FJ (2001). Dosimetry modeling of inhaled formaldehyde: Binning nasal flux predictions for quantitative risk assessment. Toxicological Sciences 64(1): 111-121.

Kutzman R. (1981). A subchronic inhalation study of Fischer 344 rats exposed to 0, 0.4, 1.4, or 4.0 ppm acrolein. National Toxicology Program: Interagency Agreeement No. 222-Y01-ES-9-0043. Brookhaven National Laboratory. Upton, NY

Kutzman RS, Popenoe EA, Schmaeler M and Drew RT (1985). Changes in rat lung structure and composition as a result of subchronic exposure to acrolein. Toxicology 34(2): 139-51.

Lam CW, Casanova M and Heck HD (1985). Depletion of nasal mucosal glutathione by acrolein and enhancement of formaldehyde-induced DNA-protein cross-linking by simultaneous exposure to acrolein. Arch Toxicol 58(2): 67-71.

Leikauf GD, Doupnik CA, Leming LM and Wey HE (1989a). Sulfidopeptide leukotrienes mediate acrolein-induced bronchial hyperresponsiveness. J Appl Physiol 66(4): 1838-45.

Leikauf GD, Leming LM, O'Donnell JR and Doupnik CA (1989b). Bronchial responsiveness and inflammation in guinea pigs exposed to acrolein. J Appl Physiol 66(1): 171-8.

Linhart I, Frantik E, Vodickova L, Vosmanska M, Smejkal J and Mitera J (1996). Biotransformation of acrolein in rat: excretion of mercapturic acids after inhalation and intraperitoneal injection. Toxicol Appl Pharmacol 136(1): 155-60.

Lyon JP, Jenkins LJ, Jr., Jones RA, Coon RA and Siegel J (1970). Repeated and continuous exposure of laboratory animals to acrolein. Toxicol Appl Pharmacol 17(3): 726-32.

Mahut B, Delacourt C, de Blic J, Mani TM and Scheinmann P (1993). Bronchiectasis in a child after acrolein inhalation. Chest 104(4): 1286-7.

Mannino DM, Homa DM, Pertowski CA, Ashizawa A, Nixon LL, Johnson CA, Ball LB, Jack E and Kang DS (1998). Surveillance for asthma--United States, 1960-1995. MMWR CDC Surveill Summ 47(1): 1-27.

McNulty M, Heck HD and Csasnova-Schmitz M (1984). Depletion of glutathione in rat respiratory mucosa by inhaled acrolein. Fed Proc 43: 575, abstract 1695.

MDH (2002). Health Risk Values for Air - Subchronic HRVs March 2002. Minnesota Department of Health,. http://www.health.state.mn.us/divs/eh/air/hrvtable.htm#sub.

Morris JB, Symanowicz PT, Olsen JE, Thrall RS, Cloutier MM and Hubbard AK (2003). Immediate sensory nerve-mediated respiratory responses to irritants in healthy and allergic airway-diseased mice. J Appl Physiol 94(4): 1563-71.

Nazaroff WW and Singer BC (2004). Inhalation of hazardous air pollutants from environmental tobacco smoke in US residences. J Expo Anal Environ Epidemiol 14 Suppl 1: S71-7.

NTIS. (1981). Acrolein health effects. Final task 6 report. Contract # 68-03-2928. National Technical Information Service. Arlington (VA)

OEHHA. (2001). *Prioritization of toxic air contaminants - children's health protection act - final report*. Available on-line at: <http://www.oehha.ca.gov/air/toxic_contaminants/SB25finalreport.html>.

Parent RA, Paust DE, Schrimpf MK, Talaat RE, Doane RA, Caravello HE, Lee SJ and Sharp DE (1998). Metabolism and distribution of [2,3-14C]acrolein in Sprague-Dawley rats. II. Identification of urinary and fecal metabolites. Toxicol Sci 43(2): 110-20.

Penn A, Nath R, Pan J, Chen L, Widmer K, Henk W and Chung FL (2001). 1,N(2)-propanodeoxyguanosine adduct formation in aortic DNA following inhalation of acrolein. Environ Health Perspect 109(3): 219-24.

Prentiss A (1937). Chemicals in War. In. McGraw-Hill. New York: 139-140.

Roemer E, Anton HJ and Kindt R (1993). Cell proliferation in the respiratory tract of the rat after acute inhalation of formaldehyde or acrolein. J Appl Toxicol 13(2): 103-7.

Roux E, Hyvelin JM, Savineau JP and Marthan R (1999). Human isolated airway contraction: interaction between air pollutants and passive sensitization. Am J Respir Crit Care Med 160(2): 439-45.

Slott VL and Hales BF (1986). The embryolethality and teratogenicity of acrolein in cultured rat embryos. Teratology 34(2): 155-63.

Takabe W, Niki E, Uchida K, Yamada S, Satoh K and Noguchi N (2001). Oxidative stress promotes the development of transformation: Involvement of a potent mutagenic lipid peroxidation product, acrolein. Carcinogenesis (Oxford) 22(6): 935-941.

U.S.EPA. (2003). Toxicological review of acrolein. EPA/635/R-03/003. U.S. Environmental Protection Agency. Washington DC

Weber-Tschopp A, Fischer T, Gierer R and Grandjean E (1977). [Experimentally induced irritating effects of acrolein on men (author's transl)]. Int Arch Occup Environ Health 40(2): 117-30.

WHO. (1992). *Environmental health criteria 127. Acrolein*. World Health Organization. www.inchem.org/documents/ehc/ehc/ehc127.htm#SectionNumber:10.1.

Yang XM, Wu XL, Choi YE, Kern JC and Kehrer JP (2004). Effect of acrolein and glutathione depleting agents on thioredoxin. Toxicology 204(2-3): 209-218.

Inorganic Arsenic Reference Exposure Levels

1. Summary

Acute, 8-hour and chronic reference exposure levels (RELs) were derived for inorganic arsenic including arsine. Inorganic arsenic causes a wide variety of toxic effects in humans and experimental animals including effects on development, the vascular system, the nervous system, blood, lung, and skin. The most sensitive acute effects were seen in mice (fetal development) whereas the most sensitive 8-hour and chronic effects were decreased intellectual function in children. The key values are summarized below.

1.1 Inorganic Arsenic Acute REL

Reference Exposure Level	**0.2 μg As/m³**
Critical effect(s)	Decreased fetal weight in mice
Hazard Index target(s)	Development (teratogenicity); cardiovascular system; nervous system

1.2 Inorganic Arsenic 8-Hour REL

Reference Exposure Level	**0.015 μg/ As/m³**
Oral Reference Exposure Level	0.0035 μg/kg bw-day
Critical effect(s)	Decreased intellectual function in 10 year old children
Hazard Index target(s)	Development (teratogenicity); cardiovascular system; nervous system; lung; skin

1.3 Inorganic Arsenic Chronic REL

Reference Exposure Level	**0.015 μg As/m³**
Oral Reference Exposure Level	0.0035 μg/kg bw-day
Critical effect(s)	Decreased intellectual function in 10 year old children
Hazard Index target(s)	Hematologic

2. Physical & Chemical Properties

Table 2.1 Arsenic and Arsenic Species*

Molecular formula	Molecular weight	Percent As by weight	Synonyms	CAS Registry Number
As	74.92	100%	Arsenic black, metallic arsenic	7440-38-2
As_2O_3 As_4O_6	197.82 395.68	75.7%	Arsenious oxide, arsenic (III) trioxide, arsenic oxide, arsenous acid, arsenous acid anhydride, Crude Arsenic, White Arsenic	1327-53-3
$AsCl_3$	181.28	41.3%	Arsenic butter, trichloroarsine, arsenious chloride	7784-34-1
As_2O_5	229.82	65.2%	Arsenic pentoxide, arsenic anhydride, arsenic oxide, arsenic acid anhydride	1303-28-2
$AsHNa_2O_4$	185.91	40.3%	Arsenic acid disodium salt, disodium arsenate, sodium arsenate dibasic	7778-43-0
$AsHNa_2O_3$	130.92	57.2%	Arsenous acid disodium salt, arsenious acid sodium salt	7784-46-5
AsH_3	77.94	96.12	Arsine, arsane, arsenic hydride, arsenous hydride, hydrogen arsenide, arsenic trihydride	7784-42-1
$As(OH)_3$			Arsenous acid	13464-58-9
$AsO(OH)_3$	141.93	52.78	Arsenic acid, orthoarsenic acid	7778-39-4
As_4S_4	427.92	70.03	Arsenic disulfide, realgar, red arsenic sulfide	
$CH_3AsO(OH)_2$	139.97	53.51	Monomethylarsonic acid	124-58-3
$CH_3As(OH)_2$	123.77	60.41	Monomethylarsonous acid	25400-23-1
$(CH_3)_2AsO(OH)$	137.99	54.28	Dimethylarsinic acid, cacodylic acid	75-60-5
$(CH_3)_2AsOH$	121.99	61.40	Dimethylarsinous acid	55094-22-9
$(CH_3)_3AsO$	136.02	55.06	Trimethylarsine oxide	4964-14-1

*Note: Methylated arsenic species occurring naturally and as metabolites (IARC, 2004)

2.1 Arsenic (Metallic) (ATSDR, 2000)

Description	Yellow, black or gray solid
Molecular formula	see Table 2.1
Molecular weight	see Table 2.1
Specific gravity (water = 1)	5.778 g/cm^3 @ 25°C
Boiling point	613°C (sublimes) at 760 mm Hg
Vapor pressure	7.5 x 10^{-3} mmHg at 280 °C
Flashpoint	not applicable
Explosive limits	not applicable
Solubility	soluble in nitric acid, insoluble in water
Odor threshold	not applicable
Odor description	odorless
Metabolites	dimethylarsinic acid, methylarsonic acid
Conversion factor	not applicable for As

2.2 Arsenic Trioxide (ATSDR, 2000)

Description	As_2O_3: White solid, glassy, amorphous lumps or crystal
Molecular formula	See Table 2.1
Molecular weight	197.84
Density	As_2O_3: 3.865 g/cm^3
Boiling point	As_2O_3: 460°C
Melting point	As_2O_3: 274°C
Solubility	Oxides: slightly soluble in water 17g/L, insoluble in alcohol, chloroform, ether.
Metabolites	Dimethylarsinic acid, methylarsonic acid

2.3 Arsine (U.S. EPA, 2006a)

Description	Colorless gas
Molecular formula	AsH_3
Molecular weight	77.93
Specific gravity (Water = 1)	1.689 @ 84.9°C
Boiling point	-62.55°C
Melting point	-117°C
Vapor pressure	Greater than 1 atm
Vapor density (Air = 1)	2.695
Solubility	soluble in chloroform and benzene, slightly soluble in water (20 mL/100 mL at 20 C), ethyl alcohol and in alkalis
Odor threshold	0.5 ppm
Odor description	garlic-like or fishy odor
Metabolites	oxidation to arsenite, arsenate, other unidentified (Landrigan et al., 1982; Carter et al., 2003)
Conversion factor	1 ppm = 3.19 mg/m³ @ 25°C

3. Occurrence and Major Uses

Arsenic is ubiquitous and is found in small amounts in soils and water throughout the world and also in foods, particularly seafood (NIOSH, 1975). Ore refining processes, including the smelting of copper and lead, are the major sources of release of arsenic dust and inorganic arsenic compounds. Arsenic trioxide is the form of inorganic arsenic most commonly produced. It is used as a raw material for the production of other inorganic arsenic compounds, alloys, and organic arsenic compounds (Grayson, 1978).

Pesticides have historically constituted the largest single use (50%) of arsenic compounds (HSDB, 1995). The major arsenic herbicides manufactured are monosodium methyl arsonate (MSMA), disodium methyl arsonate (DSMA), and dimethyl arsenic acid (cacodylic acid). Inorganic arsenic compounds are also used as herbicides (arsenite), insecticides (arsenic trioxide, calcium and other arsenates), or rodenticides (sulfides) (ACGIH, 1992). Arsenic trichloride, for example, is used mainly as a chemical intermediate in the production of insecticides, but has other applications in the ceramics and pharmaceutical industries (HSDB, 1995). Arsenic was used as a pesticide to treat tobacco; thus, cigarette smoke was another common source of exposure (U.S.EPA, 1984). The use of arsenic compounds in agriculture has reduced in recent years and U.S. EPA is considering ending their uses under the pesticide reregistration program (U.S. EPA, 2006b).

Arsenic-based wood preservatives have constituted the next largest use (40%) of arsenic compounds (HSDB, 1995). In December 2003 the U.S. EPA terminated all residential uses of wood preservatives containing arsenic limiting such products to restricted use by certified pesticide applicators (U.S. EPA, 2002).

The highly toxic trivalent arsenic compounds, such as arsenic trioxide, are typically introduced into the environment as a result of industrial processes including the smelting of metal ores. Pentavalent arsenic compounds are generally considered to be less toxic and are most frequently found naturally.

Processes such as smelting, galvanizing, soldering, and etching, that require the treatment of metal with strong acids, are possible sources of arsine gas. Acid treatment of metals contaminated with arsenic can result in the release of arsine gas. Arsine is used to provide arsenic as a dopant in the semiconductor industry. Combustion of fossil fuels may produce arsine gas.

4. Toxicokinetics

A knowledge of the metabolism of inorganic arsenic has long been thought to be essential to understanding the mode(s) of action of inorganic arsenic toxicity. Trivalent (+3) arsenic species (e.g., arsenite) have often exhibited greater acute toxicity than pentavalent (+5) species (e.g., arsenate). Since the metabolism of inorganic arsenic in mammalian species generally proceeds via alternate reductive and oxidative methylation steps to

mono- (MMA) and dimethyl (DMA) arsenic acids, it was believed that methylation represented detoxication of inorganic arsenic. However, recent evidence supports the idea that trivalent methylated species are in some cases more toxic than inorganic precursors and may play a key role in arsenic toxicity for selected endpoints. The metabolism of arsine (-3), while less studied, appears to progress similarly after its oxidation to arsenite (+3) and is in part the basis for including arsine in the RELs for inorganic arsenic.

Several comprehensive reviews of the absorption, distribution, metabolism and elimination of arsenic have been published (Vahter, 1983; Thompson, 1993; ATSDR, 2000; NRC, 2001). Most information on the toxicokinetics of arsenic derives from oral exposure studies. The kinetics of arsenic varies depending on the chemical form of arsenic and on the animal species. The following discussion is limited to the oxidized forms found in water and air and forms that are ingested via the aquatic food chain. These include the inorganic, soluble forms of arsenite (As^{III}) and arsenate (As^{V}), as well as the organic methyl arsonate (MMA), dimethylarsinic acid (DMA), trimethylarsine (TMA), and or arsenobetaine (in fish).

4.1 Inorganic Arsenic Oxides

4.1.1 Absorption and Distribution

Owen (1990) reported inhalation absorption of 32 percent (range 30 to 34 %) from arsenic containing aerosols, however it is uncertain if this figure included the gastrointestinal absorption of arsenic particles from the upper respiratory tract. The International Commission on Radiological Protection Human Respiratory Tract Model (ICRP, 1994) gives total deposition fractions for 10 yr old children inhaling 1 μm activity median thermodynamic diameter particles at 0.31 to 2.03 m^3/hr of 0.42 to 0.58. There are relatively few data on the kinetics of airborne arsenic excretion. Mann et al. (1996a) modeled inhalation exposures based on the occupational data of Vahter et al. (1986) and Offergelt et al. (1992). For simulated occupational exposures of 10 μg/m^3 of arsenic aerosol of MMAD of 5.0 μm, GSD of 2.1, 1.2 L tidal volume and a breathing rate of 16 /min, urinary excretion increased over the work week's exposure from 7 to 25 μg As/g creatinine. Model predictions of arsenic metabolites (Asi, MMA, DMA) in postshift urine generally fell within the range of observations for 18 workers in the exposure range of 10-1000 μg As/m^3. After daily inhalation exposure of 100 μg As (III)/m^3 for three weeks, the model predictions for urinary metabolite distribution closely matched observed values (predicted/observed means: Asi, 1.05; MMA, 1.0; DMA, 1.0). From the model, Mann et al. (1996b) derived a fitted lung absorption first order rate constant for arsenic trioxide dust of 0.01/cm^2-hr.

In general, investigations that have monitored arsenic excretion of experimental animals following parenteral administration have demonstrated that only a small fraction of the administered arsenic is excreted in the feces. Thus, to estimate the amount of inorganic arsenic absorbed following oral administration, most kinetic and metabolic studies have

monitored the urine. Soluble compounds of inorganic arsenic, whether in the trivalent or pentavalent form, are readily absorbed (80-90 percent) in most animal species following oral administration (Charbonneau et al., 1978; Vahter, 1981; Hughes et al., 1994; Freeman et al., 1995). However, only about 40-50 percent absorption has been reported in hamsters (Yamauchi and Yamamura, 1985; Marafante and Vahter, 1987). Absorption of orally administered inorganic arsenic in humans has been shown to range between 54-80 percent (Tam et al., 1979; Buchet et al., 1981b; a; Kurttio et al., 1998).

Inorganic arsenic compounds are poorly absorbed through the skin (Ca.1-5%); the trivalent is more rapidly absorbed than the pentavalent (Wester et al., 1993; Wester et al., 2004).

Organic forms of arsenic are also extensively absorbed from the gastrointestinal tract. Experimental studies examining the absorption of MMA, DMA, TMA and arsenobetaine in humans have demonstrated 75-92 percent absorption. At low-level exposures, excretion of arsenic and its metabolites seems to balance absorption of inorganic arsenic. With increasing arsenic intake, there is suggestive evidence that methylation appears less complete. Studies, which examine the effect of dose on excretion patterns, have been conducted in mice and humans (Buchet et al., 1981b; a; Vahter, 1981). As the dose of inorganic arsenic increases, the percent of arsenic excreted as DMA decreases, accompanied by an increased excretion in the percent as inorganic arsenic. The percent excreted as MMA remains virtually unchanged. *In vitro* metabolism studies on the methylation of inorganic arsenic have demonstrated that the liver is the site of methylating activity and that S-adenosylmethionine and reduced glutathione are required as methyl donors (Buchet and Lauwerys, 1985; 1987).

While absorption from the gastrointestinal tract is the most important route of exposure for waterborne arsenic, some potential for dermal absorption has been reported. Rahman et al. (1994) conducted *in vitro* studies with sodium [^{74}As] arsenate and clipped full-thickness mouse skin in a flow-through system. Doses of 5, 50, 500, or 5000 ng were applied to 0.64 cm^2 of skin as a solid, in aqueous vehicle, or in soil. Absorption of sodium arsenate increased linearly with applied dose from all vehicles. The maximum absorption of 62 percent of applied dose was obtained with the aqueous vehicle and the least (0.3 percent) with soil. Wester et al. (1993) evaluated the percutaneous absorption of [^{73}As] arsenate from soil or water *in vivo* in Rhesus monkeys and *in vitro* in human cadaver skin. Water solutions of [^{73}As] arsenate at low (0.024 ng/cm^2) or high (2.1 $\mu g/cm^2$) surface concentrations were compared. With topical administration for 24 hr, *in vivo* absorption in the Rhesus monkey was 6.4 ± 3.9 (SD) percent from the low dose and 2.0 ± 1.2 (SD) percent from the high dose. *In vitro* percutaneous absorption of the low dose from water in human skin was 0.93 ± 1.1 percent in receptor fluid and 0.98 ± 0.96 percent in the washed skin; the total was about 1.9 percent. Absorption from soil (0.4 ng/cm^2) was less, at 6.4 percent in the monkey *in vivo* and 0.8 percent in human skin *in vitro*.

The retention and distribution patterns of arsenic are in part determined by its chemical properties. Arsenite (As^{III}) reacts and binds to sulfhydryl groups while arsenate (As^{V}) has

chemical properties similar to those of phosphate. As^V also has affinity for sulfhydryl groups; however, its affinity is approximately 10-fold less than As^{III} (Jacobson-Kram and Montalbano, 1985). The distribution and retention patterns of As^{III} and As^V are also affected by species, dose level, methylation capacity, valence form, and route of administration.

Vahter et al. (1984) studied tissue distribution and retention of ^{74}As-DMA in mice and rats. About 80 percent of an oral dose of 0.4 mg As/kg was absorbed from the gastrointestinal tract. In mice >99 percent of the dose was excreted within 3 d compared to only 50 percent in rats, due largely to accumulation in blood. Tissue distribution in mice showed the highest initial (0.5-6 hr) concentrations in kidneys, lungs, intestinal mucosa, stomach, and testes. Tissues with the longest retention times were lungs, thyroid, intestinal walls, and lens.

The effect of dose on arsenate disposition was evaluated in adult female $B6C3F_1$ mice dosed orally with 0.5 to 5000 μg/kg [^{73}As]-arsenate in water (Hughes et al., 1994). Urine was collected at several time points over a 48-hr period, and feces at 24 and 48 hr post-exposure. The recovery of As-derived radioactivity in excreta and tissues ranged from 83.1 to 89.3 percent of dose. As-derived radioactivity was detected in several tissues (urinary bladder, gall bladder, kidney, liver, lung) although the sum for each exposure level was very low (<0.5 percent of dose). The principal depot was the liver, followed by the kidneys. As the dose of arsenate increased there was a significant increase in the accumulation of radioactivity in the urinary bladder, kidney, liver, and lungs. The greatest concentration of As radioactivity was in the urinary bladder.

4.1.2 Metabolism

Most studies of arsenic metabolism have involved administration of inorganic arsenic (Asi) as arsenate (As^V) or arsenite (As^{III}) to an experimental animal or a human, and detection of Asi and the methylated metabolites methylarsonic acid (MMA^V) and dimethylarsinic acid (DMA^V) in urine, feces, and tissues.

Thompson (1993) conducted an extensive review and analysis of the mammalian metabolic data on arsenic. The metabolism of arsenate can be viewed as a cascade of reductive and oxidative methylation steps leading successively to As^{III}, MMA^V, MMA^{III}, DMA^V, DMA^{III}, $TMAO^V$, and TMA as outlined in Scheme 1. Recently Hayakawa et al. (2005) proposed a new metabolic pathway for arsenite, which does not involve oxidative methylation but rather is mediated by As-glutathione complexes, S-adenosylmethionine (SAM) and human arsenic methyltransferase Cyt19. In this pathway arsenic triglutathione ($As(SG)_3$) is converted to monomethyl-(MADG) and dimethyl-(DMAG) conjugates which are hydrolyzed to MMA^{III} and DMA^{III}, respectively. Thus pentavalent methylated metabolites might arise via oxidation of their trivalent forms rather than the reverse as shown in Scheme 1.

Scheme 1. Biomethylation of Arsenic Involving Alternate Reduction of Pentavalent Arsenic to Trivalent Arsenic Followed by Oxidative Addition of a Methyl Group (after Jiang et al. (2003))

$$\underset{As^{V}}{\mathbf{As^{V}O(OH)_3}} \xrightarrow{+2e} \underset{As^{III}}{\mathbf{As^{III}(OH)_3}} \xrightarrow{+CH_3^+} \underset{MMA^{V}}{\mathbf{CH_3As^{V}O(OH)_2}} \xrightarrow{+2e} \underset{MMA^{III}}{\mathbf{CH_3As^{III}(OH)_2}} \xrightarrow{+CH_3^+}$$

$$\underset{DMA^{V}}{\mathbf{(CH_3)_2As^{V}O(OH)}} \xrightarrow{+2e} \underset{DMA^{III}}{\mathbf{(CH_3)_2As^{III}OH}} \xrightarrow{+CH_3^+} \underset{TMAO}{\mathbf{(CH_3)_3As^{V}O}} \xrightarrow{+2e} \underset{TMA^{III}}{\mathbf{(CH_3)_3As^{III}}}$$

MMA^{III} and DMA^{III} have only recently been detected as stable urinary metabolites in human subjects (Aposhian et al., 2000a; Aposhian et al., 2000b; Le et al., 2000a; Le et al., 2000b), and trimethylarsine oxide (TMAO) and trimethylarsine (TMA) are rarely seen and are very minor metabolites in most mammals if found at all. Few data are available on the tissue concentrations of trivalent methylated As species (Kitchin, 2001). Gregus et al. (2000) found that in bile duct-cannulated rats, As^{III} and its metabolites were preferentially excreted into bile (22 percent) versus eight percent into urine in two hr. Arsenite appeared in bile rapidly and constituted the large majority in the first 20 min. Thereafter As^{III} declined and MMA^{III} output gradually increased. From 40 min after i.v. As^{III} administration, MMA^{III} was the dominant form of biliary arsenic. Within two hr 9.2 percent of the dose was excreted in the bile as MMA^{III}. Injection of arsenate produced a mixture of As^{V}, As^{III} and MMA^{III} in the bile. Curiously, rats injected with MMA^{V} did not excrete MMA^{III}.

The metabolism results of Styblo et al. (1995) in rat liver cytosol *in vitro* seem to support the overall metabolic scheme noted above; MMA^{III} and MMA^{III}-diglutathione complex are more rapidly methylated to the dimethyl forms than MMA^{V}. Thompson also suggests that the data support the presence of two inhibitory loops: (1) competitive inhibition by MMA^{III} of the $As^{III} \rightarrow MMA^{V}$ step catalyzed by MMTase; and (2) possibly noncompetitive inhibition by As^{III} of the $MMA^{III} \rightarrow DMA^{V}$ step catalyzed by DMTase.

Styblo et al. (1996) observed 50 μ*M* arsenite inhibition of DMA^{V} production in rat liver cytosol *in vitro*. Healy et al. (1998) studied the activity of MMTase in tissues of mice. The activity was determined with sodium arsenite and *S*-[methyl-^{3}H]-adenosyl-L-methionine by measuring the formation of [methyl-^{3}H] monomethylarsonate. The mean MMTase activities (units/mg ± SEM) measured in cytosol of mouse tissues were: liver, 0.40 ± 0.06; testis, 1.45 ± 0.08; kidney, 0.70 ± 0.06; and lung, 0.22 ± 0.01. When mice were given arsenate in drinking water for 32 or 92 days at 25 or 2500 μg As/L, the MMTase activities were not significantly increased compared to controls. MMTases and DMTases have been partially purified from the livers of rabbits (Zakharyan et al., 1995), Rhesus monkeys (Zakharyan et al., 1996) and hamsters (Wildfang et al., 1998). All of the enzyme preparations exhibited Michaelis-Menten enzyme kinetics with Km values ranging from 8×10^{-4} *M* for hamster DMTase to 1.8×10^{-6} *M* for hamster MMTase. Vmax values ranged from 0.007 pmol/mg protein/hr for hamster DMTase to 39.6 pmol/mg

protein/hr for rabbit MMTase. Comparative studies have shown several species to be deficient in methyltransferase activities, notably New World monkeys, marmosets, tamarin, squirrel, chimpanzee, and guinea pig (Vahter et al., 1995b; Aposhian, 1997). While comparisons with human arsenic methyl transferase are limited by lack of a purified human enzyme, based on excretion profiles of urinary metabolites the rabbit and hamster appear most pharmacokinetically similar to humans than the other species studied. Walton et al. (2003) compared the methylation of arsenite by rat and human primary hepatocytes in vitro (control values in their Tables 1 and 2). For the rat the methylation rate after a 3 hr incubation with 0.1 μM arsenite was 99.3 ± 1.87 pmol CH_3/hr/10^6 cells (mean ± SD, N =4). The human hepatocytes similarly exposed for 24 hr had a methylation rate of 1.68 ± 0.24 pmol CH_3/hr/10^6 cells, over a 50-fold difference in apparent methylation rate.

While the reduction of arsenate and MMA^V can be accomplished nonenzymatically *in vitro*, and arsenate reduction by glutathione occurs in mammalian blood *in vivo* (Vahter and Envall, 1983; Winski and Carter, 1995), these reductive steps are most likely enzymatically mediated *in vivo*. An arsenate reductase has been partially purified from human liver and described (Radabaugh and Aposhian, 2000). The approximate mass of the enzyme was 72,000. It was specific for arsenite (i.e., did not reduce [^{14}C] MMA^V) and exhibited substrate saturation at about 300 μM. The human arsenate reductase requires a thiol and a heat-stable cofactor and is apparently distinct from those isolated from bacteria (Ji and Silver, 1992; Gladysheva et al., 1994; Krafft and Macy, 1998).

Monomethyl arsonate (MMA^V) reductases have been isolated and described for rabbit (Zakharyan and Aposhian, 1999) and hamster (Sampayo-Reyes et al., 2000). In the latter study the distribution of MMA^V reductase activity was 91.4 nmol MMA^{III}/mg protein/hr in brain and 61.8 nmol MMA^{III}/mg protein/hr in bladder. Skin, kidney and testis all had less than 15 nmol/mg/hr. Spleen, liver, lung, and heart were all between 15 and 62 nmol/mg/hr. The high activity of MMA^V reductase in brain is curious and may help explain some of the neurotoxic effects of arsenic. Due to relatively low affinity of the MMA^V reductase ($K_M = 2.2 \times 10^{-3}$ M) compared to the methyl transferases ($K_M =$ 5-9x10^{-6} M), the MMA^V reduction is thought to be the rate-limiting step in arsenic metabolism (Zakharyan and Aposhian, 1999). The partially purified human liver MMA^V reductase has been shown to be identical with human glutathione S-transferase Omega class hGSTO 1-1 (Zakharyan et al., 2001).

DMA is the main metabolite found in the tissues and urine of most experimental animals administered inorganic arsenic. Humans are also somewhat unique in that MMA has been found to be an important metabolite of inorganic arsenic in addition to DMA. Studies conducted on human volunteers given a single oral dose of inorganic arsenic demonstrated that within 4-7 days, 46-62 percent of the dose was excreted in the urine (Tam et al., 1979; Pomroy et al., 1980; Buchet et al., 1981b; a). Approximately 75 percent of the excreted arsenic is methylated, about one-third as MMA and two-thirds as DMA.

The possibility of genetic polymorphism in arsenic metabolism has been suggested by Vahter et al. (1995a), who studied native Andean women in northwestern Argentina who were exposed to a wide range of As concentrations in drinking water (2.5 to 200 μg As/L). The women exposed to the highest As concentration in water exhibited surprisingly low levels of MMA in their urine (2.3 percent of metabolites). The percentage of arsenic urinary metabolites as MMA in typical human urine ranges from 12 to 20. Chiou et al. (1997a) studied the relationships among arsenic methylation capacity, body retention, and genetic polymorphisms of glutathione-*S*-transferase (GST) M1 and T1 in 115 human subjects. Percentages of As species in urine (mean ± SE) were: Asi, 11.8 ± 1.0; MMA, 26.9 ± 1.2; and DMA, 61.3 ± 1.4. Genetic polymorphisms of GST M1 and T1 were significantly associated with As methylation. Subjects with the null genotype of GST M1 had an increased percentage of Asi in urine, while those with the null genotype GST T1 had elevated DMA in their urine samples.

Marnell et al. (2003) reported six polymorphisms in the MMA^V reductase hGSTO1 gene in DNA isolated from peripheral blood of 75 Mexican subjects. Two subjects with the same polymorphism showed 5 to 10 fold higher concentrations (μg/g creatinine) of Asi in their urine than other subjects.

Yu et al. (2003) screened DNA of 22 subjects of European ancestry (EA) and 24 of indigenous American ancestry (IA) for polymorphisms in arsenate reductase and MMA^V reductase genes. For the arsenate reductase gene (hPNP) 48 polymorphic sites were identified while 33 were found in the MMA^V reductase gene (hGSTO1-1). For the EA individuals the MMA^V reductase gene showed greater polymorphism than the arsenate reductase gene whereas the reverse was seen in the IA individuals. In the latter group only one polymorphism had a frequency of > 10%. Meza et al. (2005) screened 135 As-exposed subjects from Sonora, Mexico for polymorphisms in arsenic metabolism genes: arsenate reductase (hPNP); MMA^V reductase (hGSTO); and arsenic 3 methyltransferase (CYT19). The subjects were exposed to drinking water with 5.5 to 43.3 ppb arsenic. The screening was based on urinary DMA^V/MMA^V (D/M) ratios. The analysis revealed that all of the variation was due to a very strong association between CYT19 and D/M in children only (7-11 yr). With children removed no significant association was seen in adults (18-79 yr). This developmentally regulated association between CYT19 and arsenic metabolism raises questions about the adequacy of arsenic risk assessment for children.

4.1.3 Excretion

Several authors have studied the kinetics of As excretion in humans. Tam et al. (1979) administered ^{74}As arsenic acid (0.01 μg, ca. 6 μCi) to six adult males (age: 28-60; body weight: 64-84 kg) following an overnight fast. The urine was analyzed at 24 hr intervals for five days following As administration. In the first 24 hr period Asi excretion exceeded that of the methylated metabolites but thereafter the usual DMA > MMA > Asi pattern persisted, with DMA increasing in percentage of cumulative excretion at the later time points. A follow up study (Pomroy et al., 1980) followed ^{74}As excretion for periods

up to 103 days using a whole body counter, with measurement of excreta for the first seven days. Their results indicate that the excretion data were best represented by a three-component exponential function. The coefficients for the pooled data accounted for 65.7 percent of excretion with a half-life of 2.09 days, 30.4 percent with a half-life of 9.5 days, and 3.7 percent with a half-life of 38.4 days. A four-exponent function showed a better fit to one of the six subjects (half-lives: 0.017, 1.42, 7.70 and 44.1 days).

4.1.4 PBPK Models

Physiologically-based pharmacokinetic (PBPK) models employ data from various sources to mathematically simulate the uptake, distribution, metabolism and excretion of toxic chemicals in species of interest. Such models are used in risk assessment to estimate target tissue doses and to facilitate route-to-route and interspecies extrapolations. By contrast, pharmacodynamic (PD) models simulate biological responses to chemical exposures. A number of PBPK models for arsenic disposition and metabolism have been developed for experimental animals and humans (Mann et al., 1994; Menzel et al., 1994; Mann et al., 1996a; 1996b; Yu, 1999; Gentry et al., 2004). Although these models are based on somewhat different principles, they all seem to do a fair job in predicting the overall disposition of arsenic in animals and man. However, while the models often incorporate the latest ideas on the metabolism of inorganic arsenic with respect to oxidation state, methylated metabolites, and enzyme inhibition, due to limitations in our understanding of the modes of action of arsenic toxicity, they have yet to include representations of biological responses or pharmacodynamic (PD) capabilities, such as dosimetry linked alterations of DNA methylation, cell signaling pathways, DNA repair inhibition or generation of reactive oxygen species.

As an example of the complexity of arsenic action, Gentry et al. (2004) observed that pharmacodynamic changes occurred in mice without changes in PBPK predicted arsenic tissue dosimetry. These authors used the PBPK model of Mann et al. (1996a,b) extended to mice to evaluate possible dosimetry differences between mouse a strain susceptible to arsenic induced tumors (C57Bl/6J) and those that lacked susceptibility (e.g., Swiss CD-1, Swiss CD: NIH(S), C57Bl/6p53 (+/-)). The model was parameterized using published acute mouse data for arsenate, arsenite, MMA and DMA and validated with acute exposure data from the C57Black mouse strain. Model predictions for acute exposure were then compared with data from acute (24 hr) and chronic exposures (26 weeks). No differences were seen in the volume of distribution or tissue-plasma concentration ratios between acute and chronic exposures. Comparison of metabolite profiles in blood, liver and urine also showed little difference between acute and chronic exposures. Model predictions compared well with observed values. The authors concluded "… that pharmacokinetic factors do not provide an explanation for the difference in outcomes across the various mouse bioassays." This conclusion may be overly broad since all the metabolites of arsenic and its metabolic pathways were not included in the PBPK modeling.

4.2 Arsine

Although most studies of arsenic metabolism have centered on arsenate and arsenite, other forms of arsenic are also metabolized in humans. Apostoli et al. (1997) reported on the metabolism of arsine gas ($As^{-III}H_3$) in an occupationally exposed worker. Arsenic species were analyzed in urine over a five-day post-exposure period by liquid chromatography and inductively coupled plasma mass spectroscopy. The As species most excreted were MMA, DMA, As^{III}, arsenobetaine (AsB), and to a lesser extent As^{V}. The data indicate a capability to oxidize As^{-III} to As^{V} species probably via arsenite $As(OH)_3$. Arsenobetaine, an important form of arsenic in food, does not undergo subsequent biotransformation and is excreted via the urine. Curiously, arsenobetaine does not appear to be a metabolite of arsine in rats exposed for 1 hour to 4 to 80 mg/m^3 arsine (Buchet et al., 1998). The apparent similarity of the metabolism of arsine and arsenite is important and supports the use of the inorganic arsenic RELs for arsine.

Carter et al. (2003) have reviewed the metabolism of arsenic oxides, gallium arsenide and arsine. These authors describe three reactions that appear to occur in aqueous solutions of arsine (-III): (1) the formation of elemental As^0 and hydrogen; (2) reaction of AsH_3 with oxidized thiols to form diarsine AsH_2-AsH_2 (proposed) and reduced thiol RSH; and (3) possible reaction between arsine and oxygen species, producing arsine hydroperoxide H_2AsOOH (Hatlelid et al., 1995; 1996). Relatively few studies of arsine metabolism have been conducted in experimental animals. In vitro studies indicate that arsine was rapidly distributed to red blood cells. In plasma arsine appeared to decompose over a few hours. Arsine apparently undergoes rapid oxidative metabolism although the intermediary metabolites have not been identified and apparently are not identical with those shown above for arsenite metabolism (Scheme 1) (Carter et al., 2003). A hypothetical scheme based on the same alternate application of oxidative methylation and reduction steps might look as follows with double arrows indicating four electron oxidation steps and single arrows two electron reduction steps:

$$\mathbf{As^{-III}H_3 \rightarrow\rightarrow H_2As^{I}(O)CH_3 \rightarrow H_2As^{-I}CH_3 \rightarrow\rightarrow HAs^{III}(O)(CH_3)_2 \rightarrow HAs^{I}(CH_3)_2 \rightarrow\rightarrow As^{V}(O)(CH_3)_3}$$

Arsine **TMAO**

According to this scheme the intermediary metabolites would include methylated arsine and arsine oxide species. Alternatively nonmethylative oxidation of arsine could lead to arsenite and arsenate via hydroxylated arsine species. Other metabolites possibly based on the oxidation of elemental As or arising via the postulated arsine hydroperoxide are also possible.

5. Acute Toxicity of Arsenic and Arsenic Compounds

5.1 Acute Toxicity to Adult Humans

The relative acute toxicity of arsenic compounds decreases as follows: arsine (-III) > organo-arsine derivatives > arsenites (III) > arsenoxides (III) > arsenates (V) > pentavalent organic compounds (V) > arsonium metals (I) > metallic arsenic (0), where the Roman numeral indicates the oxidation state (HSDB, 1995).

Acute inhalation exposure may result in severe irritation of the mucous membranes of the upper and lower respiratory tract with symptoms of cough, dyspnea, and chest pain (Friberg et al., 1986). These may be followed by garlicky breath and gastrointestinal symptoms including vomiting and diarrhea (HSDB, 1995). Signs of acute poisoning are dermatitis, nasal mucosal irritation, laryngitis, mild bronchitis, and conjunctivitis (Friberg et al., 1986). The acute toxic symptoms of trivalent arsenic poisoning are due to severe inflammation of the mucous membranes and increased permeability of the capillaries (HSDB, 1995). Ingestion of 2 grams of As_2O_3 was fatal to an adult male (Levin-Scherz et al., 1987).

5.2 Acute Toxicity to Infants and Children

Relatively little data are available on acute toxicity of arsenic compounds to children. Childhood poisonings due to arsenic have been reported in the medical literature, often with little dosimetry. Campbell & Oates (1992) surveyed 200 child poisonings and found of the four deaths reported one was due to arsenic-containing weed killer (probably cacodylic acid). Alternatively, the use of arsenic trioxide in cancer chemotherapy seems well tolerated. George *et al*. (2004) reported the treatment of 11 children with acute promyelocytic leukemia with i.v. 0.15 mg As_2O_3/kg-d (8 treatment cycles over a period of 12 months). The toxic effects noted, including leukocytosis and skin hyperpigmentation, were considered minimal. Relapse-free survival was 81%.

5.3 Acute Toxicity to Experimental Animals

5.3.1 Inahation Exposure

The lethal concentration low (LC_{Lo}) for $AsCl_3$ in the cat for a 20-minute inhalation exposure is 100 ppm (740 mg/m³) (Flury, 1921). In the mouse, the LC_{Lo} of $AsCl_3$ for a 10-minute exposure is 338 ppm (2500 mg/m³) (Flury, 1931).

A single intratracheal instillation of 17 mg As_2O_3/kg in rats resulted in multifocal interstitial pneumonia and focal proliferative bronchiolitis and alveolitis observed at necropsy 14 days post-exposure (Webb et al., 1986). The authors suggest that As_2O_3 induced an acute fibrogenic response.

5.3.2 Immunotoxicity

Changes in host resistance from inhalation exposure to As_2O_3 aerosol were examined in female CD1 mice using a streptococcus infectivity model and an assay for pulmonary

bactericidal activity (Aranyi et al., 1981; Aranyi et al., 1985). Mice (100-200/group) were exposed to As_2O_3 aerosol (or filtered air) for 3 hours/day, 5 days/week, for 1, 5 or 20 days. Aerosol exposed and control mice were then combined before challenge with *Streptococcus zoopidemicus* aerosol (4-8 replicate exposures). Statistically significant increases in mortality ($P < 0.05$) were observed in mice exposed: (1) once to 271, 496, and 940 μg As/m^3; (2) 5 times to 519 μg As/m^3; and (3) 20 times to 505 μg As/m^3. Multiple exposures at a given exposure level did not correlate with increased mortality, suggesting an adaptation mechanism. Single exposures did, however, show a dose-response for increased mortality with increasing level of arsenic exposure. Bactericidal activity was evaluated by measuring the ratio of viable bacteria count to radioactive count in the lung 3 hours after infection with ^{35}S-labeled *Klebsiella pneumoniae*. A single exposure to 271, 496, and 940 μg As/m^3, but not 123 μg As/m^3, resulted in significantly decreased bactericidal activity. Five exposures to 519 μg As/m^3 and twenty exposures to both 245 and 505 μg As/m^3 resulted in decreased bactericidal activity. The studies indicate a NOAEL for immunotoxicity of 123 μg As/m^3. This study provides a partial mode of action of arsenic-induced increase in mortality due to experimental lung infections with the mouse pathogen *S.zooepidemicus*. The second bactericidal assay with radiolabelled *K.pneumoniae* provides a plausible explanation, namely that arsenic exposure above 123 μg/m^3 inhibits normal immune bactericidal response in the lung.

5.3.3 Organ and Enzyme Effects

Among the other adverse effects of inorganic arsenic noted in experimental animals, the most interesting and relevant to the 8-hour and chronic RELs are those on the brain and nervous system. These include changes in brain histology and conditioned reflexes, changes in locomotor activity, and decreased acetyl cholinesterase, GAD, and GABA levels in the hypothalamus, brain stem and cerebellum. Arsenic induced alterations of brain structure and function are consistent with the more subtle neuro-developmental effects seen in children exposed to inorganic arsenic at lower environmental levels.

5.4 Developmental and Reproductive Toxicity

Arsenic is listed under California Proposition 65 (Cal/EPA, Safe Drinking Water and Toxic Enforcement Act of 1986) as a developmental toxicant. The oxidation state of arsenic determines the teratogenic potential of its inorganic compounds; trivalent (III) arsenic compounds possess greater teratogenic potential than pentavalent (V) compounds. In hamsters, a single maternal intravenous injection of 20 mg/kg sodium arsenate (V) ($AsHNa_2O_4$) on gestation day 8 was lethal to 44% of all embryos (Willhite and Ferm, 1984). A smaller dose (10 mg/kg) of sodium arsenite (III) ($AsHNaO_2$) administered in the same manner resulted in 90% embryonic lethality.

Fetal malformations, including exencephaly, resulted from an intravenous injection of $AsH_3Na_2O_4$ (V) into pregnant hamsters on gestation day eight (Ferm and Carpenter, 1968). The reproductive NOAEL in this experiment was 5 mg/kg. A significant reduction in fetal body weight, but no malformations were observed following a maternal

dose of 5 mg/kg AsH_2NaO_3 (III) by the same route on gestation day eleven or twelve (Harrison and Hood, 1981).

A significant increase in pre-implantation mortality followed exposure of pregnant rats to aerosolized As_2O_3 at 1 mg/m³ for 5 months; no maternal toxicity was observed (Kamkin, 1982). At the LOAEL, 0.3 mg/m³, slightly elevated pre-implantation lethality was observed. The validity of this report cannot be evaluated, however, because key experimental details were not reported

A significant decrease in spermatozoa motility was observed in male rats following continuous exposure to As_2O_3 at a concentration of 40 mg/m³ for 48 hours (Kamil'dzhanov, 1982). Intravenous injection of radioactive arsenate (V) or arsenite (III) in several rodent species, including mice and hamsters, resulted in accumulation of arsenic in the lumen of the epididymal duct, which suggests that long term exposure of sperm may occur *in vivo* following acute exposure to As (Danielsson et al., 1984).

Nagymajtenyi et al., (1985) exposed pregnant CFLP mice (8-11 females/group) to As_2O_3 aerosol for 4 hours/day on gestational days 9-12 at concentrations of 0, 0.26, 2.9, or 28.5 mg As_2O_3/m^3 (~0.2, 2.2, and 21.6 mg As/m^3. The aerosol was generated by spraying an aqueous solution of As_2O_3. On the 18th day of gestation the mice were sacrificed and the fetuses removed. The numbers of live and dead fetuses were recorded, weighed, and examined microscopically. Fifty fetuses were stained with Alizarin red-S for skeletal examination. Chromosome preparations were made from livers of 10 fetuses per exposure group. Twenty mitoses in each fetus (200/group) were scored for chromosomal damage and 10 percent of these were karyotyped. The data were analyzed with either Fisher's exact test or in the case of fetal weights with the Dunnett multiple comparison t-test.

A statistically significant decrease in fetal weight was observed in all of the dose groups ($P < 0.05$), with a 3, 9, and 29% reduction in average fetal weight with increasing dose (Table 6.4.1). Significantly delayed bone maturation (ossification defects) was observed only in the highest dose group (sternum 14/50; limbs 32/50, both $p < 0.05$). However, an apparent positive dose-related trend in the number of fetuses with skeletal malformations was observed (2 [control], 3, 7, 31, respectively). A similar dose-related trend in chromosome aberrations in liver cells was also observed in the number of cells with damage (6[control], 10, 13, 24), chromatid gaps, chromatid breaks, chromosome fragments, and chromosome breaks (5[control], 10, 13, 27). Only the number of damaged cells and chromosome breaks at the high dose were significantly different from the control ($p < 0.05$).

Table 6.4.1 Data from Table 1 of Nagymajtényi et al. (1985).

As_2O_3 (mg/m^3)	Number of litters	Living fetuses per mother	Number of fetuses examined	% dead fetuses	Average fetal weight (grams)
28.5±0.3	11	9.6	100	29	0.981±0.04*
2.9±0.04	8	12.8.	100	13	1.146±0.03*
0.26±0.01	8	12.5	100	12	1.225±0.03*
0	8	12.5	100	8	1.272±0.02

* Significantly different from control ($p<0.05$)

This study demonstrates that inhalation exposure to inorganic arsenic is markedly fetotoxic. Arsenic concentrations of 28.5 mg/m^3 caused a reduction in the number of live fetuses, in fetal weight, and an increase in fetuses with delayed osteogenesis.

Rats exposed to 1 μg As_2O_3/m^3 (0.76 μg As/m^3) for 5 months showed increased preimplantation mortality and delayed ossification in fetuses (Kamkin, 1982). Experimental detail was not presented, thus limiting the usefulness of this study.

A significant decrease in spermatozoa motility was observed in male rats following continuous exposure to 32.4 mg As_2O_3/m^3 for 48 hours (Kamil'dzhanov, 1982). Similarly, motility was decreased after: (1) a 120-hour exposure to 7.95 mg/m^3; (2) a 252-hour exposure to 1.45 mg/m^3; and (3) an 800-hour exposure to 0.36 mg/m^3.

Holson et al. (1999) administered arsenic trioxide (As_2O_3) by whole body inhalation to groups of 25 Crl:CD (SD)BR female rats every day for six hours per day, beginning fourteen days prior to mating and continuing throughout mating. The target exposure levels were 0.3, 3.0, and 10.0 mg As_2O_3/m^3 (measured means: 0.24, 2.6, 8.3 mg As/m^3). Maternal toxicity evidenced by the occurrence of rales, a decrease in net body weight gain, and decreased food intake during pre-mating and gestation exposure, was observed only at the high dose. The NOAEL for maternal toxicity was 2.6 mg As/m^3 (3.4 mg As_2O_3/m^3). No treatment-related malformations or developmental variations were observed at any exposure level. The NOAEL for developmental toxicity was 8.3 mg As/m^3 (11 mg As_2O_3/m^3). The median mass aerodynamic diameter of particle sizes generated in the exposure chambers ranged from 1.9 to 2.2 μm for the three doses indicating that the dusts were respirable. However there were no blood or urine arsenic analytical data to assess delivered doses.

Nemec et al. (1998) evaluated the developmental toxicity of inorganic arsenic in mice and rabbits. CD-1 mice (25/dose group) and New Zealand White rabbits (20/dose group) were gavaged with aqueous arsenic acid (H_3AsO_4) doses of 0, 7.5, 24, or 48 mg/kg-d on gestation days (GD) six through 15 (mice) or 0, 0.19, 0.75, or 3.0 mg/kg-d on GD six through 18 (rabbits). The animals were examined at necropsy (GD 18, mice; GD 29, rabbits). Treatment related maternal toxicity including mortality (2/25) was observed only in the highest dose administered to mice. Effects on maternal weight gain were noted only on GD 6-9 ($P < 0.01$) and GD 15-18 ($P < 0.05$) of the mid dose and on GD 6-9

($p < 0.05$) of the low dose. While overall maternal weight gains were statistically significantly reduced only at the top dose there was an apparent negative trend in decreased GD18 body weights with increasing dose (56.2 g control, 54.9 g, 52.7g, 46.7g, respectively). While the authors identified a NOAEL for maternal toxicity of 7.5 mg/kg-d, the apparent negative trend noted above suggests that this may be a LOAEL (4.0 mg As/kg-d).

Statistically significant adverse effects on offspring growth or survival were seen only at the highest dose of 48 mg/kg-d. However, there was an apparent negative trend in the number of live fetuses per litter with increasing dose (12.3 control, 11.6, 11.0, 6.6, respectively). An increased incidence of resorptions per litter was seen in the 48 mg/kg-d dose group ($P \leq 0.01$), (mainly early resorptions). Early and total resorptions showed an apparent positive trend (6.4% total control, 6.1%, 9.6%, 41.9%, respectively). Mean fetal weight showed an apparent negative trend (1.3 g control, 1.32 g, 1.23 g, 0.99 g, respectively). There were no statistically significant dose-related increases in the overall incidence of fetal malformations; however, the mean percent of litter malformation was about three-fold higher in the 48 mg/kg-d dose group than in the lower doses and control. The NOAEL for developmental toxicity would appear to be 7.5 mg/kg-d (4.0 mg As/kg-d).

Maternal toxicity in rabbits, including mortality, slight body weight loss, and clinical signs (decreased urination and defecation, occasional prostration and ataxia), occurred only at the high arsenic acid dose of 3.0 mg/kg-d. The number of does with decreased urination and defecation appeared to be slightly higher in the mid- and low-dose groups, but these effects may not have been treatment related and no effects on body weight were seen. At sacrifice on GD 29 maternal body weight appeared to be reduced in the high dose group. A significant loss in mean maternal gravid body weight occurred during the first six days of high-dose treatment (GD 6-12) ($p \leq 0.01$). This effect persisted and was significantly different from controls for the entire treatment interval (GD 6-18). There were no statistically significant increases in the incidences of any developmental parameters, including malformations. Fetal survival, mean fetal weight, and sex ratio on GD 29 were not affected by the treatment. The number of live fetuses per litter was reduced and resorptions per litter increased in the high-dose group. The latter findings were mainly due to one doe with a totally resorbed litter. The overall values were the range from laboratory historical controls. The authors identified a NOAEL of 0.75 mg/kg-d (0.4 mg As/kg-d) for both maternal toxicity and developmental toxicity.

Stump et al. (1999) administered either sodium arsenate (As^{V}) i.p. or arsenic trioxide (As^{III}) i.p. or by gavage on GD 9 to 25 Crl:CD (SD) BR rats. The doses of sodium arsenate were 0, 5, 10, 20, and 35 mg/kg (0, 1.2, 2.4, 4.8, 8.4 mg As/kg). The doses of arsenic trioxide were: i.p. 0, 1, 5, 10, and 15 mg/kg (0, 0.8, 3.8, 7.6, and 11.4 mg As/kg); and by gavage (p.o.) 0, 5, 10, 20, 30 mg/kg (0, 3.8, 7.6, 15.2, 22.7 mg As/kg). Sodium arsenate (i.p.) caused decreased maternal food consumption (GD 9-20), decreased body weights and body weight gains at the highest dose of 35 mg/kg. Decreased food consumption was also seen in the 20 mg/kg dose group at GD 9-10 and GD 9-20. Arsenic trioxide (i.p.) resulted in excessive mortality in the highest dose-group (19/25)

and significant reductions in maternal food consumption, body weight at GD20, body weight change, and net body weight in the next highest dose-group (10 mg/kg). Arsenic trioxide (p.o.) resulted in less mortality in the highest dose-group (7/25). Clinical signs were noted in the 20 and 30 mg/kg dose-groups including changes in fecal consistency and decreased defecation. Food consumption (GD 9-10) was decreased in a dose-dependent manner across As treatment groups. The study identified single dose maternal effects NOAELs of 2.4 mg As/kg for sodium arsenate (i.p.) and 3.8 mg As/kg for arsenic trioxide i.p. A LOAEL of 3.8 mg As/kg was identified for arsenic trioxide p.o.

Intraperitoneal administration of sodium arsenate or arsenic trioxide caused neural tube and ocular defects (exencephaly, microphthalmia/anophthalmia, and other craniofacial defects) in the offspring of treated rats. These effects were statistically significant only at doses causing maternal toxicity or mortality (35 and 10 mg/kg, respectively). Oral administration of arsenic trioxide caused no treatment-related malformations. The study identified single dose developmental NOAELs of 2.4 mg As/kg for sodium arsenate i.p., 3.8 mg As/kg for arsenic trioxide i.p., and 15.2 mg As/kg for arsenic trioxide p.o.

DeSesso et al. (1998), in a comprehensive review of the developmental toxicity of inorganic arsenic, concluded that cranial neural tube defects (NTDs) were induced in rodents only when exposure occurred early in gestation, at high maternally toxic doses, and by parenteral routes of administration. They argued that such NTD effective doses are unlikely to be achieved by the oral, inhalation, or dermal routes in rodents, and that inorganic arsenic does not represent a realistic developmental risk in humans subjected to any environmentally relevant exposure scenarios.

Male and female Charles River CD mice (10/group) were treated with 0 or 5 ppm arsenite in drinking water continuously through three generations (Schroeder and Mitchener, 1971). Endpoints examined included the interval between litters, the age at first litter, the ratio of males to females, the number of runts, stillborn offspring, failures to breed, and congenital abnormalities. The study showed an alteration in the number of small litters in the arsenic exposed group.

Female CD-1 mice (8-15/group) were treated by oral gavage with 0, 20, 40, or 45 mg sodium arsenite/kg on a single day of gestation between days 8 and 15 (Baxley et al., 1981). Maternal mortality, fetal malformations, and increased prenatal death were observed among animals treated with 40 and 45 mg sodium arsenite/kg.

Pregnant golden hamsters (>10/group) were treated by oral gavage with a single administration of 0, 20, or 25 mg/kg sodium arsenite on one of gestational days 8-12 (Hood and Harrison, 1982). Prenatal mortality was increased among animals receiving 25 mg/kg on gestational days 8 and 12 and fetal weights were decreased among animals receiving 25 mg/kg on gestational day 12. One dam died following administration of 20 mg/kg.

Intravenous injection of radioactive arsenate (V) or arsenite (III) in several rodent species, including mice and hamsters, resulted in accumulation of arsenic in the lumen of

the epididymal duct, which suggested that long term exposure of sperm to arsenic may occur *in vivo* following acute exposure (Danielsson et al., 1984).

6. Chronic Toxicity of Arsenic and Arsenic Compounds

6.1 Chronic Toxicity to Adult Humans

Arsenic in drinking water is carcinogenic to humans (Group 1, IARC, 2004). Arsenic compounds show limited to sufficient evidence of carcinogenicity in experimental animals (IARC, 2004). The U.S. Environmental Protection Agency has classified arsenic as Group A; a human carcinogen, based on sufficient evidence from human data including increased lung cancer mortality in multiple human populations exposed primarily through inhalation, increased mortality from multiple internal organ cancers (liver, kidney, lung, bladder), and increased skin cancers observed in populations exposed to arsenic in drinking water (IRIS online file www.epa.gov/iris/subst/0278.htm). Since this document deals with noncancer risks, the carcinogenicity of arsenic is not covered here in any detail (see OEHHA (1999)).

6.1.1 Inhalation Exposure

Smelter workers, exposed to concentrations of arsenic up to 7 mg As/m^3, showed an increased incidence in nasal septal perforation, rhinopharyngolaryngitis, tracheobronchitis, and pulmonary insufficiency (Lundgren, 1954).
In a case-control study, copper smelter workers (n = 47) exposed to arsenic for 8-40 years (plus 50 unexposed controls matched for age, medical history, and occupation) were examined by electromyography and for nerve conduction velocity in the arms and legs (Blom et al., 1985). The workers were found to have a statistically significant correlation between cumulative exposure to arsenic and reduced nerve conduction velocities in three peripheral nerves (upper and lower extremities). Slightly reduced nerve conduction velocity in 2 or more peripheral nerves was reported as “more common” among arsenic exposed workers. Minor neurological and electromyographic abnormalities were also found among exposed workers. Occupational exposure levels were estimated to be 0.05-0.5 mg As/m^3, with As_2O_3 the predominant chemical form. Except for three arsenic exposed workers who had long-term exposure to lead, exposure to other heavy metals was insignificant.

The smelter workers described by Blom et al. (1985) (number of controls reduced to 48) were further examined for prevalence of Raynaud’s phenomenon and for vasospastic tendency by measurement of finger systolic pressure at 10°C and/or 15°C relative to that at 30°C (FSP%) (Lagerkvist et al., 1986). The FSP% was found to covary with the duration of exposure to arsenic, and the prevalence of Raynaud’s phenomenon was significantly increased among exposed workers. Daily arsenic uptake was estimated at less than 300 µg/day and was confirmed with urinary excretion data.

Hyperpigmentation and hyperkeratinization were observed in workers exposed to 0.4 - 1 mg/m^3 inorganic arsenic for two or more years (Perry et al., 1948).

6.1.2 Oral Exposure

Most of the relevant epidemiological data on arsenic adverse effects comes from studies of arsenic exposure via drinking water. These studies are relevant because arsenic exerts similar toxic effects once it enters the body. For example, arsenic causes lung cancer in humans by both oral and inhalation routes. The adverse effects summarized below include skin lesions(keratosis and altered pigmentation), vascular effects on the heart, brain and peripheral vasculature, peripheral neuropathy, and lung disease.

6.1.2.1 Skin Effects

Mazumder et al. (1998) investigated arsenic-associated skin lesions of keratosis and hyperpigmentation in 7683 exposed subjects in West Bengal, India. While water arsenic concentrations ranged up to 3400 μg/L, over 80% of the subjects were consuming water with < 500 μg/L. The age-adjusted prevalence of keratosis was strongly related to water As concentration, rising from zero in the lowest exposure level (< 50 μg/L) to 8.3% for females drinking water containing >800 μg As/L, and from 0.2 to 10.7% in males, respectively. A similar dose-response was observed for hyperpigmentation: 0.3 to 11.5% for females; and 0.4 to 22.7% for males. Overall males had 2-3 times the prevalence of both keratosis and hyperpigmentation than females apparently ingesting the same doses of arsenic per body weight. Subjects that were more than 20% below standard body weight for their age and sex had a 1.6-fold increase in the prevalence of keratoses, suggesting that malnutrition may play a role in increasing susceptibility.

Rahman et al. (2006) evaluated arsenic exposure and age- and sex-specific risk for skin lesions in a population-based case-referent study in Bangladesh. The entire population over four years of age of Matlab, Bangladesh (N = 166,934) was screened for skin lesions. Skin lesions were classified as hyperpigmentation (melanosis), hypopigmentation (leukomelanosis), or keratosis. A total of 504 cases with skin lesions were identified. A randomly selected referent group of 1830 subjects was included in the study. Arsenic exposure was assessed by personal history of tube well use since 1970 or year of birth if later. Water samples from all functioning tube wells were measured for arsenic concentration by hydride-generation atomic absorption spectroscopy. A dose-response relationship was observed for increased skin lesions and arsenic exposure for both sexes ($P < 0.001$). For males using the metric of As μg/L the highest exposure quintile (≥ 300 μg/L) gave an adjusted odds ratio (OR) of 9.56 (95% CI = 4.20-21.8). Females gave a corresponding OR of 6.08 (3.06-15.5). The cumulative As exposure metric (μg/L x years) gave OR's of 10.4 and 9.19, respectively. In an analysis with males and females combined, adjusted for age and socioeconomic status, males had significantly higher risk of As-related skin lesions than females, when females' lowest average exposure quintile was used as the reference. For the highest quintile, the males OR was 10.9 (5.8-20.4) and the females OR was 5.78 (3.10-10.8), $P = 0.005$.

Dermatitis and irritation of the mucous membranes have been observed in arsenic-exposed workers (Vallee et al., 1960). Hepatic fatty infiltration, central necrosis, and cirrhosis were observed in two patients who ingested As_2O_3 (1% in Fowler's solution) for three or more years (Morris et al., 1974). Daily consumption of 0.13 mg As/kg in contaminated well water resulted in the chronic poisoning and death of four children; at

autopsy, myocardial infarction and arterial thickening were noted (Zaldivar and Guillier, 1977).

6.1.2.2 Vascular Disease

Vascular diseases have long been noted to be associated with chronic arsenic exposures among German vineyard workers (Grobe, 1976) and inhabitants of Antofagasta, Chile (Borgono et al., 1977). Peripheral vascular diseases have been reported to be associated with the occurrence of arsenic in well waters in Taiwan (Chen and Wu, 1962; Chi and Blackwell, 1968; Tseng, 1977; Chen et al., 1988). Concentrations in one study were characterized as 0.10 – 1.8 ppm (Yu et al. ,1984). The term arseniasis or arsenosis connotes vascular disease associated with chronic exposure to arsenic, specifically blackfoot disease (BFD). BFD is characterized by progressive narrowing of the peripheral arteries, particularly those of the lower extremities. This can lead to ulceration, gangrene and amputation. The etiology of BFD is unclear but arsenic is thought to be the principal cause. The term arsenicosis refers to arsenic induced skin lesions ranging in severity over four stages, seven grades and 20 sub-grades from diffuse melanosis (skin pigmentation or depigmentation) to aggressive skin and internal malignancy (Saha, 2003).

Wu et al. (1989) found significant trends of mortality rates from peripheral vascular diseases and cardiovascular diseases with concentrations of arsenic in well water. However, no significant association was observed for cerebrovascular accidents. Engel and Smith (1994) evaluated arsenic in drinking water and mortality from vascular disease in 30 U.S. counties from 1968 to 1984. Mean As levels in drinking water ranged from 5.4 to 91.5 μg/L. Standardized mortality ratios (SMRs) for diseases of arteries, arterioles, and capillaries (DAAC) for counties exceeding 20 μg/L were 1.9 (90% C.I. = 1.7-2.1) for females and 1.6 (90% C.I. = 1.5-1.8) for males. SMRs for three subgroups of DAAC including arteriosclerosis and aortic aneurysm were also elevated as were congenital abnormalities of the heart and circulatory system.

Tseng et al. (1996) studied the dose relationship between peripheral vascular disease (PVD) and ingested inorganic arsenic in blackfoot disease endemic villages in Taiwan. A total of 582 adults (263 men and 319 women) underwent Doppler ultrasound measurement of systolic pressures on bilateral ankle and brachial arteries and estimation of long-term arsenic exposure. The diagnosis of PVD was based on an ankle-brachial index of < 0.9 on either side. Multiple logistic regression analysis was used to assess the association between PVD and As exposure. A dose-response relationship was observed between the prevalence of PVD and long-term As exposure. The odds ratios (95% confidence intervals) after adjustment for age, sex, body mass index, cigarette smoking, serum cholesterol and triglyceride levels, diabetes mellitus and hypertension were 2.77 (0.84-9.14), and 4.28 (1.26-14.54) for those who had cumulative As exposures of 0.1 to 19.9 and ≥ 20 (mg/L) x yr, respectively. A follow up study (Tseng et al., 1997) indicated that PVD was correlated with ingested As and not with abnormal lipid profiles. The lipid profiles studied were total cholesterol, triglyceride, high-density lipoprotein cholesterol (HDL-c) and low-density lipoprotein cholesterol (LDL-c), apolipoprotein AI, and apolipoprotein B. Other lipids such as modified LDL, subclasses of LDL and HDL, and

other lipoproteins such as lipoprotein (a), which may track as better indicators of atherosclerosis, were not included. Also, the roles of platelet aggregation and coagulation profiles were not studied.

Chen et al. (1996) evaluated the dose-response relationship between ischemic heart disease (ISHD) mortality and long-term arsenic exposure. Mortality rates from ISHD among residents in 60 villages in an area of Taiwan with endemic arseniasis from 1973 through 1986 were analyzed for association with As concentrations in drinking water. Based on 1,355,915 person-years and 217 ISHD deaths, the cumulative ISHD mortalities from birth to age 79 yr were 3.4%, 3.5%, 4.7%, and 6.6% for the median As concentrations of < 0.1, 0.1-0.34, 0.35-0.59, and ≥ 0.6 mg/L, respectively. Multivariate-adjusted relative risks (RRs (95% C.I.)) associated with cumulative arsenic exposure from well water were 2.46 (0.53-11.36), 3.97 (1.01-15.59), and 6.47 (1.88-22.24) for 0.1-9.9, 10.0-19.9, and 20+ (mg/L)-yr, respectively, compared with those without As exposure.

Chiou et al. (1997b) evaluated the dose-response relationship between prevalence of cerebrovascular disease and ingested arsenic among residents of the Lanyang Basin in northeast Taiwan. A total of 8102 adults from 3901 households were recruited for the study. Arsenic in well water of each household was determined by hydride generation and atomic absorption spectrometry. Logistic regression analysis was used to estimate multivariate-adjusted odds ratios and 95% confidence intervals for various risk factors of cerebrovascular disease. A significant dose-response relationship was observed between As concentration in well water and prevalence of cerebrovascular disease after adjustment for age, sex, hypertension, diabetes mellitus, cigarette smoking, and alcohol consumption. The dose-response relationship was even more prominent for cerebral infarction with multivariate-adjusted odds ratios (95% C.I.) of 1.0, 3.4 (1.6-7.3), 4.5 (2.0-9.9), and 6.9 (3.0-16), respectively, for those who consumed well water with As concentrations of 0, 0.1-50.0, 50.1-299.9, and > 300 μg/L. For cumulative arsenic exposures of <0.1, 0.1-4.9, and ≥ 5.0 (mg/L)-yr, the odds ratios were 1.00, 2.26, and 2.69 for cerebrovascular disease and 1.00, 2.66, and 3.39 for cerebral infarction, respectively. All of the values above for As exposed groups were significantly greater than unexposed at $P < 0.05$.

Chen et al. (1995) also investigated the association between long-term exposure to inorganic arsenic and the prevalence of hypertension. A total of 382 men and 516 women were studied in villages where arseniasis was endemic. Hypertension was defined as a systolic blood pressure of 160 mm Hg or greater, or a history of hypertension treated with antihypertensive drugs. The long-term arsenic exposure was calculated from the history of artesian well water consumption obtained through subject questionnaires and the measured arsenic concentration in well water. Residents in villages where long-term arseniasis was endemic had a 1.5-fold increase in age- and sex-adjusted prevalence of hypertension compared with residents in nonendemic areas. The duration of well water consumption, average As water concentration, and cumulative As exposure were all significantly associated with hypertension. For the cumulative As exposure in (mg/L)-yr, the percent prevalence values were: 0, 5.0%; 0.1-6.3 (mg/L)-yr, 4.9%; 6.4-

10.8 (mg/L)-yr, 12.8%; 10.9-14.7 (mg/L)-yr, 22.1%; 14.8-18.5 (mg/L)-yr, 26.5%; > 18.5 (mg/L)-yr, 29.2%.

As part of a study of arsenic exposure via drinking water and mortality outcome in Millard County, Utah, Lewis et al. (1999) found a statistically significant association with mortality from hypertensive heart disease. Median drinking water concentration of arsenic ranged from 14 to 166 µg/L for the 946 subjects in the study. The standard mortality ratios (SMR) without regard to specific exposure levels were SMR = 2.20 (95% C.I., 1.36-3.36) for males and SMR = 1.73 (95% C.I., 1.11-2.58) for females. When analyzed by cumulative exposure groups of low (< 1.0 (mg/L)-yr), medium (1.0-4.9 (mg/L)-yr), and high (≥ 5.0 (mg/L)-yr), there was no apparent dose response relationship. However the cumulative dose estimates in this study were lower than in the Chen et al. (1995) discussed above so the results of the two studies are not inconsistent.

Chen *et al*. (2006) conducted a cross-sectional analysis of the association of arsenic exposure from drinking water and blood pressure in 10,910 subjects. Time-weighted well arsenic concentrations (TWA) based on current and past well usage were derived. Odds ratios (OR's) for high pulse pressure (systolic – diastolic pressure ≥ 55 mmHg) by increasing TWA quintiles (≤ 8, 8.1-40.8, 40.9-91.0, 91.1-176.0, 176.1-864.0 µg/L) were: 1.00 (referent); 1.39 (95% C.I. 1.14, 1.71); 1.21 (0.9, 1.49); 1.19 (0.97, 1.45); 1.19 (0.97,1.46). OR's for systolic hypertension (≥ 140 mmHg) suggested a similar but weaker association. Participants with lower than average intake of B vitamins and folate showed somewhat higher OR's. No associations were apparent for TWA and diastolic hypertension.

In a study related to those above, Lai et al. (1994) studied inorganic arsenic ingestion and the prevalence of diabetes mellitus. A total of 891 adult residents of villages in southern Taiwan where arseniasis is endemic were included in the study. Diabetes status was determined by an oral glucose tolerance test and a history of diabetes regularly treated with sulfonylurea or insulin. Cumulative arsenic exposure in ppm-yr was determined from the detailed history of drinking artesian well water. There was a dose-response relation between cumulative arsenic exposure and prevalence of diabetes mellitus. The relation remained significant after adjustment for age, sex, body mass index, and activity level at work by a multiple logistic regression analysis giving multivariate-adjusted odds ratios of 6.61 and 10.05, respectively, for exposures of 0.1-15 ppm-yr and > 15.0 ppm-yr versus an unexposed group. In an effort to confirm this association between diabetes mellitus and arsenic observed for drinking water in Taiwan, Rahman and Axelson (1995) reviewed 1978 case-control data from a Swedish copper smelter. Twelve cases of diabetes mellitus (death certificate) were compared with 31 controls without cancer, cardiovascular and cerebrovascular disease. The odds ratios for diabetes mellitus with increasing arsenic exposure categories were 1.0 (reference level), 2.0, 4.2, and 7.0 with the 95% confidence level including unity. The trend was weakly significant, $p = 0.03$. Albeit with limited numbers, the study provides some support for a role of arsenic exposure in the development of diabetes mellitus.

6.1.2.3 Neurological Disease
Hafeman et al. (2005) evaluated the association between arsenic exposure and peripheral neuropathy in a cross-sectional study of 137 adults in Bangladesh. Exposure measures included individual arsenic water concentration, cumulative arsenic index (CAI), and urinary arsenic concentration. Experimental measures were primarily vibrotactile threshold testing of the index finger (IVT) and toe (TVT) and secondarily tapping speed, grip strength, ankle reflex, and proprioception. The cumulative arsenic index and urinary arsenic were both significantly associated with elevated TVT (P = 0.02 and P = 0.009, respectively) after adjustment for age and gender. While dose-response relations were difficult to define, a linear regression analysis of TVT (vibration units) versus the continuous measures of urinary arsenic and CAI gave slopes of 0.02 and 0.0025 TVT units/50 μg As/mg urinary creatinine, respectively. The association between IVT and arsenic exposure was not statistically significant. No association was found between any measure of arsenic exposure and grip strength, tapping speed, ankle reflex, or proprioception.

6.1.2.4 Lung Disease
Von Ehrenstein et al. (2005) studied the relation between lung function, respiratory symptoms, and arsenic in drinking water among 287 adults, including 132 with arsenic-induced skin lesions in West Bengal, India. Arsenic levels in drinking water and the number of male subjects with or without skin lesions were: 0-99 μg/L, 9, 36; 100-399 μg/L, 66, 34; ≥400 μg/L, 18, 15, respectively. For respiratory symptoms of "shortness of breath at night" and "morning cough", the odds ratios (ORs) for men with skin lesion versus those without was 2.8 with 95% confidence intervals (C.I.) of (1.1, 7.6) and (1.2, 6.6), respectively. For men with skin lesions, the average forced expiratory volume in one second (FEV_1) was reduced by 256.2 mL (95% C.I.; 113.9, 398.4) P < 0.001. Average forced vital capacity (FVC) was reduced by 287.8 mL (95% C.I.; 134.9, 440.8) P < 0.001. In men a 100 μg/L increase in arsenic level was associated with a 45.0 mL decrease (95% C.I.; 6.2, 83.9) in FEV_1 (P = 0.02) and a 41.4 mL decrease (95% C.I.; -0.7, 83.5) in FVC (P = 0.054). Women participating in the study (N = 109) had a lower risk of developing skin lesions than men and exhibited few respiratory symptoms.

6.2 Chronic Toxicity to Infants and Children

The adverse effects of inorganic arsenic exposure reported in children include skin lesions, neurodevelopmental effects (IQ and related effects), lung disease expressed in later years, and reproductive effects (decreased birth weight, spontaneous abortion, neonatal death).

6.2.1 Skin Effects

As noted above Mazumder et al. (1998) observed a dose-response for arsenic-associated skin lesions in a cross-sectional survey of 7683 subjects in West Bengal, India. The study population was divided by age decades such that the effect on young children (≤ 9 yr) and adolescents (10-19 yr) could be analyzed separately. The prevalence of keratosis in females and males was 0.2 and 0.5 percent in young children and 1.0 and 1.7 percent in adolescents, respectively. The comparable values for hyperpigmentation were 1.7 and 2.0 percent and 2.2 and 3.5 percent, respectively. Overall 1149 young children and 1599

adolescents were surveyed. The low- to mid-dose quantal responses for combined skin lesions in young children using the mid points of the arsenic concentration ranges (μg/L) were: 25, 0/414; 75, 0/95; 125, 4/118; 175, 2/50; 275, 6/161; 425, 11/101. For the adolescents the comparable values were: 1/730; 2/147; 2/107; 7/110; 26/213; 9/58.

6.2.2 Neurodevelopmental Effects

The adverse effects of inorganic arsenic on the developing intellectual function of exposed children have been reported in several studies summarized in this section. While some of the studies have deficiencies, as a group they indicate that arsenic exposure, like lead exposure, presents a higher risk to children than adults. The neurodevelopmental endpoint has been selected by OEHHA as the critical effect for deriving 8-hour and chronic RELs for inorganic arsenic.

Calderon et al. (2001) conducted a cross-sectional study to examine the effects of chronic exposure to lead (Pb) and arsenic (As), and also nutrition, on the neuropsychological development of children. Two populations of children aged six to nine years (N = 41, 39) with differing As exposure levels (63 vs. 40 μg/g) but similar Pb exposures (8.9 vs. 9.7 μg Pb/dL blood, respectively) were compared using the Wechsler Intelligence Scale for Children (WISC) Revised Version for Mexico. After controlling for significant potential confounders verbal IQ was observed to decrease with increasing urinary arsenic ($P < 0.01$). Language, verbal comprehension and long-term memory also appeared to be adversely affected by increasing arsenic exposure (concepts and knowledge factors, $P < 0.05$ each). Blood lead was significantly associated with a decrease in attention (sequential factor, $P < 0.05$). However since blood lead is an imprecise measure of lead burden there could be some residual confounding in this study.

The relationship between arsenic exposure via drinking water and neurological development as indicated by intelligence (IQ) was assessed in Thailand (Siripitayakunkit et al., 1999) in 529 children aged six to nine years using a cross-sectional design. Arsenic levels in hair were used to assess exposure and the WISC test for children was used to assess IQ. The range of arsenic concentrations in hair was 0.48 to 26.94 μg/g (mean = 3.52, SD = 3.58). The mean IQ of the study was 90.44 (range 54 to 123). Most of the IQs were classified as average (45.7%) or dull normal (31.6%). Approximately 14% and 3% of the children were in the borderline and mental defective groups, respectively. The percentage of children in the average IQ group decreased significantly from 57 percent to 40 percent with increasing arsenic exposure. The percentage in the lower IQ group increased with increasing As (23% to 38%) and in the low IQ group (zero to six percent). In a comparison of IQ between children with As hair levels ≤ two ppm or > two ppm, arsenic was found to explain 14 percent of the variance in IQ after controlling for father's occupation, mother's intelligence score, and family income. Arsenic levels in hair above 2 ppm were associated with a 0.75-point decrease in IQ below the grand mean and As levels above 5 ppm with a two point decrease. Although the cross-sectional study design does not allow for establishment of the time precedence of exposure to arsenic, the investigators stated that the subjects of the study were born in a period of chronic arsenic poisoning and that this cohort has been continuously exposed since birth due to their non-mobility. The study suffers from small numbers of children exposed to low arsenic (hair

arsenic ≤ 1 ppm) so this group could not be compared to the high arsenic children. Also the possible exposure to chemical confounders like lead was not discussed.

In a parallel cross-sectional study (Siripitayakunkit et al., 2001) the 529 children (above) were subjected to the Motor-Free Visual Perception Test (MVPT) and the Visual-Motor Integration Test (VMI). The visual perception score of each child was compared with the score of children in a control sub-district of the same age. The cutoff point for poor perception was the mean minus one standard deviation (SD) in each age level. Among arsenic-exposed children, 21 percent had poor visual perception and 17.6 percent had poor VMI. The comparable values in the control population were 16.5 percent and 15.8 percent, respectively. Potential confounders were controlled by multiple classification analysis. Only five percent of the variance in visual perception of children was significantly explained by arsenic (P = 0.01). The grand mean perception score was 20.57 and the adjusted values at low, medium and high hair As were 20.92, 20.51, and 20.03, respectively. Alternatively, these authors did not find an effect of arsenic on visual-motor integration.

Like the study of IQ decrements noted above, this study has the advantage of associating an adverse effect in children with a metric of chronic arsenic exposure, hair arsenic concentration. Disadvantages include a limited level of reporting and possible confounding with exposure to other metals.

Tsai et al. (2003) performed a cross-sectional study of the effect of arsenic exposure on the development of cognitive function among adolescents. Forty-nine 13-year old students were divided into low and high exposure groups and were compared with 60 13-year old unexposed children. Four neurobehavioral tests were conducted: continuous performance test (CPT); symbol digit (SD); pattern memory (PM); and switching attention (SA). Exposure in terms of As concentration in drinking water averaged 0 (<0.15), 131.2, and 185.0 ppb for control and exposure groups, respectively. Average cumulative arsenic exposures were 0, 252.1, and 768.2 mg (e.g., 184.99 ppb x 1008.6 cm^3/d x 11.28 yr x 365 d/yr x 10^{-3}). Neurobehavioral analysis revealed significant dose-response effects of arsenic exposure on CPT (P = 0.005), PM (P = 0.009) and SA (P = 0.0001), but not on SD (P = 0.23). A multiple linear regression analysis of the dose-response relationship between cumulative arsenic exposure and neurobehavioral endpoints showed a strong arsenic effects for CPT (low exposure group, P = 0.001), PM (high exposure group, P = 0.003) and SA (high and low exposures, P = 0.0001). This study is limited by low numbers but seems in line with other findings of As-induced CNS effects. The authors note that "the central nervous system of child and adolescents might be more vulnerable than adult to neurotoxicant". Although no dose-response relationship between As exposure and nerve conduction velocities was observed, the authors could not exclude the possibility of peripheral nerve dysfunction.

Wasserman et al. (2004) conducted a cross-sectional study of intellectual function in 201 As-exposed 10-year old children in Bangladesh. Children's intellectual function was assessed with tests drawn from the Wechsler Intelligence Scale for Children version III including Verbal, Performance, and Full-Scale raw scores. Children provided urine for

arsenic and creatinine and blood samples for blood lead and hemoglobin measurements. After adjustment for sociodemographic covariates and waterborne levels of manganese (Mn), As in drinking water was associated with reduced intellectual function, in a dose-dependent manner. Children exposed to water arsenic of > 50 μg/L had significantly lower Performance and Full-Scale scores than did children with water As levels < 5.5 μg/L. Using the Full-Scale raw score, As water concentrations of 10 and 50 μg/L were associated with decrements of 3.8 and 6.4 points, respectively. The relationships between urinary arsenic concentration (μg As/g creatinine) and child intellectual function were not statistically significant but were in the expected (negative) direction (Full-Scale, P = 0.09; Performance, P = 0.14; Verbal, P = 0.11). Since there was no standard of intelligence for use in Bangladesh these decrements could not be directly equated with U.S. standard IQ points. However, "other simpler predictors of child intellectual function, such as maternal education and child height and head circumference, were significantly related to intellectual raw scores in the expected directions." In this study, as in others of this type exposure is inferred from water concentration.

6.2.3 Lung Effects

Smith et al. (1998) studied lung and urinary bladder cancer mortality in a region of northern Chile (Antofagasta) where the residents were exposed to arsenic in their drinking water. Arsenic levels ranged from a population weighted average of 570 μg/L between 1955 and 1969 to 100 μg/L by 1980. The mortality ratios (observed/expected deaths) for bladder, kidney, liver, and skin cancers, and all other cancers combined, were not related to age in either sex. However, lung cancer mortality ratios were particularly high in younger men aged 30-39 yr (SMR = 11.7, 95 percent C.I. 6.4-19.6, P < 0.001). Also observed was a decreasing trend in chronic obstructive pulmonary disease deaths (COPD), with higher rates among younger men, particularly those aged 30-39. Four COPD deaths were reported among men (0.8 expected), and six deaths among women (0.1 expected). These ten individuals who died of COPD would have been young children at the time of peak arsenic water levels in 1955-1970.

In a later study Smith et al. (2006) reported increased mortality from lung cancer and bronchiectasis in young adults following arsenic exposures *in utero* and in early childhood. For subjects born just before the high exposure period (1950-1957) and exposed in early childhood the SMR for bronchiectasis was 12.4 (95% C.I., 3.3-31.7; P < 0.001). For those born during the high exposure period (1958-1970) with likely *in utero* and early childhood exposure the SMR for bronchiectasis was 46.2 (C.I., 21.1-87.7; P < 0.001). The authors conclude that "exposure to arsenic in drinking water during early childhood or *in utero* has pronounced pulmonary effects, greatly increasing subsequent mortality in young adults form both malignant and nonmalignant lung disease."

Additional evidence supporting a link between childhood arsenic exposure and subsequent lung disease comes from autopsies of children in the affected area. The results of five autopsies of children, who died in 1968 and 1969 in Antofagasta and showed skin lesions and other evidence of arsenic poisoning, also showed lung abnormalities in four of the children. Two of these cases exhibited interstitial fibrosis (Rosenberg, 1974). Also, a survey of 144 children in Antofagasta with skin pigmentation

due to arsenic exposure reported a history of bronchopulmonary disease 2.5-fold more frequent than children with normal skin (15.9 vs. 6.2 percent, respectively) (Borgono et al., 1977).

6.2.4 Reproductive and Other Effects

Chronic exposure to arsenic has been associated with decreased birth weight and an increased rate of spontaneous abortion in female smelter workers. However, this association is confounded by the presence of other toxicants in the smelting process, including lead (Nordstrom et al., 1979). Anemia and leukopenia have been reported in infants ingesting approximately 3.5 mg As/day in contaminated milk over a period of 33 days (Hammamoto, 1955).

Premature birth and subsequent neonatal death was reported in a single individual following ingestion of arsenic (Lugo et al., 1969).

Ihrig et al. (1998) conducted a hospital-based case-control study of stillbirths and environmental arsenic exposure using an atmospheric dispersion model linked to a geographical information system. They collected data on 119 cases and 267 controls in a central Texas area including a facility with 60-year history of arsenic-based agricultural product manufacture. Four exposure groups were categorized (0; < 10 ng/m^3; 10-100 ng/m^3; and > 100 ng/m^3). For the period 1983-93 they fit a conditional logistic regression model including maternal age, race/ethnicity, parity, income group, exposure as a categorical variable, and exposure-race/ethnicity interaction. Effects were only seen in the Hispanic group with the medium exposure group having a prevalence odds ratio and 95% confidence interval of 1.9 (0.5-6.6) and the high exposure group 8.4 (1.4-50.1). The authors postulate a possible influence of a genetic polymorphism affecting folate metabolism in Hispanic populations possibly leading to increased neural tube defects and stillbirths. Small numbers limits this study; for example, there were only seven cases in the high exposure group and five of these were Hispanic.

Von Ehrenstein et al. (2006) studied pregnancy outcomes, infant mortality, and arsenic exposure via drinking water in West Bengal, India. The reproductive histories of 202 women were reviewed including measurements of 409 drinking water wells. The total number of pregnancies was 660 and the number of live births plus stillbirths was 558. Odds ratios for spontaneous abortion, stillbirth, neonatal mortality (death in the first month) and infant mortality (death in the first year) were estimated by logistic regression. Exposure to arsenic concentrations ≥ 200 μg/L during pregnancy was associated with a six-fold increased risk of stillbirth after adjustment for potential confounders (OR = 6.07; 95% C.I. 1.24-24.0, p = 0.01). The odds ratio for neonatal death was 2.81 (95% C.I. 0.73-10.8). No significant associations were found for arsenic exposure and spontaneous abortion (OR = 1.01; 95% C.I. 0.38-2.70) or overall infant mortality (OR = 1.33; 95% C.I. 0.43-4.04). Arsenic related skin lesions were observed in12 women who had increased risk of stillbirth (OR = 13.1; 95% C.I. 3.17-54.0).

6.3 Subchronic and Chronic Toxicity to Experimental Animals

Female albino rats (20 per group) were exposed to 0, 1.3, 4.9, or 60.7 μg As_2O_3/m^3 as aerosol continuously for 3 months (Rozenshtein, 1970). Decreased whole blood sulfhydryl group content, histological changes in the brain, bronchi, and liver, changes in conditioned reflexes, and changes in chronaxy ratio were observed in both the high- and mid-dose groups. Among animals in the high dose group, eosinophilia, decreased blood cholinesterase activity, decreased serum sulfhydryl content, and increased blood pyruvic acid were observed. No significant changes were observed in the low-dose group.

Male mice (8-10 per group) were exposed to 0, 0.5, 2.0, or 10.0 ppm sodium arsenite in drinking water for 3 weeks followed by a 28-day recovery period (Blakley et al., 1980). The primary immune response of the spleen (as indicated by changes in IgM-production assayed by plaque-formation) was suppressed at all dose levels. The secondary immune response was also suppressed at all dose levels as indicated by a decrease in the number of IgG producing cells.

Male Sprague-Dawley rats (7-28 per group) were exposed to 0, 40, 85, or 125 ppm sodium arsenate in drinking water for 6 weeks (Brown et al., 1976). Rats from all arsenic exposed groups showed increased relative kidney weights, decreased renal mitochondrial respiration, and ultrastructural changes to the kidney.

Male ddY mice (number not stated) received 0, 3, or 10 mg As_2O_3/kg/day orally for 14 days and were examined for changes in concentrations of monoamine-related substances in various brain regions and for changes in locomotor activity (Itoh et al., 1990). Locomotor activity was increased in the low-dose group and decreased in the high-dose group. Several monoamine-related compounds were altered in both dose groups in the cerebral cortex, hippocampus, hypothalamus, and corpus striatum. The study indicates an effect of arsenite on brain chemistry but is inconclusive with respect to dose response.

Male and female Wistar rats (7-10 per group) were treated from age 2 to 60 days by oral gavage with daily administration of 0 or 5 mg As/kg body weight (as sodium arsenate) (Nagaraja and Desiraju, 1993; 1994). After 160 days, body weights, brain weights, and food consumption were decreased in the arsenic exposed group. Acetylcholinesterase (AChE) and GAD activity and GABA levels were decreased in the hypothalamus, brain stem, and cerebellum during the exposure period; all but AChE activity returned to normal during the post-exposure period. Changes in operant conditioning were also observed among the exposed animals.

Female Holtzman rats (>5 per group) were treated with 0, 100, 500, 1000, 2000, or 5000 ppm As_2O_3 in feed for 15 days (Wagstaff, 1978). Hexibarbitone sleeping time was altered in all arsenic exposed groups. Body weight and feed consumption were decreased among animals in the groups exposed to ≥ 500 ppm As_2O_3. Clinical signs of toxicity observed among arsenic exposed animals included roughened hair, diarrhea, and decreased physical activity.

Male Sprague-Dawley rats and C57 black mice (12 per group) were treated with 0, 20, 40, or 85 ppm sodium arsenate in drinking water for up to 6 weeks (Woods and Fowler, 1978). Among arsenic exposed rats, heme synthetase activity was decreased in all exposed groups. Among animals exposed to ≥ 40 ppm sodium arsenate, hepatic ALA synthetase activity was decreased and urinary uroporphyrin and coproporphyrin were increased. Among exposed mice, heme synthetase activity was decreased and uroporphyrinogen I synthetase activity was increased in all exposed groups. Among animals exposed to ≥ 40 ppm sodium arsenate, urinary uroporphyrin and coproporphyrin were increased.

Administration of 3.7 mg As_2O_3/kg/day to Rhesus monkeys for 12 months did not result in any neurologic change detectable by an EEG (Heywood and Sortwell, 1979). Two of the 7 animals exposed to this concentration died before the conclusion of the 52-week period. Of the surviving animals, two were retained for a 52-week recovery period after which they were sacrificed and necropsied. No significant changes in organ weights or gross appearance were noted.

7. Toxicity of Arsine

7.1 Toxicity to Adult Humans

Numerous case reports of accidental arsine poisoning exist in the literature, but reliable estimates of concentrations following acute human intoxication do not exist. This is due in large part to the insidious nature of arsine toxicity - arsine is a colorless gas, has a mild odor at low concentrations, produces no mucous membrane irritation, and usually results in delayed symptoms of toxicity (Klimecki and Carter, 1995). In mammalian systems, arsine primarily targets the erythrocyte and causes hemolysis and methemoglobinemia with acute exposure (NRC, 1984). Jaundice, hemoglobinuria, anuria, hepatic and renal damage, anoxia, and anemia are secondary effects resulting from hemolysis. Before the advent of dialysis, there were no reports of patients surviving if renal failure developed (Buchanan, 1962). Other acute symptoms reported include headache, weakness, dizziness, dyspnea, nausea, vomiting, diarrhea, and abdominal cramping (Klimecki and Carter, 1995). Central and peripheral nervous systems may be affected by acute arsine exposure, leading to agitation, disorientation, hallucinations, psychopathologic abnormalities, and peripheral nerve degeneration (Frank, 1976; Klimecki and Carter, 1995). The psychopathologic and peripheral abnormalities are thought to be secondary to the conversion of arsine to arsenate or arsenite. The first signs and symptoms of toxicity, hemoglobinuria and/or nausea, are usually delayed 2 to 24 hours following exposure (Kleinfeld, 1980).

A case report documents hemolytic anemia, hematuria, and renal failure following intermittent exposure to arsine gas over 2.5 hours (Parish et al., 1979). Symptoms of gastrointestinal distress, headache, and malaise were also reported following this exposure. The concentration of arsine gas sampled 3 days after exposure was 0.1 ppm (0.3 mg/m^3), but the concentration at the time of poisoning was unknown. Another typical accidental poisoning resulted when 2 men were exposed to arsine gas in a metal smelting works (Coles et al., 1969). Symptoms included nausea, vomiting, red urine, generalized

aching, shivering, epigastric pain, and jaundice. However, the more severely affected worker developed symptoms within 1 hour of exposure while the other did not develop symptoms for 24 hours. The more severely affected worker developed acute renal failure that required peritoneal dialysis.

In an occupational study, the highest average concentration of arsine recorded in a battery formation area of a battery manufacturing plant was 20.6 µg/m³ (0.006 ppm) (Landrigan et al., 1982). Elevated levels of urinary arsenic were observed in some workers but effects on the hematopoietic system were apparently not examined.

A study by Williams et al. (1981) collected personal and area air samples after 2 workers exhibited symptoms of arsine poisoning while restoring a large 19th century painting. Symptoms included headaches, nausea, weakness, vomiting, and red urine. The blank-corrected air concentration of arsine ranged from 0.010 to 0.067 mg/m³. While these concentrations are below the OSHA PEL 8-hour TWA of 0.2 mg/m³, the results may indicate that these workers are sensitive responders or that humans in general may be more sensitive to the effects of arsine than experimental animals. However, the air samples may not represent the actual concentration of arsine that caused the symptoms of poisoning in the workers since the workplace air was not analyzed for arsine until after symptoms were reported. The study also notes that 'appreciable concentrations' of lead and arsenic were found in the workplace air.

No studies were identified addressing the chronic toxicity of arsine in humans.

7.2 Toxicity to Infants and Children

No studies were identified allowing quantitative assessment of arsine toxicity in infants and children. Arsine's mode of toxic action is not completely understood but appears to involve binding to erythrocyte sulfhydryl groups followed by intracellular ion loss and hemolysis (Rael et al., 2000). Clinical treatment of arsine poisoning usually involves exchange transfusion. It seems plausible that infants and children would be more sensitive to the irreversible hematotoxicity of arsine than adults due to their greater breathing rate per unit body weight.

7.3 Toxicity to Experimental Animals

A number of studies were reviewed to understand the time-concentration relationship of arsine lethality. The most complete and relevant study was the IRDC (1985), which allowed determination of 1% and 5% lethality benchmark doses for exposure durations of 0.5 to 4 hours in rats. The most important acute non-lethal effects noted were hemolysis and reticulocytosis (Peterson and Bhattacharyya, 1985). Longer term effects of arsine also involved significant changes in hematological parameters (hemoglobin and mean corpuscular volume) (Blair, 1990).

LC_{50} values reported by Gates (1946) are as follows: 120-210 ppm (380-670 mg/m³) for 10 minutes in rats, 110 ppm (350 mg/m³) for 30 minutes in dogs (equivalent to 190 ppm (610 mg/m³) for 10 minutes), and 200-300 ppm (640-960 mg/m³) for 10 minutes in rabbits. An LC_{50} in mice was reported as 31 ppm (99 mg/m³) for a 50-minute

exposure (Levvy, 1947). The survival time of the fatalities (4 days) was reported to be more or less independent of exposure concentration (2500 mg/m³ to 25 mg/m³) and exposure duration.

The study by Levvy (1947) varied exposure durations for each given concentration of arsine. Because the mortality data were not presented in conventional form by the standard LC_{50} method, the data were normalized to a 1-hour exposure using the modified form of Haber's equation (as described in Section 5.7.1 of the TSD):

$$C^n \times T = K,$$

where C = concentration, T = time, K = a constant determined at a given C, T and the exponent n is a constant determined experimentally. The exponent "n" of 1.8 was determined by varying the term n in a log-normal probit analysis (Crump and Howe, 1983; Crump, 1984) until the lowest chi-square value was achieved. Fifty-four data points were used to estimate the exponent n because these points were of sufficient duration ($\geq$ 5 minutes) and resulted in the best chi-square fit for the line and obvious heterogeneity (Table 7.3.1). This relationship indicates that the toxicity of arsine varies approximately with the product of the square of concentration times time rather that simply concentration times time.

Table 7.3.1 Arsine Mortality in Mice: Results from Levvy (1947) and 1-Hour Adjusted Concentrations Using Haber's Equation ($C^n \times T = K$, where n = 1.8).

Concentration (ppm)	Exposure Duration (min)	Mortality (no. died/total)	1-Hour Adjusted Concentration (ppm)
157*	10	30/30	58
	5	28/30	39
	2.5	17/30	27
	1.7	0/30	22
78.4*	15	21/30	36
	9	10/30	27
31.4	70	30/30	34
	50	15/30	28

** Shaded rows include data used for determination of the ED_{05} and BD_{05}*

Craig and Frye (1988) reported a 4-hour LC_{50} of 42.6 ppm in rats. However, when the rats were separated by sex for statistical purposes, there was slightly greater mortality among females than males (38.9 ppm LC_{50} for females vs. 46.8 ppm LC_{50} for males). No abnormalities were seen at necropsy except red discharge from nose, mouth, and genitalia at the higher concentrations. A concentration-related suppression of body weight gain was observed during the first week of the 14-day post-observation period.

The most comprehensive arsine lethality study was undertaken by IRDC (1985). LC_{50}s of 240, 178, and 45 ppm were determined in rats (10 rats/sex/group) for 30 minute, 1 hour, and 4-hour exposures, respectively. Deaths generally occurred within 3 days

following 30-minute exposure to arsine. As in the previous study (Craig and Frye, 1988), there was slightly greater mortality in females than males. Adverse effects noted during exposure included dyspnea, while effects noted post-exposure included a concentration-related increase in hematuria, dark material around the head or the anogenital area, and pallor of ears, eyes, and feet. The higher concentrations resulted in weight loss immediately following exposure, suppressed weight gain during the first week and compensatory weight gains during the second week post-exposure. Necropsy on animals that died showed red, yellow or orange fluid in the bladder, stomach, or intestine, and discoloration of the kidneys, lungs, and liver.

Data in the IRDC (1985) report were used to determine the exponent "n" in the equation C^n x T = K. This was done by varying the term n in a log-normal probit analysis (Crump, 1984; Crump and Howe, 1983) until the lowest chi-square value was achieved. The value of "n" for extrapolation to 1-hour exposure was dependent on exposure duration. For extrapolation from 30 minutes to 1-hour exposure, n = 2.2; for extrapolation from 4-hours to 1-hour exposure, n = 1.0.

Table 7.3.2 contains the studies which provided adequate raw mortality data from which a maximum likelihood estimate corresponding to 5% lethality (ED_{05}) and benchmark dose at the 95% lower confidence interval of the ED_{05} and ED_{01} (BD_{05} and BD_{01}, respectively) could be determined.

Table 7.3.2 Animal Lethality Benchmark Dose Determinations in ppm for Arsine

Reference	Species	Exposure Time (min)	LC_{50} 60 min[1]	ED_{05} 60 min[1]	BD_{05} 60 min[1]	BD_{01} 60 min[1]
IRDC, 1985	rat	30	175	120	105	86
	rat	60	178	112	88	66
	rat	240	181	118	101	80
Craig and Frye, 1988	rat	240	170	125	102	84
Levvy, 1947	mice	varied[2]	29	20	16	13

[1] Exposure time was extrapolated to 60 minutes, if needed, using a modification of Haber's equation (C^n * T = K). For rats, n = 2.2 for extrapolation from 30 minutes to 1-hour, or n = 1.0 for extrapolation from 4 hours to 1-hour; for mice, n = 1.8.

[2] Lethality data for 5 exposure durations were pooled and normalized to a 1-hour exposure using the equation C^n x T = K (see Table 1).

In other experimental animal studies, a reduction in hematocrit as a function of arsine concentration was observed in mice following a 1-hour exposure (Peterson and Bhattacharyya, 1985). A LOAEL of 9 ppm (29 mg/m³) and a NOAEL of 5 ppm (16 mg/m³) were reported. The demarcation between the NOAEL and LOAEL for this non-lethal effect was well defined, not only among the exposure groups (5 ppm vs. 9 ppm), but also among individual mice in each exposure group (Peterson, 1990). Hematologic recovery of the surviving mice was gradual but nearly complete within 11 days after exposure (Peterson and Bhattacharyya, 1985). The study also reported a

NOAEL of 15 ppm (100% survival) and LOAEL of 26 ppm (100% lethality) for lethality.

A continuous benchmark dose analysis of these data was performed. The full data set on hematocrit reduction 24 hours after exposure gave a BMD_{05} of 7.81 ppm and a $BMDL_{05}$ of 5.2 ppm (quadratic continuous model fit P= 0.16). The only other data sets that were adequately fit were the 24 hour increase in reticulocyte count (%) with the 11 and 26 ppm outliers removed (power continuous model, P = 0.50) and the 5 days values with the 9 ppm outlier removed (cubic continuous model, AIC = 61.8). Several response levels were evaluated including 25% relative, 1 and 2 % absolute increases and 1 and 2 standard deviations. The latter SD levels were closest to the minimal significant increase levels and exceeded the control plus one control SD values of 0.88 ppm (24 hr) and 2.0 (5 days). For a 1 SD response level at 24 hours the BMD_{1SD} = 3.29 ppm and the $BMDL_{1SD}$ = 2.17 ppm. The values for 2SD were BMD_{2SD} = 4.69 ppm and $BMDL_{2SD}$ = 3.50 ppm. For the 5 days data set the BMD_{2SD} = 4.32 ppm and the $BMDL_{2SD}$ = 2.70 ppm. Reticulocytosis may be a more sensitive indicator of adverse hematologic effects of arsine exposure than hematocrit reduction.

A subchronic study in male and female rats and female mice (Fowler et al., 1989) supports the sharp demarcation in dose-response noted by Peterson and Bhattacharyya (1985). All treatment groups exposed to arsine (6 hr/day, 5 days/week) at concentrations of 10 ppm and above showed 100 percent mortality within 4 days while those exposed to 5 ppm or less showed no mortality or overt signs of toxicity. Other effects observed included a dose-related increase in spleen weight and a slight increase in liver weight. Blood samples taken at necropsy showed a slight dose-related decrease in hematocrit and a marked dose-related increase in the activity of red blood cell ALAD (δ-aminolevulinic acid dehydratase).

In a 90-day study, male and female mice were exposed to 0, 0.025, 0.5, and 2.5 ppm arsine gas for 6 hours/day, 5 days/week (Blair et al., 1990). After 5, 15, and 90 days, blood was collected for hematologic analysis. Exposure to 2.5 ppm had significant effects on all hematological parameters for nearly the entire exposure period, while 0.5 ppm caused only a few significant changes in hematological parameters at day 90 of exposure (decreased hemoglobin in males and increased MCV in females). Exposure to 0.025 ppm was without effect.

A continuous benchmark dose analysis was performed on the data sets of Blair et al. 1990. Adequate fits to the hematocrit data were obtained with the linear and quadratic models with $BMDL_{025}$ (relative risk) values ranging from 0.128 to 0.894 ppm (P values for model fits of 0.11 to 0.96). Absolute reticulocyte count increases gave continuous $BMDL_{10}$'s ranging from 0.22 to 0.68 ppm with linear and quadratic models (P values of 0.31 to 0.99). However, due to the poor dose spacing, essentially a missing dose level between 0.025 and 0.5 ppm, these results are considered inconclusive in determining an alternative NOAEL to 0.025 ppm.

7.4 Developmental and Reproductive Toxicity

In an unpublished study, workers in one semiconductor plant were reported to have a 39% rate of miscarriage, almost twice the national average (Sanger, 1987). Workers were exposed to unidentified levels of arsine gas, but other possible exposures were not identified.

A developmental toxicity study exposed pregnant rats and mice to 0.025, 0.5, or 2.5 ppm (0.079, 1.5, or 7.9 mg/m³) arsine for 6 hours per day on gestation days 6 through 15 (Morrissey et al., 1990). The rats exposed to 2.5 ppm exhibited a significant increase in fetal body weight, but no other endpoints of developmental toxicity were observed. The incidence of malformations observed in arsine exposed mice at 0.025 ppm (exencephaly) and at 2.5 ppm (unfused eyelids) was not significantly different from control mice.

8. Derivation of Reference Exposure Levels

8.1 Acute Reference Exposure Level for Inorganic Arsenic

Study	Nagymajtenyi et al., 1985
Study population	pregnant mice
Exposure method	maternal inhalation exposure
Exposure continuity	
Exposure duration	4 hours per day on gestation days 9, 10, 11, and 12
Critical effects	decreased fetal weight
LOAEL	0.26 mg/m³ As_2O_3 (0.197 mg As/m³)
NOAEL	not observed
Benchmark concentration	not derived
Time-adjusted exposure	n/a
Human Equivalent Concentration	n/a
LOAEL uncertainty factor (UF_L)	10 (no NOAEL)
Subchronic uncertainty factor (UFs)	n/a
Interspecies Uncertainty Factor	
Toxicokinetic (UF_{A-k})	√10 (animal study)
Toxicodynamic (UF_{A-d})	√10 (animal study)
Intraspecies Uncertainty Factor	
Toxicokinetic (UF_{H-k})	√10 (remaining interindividual variation: study considered effects on fetus or infant)
Toxicodynamic (UF_{H-d})	√10 (interindividual variation)
Cumulative uncertainty factor	1,000
Reference Exposure Level	**0.0002 mg As/m³ (0.20 µg As/m³,)**

Acute Reference Exposure Levels are levels at which intermittent one-hour exposures are not expected to result in adverse health effects (see Section 5 in the Technical Support Document). The most appropriate study for the basis of an acute REL for arsenic is

Nagymajtenyi et al. (1985). This study was selected since it measured a sensitive toxicological endpoint with a relevant route of exposure, and the experimental design and reporting were considered adequate (as specified in the Non-cancer Risk Assessment technical support document, Section 4.1.1). It involved a significant number of animals exposed by inhalation to three dose levels plus a control. Unfortunately, no NOAEL was obtained. However, a significant dose-related reduction in fetal weight and increased incidences of intrauterine growth retardation, skeletal malformations, and hepatocellular chromosomal aberrations were observed in mice following maternal inhalation exposure to 200 μg As/m^3 (260 μg As_2O_3/m^3) for 4 hours on gestation days 9, 10, 11, and 12 ($p<0.05$). The most sensitive effect, decreased fetal weight, was observed at 200 μg As/m^3, so 200 μg As/m^3 was taken as a LOAEL. Maternal toxicity data were not reported. This study is used as the basis of the acute REL:

0.2 mg/m^3/1000 = 0.0002 mg/m^3 = 0.2 μg As/m^3 (equivalent to 0.065 ppb arsine gas)

No temporal adjustment was made for the critical study since the critical period of exposure for a developmental effect may be very short relative to the study duration (OEHHA, 2007). The study concentration with appropriate uncertainty factors is a "not to exceed" value. An uncertainty factor of 10 (UF_L) was used to account for the lack of a no observed adverse effect level (NOAEL). A second uncertainty factor of 10 was used to account for interspecies differences between the test species and humans. This factor is the product of two components addressing pharmacokinetic (UF_{A-k}) and pharmacodynamic (UF_{A-d}) differences, each assumed to be the $\sqrt{10}$. A final uncertainty factor of 10 was applied to address human interindividual differences in pharmacokinetics (UF_{H-k}) and pharmacodynamics (UF_{H-d}) also assumed to be $\sqrt{10}$ each. The overall uncertainty of extrapolating from 4-hour exposures in mice (LOAEL) to no anticipated effects in humans is 1000 as noted in table above and the calculation of the acute REL. The rationale for the choice and value of uncertainty factors used by OEHHA is provided in the Non-cancer Risk Assessment technical support document (Section 4.4.3).

Inorganic arsenic (oxides) are listed as developmental toxicants under the California Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65). The studies reviewed in this document support the conclusion that exposure to inorganic arsenic may affect fetal weight, spontaneous abortion, neonatal death and postnatal neurological development.

In humans, the logarithm of infant mortality (death) increases linearly as birth weight decreases from 3500 to 1000 grams (Hogue et al., 1987; Rees and Hattis, 1994). This log-linear relationship exists on both sides of the weight (2500 g) conventionally used as a cutoff defining low birth weight. There is no evidence for a threshold. Thus any reduction in fetal weight is a cause for concern since it increases mortality. In the absence of certainty, OEHHA takes the health protective approach that the reduced weight effect in the animal fetuses may be biologically significant, particularly when viewed from a population perspective.

8.2 Inorganic Arsenic 8-Hour Reference Exposure Level

The 8-hour Reference Exposure Level is a concentration at or below which adverse noncancer health effects would not be anticipated for repeated 8-hour exposures which might include daily occupational, in-home or in-school exposures. (see Section 6 in the Technical Support Document).

Due to the possibility of repeated exposure and the relatively slow clearance of arsenic compounds, the 8-hour REL is taken to be equivalent to the chronic REL. The half-life of the initial exponential phase of excretion of arsenic after a single dose is typically between one and two days, but there are also several much slower excretion processes. So a single exposure to arsenic would take several days to be cleared, mainly via urinary metabolites. Repeated exposures can significantly prolong the clearance of arsenic as the internal dose accumulates, so that in terms of internal dosimetry it would be difficult to distinguish repeated periodic exposure from chronic exposure scenarios. An individual exposed daily via air and/or drinking water might show very similar urinary arsenic excretion to another individual exposed only periodically at work, school etc.

8.3 Inorganic Arsenic Chronic Reference Exposure Level

Study	Wasserman et al. (2004); Tsai et al. (2003)
Study population	201 children 10 years of age
Exposure method	drinking water
Exposure continuity	continuous
Exposure duration	9.5 to 10.5 years
Critical effects	Decrease in intellectual function, adverse effects on neurobehavioral development
LOAEL	0.23 µg As/m^3 based on est. LOAEL of 2.27 µg/L (Wasserman et al., 2004; see Section 8.3.1.1)
NOAEL	not observed
Benchmark concentration	not derived
Time-adjusted exposure	117.8 µg As/L (0.094 to 790 µg As/L)
Human DAF concentration	
LOAEL uncertainty factor (UF_L)	3 (LOAEL estimated by quantitative analysis of study data)
Subchronic uncertainty factor (UFs)	1 (default: duration >8% of lifetime)
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	1 (default: human study)
Toxicodynamic (UF_{A-d})	1 (default: human study)
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	√10 (remaining interindividual variation: study considered effects on 10 year-old but not infant)
Toxicodynamic (UF_{H-d})	√10 (default, interindividual variation)
Cumulative uncertainty factor	30
Inhalation Reference Exposure Level	**0.015 µg As/m^3**
Oral Reference Exposure Level	0.0035 µg/kg-d

The chronic Reference Exposure Level is a concentration at which adverse noncancer health effects would not be expected from chronic exposures (see Section 7 in the Technical Support Document).

8.3.1 Review and Analysis of Studies Supporting a Chronic REL

8.3.1.1 Child Based Values

A number of studies have indicated potentially greater toxicity of arsenic exposure during childhood (see below). Although some PBPK modeling has been applied to inorganic arsenic and its methyl metabolites, the modes of toxic action and relevant internal dosimetry are not sufficiently understood at present to use this modeling directly in REL development. In this section we compare quantitative analyses of dose-responses and LOAELs in key studies involving arsenic exposures in children. Health protective exposure levels derived from these analyses will be compared with similar analyses from studies in adults in the following section.

The study of Wasserman et al. (2004) indicated a dose-response of decreasing Full-Scale intellectual function raw scores with increasing drinking water arsenic exposure in 10-year olds. The values in their Fig.2 give an exact fit to a quadratic model ($Y = Y_0 + aX + bX^2$; Y_0 intercept = 0, a = -0.443, b = 0.0063, $R^2 = 1.0$) with a low dose slope of –0.44 points/μg/L. Assuming an adverse effect level of one point loss, then the corresponding arsenic concentration can be calculated as:

$$-1\text{point}/-0.44\ \text{point}/\mu\text{g/L} = 2.27\ \mu\text{g/L}.$$

This level might be equivalent to a LOAEL. Further, assuming water intake of 1 Liter/day (L/d) and essentially complete intestinal absorption, this can be converted to an intake of 2.3 μg/d. If we assume a drinking water intake based on the 95% upper confidence level (UCL) for U.S. children aged 1 to 10 years of 1564 mL/day the intake would be somewhat higher at 3.6 μg/d (OEHHA, 2000; Table 8.3). Since 10-year old males would inhale about 9.9 m^3/d (OEHHA, 2000), if airborne arsenic were 100% absorbed, this oral effect level would be equivalent to an inhalation level of 2.3 μg/day/9.9 m^3/day = 0.23 μg/m^3. Assuming a more realistic inhalation absorption of 50 % would give a value of 0.46 μg/m^3. Applying a 3-fold UF for an estimated LOAEL based on a quantitative dose response analysis (a higher value would be used without a dose response analysis) and 10-fold for inter-individual variation since only 10-year olds were studied, a health protective air concentration of 0.015 μg/m^3 can be calculated. An oral value based on the average study body weight of 21.9 kg and 100% oral absorption would be 2.3 μg/d/21.9 kg = 0.105 μg/kg-day. Applying the same overall uncertainty factor of 30 the oral health protective value would be 0.105 μg/kg-day/30 = 0.0035 μg/kg-day.

The data of Tsai *et al*. (2003) for 13 year old children gave dose response relationships for arsenic exposure metrics of ppb As in drinking water and cumulative arsenic intake (mg) vs. the pattern memory (PM) and switching attention (SA) endpoints (ms). A continuous benchmark response analysis for ppb As vs. ms test duration was conducted

for PM (BMD_{05} = 49.75; $BMDL_{05}$ = 31.2 ppb) and SA (BMD_{05} = 28.81; $BMDL_{05}$ = 19.73 ppb) both using a linear model. For cumulative As intake the PM endpoint data were similarly fit by a linear model (BMD_{05} = 194.1; $BMDL_{05}$ = 122.7 mg) and the SA data by a polynomial (quadratic) model (BMD_{05} = 39.1; $BMDL_{05}$ = 25.4 mg; see Fig. 1). The SA endpoint appears to be the most sensitive. Based on the SA $BMDL_{05}$ of 19.7 ppb and 1 L/d drinking water intake a minimum effect level of 19.7 μg/d is estimated. If we assume a drinking water intake, based on the 95% UCL for U.S. children aged 11 to 19 years of 2.143 L/d the intake would be 2-fold higher at 42.2 μg/day (OEHHA, 2000; Table 8.3). Using uncertainty factors of 10 for interindividual variation and 3 for extrapolation from a minimum to a no effect level, a health protective intake of 19.7 μg/d/30 = 0.658 μg/d is calculated. Assuming inhalation of 10 m^3/d and 50 % absorption (default) this value can be converted to an inhalation value of 0.658 μg/day/(0.50 x 10 m^3/day) = 1.32 μg/m^3. Using the SA cumulative $BMDL_{05}$ of 25.4 mg As and 10 years exposure, an effect level of 25.4 mg/(10 yr x 365days/yr) = 6.96 μg/day is calculated. Using the same assumptions and UFs as above, an inhalation value of 0.044 μg/m^3 can be derived based on As concentration. The cumulative dose metric is a more accurate estimate of arsenic exposure than As water concentration, so the value of 0.046 μg/m^3 or 0.05 μg/m^3 (rounded) is preferred over the concentration based value. An oral value based on an average body weight for a 13-14 year old child (OEHHA, 2000) of 50 kg is 6.96 μg/day/50 kg = 0.139 μg/kg-d. Applying the same overall uncertainty factor of 30 would give 0.139 μg/kg-day/30 = 0.0046 μg/kg-day.

The quantal responses for skin lesions in young children (≤ 9 yr) and adolescents (10-19 yr) from Mazumder et al. (1998) were subjected to benchmark dose analysis. For young children, the quantal linear model adequately fit the data (X^2 = 6.1, P = 0.30) with a BMD_{01} = 54.4 μg/L and a $BMDL_{01}$ = 39.3 μg/L. For adolescents, the best fitting model was the log probit (X^2 = 0.77, P = 0.68) with a BMD_{01} = 77.3 μg/L and a $BMDL_{01}$ = 47.4 μg/L. These values are similar to the analysis of all age groups combined (above) and application of a 10-fold UF for intraspecies variation seems adequate for these data. Thus the health protective intake for children for skin effects would be in the range of 3.9 to 4.7 μg/d for one Liter/day water intake. For conversion to inhalation equivalent, young children are assumed to inhale 9.9 m^3/day and adolescents 14 m^3/day (OEHHA, 2000). It is further assumed that 50 percent of inhaled arsenic is absorbed via the pulmonary and gastro-intestinal routes. The resulting health protective values would be 0.68 to 0.79 μg/m^3.

A study in Thailand (Siripitayakunkit et al., 1999) related drinking water arsenic exposure, indicated by hair arsenic, to IQ in 529 six to nine year old children. A continuous benchmark dose response analysis of this data set gave a BMD_{05} = 0.035 μg As/g hair and $BMDL_{05}$ = 0.0155 μg As/g (polynomial model). A slope of –3.2 IQ points/μg/g was derived from the $BMDL_{05}$. Using the conversion factor of 0.01μg As/g hair/μg As/Liter of water (Kurttio et al. 1998), a decrease of 1 IQ point would be equivalent to chronic consumption of 30 μg As/L water (OEHHA, 2004). At one liter/day water consumption the 30 μg/d value is over an order of magnitude higher than the analogous estimate indicated by the Wasserman et al. (2004) study above. An

inhalation value was derived as above: 30 μg/day/(10 m^3/day x 0.50 x 30UF) = 0.20 μg/m^3.

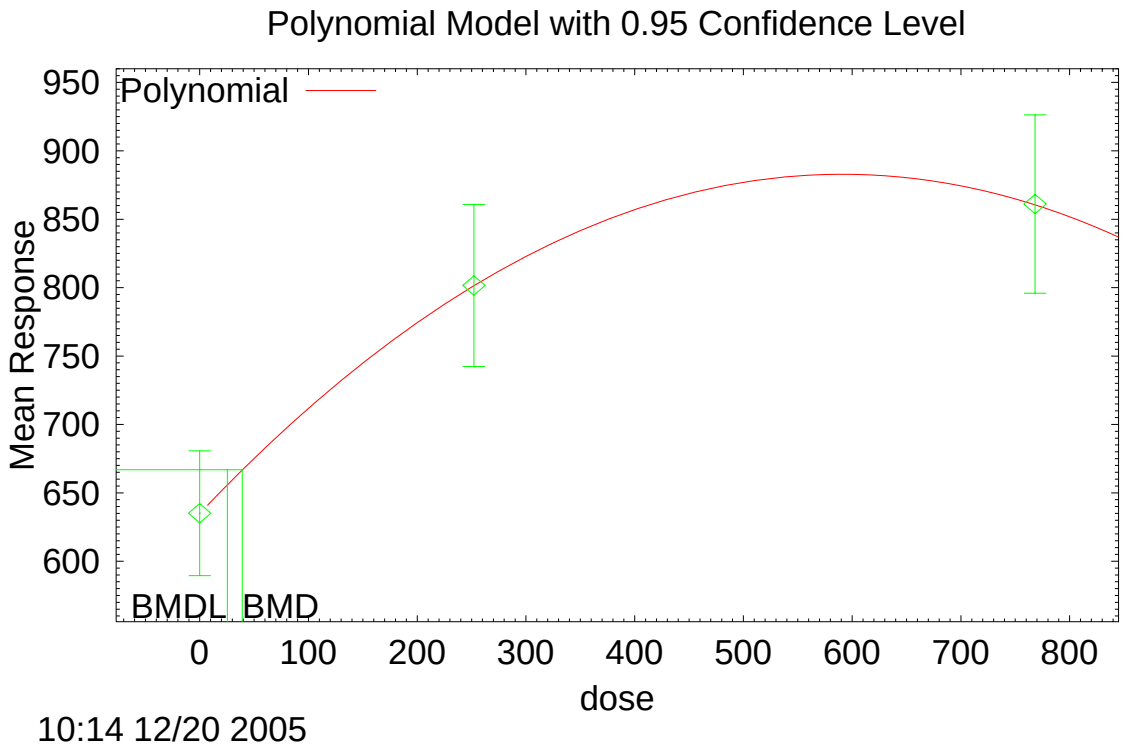
The visual perception data from Siripitayakunkit et al. (2001) was subjected to continuous benchmark dose analysis. The $BMDL_{035}$ of 2.40 μg/g hair (polynomial model) was near the low level mean minus one SD score (20.5), presumably an adverse effect level on visual perception as defined by the authors. The linear model gave a higher value (3.69 μg/g) but did not fit the data as well in the low exposure range. Using the conversion factor above, one liter per day water consumption, and a 30-fold cumulative UF results in a presumptive health protective intake of 8 μg/d for this endpoint (2.40 μg/g ÷ 0.01 μg/g/μg/Liter x 1 Liter/day ÷ 30UF = 8.00 μg/d). An inhalation value was derived as above: 8.0 μg/day/(10 m^3/day x 0.50) = 1.6 μg/m^3.

Chronic arsenic exposure appears to have adverse effects on intellectual development and visual perception in children. While the quantitation of these effects and the toxicological significance of the criteria selected are somewhat uncertain, OEHHA thinks they are sufficient to support a cREL. It is uncertain whether neurological effects are the most sensitive caused by chronic arsenic exposure in children. Additional studies in exposed children are needed to adequately quantify adverse effects. The values above are summarized in Table 8.3.1. The child-based values range from 0.015 to1.6 μg/m^3. The geometric mean of the three cognitive endpoint values is 0.053 μg/m^3.

Table 8.3.1. Inhalation Values Derived from Human Child Studies

Study	Toxic Endpoint	Criterion	Value	Derived cREL, μg/m^3
Wasserman et al. (2004)	Intellectual function	One point loss	2.27 μg/d	0.015
Siripitayakunkit et al. (1999)	IQ	One point loss	-3.2 IQ/μg/g hair As	0.20
Siripitayakunkit et al. (2001)	Visual perception loss	LOAEL	240 μg/d	1.6
Mazumder et al. (1998)	Skin Lesions	LED_{01}	39-47 μg/d	0.68-0.79
Tsai et al. (2003)	Neurobehavioral effects	LED_{05}	7 μg/d	0.05

Figure 8.3.1 Switching attention (ms) in 13-year old children versus cumulative arsenic intake in mg (Tsai et al., 2003).



Inorganic arsenic is apparently more potent in its neurotoxic effects in humans than in experimental animals. The values of 2.27 µg/day in Wasserman *et al* (2004) and 7 µg/day in Tsai *et al* (2003) for cognitive effects in 10-13 year-old children are much lower than brain effects seen in animals e.g., 5 mg/kg-day in rats (Nagaraja and Desiraju, 1993; 1994) and 3.7 mg/kg-day in Rhesus monkeys (Heywood and Sortwell, 1979).

8.3.1.2 Adult Based Values

In this section we review toxicological criteria from studies in adults that may serve as the basis for a chronic REL for inorganic arsenic or otherwise provide supporting information.

Studies in experimental animals show that inhalation exposure to arsenic compounds can produce immunological suppression, developmental defects, and histological or biochemical effects on the nervous system and lung, thus providing supportive evidence of the types of toxicity observed in humans. Among the inhalation studies, the lowest adverse effect level (LOAEL) was quite consistent:

245 µg As/m^3 for decreased bactericidal activity in mice (Aranyi et al., 1985);
200 µg As/m^3 for decreased fetal weight in mice (Nagymajtenyi et al., 1985); and
270 µg As/m^3 for decreased sperm motility in rats (Kamil'dzhanov, 1982).

Reports of human inhalation exposure to arsenic compounds, primarily epidemiological studies of smelter workers, indicate that adverse health effects occur as a result of chronic exposure. Among the targets of arsenic toxicity are the respiratory system (Lundgren, 1954), the circulatory system (Lagerkvist et al., 1986), the skin (Perry et al., 1948), the nervous system (Blom et al., 1985), and the reproductive system (Nordstrom et al., 1979). Occupational exposure levels associated with these effects ranged from 50 to 7000 μg As/m^3. These epidemiological studies suffer, however, from confounding as a result of potential exposure to other compounds, which limits their usefulness in the development of the chronic REL.

A single study showed effects occurring at 4.9 μg As_2O_3/m^3 (Rozenshtein, 1970). However, lack of detail with respect to endpoints and experimental design limits this study's usefulness for developing a Reference Exposure Level.

The cerebrovascular disease (CVD) and cerebrovascular infarct (CI) data of Chiou et al. (1997b) were subjected to benchmark dose analysis (BMD). The data were best fit using the quantal linear regression (QL) dose-response equation. Since the responses were of the order of 0.1 to 2 percent, the values calculated were for the 1 percent response (ED_{01}) and its 95% lower confidence limit (LED_{01}), rather than the usual 5 percent response values for the analysis of animal study data.

The values for CI were marginally better fit by the dose-response equation than those for CVD. Also the QL models gave better fits to the unadjusted data sets for both endpoints. The unadjusted ED_{01} and LED_{01} values with goodness of fit P value meeting the acceptable fit criterion of $P \geq 0.1$ were 359 and 189 μg/L for CVD and 268 and 166 μg/L for CI, respectively. Using the cumulative dose metric these values were 5.1, 3.0, 5.9, and 3.5 (mg/L)-yr, respectively. Due to the severity of these and other endpoints analyzed below, the uncertainty in the dose assignments (range mid-points instead of averages), and the fact that the chosen points of departure or LEDs were generally two-fold or more above concurrent control levels, the LED_{01} should be considered equivalent to a LOAEL for the purposes of risk assessment. Due to the severity of the CI endpoint, a 100 UF was used to derive a health protective water concentration of 0.1 to 0.3 μg/L based on the two dose metrics. For CVD with a 30 UF the corresponding values were 0.28 to 1.3 μg/L (for details of analysis see OEHHA, 2004). Assuming 20 m^3/day inhalation, 2 Liters/day water consumption and 50 percent inhalation absorption, the corresponding inhalation values for these vascular effects would be for CI 0.10 to 0.33 μg/m^3 and for CVD 0.28 to 1.26 μg/m^3.

BMD analysis of the ISHD data from Chen et al. (1996) showed that these data were well fit by the QL dose-response equation (ED_{01} = 8.27 (mg/L)-yr, X^2 = 0.26, P = 0.88). The LED_{01} of 5.53 (mg/L)-yr should be considered an effect level for this endpoint. In this analysis the cumulative arsenic dose metric of (mg/L)-yr and resultant benchmark doses were divided by 70 yr to yield comparable lifetime drinking water concentrations of arsenic. Using a cumulative uncertainty factor of 100, a health protective concentration of 0.16 μg/L can be derived (OEHHA, 2004). Assuming 20 m^3/day inhalation, 2 Liters/day

water consumption and 50 percent inhalation absorption the corresponding health protective inhalation value for ISHD would be 0.16 μg/m^3.

The Chen et al. (1995) data on the association of hypertension (HT) and cumulative arsenic intake via drinking water were subjected to BMD analysis. The QL dose-response equation fit the unadjusted data well but was somewhat less than adequate for the adjusted prevalence values. The acceptable criterion for the X^2 goodness of fit test for the benchmark dose is $P \geq 0.10$. In the case of arsenic induced hypertension, the 10 percent effect level was chosen due to the higher background and greater dose response range. For HT the LED_{10} is considered an appropriate LOAEL for risk assessment. In the case of the adjusted data set, removal of the highest cumulative dose allows an acceptable fit of the QL equation with an LED_{10} of 7.4 (mg/L)-yr. The data of Rahman et al. (1999) were also analyzed. Both crude and adjusted data sets were well fit by the QL model with P values much greater than 0.1. The unadjusted LED_{10} value of 6.3 (mg/L)-yr from Bangladesh is quite similar to comparable value of 7.2 (mg/L)-yr from the Taiwan study (OEHHA, 2004). Health protective drinking water concentrations with a cumulative uncertainty factor of 30 ranged from 0.55 to 0.68 μg/Liter. Assuming 20 m^3/day inhalation, 2 Liters/day water consumption and 50 percent inhalation absorption the corresponding health protective inhalation value for HT would be 0.55 to 0.70 μg/m^3.

The data of Chen *et al*. (2006) indicate a supralinear dose-response. The data were analyzed for benchmark response using metrics of time weighted average (TWA) and cumulative arsenic exposure of TWA times years of exposure or (mg/L)-yr. Systolic hypertension quantal responses of the first four quintiles of the overall population (N = 8726) were fit by the log-logistic model of BMDS (v 1.4.1). The $BMDL_1$ values (1% response) of 71.5 μg/L and 0.66 (mg/L)-yr were obtained ($X^2 = 3.8$, P = 0.15, d.f. = 2). The pulse hypertension data were similarly fit using the longer-term exposure subpopulation (N = 6319). In this case the 10% response level was used for $BMDL_{10}$'s of 0.49 μg/L and 0.004 (mg/L)-yr ($X^2 = 4.45$, P = 0.11, d.f = 2). TWA BMDLs for systolic and pulse hypertension in arsenic exposed subpopulations with lower intakes of B vitamins were also evaluated. The $BMDL_{10}$ values for populations with low dietary folate ranged from 62 to 405 μg/L TWA. The results indicate a higher sensitivity of the pulse hypertension effect to low level arsenic than the systolic hypertension effect. The supralinearity of dose-response makes comparison with earlier studies problematic. For example, projected 10^{-4} extra risk levels for pulse and systolic hypertension from this study are at least an order of magnitude less than values seen earlier with Chen *et al*. (1995) or Rahman *et al*. (1999) although cumulative arsenic exposures were 5-10 times higher in the latter studies (Table 6).

Similarly, the diabetes mellitus (DM) data of Lai et al. (1994) and Rahman et al.(1998) were analyzed. In this case, the QL dose-response model adequately fit both unadjusted and multivariate-adjusted prevalences. EDs and LEDs were determined for the 1 and 5 percent response levels. The LED_{05} for the adjusted values appear the best choice for a chronic criterion for arsenic-induced diabetes mellitus, i.e., 8.8 (mg/L)-yr from Lai et al. and 0.21 mg/L from Rahman et al. The health protective drinking water derived from these values with a cumulative UF of 30 were 0.84 and 1.4 μg/L, respectively (OEHHA,

2004). Assuming 20 m^3/day inhalation, 2 Liters/day water consumption and 50 percent inhalation absorption, the corresponding health protective inhalation values for diabetes mellitus would be 0.85 to 1.4 μg/m^3.

In addition to the values noted above, an estimated LOAEL of 20 (mg/L)-yr for peripheral vascular disease from Tseng et al. (1996) was also included in this analysis. Using a cumulative UF of 30, a drinking water value of 1.9 μg/L was derived (OEHHA, 2004). Assuming 20 m^3/day inhalation, 2 Liters/day water consumption and 50 percent inhalation absorption, the corresponding health protective inhalation value for peripheral vascular disease would be 1.9 μg/m^3. The study of Wang et al. (2002) on arsenic induced carotid atherosclerosis (subclinical) also gave an estimated LOAEL of 20 (mg/L)-yr and would yield the same health protective values.

The arsenic-induced skin keratosis and hyperpigmentation data of Mazumder et al. (1998) were analyzed as above (OEHHA, 2004). For both male and female skin keratosis data sets, adequate fits were obtained by the QL model with lower bound values (LED_{01}) of 49.6 μg/L for males and 124 μg/L for females. Adequate fits could not be obtained for both hyperpigmentation data sets with the models available in the benchmark dose program; however, the dose-response graphs appeared to be linear in the lower exposure groups with respective LED_{01}s of 18.9 and 34.7 μg/L. It appears that a single dose level outlier (125 μg/L) was largely responsible for the failure of the statistical test. Mazumder also included an assessment of skin keratosis and hyperpigmentation prevalence by dose per body weight. Using the dose metric of μg/kg-day, the skin hyperpigmentation data were still unable to be fit by the BMDS models. Therefore only the skin keratosis endpoint appears suitable for the development of a health protective value for arsenic-induced noncancer effects. Using a cumulative UF of 30, a drinking water value of 1.7 μg/L was derived. Assuming 20 m^3/day inhalation, 2 Liters/day water consumption and 50 percent inhalation absorption, the corresponding health protective inhalation value for skin keratosis would be 0.34 μg/m^3.

The skin lesion data of Rahman *et al*. (2006) was analyzed for benchmark response. The unadjusted data reported in Rahman's Table 3 was used with the mid points of the exposure concentration ranges (e.g, 5, 30, 100, 224, 450 μg/L) and the mean As exposures in Rahman's Table 4 (e.g., 9.8, 59.3, 127, 199, 344 μg/L). For the unadjusted male data, no adequate fit could be obtained. The female data was adequately fit by the quantal linear (P = 0.43) and log-logistic (P = 0.51) models. The latter giving a $BMDL_{10}$ of 6.28 μg/L with mid-point based exposure estimates, and the former giving a $BMDL_{10}$ of 108.2 μg/L with mean As concentrations. Similarly, for the cumulative As dose metric of (mg/L)-yr no adequate fit was obtained with the male data, while the female data were best fit by the log-probit model (P = 0.86) for a $BMDL_{10}$ of 2.80 (mg/L)-yr. Using the age and asset adjusted data with the average As concentrations, an adequate fit to the male data could be obtained with the multistage model if the top dose group was removed, $BMDL_{10}$ = 96.0 μg/L (X^2 = 0.60, P = 0.74). The female adjusted data set gave a lower BMDL of 65.4 μg/L despite the authors' finding that the males were more sensitive. This may simply reflect the difficulty of fitting the male data. In almost all

cases, the BMDL values are lower (indicating higher risk) than seen in the earlier study by Mazumder *et al*. (1998) analyzed above.

The inhalation values derived from oral human exposure studies above are summarized in Table 8.3.2. With the exception of the very low value derived from the pulse hypertension endpoint, the derived health protective inhalation values range over approximately one order of magnitude from 0.16 to 1.7 μg/m^3. These adult values exceed the child-based values (range 0.015 to 1.6 μg/m^3). Therefore the proposed chronic REL value of 0.015 μg/m^3 is derived from the child arsenic exposure studies evaluated above and the adult studies provide supporting information.

Table 8.3.2 Inhalation Values Derived from Adult Human Drinking Water Studies

Study	**Toxic Endpoint**	**Criterion**	**Value**	**Derived chronic REL, (μg/m^3)**
Chiou et al. (1997b)	Cerebrovascular disease	LED_{01}	378 μg/d	1.26
Chiou et al. (1997b)	Cerebrovascular infarct	LED_{01}	332 μg/d	0.33
Chen et al. (1996a)	Ischemic Heart Disease Mortality	LED_{01}	5.53 (mg/L)-yr	0.16
Chen et al. (1995)	Hypertension	LED_{10}	5.8 (mg/L)-yr	0.55
Chen et al. (2006)	Systolic and pulse hypertension	SHT LED_{01} PHT LED_{10}	71.5 μg/L 0.49 μg/L	1.43 0.0033
Lai et al. (1994)	Diabetes mellitus	LED_{05}	8.8 (mg/L)-yr	0.85
Rahman et al. (1998)	Diabetes mellitus	LED_{05}	0.21 mg/L	1.4
Mazumder et al. (1998)	Skin keratosis	LED_{01}	50 μg/L	0.33
Rahman et al. (2006)	Skin keratosis or altered pigmentation	LED_{10}	65.4 μg/L	0.44
Tseng et al. (1996)	Peripheral vascular disease	est. LOAEL	20 (mg/L)-yr	1.69
Wang et al. (2002)	Carotid atherosclerosis	est. LOAEL	20 (mg/L)-yr	1.69

In addition to being inhaled, airborne arsenic can settle onto crops and soil and enter the body by ingestion. Thus an oral chronic reference exposure level for arsenic of 0.0035 μg/kg-day is also proposed.

9. Arsine Based Calculations

The NAC/NRC (National Advisory Committee on Acute Exposure Guideline Levels for Hazardous Substances/National Research Council Subcommittee on Acute Exposure Guideline Levels) derived an Acute Exposure Guidance Level-2 (AEGL-2, disabling) of 0.17 ppm (500 μg/m^3) for one-hour exposure to arsine based on the hemolysis mouse data of Peterson and Bhattacharyya (1985) (Thomas and Young, 2001). Due to the steepness of the dose response the derivation of an AEGL-1 (Non-disabling) was considered inappropriate. Also the reliance on animal data was considered more "scientifically valid than AEGLs estimated from limited anecdotal human data". The panel used a total UF of 30 (10 for interspecies differences and 3 for intraspecies differences).

Based on the same study data, OEHHA calculated a continuous $BMDL_{1SD}$ of 2.17 ppm (6.9 mg/m^3) for reticulocytosis. When this value was adjusted with uncertainty factors of 10 for interspecies and 30 for intraspecies differences (including 10 for the intraspecies toxicokinetic sub-factor, as proposed in OEHHA, 2007 draft) the potential aREL for a one hour exposure was 2.17 ppm/300 = 0.0072 ppm (23 μg/m^3).

Despite the additional 10-fold margin of safety and more sensitive endpoint incorporated in the OEHHA derivation summarized above, there is still residual uncertainty in this comparison aREL value for arsine. There is particular concern with respect to the lack of adequate human data, given that rodents appear more resistant to the effects of acute exposure to various inorganic forms of arsenic than humans. The analogy between arsine and other inorganic forms of arsenic is supported by the observation that arsine exposure in humans and experimental animals results in similar metabolites excreted in urine as result from other inorganic arsenic exposure (Landrigan et al., 1982; Buchet et al., 1998). A further source of concern with a REL based on the Peterson and Bhattacharyya (1985) study is that while the margin of exposure for hemolysis is greater than 1000, the margin for total lethality is less than 4000. Although a steep dose-response slope for acute lethality is not unprecedented, it is a problematic feature when combined with the uncertainty in animal-to human extrapolation noted above. Thus, OEHHA staff have low confidence in using the Peterson and Bhattacharyya study as a basis of an aREL value for arsine and instead will rely on the aREL based on arsenic trioxide inhalation in mice (0.2 μg/m^3 arsenic, equivalent to 0.065 ppb arsine), which is sufficiently protective for all inorganic arsenic species.

A comparison of various possible values for an 8-hour REL for arsine is shown in Table 8.3.3. Adjustment of the one-hour NOAEL from Peterson and Bhattacharyya (1985) to eight hours using the modified Haber equation for mice gives a value of 1.6 ppm (4.98 mg/m^3)/300UF = 0.053 ppm (17 μg/m^3). This value is much higher than the values observed by Williams et al. (1981) in workers exposed to arsine concentrations estimated at 0.01 to 0.07 mg/m^3. The adverse effects noted included headache, nausea, weakness and vomiting. Although based on only a couple of subjects, the Williams et al. study

would indicate an 8-hour value of about 0.04 mg/m^3/30 UF = 0.001 mg/m^3 or 1μg/m^3. Alternatively, the 90-day study of Blair et al. (1990) gives a NOAEL for hematologic effects in mice of 0.025 ppm arsine at 6 hours/day, 5 days/week. Applying the same 300 UF as above gives 0.083 ppb or 0.26 μg/m^3. This latter figure seems more in line with the limited human observations and more suitable for potentially repeated 8-hour exposures to arsine. The intraspecies extrapolation includes additional uncertainty factors (PK + PD UF) for exposure of infants and children to arsine.

Table 8.3.3. Development of Health Protective Values for Arsine

Study	Toxic Endpoint	NOAEL/LOAEL/ BMDL	Derived REL μg/m^3
Peterson and Bhattacharyya, 1985	**Reticulocytosis in mice 1 hour exposure**	**$BMDL_{1SD}$ 2.17 ppm 6.9 mg/m^3**	**Acute 23**
Peterson and Bhattacharyya, 1985	**As above with 8-hour adjustment**	**1.6 ppm 4.98 mg/m^3**	**8-hour 17**
Williams *et al.*, 1981	**Headache, nausea,weakness, and vomiting in exposed workers**	**0.01 to 0.07 mg/m^3, average 0.04 mg/m^3 LOAEL.**	**8-hour 1.0**
Blair *et al.*, 1990	**Hematologic effects**	**NOAEL 0.025 ppm 6 hr/day**	**8-hour 0.26**

PBPK modeling of arsenic species in experimental animals and humans is presently considered inadequate to apply directly to the derivation of RELs for repeated arsine exposures.

Arsine exposure at atmospheric concentrations that caused adverse maternal effects did not adversely affect endpoints of developmental toxicity in mice or rats (Morrissey *et al*., 1990). In the absence of neurodevelopmental studies with arsine, it is assumed that such an effect would be comparable to those of other inorganic forms of arsenic. In view of the observed effect levels for hematological effects noted in the animal studies, both 8 hour and chronic effects of arsine are considered to be adequately covered by the respective cREL for inorganic arsenic based on neurodevelopmental effects observed in children (i.e., 0.015 μg/m^3 arsenic, equivalent to 0.005 ppb arsine)). In view of the concern over neurodevelopmental effects for all inorganic forms of arsenic, OEHHA concludes that it is appropriate to apply this value for 8-hour and chronic exposures to arsine.

10. Arsenic as a Toxic Air Contaminant that Disproportionately Impacts Children

In view of the neurodevelopmental toxicity studies discussed above, it is clear that infants and children are more susceptible to the toxicity of arsenic than adults. OEHHA recommends that inorganic arsenic and arsine be identified as a Toxic Air Contaminant

the disproportionately impacts children under the California Health and Safety Code Section 39699.5.

11. References

ACGIH (1992). Documentation of the threshold limit values and biological exposure indices. Cincinnati (OH): American Conference of Government Industrial Hygienists, Inc.

Aposhian HV (1997). Enzymatic methylation of arsenic species and other new approaches to arsenic toxicity. Annu Rev Pharmacol Toxicol 37: 397-419.

Aposhian HV, Gurzau ES, Le XC, Gurzau A, Healy SM, Lu X, Ma M, Yip L, Zakharyan RA, Maiorino RM, Dart RC, Tircus MG, Gonzalez-Ramirez D, Morgan DL, Avram D and Aposhian MM (2000a). Occurrence of monomethylarsonous acid in urine of humans exposed to inorganic arsenic. Chem Res Toxicol 13(8): 693-7.

Aposhian HV, Zheng B, Aposhian MM, Le XC, Cebrian ME, Cullen W, Zakharyan RA, Ma M, Dart RC, Cheng Z, Andrewes P, Yip L, O'Malley GF, Maiorino RM, Van Voorhies W, Healy SM and Titcomb A (2000b). DMPS-arsenic challenge test. II. Modulation of arsenic species, including monomethylarsonous acid (MMA(III)), excreted in human urine. Toxicol Appl Pharmacol 165(1): 74-83.

Apostoli P, Alessio L, Romeo L, Buchet JP and Leone R (1997). Metabolism of arsenic after acute occupational arsine intoxication. J Toxicol Environ Health 52(4): 331-42.

Aranyi C, Bradof JN, Fenters JD, Graham JA and Miller FJ (1981). Effects of inhalation of arsenic trioxide aerosols in the pulmonary defenses of mice. In: International Conference. Heavy Metals in the Environment. Commission of the European Communities and the World Health Organization. 450-453.

Aranyi C, Bradof JN, O'Shea WJ, Graham JA and Miller FJ (1985). Effects of arsenic trioxide inhalation exposure on pulmonary antibacterial defenses in mice. J Toxicol Environ Health 15(1): 163-72.

ATSDR. (2000). *Arsenic. Toxicological Profile (Update)*. U.S. DHHS, Atlanta GA. http://www.atsdr.cdc.gov/toxprofiles/fp2-c4.pdf.

Baxley MN, Hood RD, Vedel GC, Harrison WP and Szczech GM (1981). Prenatal toxicity of orally administered sodium arsenite in mice. Bull Environ Contam Toxicol 26(6): 749-56.

Blair PC, Thompson MB, Bechtold M, Wilson RE, Moorman MP and Fowler BA (1990). Evidence for oxidative damage to red blood cells in mice induced by arsine gas. Toxicology 63(1): 25-34.

Blakley BR, Sisodia CS and Mukkur TK (1980). The effect of methylmercury, tetraethyl lead, and sodium arsenite on the humoral immune response in mice. Toxicol Appl Pharmacol 52(2): 245-54.

Blom S, Lagerkvist B and Linderholm H (1985). Arsenic exposure to smelter workers. Clinical and neurophysiological studies. Scand J Work Environ Health 11(4): 265-9.

Borgono JM, Vicent P, Venturino H and Infante A (1977). Arsenic in the drinking water of the city of Antofagasta: epidemiological and clinical study before and after the installation of a treatment plant. Environ Health Perspect 19: 103-5.

Brown MM, Rhyne BC and Goyer RA (1976). Intracellular effects of chronic arsenic administration on renal proximal tubule cells. J Toxicol Environ Health 1(3): 505-14.

Buchanan WD (1962). Arsine. In: Toxicity of Arsenic Compounds. Browning E. Elsevier. New York (NY): 67-75.

Buchet JP, Apostoli P and Lison D (1998). Arsenobetaine is not a major metabolite of arsine gas in the rat. Arch Toxicol 72(11): 706-10.

Buchet JP and Lauwerys R (1985). Study of inorganic arsenic methylation by rat liver in vitro: relevance for the interpretation of observations in man. Arch Toxicol 57(2): 125-9.

Buchet JP and Lauwerys R (1987). Study of factors influencing the in vivo methylation of inorganic arsenic in rats. Toxicol Appl Pharmacol 91(1): 65-74.

Buchet JP, Lauwerys R and Roels H (1981a). Comparison of the urinary excretion of arsenic metabolites after a single oral dose of sodium arsenite, monomethylarsonate, or dimethylarsinate in man. Int Arch Occup Environ Health 48(1): 71-9.

Buchet JP, Lauwerys R and Roels H (1981b). Urinary excretion of inorganic arsenic and its metabolites after repeated ingestion of sodium metaarsenite by volunteers. Int Arch Occup Environ Health 48(2): 111-8.

Calderon J, Navarro ME, Jimenez-Capdeville ME, Santos-Diaz MA, Golden A, Rodriguez-Leyva I, Borja-Aburto V and Diaz-Barriga F (2001). Exposure to arsenic and lead and neuropsychological development in Mexican children. Environ Res 85(2): 69-76.

Campbell D and Oates RK (1992). Childhood poisoning--a changing profile with scope for prevention. Med J Aust 156(4): 238-40.

Carter DE, Aposhian HV and Gandolfi AJ (2003). The metabolism of inorganic arsenic oxides, gallium arsenide, and arsine: a toxicochemical review. Toxicol Appl Pharmacol 193(3): 309-34.

Charbonneau SM, Spencer K, Bryce F and Sandi E (1978). Arsenic excretion by monkeys dosed with arsenic-containing fish or with inorganic arsenic. Bull Environ Contam Toxicol 20(4): 470-7.

Chen CJ, Chiou HY, Chiang MH, Lin LJ and Tai TY (1996). Dose-response relationship between ischemic heart disease mortality and long-term arsenic exposure. Arterioscler Thromb Vasc Biol 16(4): 504-10.

Chen CJ, Hsueh YM, Lai MS, Shyu MP, Chen SY, Wu MM, Kuo TL and Tai TY (1995). Increased prevalence of hypertension and long-term arsenic exposure. Hypertension 25(1): 53-60.

Chen CJ, Wu MM, Lee SS, Wang JD, Cheng SH and Wu HY (1988). Atherogenicity and carcinogenicity of high-arsenic artesian well water. Multiple risk factors and related malignant neoplasms of blackfoot disease. Arteriosclerosis 8(5): 452-60.

Chen KP and Wu HY (1962). Epidemiologic studies on blackfoot disease: II. A study of source of drinking water in relation to the disease. J Formosan Med Assoc 61: 611-618.

Chen Y, Factor-Litvak P, Howe GR, Graziano JH, Brant-Rauf P, Parvez F, Van Geen A, Ahsan H. (2006). Arsenic exposure from drinking water, dietary intakes of B vitamins and folate, and risk of high blood pressure in Bangladesh: A population-based, cross-sectional study. Am J Epidemiol 165:541-552.

Chi IC and Blackwell RQ (1968). A controlled retrospective study of blackfoot disease, an endemic peripheral gangrene disease in Taiwan. Am J Epidemiol 88: 7-24.

Chiou HY, Hsueh YM, Hsieh LL, Hsu LI, Hsu YH, Hsieh FI, Wei ML, Chen HC, Yang HT, Leu LC, Chu TH, Chen-Wu C, Yang MH and Chen CJ (1997a). Arsenic methylation capacity, body retention, and null genotypes of glutathione S-transferase M1 and T1 among current arsenic-exposed residents in Taiwan. Mutat Res 386(3): 197-207.

Chiou HY, Huang WI, Su CL, Chang SF, Hsu YH and Chen CJ (1997b). Dose-response relationship between prevalence of cerebrovascular disease and ingested inorganic arsenic. Stroke 28(9): 1717-23.

Coles GA, Davies HJ, Daley D and Mallick NP (1969). Acute intravascular haemolysis and renal failure due to arsine poisoning. Postgrad Med J 45(521): 170-2.

Craig DK and Frye J. (1988). Acute LC50 nose only inhalation toxicity studies of arsine in rats (final report). N0512-4700. Batelle Columbus Labs. Columbus (OH)

Crump KS (1984). A new method for determining allowable daily intakes. Fundam Appl Toxicol 4(5): 854-71.

Crump KS and Howe R. (1983). Probit - A computer program to extrapolate quantile animal toxicological data to low doses. Crump K.S. & Company Inc.

Danielsson BR, Dencker L, Lindgren A and Tjalve H (1984). Accumulation of toxic metals in male reproduction organs. Arch Toxicol Suppl 7: 177-80.

DeSesso JM, Jacobson CF, Scialli AR, Farr CH and Holson JF (1998). An assessment of the developmental toxicity of inorganic arsenic. Reprod Toxicol 12(4): 385-433.

Engel RR and Smith AH (1994). Arsenic in drinking water and mortality from vascular disease: an ecologic analysis in 30 counties in the United States. Arch Environ Health 49(5): 418-27.

Ferm VH and Carpenter SJ (1968). Malformations induced by sodium arsenate. J Reprod Fertil 17(1): 199-201.

Flury F (1921). [Uber Kampfgasvergiftungen. IX. Lokal reizende Arsenverbindungen]. Zeichschrift fur die Gesamte Experimentelle Medizin 13: 527-528.

Flury F (1931). Schadliche Gase - dampfe, nebel, rauch - und staubarten. Berlin: Verlag von Julius Springer.

Fowler BA, Moorman MP, Adkins BJ, Bakewell WEJ, Blair PC and Thompson MB (1989). Arsine: toxicity data from acute and short-term inhalation exposures. In: ACGIH. Hazard assessment and control technology in semiconductor manufacturing. Lewis Publishers. Chelsea (MI): 85-89.

Frank G (1976). [Neurological and psychiatric disorders following acute arsine poisoning (author's transl)]. J Neurol 213(1): 59-70.

Freeman GB, Schoof RA, Ruby MV, Davis AO, Dill JA, Liao SC, Lapin CA and Bergstrom PD (1995). Bioavailability of arsenic in soil and house dust impacted by smelter activities following oral administration in cynomolgus monkeys. Fundam Appl Toxicol 28(2): 215-22.

Friberg L, Nordberg GF and Vouk VB, eds. (1986). Handbook on the Toxicology of Metals. Elsevier Amsterdam. p 59

Gates M, Williams J and Zapp JA. (1946). *Arsenicals. Summary technical report of Division 9, National Defense Research Committee. Vol. 1. Chemical warfare agents and related chemical problems.*: Office of Scientific Research and Development.

Gentry PR, Covington TR, Mann S, Shipp AM, Yager JW and Clewell HJ, 3rd (2004). Physiologically based pharmacokinetic modeling of arsenic in the mouse. J Toxicol Environ Health A 67(1): 43-71.

George B, Mathews V, Poonkuzhali B, Shaji RV, Srivastava A and Chandy M (2004). Treatment of children with newly diagnosed acute promyelocytic leukemia with arsenic trioxide: a single center experience. Leukemia 18(10): 1587-90.

Gladysheva TB, Oden KL and Rosen BP (1994). Properties of the arsenate reductase of plasmid R773. Biochemistry 33(23): 7288-93.

Grayson M, ed. (1978). Kirk-Othmer Encyclopedia of Chemical Technology. John Wiley and Sons New York. 247-251

Gregus Z, Gyurasics A and Csanaky I (2000). Biliary and urinary excretion of inorganic arsenic: monomethylarsonous acid as a major biliary metabolite in rats. Toxicol Sci 56(1): 18-25.

Grobe VJ (1976). [Peripheral circulatory disorders and acrocyanosis in arsenic exposed Moselle wine-growers]. Berufsdermatosen 24(3): 78-84.

Hafeman DM, Ahsan H, Louis ED, Siddique AB, Slavkovich V, Cheng Z, van Geen A and Graziano JH (2005). Association between arsenic exposure and a measure of subclinical sensory neuropathy in Bangladesh. J Occup Environ Med 47(8): 778-84.

Hammamoto E (1955). [Infant arsenic poisoning by powdered milk]. Japanese Medical Journal 1649: 2-12 [cited in ATSDR, 2006].

Harrison WP and Hood RD (1981). Prenatal effecrs following exposure of hamsters to sodium arsenite by oral or intraperitoneal routes [abstract]. Teratology 23: 40A [cited in Willhite and Ferm, 1984].

Hatlelid KM, Brailsford C and Carter DE (1995). An in vitro model for arsine toxicity using isolated red blood cells. Fundam Appl Toxicol 25(2): 302-6.

Hatlelid KM, Brailsford C and Carter DE (1996). Reactions of arsine with hemoglobin. J Toxicol Environ Health 47(2): 145-57.

Hayakawa T, Kobayashi Y, Cui X and Hirano S (2005). A new metabolic pathway of arsenite: arsenic-glutathione complexes are substrates for human arsenic methyltransferase Cyt19. Arch Toxicol 79(4): 183-91.

Healy SM, Casarez EA, Ayala-Fierro F and Aposhian H (1998). Enzymatic methylation of arsenic compounds. V. Arsenite methyltransferase activity in tissues of mice. Toxicol Appl Pharmacol 148(1): 65-70.

Heywood R and Sortwell RJ (1979). Arsenic intoxication in the rhesus monkey. Toxicol Lett 3: 137-144.

Hogue CJ, Buehler JW, Strauss LT and Smith JC (1987). Overview of the National Infant Mortality Surveillance (NIMS) project--design, methods, results. Public Health Rep 102(2): 126-38.

Holson JF, Stump DG, Ulrich CE and Farr CH (1999). Absence of prenatal developmental toxicity from inhaled arsenic trioxide in rats. Toxicol Sci 51(1): 87-97.

Hood RD and Harrison WP (1982). Effects of prenatal arsenite exposure in the hamster. Bull Environ Contam Toxicol 29(6): 671-8.

HSDB (1995). Hazardous Substances Data Bank. National Library of Medicine. http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB.

Hughes MF, Menache M and Thompson DJ (1994). Dose-dependent disposition of sodium arsenate in mice following acute oral exposure. Fundam Appl Toxicol 22(1): 80-9.

IARC. (2004). *Some Drinking -water Disinfectants and Contaminants, including Arsenic*. 84. International Agency for Research on Cancer, World Health Organization, Lyon, France.

ICRP (1994). Human Respiratory Tract Model for Radiological Protection. International Commission on Radiological Protection. Publication 66 Annals of the ICRP. Tarrytown (NY): Elsevier Science Inc. 24(1-3).

Ihrig MM, Shalat SL and Baynes C (1998). A hospital-based case-control study of stillbirths and environmental exposure to arsenic using an atmospheric dispersion model linked to a geographical information system. Epidemiology 9(3): 290-4.

IRDC. (1985). Three acute inhalation toxicity studies of arsine on rats (final reports). 533-001, 533-002, 533-003. International Research and Development Corporation. Mattawan (MI)

Itoh T, Zhang YF, Murai S, Saito H, Nagahama H, Miyate H, Saito Y and Abe E (1990). The effect of arsenic trioxide on brain monoamine metabolism and locomotor activity of mice. Toxicol Lett 54(2-3): 345-53.

Jacobson-Kram D and Montalbano D (1985). The reproductive effects assessment group's report on the mutagenicity of inorganic arsenic. Environ Mutagen 7(5): 787-804.

Ji G and Silver S (1992). Reduction of arsenate to arsenite by the ArsC protein of the arsenic resistance operon of Staphylococcus aureus plasmid pI258. Proc Natl Acad Sci U S A 89(20): 9474-8.

Jiang G, Gong Z, Li XF, Cullen WR and Le XC (2003). Interaction of trivalent arsenicals with metallothionein. Chem Res Toxicol 16(7): 873-80.

Kamil'dzhanov AX (1982). Hygiene basis for the maximum permissible concentration of arsenic trioxide in the ambient air. Gig Sanit 2: 74-75.

Kamkin AB (1982). [Review of the maximum permissible concentration of arsenic trioxide in the atmosphere of populated places]. Gig Sanit(1): 6-9.

Kitchin KT (2001). Recent advances in arsenic carcinogenesis: modes of action, animal model systems, and methylated arsenic metabolites. Toxicol Appl Pharmacol 172(3): 249-61.

Kleinfeld MJ (1980). Arsine poisoning. J Occup Med 22(12): 820-1.

Klimecki WT and Carter DE (1995). Arsine toxicity: chemical and mechanistic implications. J Toxicol Environ Health 46(4): 399-409.

Krafft T and Macy JM (1998). Purification and characterization of the respiratory arsenate reductase of Chrysiogenes arsenatis. Eur J Biochem 255(3): 647-53.

Kurttio P, Komulainen H, Hakala E, Kahelin H and Pekkanen J (1998). Urinary excretion of arsenic species after exposure to arsenic present in drinking water. Arch Environ Contam Toxicol 34(3): 297-305.

Lagerkvist B, Linderholm H and Nordberg GF (1986). Vasospastic tendency and Raynaud's phenomenon in smelter workers exposed to arsenic. Environ Res 39(2): 465-74.

Lai MS, Hsueh YM, Chen CJ, Shyu MP, Chen SY, Kuo TL, Wu MM and Tai TY (1994). Ingested inorganic arsenic and prevalence of diabetes mellitus. Am J Epidemiol 139(5): 484-92.

Landrigan PJ, Costello RJ and Stringer WT (1982). Occupational exposure to arsine. An epidemiologic reappraisal of current standards. Scand J Work Environ Health 8(3): 169-77.

Le XC, Lu X, Ma M, Cullen WR, Aposhian HV and Zheng B (2000a). Speciation of key arsenic metabolic intermediates in human urine. Anal Chem 72(21): 5172-7.

Le XC, Ma M, Cullen WR, Aposhian HV, Lu X and Zheng B (2000b). Determination of monomethylarsonous acid, a key arsenic methylation intermediate, in human urine. Environ Health Perspect 108(11): 1015-8.

Levin-Scherz JK, Patrick JD, Weber FH and Garabedian C, Jr. (1987). Acute arsenic ingestion. Ann Emerg Med 16(6): 702-4.

Levvy GA (1947). A study of arsine poisoning. Quart J Exp Physiol 34: 47-67.

Lewis DR, Southwick JW, Ouellet-Hellstrom R, Rench J and Calderon RL (1999). Drinking water arsenic in Utah: A cohort mortality study. Environ Health Perspect 107(5): 359-65.

Lugo G, Cassady G and Palmisano P (1969). Acute maternal arsenic intoxication with neonatal death. Am J Dis Child 117(3): 328-30.

Lundgren KD (1954). [Damage to respiratory organs in workers in a smelting plant] cited in U.S. EPA, 1984. Nord Hyg Tidskr 3-4: 66-82.

Mann S, Droz PO and Vahter M (1994). A physiologically based pharmacokinetic model for four major arsenic species in mammals. In: Arsenic Exposure and Health Effects. Special Issue of Environmental Geochemistry and Health. Science and Technology Letters

Chappell W. R., Abernathy C. O. and Cothern C. R. Northwood. Middlesex, England: 16: 219-231.

Mann S, Droz PO and Vahter M (1996a). A physiologically based pharmacokinetic model for arsenic exposure. I. Development in hamsters and rabbits. Toxicol Appl Pharmacol 137(1): 8-22.

Mann S, Droz PO and Vahter M (1996b). A physiologically based pharmacokinetic model for arsenic exposure. II. Validation and application in humans. Toxicol Appl Pharmacol 140(2): 471-86.

Marafante E and Vahter M (1987). Solubility, retention, and metabolism of intratracheally and orally administered inorganic arsenic compounds in the hamster. Environ Res 42(1): 72-82.

Marnell LL, Garcia-Vargas GG, Chowdhury UK, Zakharyan RA, Walsh B, Avram MD, Kopplin MJ, Cebrian ME, Silbergeld EK and Aposhian HV (2003). Polymorphisms in the human monomethylarsonic acid (MMA V) reductase/hGSTO1 gene and changes in urinary arsenic profiles. Chem Res Toxicol 16(12): 1507-13.

Mazumder DNG, Haque R, Ghosh N, De BK, Santra A, Chakraborty D and Smith AH (1998). Arsenic levels in drinking water and the prevalence of skin lesions in West Bengal, India. Int J Epidemiol 27(5): 871-7.

Menzel DB, Ross M, Oddo SV, Bergstrom PD, Greene H and Roth RN (1994). A physiologically based pharmacokinetic model for ingested arsenic. In: Arsenic Exposure and Health Effects. Special Issue of Environmental Geochemistry and Health. Science and Technology Letters. Chappell W. R., Abernathy C. O. and Cothern C. R. Northwood

Middlesex, England: 16: 209-218.

Meza MM, Yu L, Rodriguez YY, Guild M, Thompson D, Gandolfi AJ and Klimecki WT (2005). Developmentally restricted genetic determinants of human arsenic metabolism: association between urinary methylated arsenic and CYT19 polymorphisms in children. Environ Health Perspect 113(6): 775-81.

Morris JS, Schmid M, Newman S, Scheuer PJ and Sherlock S (1974). Arsenic and noncirrhotic portal hypertension. Gastroenterology 66(1): 86-94.

Morrissey RE, Fowler BA, Harris MW, Moorman MP, Jameson CW and Schwetz BA (1990). Arsine: absence of developmental toxicity in rats and mice. Fundam Appl Toxicol 15(2): 350-6.

Nagaraja TN and Desiraju T (1993). Regional alterations in the levels of brain biogenic amines, glutamate, GABA, and GAD activity due to chronic consumption of inorganic arsenic in developing and adult rats. Bull Environ Contam Toxicol 50(1): 100-7.

Nagaraja TN and Desiraju T (1994). Effects on operant learning and brain acetylcholine esterase activity in rats following chronic inorganic arsenic intake. Hum Exp Toxicol 13(5): 353-6.

Nagymajtenyi L, Selypes A and Berencsi G (1985). Chromosomal aberrations and fetotoxic effects of atmospheric arsenic exposure in mice. J Appl Toxicol 5(2): 61-3.

Nemec MD, Holson JF, Farr CH and Hood RD (1998). Developmental toxicity assessment of arsenic acid in mice and rabbits. Reprod Toxicol 12(6): 647-58.

Nordstrom S, Beckman L and Nordenson I (1979). Occupational and environmental risks in and around a smelter in northern Sweden. V. Spontaneous abortion among female employees and decreased birth weight in their offspring. Hereditas 90(2): 291-6.

NRC. (1984). *Emergency and Continuous Exposure Limits for Selected Airborne Contaminants.* . 1. National Academy Press, Washington, DC.

NRC. (2001). *Arsenic in Drinking Water 2001 Update*. National Academy Press, Washington, DC.

OEHHA. (1999). *The Air Toxics Hot Spots Program Risk Assessment Guidelines. Part II: Technical Support Document for Describing Available Cancer Potency Factors*. Air Toxicology and Epidemiology Section, Office of Environmental Health Hazard Assessment, California Environmental Protection Agency.

OEHHA. (2000). *The Air Toxics Hot Spots Program Risk Assessment Guidelines. Part IV: Technical Support Document for Exposure Assessment and Stochastic Analysis*. Air Toxicology and Epidemiology Section, Office of Environmental Health Hazard Assessment, California Environmental Protection Agency. http://www.oehha.ca.gov/air/hot_spots/pdf/May2005Hotspots.pdf.

OEHHA. (2004). *Public Health Goals for Arsenic in Drinking Water*. Pesticide and Environmental Toxicology Branch, Office of Environmental Health Hazard Assessment, California Environmental Protection Agency. http://www.oehha.ca.gov/water/phg/allphgs.html.

OEHHA. (2007). *The Air Toxics Hot Spots Program Risk Assessment Guidelines. Part V. Technical Support Document for the Derivation of Noncancer Reference Exposure Levels (5-15-07, draft).* Air Toxicology and Epidemiology Branch. Office of Environmental Health Hazard Assessment, California Environmental Protection Agency.

Offergelt JA, Roels H, Buchet JP, Boeckx M and Lauwerys R (1992). Relation between airborne arsenic trioxide and urinary excretion of inorganic arsenic and its methylated metabolites. Br J Ind Med 49(6): 387-93.

Owen BA (1990). Literature-derived absorption coefficients for 39 chemicals via oral and inhalation routes of exposure. Regul Toxicol Pharmacol 11(3): 237-52.

Parish GG, Glass R and Kimbrough R (1979). Acute arsine posioning in two workers cleaning a clogged drain. Arch Environ Health 34(4): 224-7.

Perry K, Bowler RG, Bucknell HM, Druett HA and Scilling RSF (1948). Studies in the incidence of cancer in a factory handling inorganic compounds of arsenic. II. Clinical and environmental investigations. Br J Ind Med 5: 6-15.

Peterson DP (1990). Personal communication.

Peterson DP and Bhattacharyya MH (1985). Hematological responses to arsine exposure: quantitation of exposure response in mice. Fundam Appl Toxicol 5(3): 499-505.

Pomroy C, Charbonneau SM, McCullough RS and Tam GK (1980). Human retention studies with 74As. Toxicol Appl Pharmacol 53(3): 550-6.

Radabaugh TR and Aposhian HV (2000). Enzymatic reduction of arsenic compounds in mammalian systems: reduction of arsenate to arsenite by human liver arsenate reductase. Chem Res Toxicol 13(1): 26-30.

Rael LT, Ayala-Fierro F and Carter DE (2000). The effects of sulfur, thiol, and thiol inhibitor compounds on arsine-induced toxicity in the human erythrocyte membrane. Toxicol Sci 55(2): 468-77.

Rahman M and Axelson O (1995). Diabetes mellitus and arsenic exposure: a second look at case-control data from a Swedish copper smelter. Occup Environ Med 52(11): 773-4.

Rahman M, Tondel M, Ahmad SA and Axelson O (1998). Diabetes mellitus associated with arsenic exposure in Bangladesh. Am J Epidemiol 148(2): 198-203.

Rahman M, Tondel M, Ahmad SA, Chowdhury IA, Faruquee MH and Axelson O (1999). Hypertension and arsenic exposure in Bangladesh. Hypertension 33(1): 74-8.

Rahman M, Vahter M, Sohel N, Yunus M, Wahed MA, Streatfield PK, Ekstrom EC, Persson LA. (2006). Arsenic exposure and age- and sex-specific risk for skin lesions: A population-based case-referent study in Bangladesh. Environ Health Perspect 114:1847-1852.

Rahman MS, Hall LL and Hughes MF (1994). In vitro percutaneous absorption of sodium arsenate in B6C3F1 mice. Toxicol in Vitro 8(3): 441-448.

Rees DC and Hattis D (1994). Developing quantitative strategies for animal to human exprapolation. In: Principles and Methods of Toxicology. Hayes A. W. Raven Press. New York.

Rosenberg HG (1974). Systemic arterial disease and chronic arsenicism in infants. Arch Pathol 97(6): 360-5.

Rozenshtein IS (1970). [Sanitary toxicological assessment of low concentrations pf arsenic trioxide in the atmosphere]. Gig Sanit 35: 16-21.

Saha KC (2003). Saha's grading of arsenicosis progression and treatment. In: Arsenic Exposure and Health Effects V. Chappell W. R., Abernathy C. O. and Thomas D. J. Elsevier. San Diego: 391.

Sampayo-Reyes A, Zakharyan RA, Healy SM and Aposhian HV (2000). Monomethylarsonic acid reductase and monomethylarsonous acid in hamster tissue. Chem Res Toxicol 13(11): 1181-6.

Sanger DE (1987). Unpublished study cited in Blair et al., 1990. Amherst, MA: University of MassachusettsSchool of Public Health.

Schroeder HA and Mitchener M (1971). Toxic effects of trace elements on the reproduction of mice and rats. Arch Environ Health 23(2): 102-6.

Siripitayakunkit U, Lue S and Choprapawon C (2001). Possible effects of arsenic on visual perception and visual-motor integration of children in Thailand. In: Arsenic Exposure and Health Effects. Chappell W. R., Abernathy C. O. and Calderon R. L. Elsevier Science.

Siripitayakunkit U, Visudhiphan P, Pradipasen M and Vorapongsathron T (1999). Association between chronic arsenic exposure and children's intelligence in Thailand. In: Arsenic Exposure and Health Effects. Chappell W. R., Abernathy C. O. and Calderon J. Elsevier Science.

Smith AH, Goycolea M, Haque R and Biggs ML (1998). Marked increase in bladder and lung cancer mortality in a region of Northern Chile due to arsenic in drinking water. Am J Epidemiol 147(7): 660-9.

Stump DG, Holson JF, Fleeman TL, Nemec MD and Farr CH (1999). Comparative effects of single intraperitoneal or oral doses of sodium arsenate or arsenic trioxide during in utero development. Teratology 60(5): 283-91.

Styblo M, Delnomdedieu M and Thomas DJ (1996). Mono- and dimethylation of arsenic in rat liver cytosol in vitro. Chem Biol Interact 99(1-3): 147-64.

Styblo M, Yamauchi H and Thomas DJ (1995). Comparative in vitro methylation of trivalent and pentavalent arsenicals. Toxicol Appl Pharmacol 135(2): 172-8.

Tam GK, Charbonneau SM, Bryce F, Pomroy C and Sandi E (1979). Metabolism of inorganic arsenic (74As) in humans following oral ingestion. Toxicol Appl Pharmacol 50(2): 319-22.

Thomas R and Young R (2001). Arsine. Acute exposure guideline levels. Inhal Toxicol 13 (Suppl): 43-77.

Thompson DJ (1993). A chemical hypothesis for arsenic methylation in mammals. Chem Biol Interact 88(2-3): 89-14.

Tsai SY, Chou HY, The HW, Chen CM and Chen CJ (2003). The effects of chronic arsenic exposure from drinking water on the neurobehavioral development in adolescence. Neurotoxicology 24(4-5): 747-53.

Tseng CH, Chong CK, Chen CJ and Tai TY (1996). Dose-response relationship between peripheral vascular disease and ingested inorganic arsenic among residents in blackfoot disease endemic villages in Taiwan. Atherosclerosis 120(1-2): 125-33.

Tseng WP (1977). Effects and dose--response relationships of skin cancer and blackfoot disease with arsenic. Environ Health Perspect 19: 109-19.

U.S. EPA. (2002). *Notice of Receipt of Requests to Cancel Certain Chromated Copper Arsenate (CCA) Wood Preservative Products and Amend to Terminate Certain Uses of CCA Products* OPP-66300; FRL-6826-8. U.S. Environmental Protection Agency.

U.S. EPA. (2006a). *EPA Chemical Profiles: Arsine*. U. S. Environmental Protection Agency. http://yosemite.epa.gov/oswer/ceppoehs.nsf/Profiles/1303-28-2?OpenDocument.

U.S. EPA. (2006b). *Organic herbicides (MSMA, DSMA, CAMA, and Cacodylic Acid), Registration Eligibility Decision: Notice of Availability*. Volume 71, No 153. Federal Register: August 9, 2006.

Vahter M (1981). Biotransformation of trivalent and pentavalent inorganic arsenic in mice and rats. Environ Res 25(2): 286-93.

Vahter M (1983). Metabolism of arsenic. In: Bilogical and Environmental Effects of Arsenic. Fowler B. A. Elsevier. Amsterdam: 171-198.

Vahter M, Concha G, Nermell B, Nilsson R, Dulout F and Natarajan AT (1995a). A unique metabolism of inorganic arsenic in native Andean women. Eur J Pharmacol 293(4): 455-62.

Vahter M, Couch R, Nermell B and Nilsson R (1995b). Lack of methylation of inorganic arsenic in the chimpanzee. Toxicol Appl Pharmacol 133(2): 262-8.

Vahter M and Envall J (1983). In vivo reduction of arsenate in mice and rabbits. Environ Res 32(1): 14-24.

Vahter M, Friberg L, Rahnster B, Nygren A and Nolinder P (1986). Airborne arsenic and urinary excretion of metabolites of inorganic arsenic among smelter workers. Int Arch Occup Environ Health 57(2): 79-91.

Vahter M, Marafante E and Dencker L (1984). Tissue distribution and retention of 74As-dimethylarsinic acid in mice and rats. Arch Environ Contam Toxicol 13(3): 259-64.

Vallee BL, Ulmer DD and Wacker WEC (1960). Arsenic toxicology and biochemistry. Arch Ind Health 21: 132-151.

von Ehrenstein OS, Guha Mazumder DN, Hira-Smith M, Ghosh N, Yuan Y, Windham G, Ghosh A, Haque R, Lahiri S, Kalman D, Das S and Smith AH (2006). Pregnancy outcomes, infant mortality, and arsenic in drinking water in West Bengal, India. Am J Epidemiol 163(7): 662-9.

von Ehrenstein OS, Mazumder DN, Yuan Y, Samanta S, Balmes J, Sil A, Ghosh N, Hira-Smith M, Haque R, Purushothamam R, Lahiri S, Das S and Smith AH (2005). Decrements in lung function related to arsenic in drinking water in West Bengal, India. Am J Epidemiol 162(6): 533-41.

Wagstaff DJ (1978). Alteration of hepatic detoxication enzyme activity by dietary arsenic trioxide. Food Cosmet Toxicol 16(5): 423-6.

Walton FS, Waters SB, Jolley SL, LeCluyse EL, Thomas DJ and Styblo M (2003). Selenium compounds modulate the activity of recombinant rat AsIII-methyltransferase and the methylation of arsenite by rat and human hepatocytes. Chem Res Toxicol 16(3): 261-5.

Wang CH, Jeng JS, Yip PK, Chen CL, Hsu LI, Hsueh YM, Chiou HY, Wu MM and Chen CJ (2002). Biological gradient between long-term arsenic exposure and carotid atherosclerosis. Circulation 105(15): 1804-9.

Wasserman GA, Liu X, Parvez F, Ahsan H, Factor-Litvak P, van Geen A, Slavkovich V, LoIacono NJ, Cheng Z, Hussain I, Momotaj H and Graziano JH (2004). Water arsenic exposure and children's intellectual function in Araihazar, Bangladesh. Environ Health Perspect 112(13): 1329-33.

Webb DR, Wilson SE and Carter DE (1986). Comparative pulmonary toxicity of gallium arsenide, gallium(III) oxide, or arsenic(III) oxide intratracheally instilled into rats. Toxicol Appl Pharmacol 82(3): 405-16.

Wester RC, Hui X, Barbadillo S, Maibach HI, Lowney YW, Schoof RA, Holm SE and Ruby MV (2004). In vivo percutaneous absorption of arsenic from water and CCA-treated wood residue. Toxicol Sci 79(2): 287-95.

Wester RC, Maibach HI, Sedik L, Melendres J and Wade M (1993). In vivo and in vitro percutaneous absorption and skin decontamination of arsenic from water and soil. Fundam Appl Toxicol 20(3): 336-40.

Wildfang E, Zakharyan RA and Aposhian HV (1998). Enzymatic methylation of arsenic compounds. VI. Characterization of hamster liver arsenite and methylarsonic acid methyltransferase activities in vitro. Toxicol Appl Pharmacol 152(2): 366-75.

Willhite CC and Ferm VH (1984). Prenatal and developmental toxicology of arsenicals. In: Nutritional and Toxiclogical Aspects of Food Safety. Friedman M. Plenum Publishing Corp. New York (NY): 9: 205-228.

Williams PL, Spain WH and Rubenstein M (1981). Suspected arsine poisoning during the restoration of a large cyclorama painting. Am Ind Hyg Assoc J 42(12): 911-3.

Winski SL and Carter DE (1995). Interactions of rat red blood cell sulfhydryls with arsenate and arsenite. J Toxicol Environ Health 46(3): 379-97.

Woods JS and Fowler BA (1978). Altered regulation of mammalian hepatic heme biosynthesis and urinary porphyrin excretion during prolonged exposure to sodium arsenate. Toxicol Appl Pharmacol 43(2): 361-71.

Wu MM, Kuo TL, Hwang YH and Chen CJ (1989). Dose-response relation between arsenic concentration in well water and mortality from cancers and vascular diseases. Am J Epidemiol 130(6): 1123-32.

Yamauchi H and Yamamura Y (1985). Metabolism and excretion of orally administrated arsenic trioxide in the hamster. Toxicology 34(2): 113-21.

Yu D (1999). A physiologically based pharmacokinetic model of inorganic arsenic. Regul Toxicol Pharmacol 29(2 Pt 1): 128-41.

Yu HS, Sheu HM, Ko SS, Chiang LC, Chien CH, Lin SM, Tserng BR and Chen CS (1984). Studies on blackfoot disease and chronic arsenism in southern Taiwan: with special reference to skin lesions and fluorescent substances. J Dermatol 11(4): 361-70.

Yu L, Kalla K, Guthrie E, Vidrine A and Klimecki WT (2003). Genetic variation in genes associated with arsenic metabolism: glutathione S-transferase omega 1-1 and purine nucleoside phosphorylase polymorphisms in European and indigenous Americans. Environ Health Perspect 111(11): 1421-7.

Zakharyan R, Wu Y, Bogdan GM and Aposhian HV (1995). Enzymatic methylation of arsenic compounds: assay, partial purification, and properties of arsenite methyltransferase and monomethylarsonic acid methyltransferase of rabbit liver. Chem Res Toxicol 8(8): 1029-38.

Zakharyan RA and Aposhian HV (1999). Enzymatic reduction of arsenic compounds in mammalian systems: the rate-limiting enzyme of rabbit liver arsenic biotransformation is MMA(V) reductase. Chem Res Toxicol 12(12): 1278-83.

Zakharyan RA, Sampayo-Reyes A, Healy SM, Tsaprailis G, Board PG, Liebler DC and Aposhian HV (2001). Human monomethylarsonic acid (MMA(V)) reductase is a member of the glutathione-S-transferase superfamily. Chem Res Toxicol 14(8): 1051-7.

Zakharyan RA, Wildfang E and Aposhian HV (1996). Enzymatic methylation of arsenic compounds. III. The marmoset and tamarin, but not the rhesus, monkeys are deficient in

methyltransferases that methylate inorganic arsenic. Toxicol Appl Pharmacol 140(1): 77-84.

Zaldivar R and Guillier A (1977). Environmental and clinical investigations on endemic chronic arsenic poisoning in infants and children. Zentralbl Bakteriol [Orig B] 165(2): 226-34.

Formaldehyde Reference Exposure Levels

(Methanal, oxomethane, methylene oxide)

CAS 50-00-0

$H_2C{=}O$

1. Summary

The non-cancer adverse health effects of formaldehyde are largely a manifestation of its ability to irritate mucous membranes. As a result of its solubility in water and high reactivity, formaldehyde is efficiently absorbed into the mucus layers protecting the eyes and respiratory tract where it rapidly reacts, leading primarily to localized irritation. Acute high exposure may lead to eye, nose and throat irritation, and in the respiratory tract, nasal obstruction, pulmonary edema and dyspnea. Prolonged or repeated exposures have been associated with allergic sensitization, asthma-like symptoms, histopathological changes in respiratory epithelium, and decrements in lung function. Children, especially those with diagnosed asthma, may be more likely to show impaired pulmonary function and symptoms of asthma than are adults following chronic exposure to formaldehyde.

1.1 Formaldehyde Acute REL

Reference Exposure Level	**55 μg/m³ (44 ppb)**
Critical effect(s)	Mild and moderate eye irritation
Hazard Index target(s)	Eye irritation

1.2 Formaldehyde 8-Hour REL

Reference Exposure Level	**9 μg/m³ (7 ppb)**
Critical effect(s)	Asthma-like respiratory symptoms
Hazard Index target(s)	Respiratory

1.3 Formaldehyde Chronic REL

Reference Exposure Level	**9 μg/m³ (7 ppb)**
Critical effect(s)	Asthma-like respiratory symptoms
Hazard Index target(s)	Respiratory

2. Physical & Chemical Properties

Description	Colorless gas
Molecular formula	CH_2O
Molecular weight	30.03 g/mol
Density	0.815 g/L @ -20° C
Boiling point	-19.5° C
Melting point	-92° C
Vapor pressure	3883 mm Hg @ 25° C
Flashpoint	300° C
Explosive limits	7% - 73%
Solubility	soluble in water, alcohol, ether and other polar solvents
Odor threshold	0.05-0.5 ppm
Metabolites	formic acid
Conversion factor	1 ppm in air = 1.24 mg/m^3 @ 25° C

3. Occurrence and Major Uses

Formaldehyde has four major applications: as an intermediate in the manufacture of melamine, polyacetal, and phenolic resins; as an intermediate in the production of industrial chemicals; as a bactericide or fungicide; and as a component in the manufacture of end-use consumer products. Phenol-formaldehyde resins are used in the production of plywood, particleboard, foam insulation, and a wide variety of molded or extruded plastic items. Formaldehyde is also used as a preservative, a hardening and reducing agent, a corrosion inhibitor, a sterilizing agent, and in embalming fluids. Indoor sources include upholstery, permanent press fabrics, carpets, pesticide formulations, urea-formaldehyde foam insulation, and cardboard and paper products. Outdoor sources include emissions from fuel combustion (motor vehicles), industrial fuel combustion (power generators), oil refining processes, and other uses (copper plating, incinerators, etc.). The largest portion of outdoor ambient formaldehyde results from photochemical oxidation of a number of reactive organic gases in the atmosphere (CARB, 2006). According to the California Toxics Inventory (CARB, 2005a), the mean statewide ambient level of formaldehyde in 2004 was 2.69 ppb, with the highest levels (3.76 ppb) reported for the South Coast Air Basin. CARB reported statewide emissions of 20,251 tons from stationary and mobile sources (CARB, 2005b).

4. Metabolism

Inhaled formaldehyde reacts rapidly at the site of contact and is efficiently absorbed in the respiratory tract. A portion of the formaldehyde entering the mucous layer of the respiratory tract is reversibly hydrated to methylene glycol. Both the hydrated and free formaldehyde may be absorbed into the epithelial layer where formaldehyde may bind reversibly to glutathione to form *S*-hydroxymethylglutathione. This in turn is oxidized to *S*-formylglutathione by formaldehyde dehydrogenase. Hydrolysis of *S*-formylglutathione

yields formate and glutathione. Formic acid may be eliminated in urine and feces, or dehydrogenated to CO_2 and exhaled. The presence of glutathione and formaldehyde dehydrogenase in epithelial cells of the respiratory tract varies with location and influences the amount of formaldehyde reaching the blood. While glutathione-bound formaldehyde is rapidly metabolized, free formaldehyde in cells can form DNA-protein cross-links (Franks, 2005).

5. Acute Toxicity of Formaldehyde

5.1 Acute Toxicity to Adult Humans

In small human studies, exposure to formaldehyde (1-3 ppm) has resulted in eye and upper respiratory tract irritation (Weber-Tschopp et al., 1977; Kulle et al., 1987). Most people cannot tolerate exposures to more than 5 ppm formaldehyde in air; above 10-20 ppm symptoms become severe and shortness of breath occurs (Feinman, 1988). High concentrations of formaldehyde may result in nasal obstruction, pulmonary edema, choking, dyspnea, and chest tightness (Porter, 1975; Solomons and Cochrane, 1984).

A few human case studies report severe pulmonary symptoms. A medical intern with known atopy and exposure to reportedly high (but unspecified) levels of formaldehyde over a period of 1 week developed dyspnea, chest tightness, and edema, following a subsequent 2 hour exposure to formaldehyde (Porter, 1975). Five workers exposed to formaldehyde from newly installed urea-formaldehyde chipboard in a poorly ventilated basement experienced intolerable eye and upper respiratory tract irritation, choking, marked dyspnea, and nasal obstruction (Solomons and Cochrane, 1984). However, the concentration of formaldehyde and the contribution of other airborne chemicals were unknown in both reports.

Numerous acute controlled and occupational human exposure studies have been conducted with both asthmatic and normal subjects to investigate formaldehyde's irritative and pulmonary effects (Frigas et al., 1984; Sheppard et al., 1984; Sauder et al., 1986; Schachter et al., 1986; Kulle et al., 1987; Sauder et al., 1987; Schachter et al., 1987; Witek et al., 1987; Uba et al., 1989; Harving et al., 1990; Akbar-Khanzadeh et al., 1994). Short exercise sessions during exposure on a bicycle ergometer were included in some of the studies. Concentrations of formaldehyde in the human exposure studies ranged as high as 3 ppm for up to 3 hours. The major findings in these studies were mild to moderate eye and upper respiratory tract irritation typical of mild discomfort from formaldehyde exposure.

In a human irritation study by Weber-Tschopp et al. (1977), 33 subjects were exposed to formaldehyde at concentrations ranging from 0.03-3.2 ppm (0.04-4.0 mg/m^3) for 35 minutes. Thresholds were 1.2 ppm (1.5 mg/m^3) for eye and nose irritation, 1.7 ppm (2.1 mg/m^3) for eye blinking, and 2.1 ppm (2.6 mg/m^3) for throat irritation.

Kulle et al. (1987) exposed nonasthmatic humans to up to 3.0 ppm (3.7 mg/m^3) formaldehyde in a controlled environmental chamber for 3 hours. Significant dose-response relationships were seen with odor and eye irritation (Table 5.1) as ranked on

symptom questionnaires as none, mild, moderate or severe. Irritation was assessed in this manor prior to exposure, at the end of exposure, and again 24 hour after exposure.

Table 5.1 Mean Symptom Difference (t_{180}-t_0) ± SE with Formaldehyde*
(from Kulle et al., 1987)

	Formaldehyde conc. (ppm)				P value
	0.0	1.0	2.0	3.0	
Odor sensation	0.00 ± 0.00	0.22 ± 0.15	0.44 ± 0.18	1.00 ± 0.29	<0.0001
Nose/throat irritation	0.00 ± 0.00	0.11 ± 0.11	0.33 ± 0.17	0.22 ± 0.15	0.054
Eye irritation	0.00 ± 0.00	0.44 ± 0.24	0.89 ± 0.26	1.44 ± 0.18	<0.0001
Chest discomfort	0.00 ± 0.00	0.00 ± 0.00	0.11 ± 0.11	0.00 ± 0.00	0.62
Cough	0.00 ± 0.00	0.11 ± 0.11	0.00 ± 0.00	0.00 ± 0.00	0.11
Headache	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.11 ± 0.11	0.33

*Presence and severity of symptoms scored as: 0 = none; 1 = mild (present but not annoying); 2 = moderate (annoying); 3 = severe (debilitating). n=9

At 0.5 ppm for 3 hours, none of 9 subjects had eye irritation. At 1.0 ppm, 3 of 19 subjects reported mild eye irritation and one experienced moderate irritation. At 2.0 ppm, 6 subjects reported mild and 4 reported moderate eye irritation. Measured nasal flow resistance was increased at 3.0 ppm but not at 2.0 ppm (2.5 mg/m^3). With respect to the lower respiratory tract, there were no significant decrements in pulmonary function nor increases in methacholine induced bronchial reactivity as a result of 3-hour exposures to 0.5-3.0 ppm (0.6-3.7 mg/m^3) formaldehyde at rest or during exercise, including 24 hours post exposure.

Eleven healthy subjects and nine patients with formalin skin sensitization were exposed to 0.5 mg/m^3 (0.4 ppm) formaldehyde for 2 hours (Pazdrak et al., 1993). Nasal lavage was performed prior to and 5 to 10 minutes, 4 hours, and 18 hours after exposure. Rhinitis was reported and increases in the number and proportion of eosinophils, elevated albumin and increased protein levels were noted in nasal lavage fluid 4 and 18 hours after exposure. No differences were found between patients with skin sensitization and healthy subjects.

In a study by Green et al. (1987), volunteer asthmatic and normal subjects exposed to formaldehyde displayed decrements in pulmonary function. Exposure to 3 ppm formaldehyde for 1 hour resulted in clinically significant reductions of forced expiratory volume in one second (FEV_1) (defined as > 20% or more) and FEV_1/forced vital capacity (FVC) (ratio 70% or less) in 5 individuals in the study (2 of 16 asthmatics, 2 of 22 normal subjects, and one clinically normal subject with hyperactive airways). Of these individuals, 3 had reductions of FEV_1 of 20% or more during exposure. One of 22 asthmatics had a greater than 20% reduction in FEV_1 (-25.8%) at 17 minutes into exposure following a 15 minute moderate exercise session (minute ventilation [V_E] = 30-40 l/min), which, according to the authors, was low enough to prevent exercise-induced bronchospasm. One of 22 normal subjects also exhibited a greater than 20% clinically significant reduction in FEV_1 (-24.4%) and in FEV_1/FVC, which occurred at 47 minutes

into exposure to 3 ppm formaldehyde. These reductions occurred following a second 15-minute heavy-exercise session (V_E = 60-70 l/min) near the end of the 1 hour exposure period. A third asymptomatic "normal" subject with hyperactive airways had a clinically significant reduction of FEV_1 (-20.5%) at 17 minutes, following the first heavy exercise session. This subject exhibited occult airway hyperactivity and was excluded from analysis with the other exposure groups due to his respiratory condition. Subjects exhibiting reductions in FEV_1 of greater than 20% following exposure also exhibited FEV_1/FVC ratios of less than 70%. However, none of the subjects in the study exhibited a clinically significant reduction of 50% or greater in airway conductance (SG_{aw}) during exposure to 3 ppm formaldehyde.

Kriebel et al. (2001) conducted a subchronic epidemiological study of 38 anatomy class students who, on average, were exposed to a geometric mean of 0.70 ± 2.13 ppm for 2 hours per week over 14 weeks. After class, eye, nose and throat irritation was significantly elevated compared with pre-laboratory session exposures, with a one unit increase in symptom intensity/ppm of formaldehyde. Peak expiratory flow (PEF) was found to decrease by 1%/ppm formaldehyde during the most recent exposure. Changes in PEF and symptom intensity following formaldehyde exposure were most pronounced during the first weeks of the semester but attenuated with time, suggesting partial acclimatization.

Rhinitis and a wide range of asthma-like conditions can result from exposure to formaldehyde. Some studies have reported that workers exposed to low concentrations may develop severe prolonged asthma attacks after prior exposure; this suggests that they may have become sensitized (Feinman, 1988). However, in adults, an association between formaldehyde exposure and allergic sensitization through IgE- and IgG-mediated mechanisms has been observed only inconsistently (Thrasher et al., 1987; Krakowiak et al., 1998; Wantke et al., 2000; Kim et al., 2001).

Formaldehyde provocation of human subjects, occupationally exposed to formaldehyde and suffering from asthma-like symptoms such as wheezing, shortness of breath, or rhinitis, occasionally resulted in pulmonary function decrements (2 to 33% response rate) consistent with immediate, delayed, or both immediate and delayed bronchoconstriction (Hendrick and Lane, 1977; Wallenstein et al., 1978; Burge et al., 1985; Nordman et al., 1985). While some of the concentrations of formaldehyde that elicited a positive response following provocation tests (6 to 20.7 ppm) were quite high, the authors of these studies suggested that formaldehyde-induced bronchial hyperreactivity is due to specific sensitization to the gas. However, none of these studies was able to detect antibodies to formaldehyde which would support that sensitization to formaldehyde occurs through an immunologic pathway.

In controlled studies with asthmatics from urea-formaldehyde insulated homes, formaldehyde concentrations equal to or greater than those found in indoor environments have not resulted in hematologic or immunologic abnormalities. These tests include: blood count and differential, erythrocyte sedimentation rate; lymphocyte subpopulations (E-rosetting, T3, T4, T8, B73.1, Fc receptor positive lymphocytes and large granular

lymphocytes); lymphocyte response to phytohemagglutinin and formalin-treated red blood cells; serum antibody against the Thomsen-Friedenrich RBC antigen and against formalin-RBC; and natural killer, interferon-boosted natural killer, and antibody-dependent cell-mediated cytotoxicity (Pross et al., 1987). While six of the studies cited above reported decrements in lung function associated with short-term formaldehyde exposure among at least some of the asthmatic subjects, a number of other exposure studies of patients with asthma have failed to demonstrate that exposure to formaldehyde results in onset or aggravation of the patients' asthmatic symptoms (Sheppard et al., 1984; Sauder et al., 1987; Harving et al., 1990; Krakowiak et al., 1998).

The effects of formaldehyde on asthmatics may be dependent on previous, repeated exposure to formaldehyde. Burge et al. (1985) found that 3 out of 15 occupationally exposed workers challenged with formaldehyde vapors at concentrations from 1.5 ppm to 20.6 ppm for brief durations exhibited late asthmatic reactions. Six other subjects had immediate asthmatic reactions likely due to irritant effects. Asthmatic responses (decreased PEF, FVC, and FEV_1) were observed in 12 occupationally-exposed workers challenged with 1.67 ppm (2.5 mg/m^3) formaldehyde (Nordman et al., 1985). Similarly, asthmatic responses were observed in 5 of 28 hemodialysis workers occupationally exposed to formalin and challenged with formaldehyde vapors (concentration not measured) (Hendrick and Lane, 1977). In asthmatics not occupationally exposed to formaldehyde, Sheppard et al. (1984) found that a 10-minute challenge with 3 ppm formaldehyde coupled with moderate exercise did not induce significant changes in airway resistance or thoracic gas volume.

Gorski et al. (1992) evaluated the production of active oxygen species by neutrophils in 18 persons exposed to 0.5 mg/m^3 formaldehyde for 2 hours. All 13 subjects who had allergic contact dermatitis (tested positive to formaldehyde in skin patch) exhibited significantly higher chemiluminescence of granulocytes isolated from whole blood 30 minutes and 24 hours post-exposure than the individuals who were not formaldehyde sensitive. Thus, the immune cellular response of skin-sensitized individuals to an inhalation exposure to formaldehyde indicates increased production of active oxygen species. The significance of this result is unclear but may have repercussions for toxicological effects mediated by active oxygen species.

Predisposing Conditions for Formaldehyde Toxicity

Medical: Persons with eye, skin, respiratory, or allergic conditions (especially asthma) may be more sensitive to the effects of formaldehyde (ATSDR, 1999). Asthmatics sensitized to formaldehyde may be more sensitive to formaldehyde at low concentrations than non-sensitized individuals.

5.2 Acute Toxicity to Infants and Children

No studies of the effects of acute exposure to formaldehyde in children or young experimental animals were located. However, as noted above for adults, there is evidence that following acute exposure to formaldehyde, asthmatics and others previously sensitized to formaldehyde may be more likely to show asthma-like symptoms such as wheezing, shortness of breath, rhinitis, and/or decrements in pulmonary function consistent with immediate and/or delayed bronchoconstriction (Nordman et al., 1985; Burge et al., 1985; Hendrick and Lane, 1977; Wallenstein et al., 1978). Furthermore, some asthmatics may respond with significant reductions in lung function due to the irritant effects on asthma, sensitized or not. The potential association between formaldehyde exposure and asthma is of special concern for children since, as noted in OEHHA (2001): "*OEHHA considers asthma to impact children more than adults. Children have higher prevalence rates of asthma than do adults (Mannino* et al.*, 1998). In addition, asthma episodes can be more severe due to the smaller airways of children, and result in more hospitalizations in children, particularly from the ages of 0 to 4 years, than in adults (Mannino* et al.*, 1998)."* Thus children, particularly asthmatic children, may be at greater risk from acute exposure to formaldehyde.

5.3 Acute Toxicity to Experimental Animals

Acute exposures of experimental animals to formaldehyde are associated with changes in pulmonary function (decreased respiratory rate, increased airway reactivity and resistance) at low concentrations, while pulmonary edema and death have been reported at high concentrations. Neurochemical and neurobehavioral changes have also been observed.

In 72 rats exposed to approximately 600-1,700 mg/m^3 (500-1,400 ppm) formaldehyde vapor for 30 minutes, the LC_{50} was found to be 1,000 mg/m^3 (800 ppm) (Skog, 1950). The first deaths did not occur until 6 hours after cessation of exposure. Respiratory difficulty lasted several days after exposure and the last of 49 rats died after 15 days of purulent bronchitis and diffuse bronchopneumonia. Three weeks following exposure, histological examinations of the 23 surviving animals revealed bronchitis, pulmonary microhemorrhages, and edema. No changes were seen in other organs.

A multispecies study by Salem and Cullumbine (1960) showed that a 10-hour exposure to 15.4 ppm (19 mg/m^3) formaldehyde vapor killed 3/5 rabbits, 8/20 guinea pigs, and 17/50 mice. The report stated that formaldehyde exposure resulted in delayed lethality.

Alarie (1981) determined the 10 minute LC_{50} for formaldehyde in mice to be 2,162 ppm (95% confidence interval, 1,687-2,770 ppm). The post-exposure observation period was 3 hours. From the concentration mortality graph provided in the report, an MLE_{05} and BC_{05} of 1,440 ppm and 778 ppm, respectively, could be estimated for a 10-minute formaldehyde exposure. However, as indicated in the previous reports, delayed deaths occur with formaldehyde which suggests that the 3-hour post-exposure observation period used in this study may not have been long enough.

In other lethality studies, Nagornyi et al.(1979) determined a 4-hour formaldehyde LC_{50} in rats and mice to be 588 mg/m^3 (474 ppm) and 505 mg/m^3 (407 ppm), respectively. However, the raw data for this study were not included in the report. Horton et al. (1963) observed that a 2-hour exposure of mice to 0.9 mg/l (900 mg/m^3) formaldehyde resulted in deaths from massive pulmonary hemorrhage and edema, but a 2 hour exposure to 0.14 mg/l (140 mg/m^3) did not produce signs of "substantial distress."

Swiecichowski et al., (1993) exposed groups of five to seven guinea pigs to 0.86, 3.4, 9.4, 31.1 ppm (1.1, 4.2, 11.6, 38.6 mg/m^3) formaldehyde for 2 hours, or to 0.11, 0.31, 0.59, 1.05 ppm (0.14, 0.38, 0.73, 1.30 mg/m^3) formaldehyde for 8 hours. An 8-hour exposure to $\geq$0.3 ppm ($\geq$ 0.4 mg/m^3) formaldehyde was sufficient to produce a significant increase in airway reactivity. Similar effects occurred after > 9 ppm (> 11 mg/m^3) formaldehyde for the 2-hour exposure group. Formaldehyde exposure also heightened airway smooth muscle responsiveness to acetylcholine (or carbachol) *ex vivo*. No inflammation or epithelial damage was seen up to 4 days after exposure. The researchers suggest that duration of exposure is important to the induction of airway hyperreactivity and that prolonged (8-hour), low-level exposures may generate abnormal physiologic responses in the airways not detectable after acute (2-hour) exposures.

Male F-344 rats, 7-9 weeks old, were exposed to 0.5, 2, 6 or 15 ppm formaldehyde for 6 hours per day for 1 to 4 days (Monteiro-Riviere and Popp, 1986). Effects noted in the rat nasal respiratory epithelium with 0.5 or 2 ppm were limited to altered cilia with occasional wing-like projections on the ends of the ciliary shafts. Effects noted at 6 ppm for 1 day were autophagic vacuoles in some basal cells, neutrophils in the basal and suprabasal layers, and hypertrophy of goblet and ciliated cells. Loss of microvilli in ciliated cells was noted at all exposure concentrations.

Rats were exposed to 0, 5, 10 or 20 ppm formaldehyde for 3 hours per day on 2 consecutive days (Boja et al., 1985). Decreased motor activity and neurochemical changes in dopamine and 5-hydroxytryptamine neurons were reported.

The effects of formaldehyde inhalation on open-field behavior in mice were examined by Malek et al. (2004) 2 and 24 hours after a single 2-hour exposure to 0, 1.1, 2.3 or 5.2 ppm. Two hours after exposure there were significant decreases in rearing and in several measures of exploratory behavior, with evidence of dose-dependence in all dose groups compared with controls. At 24 hours, there were still significant differences between dosed and control mice but the dose-dependence was no longer evident.

Nielson et al. (1999) analyzed the breathing patterns of Balb/c mice exposed to 0.2-13 ppm formaldehyde and found a concentration-dependent decrease in respiratory rate of 32.9%/log concentration. In the range of 0.3-4.0 ppm, the decrease in respiratory rates was attributable to sensory irritation. Above 4.0 ppm, bronchoconstriction also contributed to the decreased breathing rate. The authors suggest a NOEL of 0.3 ppm for these effects in mice.

Amdur (1960) exposed groups of 4 to 18 guinea pigs to formaldehyde at 0.05, 0.31, 0.58, 1.22, 3.6, 11.0, or 49 ppm formaldehyde for one hour. Resistance to flow and lung compliance were calculated from measures of intrapleural pressure, tidal volume, and rate of flow to the lungs at the end of exposure and one hour later. Resistance and compliance were significantly different from the control level for the 0.31 ppm exposure ($p<0.05$) and increasingly significant at higher concentrations. One hour later, only the 49 ppm exposure remained significant ($p<0.01$). In addition, the tracheas of groups of 6 to 10 guinea pigs were cannulated and exposed for one hour to 0.90, 5.2, 20, or 50 ppm formaldehyde, and 1.14 or 3.6 ppm formaldehyde with 10 mg/m^3 sodium chloride. With the protective effect of the trachea bypassed, the resistance and compliance changed substantially. The addition of sodium chloride further enhanced the effect, including a significant effect after one hour for the 1.14 ppm formaldehyde exposure. These results show that formaldehyde that reaches the lungs has a marked effect on airways resistance and compliance in addition to an effect on the upper airways.

Riedel et al. (1996) studied the influence of formaldehyde exposure on allergic sensitization in guinea pigs. Three groups of guinea pigs (12/group) were exposed to clean air or two different formaldehyde concentrations (0.13 and 0.25 ppm) over five consecutive days. Following exposure, the animals were sensitized to allergen by inhalation of 0.5% ovalbumin (OA). Three weeks later the animals were subjected to bronchial provocation with OA and specific anti-OA-IgGl (reaginic) antibodies in serum were measured. In another group of six animals, the respiratory tract was examined histologically for signs of inflammation directly after the end of formaldehyde or clean air exposure. In the group exposed to 0.25 ppm formaldehyde, 10/12 animals were found to be sensitized to OA (positive reaction on specific provocation) vs. 3/12 animals in the control group ($P < 0.01$). Furthermore, compressed air measurements of specific bronchial provocation and serum anti-OA-antibodies were significantly higher in the 0.25 ppm formaldehyde group than in controls. The median for compressed air measurement was 0.35 ml for the formaldehyde-exposed group vs. 0.09 ml for the controls ($p< 0.01$), indicating increased bronchial obstruction. The median for the anti-OA-IgGl measured in the formaldehyde-exposed group was 13 vs. less than 10 EU in the controls, ($p < 0.05$), indicating enhanced sensitization. In the group exposed to 0.13 ppm formaldehyde, no significant difference was found compared to the control group. Histological examination found edema of the bronchial mucosa, but there was no sign of inflammation of the lower airways in formaldehyde-exposed guinea pigs. The investigators concluded that short-term exposure to a low concentration of formaldehyde (0.25 ppm) can significantly enhance sensitization to inhaled allergens in the guinea pig.

6. Chronic Toxicity of Formaldehyde

6.1 Chronic Toxicity to Adult Humans

Formaldehyde primarily affects the mucous membranes of the upper airways and eyes. Exposed populations that have been studied include embalmers, residents in houses insulated with urea-formaldehyde foam, anatomy class students, histology technicians, wood and pulpmill workers, and asthmatics. A number of studies describing these effects have been briefly summarized below. For the sake of brevity, only the studies that best represent the given effects are presented.

In the study chosen for determination of the 8-hour and chronic RELs, nasal obstruction and discharge, and frequency of cough, wheezing, and symptoms of bronchitis were reported in 66 workers in a formaldehyde production plant exposed for 1 - 36 years (mean = 10 years) to a mean concentration of 0.21 ppm (0.26 mg/m^3) formaldehyde (Wilhelmsson and Holmstrom, 1992). All workers were exposed almost exclusively to formaldehyde, the concentrations of which were measured in the ambient air of the worksite with personal sampling equipment. Referents consisted of 36 office workers in a government office with exposure to a mean concentration of 0.06 ppm (0.09 mg/m^3) formaldehyde, and no industrial solvent or dust exposure. Symptom data, collected by questionnaire, were separated into general and work-related, and allowed identification of individuals with atopy and mucosal hyperreactivity. The critical effects from chronic exposure to formaldehyde in this study included nasal obstruction, lower airway discomfort, and eczema or itching. The frequency of reported lower airway discomfort (intermittent cough, wheezing, or symptoms of chronic bronchitis) was significantly higher among formaldehyde-exposed vs non-exposed workers (44 vs 14%; $p < 0.01$) (Table 6.1). Work-related nasal discomfort also was significantly higher in the formaldehyde group (53%) compared with the referent group (3%; $p < 0.001$). Similarly, work-related eye discomfort was 20% in the formaldehyde group but nonexistent among referents. The significant increase in symptoms of nasal discomfort in exposed workers did not correlate with total serum IgE antibody levels. However, two exposed workers, who complained of nasal discomfort, had elevated IgE levels. The investigators concluded that formaldehyde can induce nonspecific nasal hypersensitivity.

Table 6.1.1 Symptoms of Formaldehyde Exposure vs Reference Group
(from Wilhelmsson and Holmstrom, 1992)

	Formaldehyde	Reference	Rate difference	
	% (n=96)	% (n=36)	%	95% CI
General nasal discomfort	67	25	42	24-60
Workplace nasal discomfort	53	3	50	37-63
General lower airway discomfort	44	14	30	14-47
Workplace lower airway discomfort	33	3	28	15-40
General eye discomfort	24	6	18	6-36
General skin discomfort	36	11	25	10-41

In a cross-sectional study supportive of these results, Edling et al. (1988) reported histopathological changes in nasal mucosa of workers (n=75) occupationally exposed to formaldehyde (one wood laminating plant) or formaldehyde plus wood dust (two particle board plants). Ambient formaldehyde measurements in these three composite wood processing plants between 1975 and 1983 gave a time-weighted average (TWA) of 0.1-1.1 mg/m^3 (0.08- 0.89 ppm) with peaks of up to 5 mg/m^3 (4 ppm). The exposed workers were compared on the basis of medical and work histories, clinical examinations and nasal biopsies to 25 workers selected with regard to age and smoking habits but without occupational formaldehyde exposure.

Based on the histories, there was a high frequency of eye and upper airway symptoms among workers. Nasal symptoms (running nose and crusting) associated with formaldehyde exposure were reported in 60% of the workers, while 75% complained of lacrimation. Clinical examinations revealed grossly normal nasal mucosa in 75% of the cases while 25% had swollen or dry changes, or both, to the nasal mucosa. Histological examination (Table 6.2) revealed that only 3 of the 75 formaldehyde-exposed workers had normal, ciliated pseudostratified epithelium. Squamous metaplasia was reportedly observed in 59, while 6 showed mild dysplasia, and in 8 there was loss of ciliated cells and goblet cell hyperplasia. The histological grading showed a significantly higher score for nasal lesions among workers with formaldehyde exposure when compared with the referents (2.9 versus 1.8; $p < 0.05$). Exposed smokers had a higher, but non-significant, score than ex-smokers and non-smokers.

While the mean exposure time was 10.5 years (range 1-39 yr), there was no discernable difference among histology scores as a function of years of employment. The histology scores were also not different between workers in the particle board plants, exposed to both formaldehyde and wood dust, and workers in the laminate plant with exposure only to formaldehyde. The authors thus attribute the pathological changes in the nasal mucosa and the other adverse effects to formaldehyde alone in the 0.1-1.1 mg/m^3 range.

Table 6.1.2 Distribution of Histological Characteristics Associated with Formaldehyde Exposure (from Edling et al., 1988)

Histological characteristic	Grading score	Point score	Workers	%
Normal respiratory epithelium	0	0	3	4
Loss of ciliated cells	1	1	8	11
Mixed cuboidal/squamous epithelium, metaplasia	2	2	24	32
Stratified squamous epithelium	3	3	18	24
Keratosis	4	4	16	21
Budding of epithelium	1	5	0	0
Mild or moderate dysplasia	6	6	6	8
Severe dysplasia	7	7	0	0
Carcinoma	8	8	0	0

Histological changes in the nasal mucosa of formaldehyde-exposed workers was also reported by Boysen et al. (1990). In this study, nasal biopses were collected from 37 workers with 5 or more years of occupational formaldehyde exposure (0.5 - > 2 ppm) and compared with age-matched, unexposed controls who otherwise had similar environmental exposures and smoking habits. Histological changes in the nasal epithelium were scored as indicated in Table 6.1.3.

Table 6.1.3 Types of Nasal Epithelia and Scoring (from Boysen et al., 1990)

Types of epithelia	Histological score
Pseudostratified columnar	0
Stratified cuboidal	1
Mixed stratified cuboidal/stratified squamous	2
Stratified squamous, non-keratinizing	3
Stratified squamous, keratinizing	4
Dysplasia	5

As shown by the histological scoring in Table 6.1.4 below, metaplastic changes in the nasal epithelium were more pronounced in the formaldehyde-exposed workers although this difference did not reach statistical significance.

Table 6.1.4 Histological Scores of Nasal Epithelia

		Histological score						
	No	**0**	**1**	**2**	**3**	**4**	**5**	**Mean**
Exposed	37	3	16	5	9	1	3	1.9
Controls	37	5	17	10	5	0	0	1.4

Rhinoscopical examination revealed hyperplastic nasal mucosa in 9 of 37 formaldehyde-exposed workers but in only 4 of the controls. In addition, the incidence of subjective nasal complaints was significantly ($p < 0.01$) higher in the exposed group. While the small size of this study, and the small amount of the nasal mucosa accessible to biopsy limited its ability to detect formaldehyde- related histopathology, the results are consistent with the histopathologies reported by Edling et al. above.

In another occupational health study (Grammer et al., 1990), 37 workers, who were exposed for an unspecified duration to formaldehyde concentrations in the range of 0.003 to 0.073 ppm, reported ocular irritation. However, no significant serum levels of IgE or IgG antibodies to formaldehyde-human serum albumin were detected.

Kerfoot and Mooney (1975) reported that estimated formaldehyde exposures of 0.25-1.39 ppm evoked numerous complaints of upper respiratory tract and eye irritation among seven embalmers at six different funeral homes. Three of the seven embalmers in this study reportedly had asthma. Levine et al. (1984) examined the death certificates of 1477 Ontario undertakers. Exposure measurements taken from a group of West Virginia embalmers were used as exposure estimates for the embalming process, ranging from 0.3-0.9 ppm (average 1-hour exposure) and 0.4-2.1 ppm (peak 30-minute exposure).

Mortality due to non-malignant diseases was significantly elevated due to a two-fold excess of deaths related to the digestive system. The authors suggest increased alcoholism could have contributed to this increase.

Ritchie and Lehnen (1987) reported a dose-dependent increase in health complaints (eye and throat irritation, and headaches) in 2000 residents living in 397 mobile and 494 conventional homes. Complaints of symptoms of irritation were noted at concentrations of 0.1 ppm formaldehyde or above. Similarly, Liu et al. (1991) found that exposure to 0.09 ppm (0.135 mg/m^3) formaldehyde exacerbated chronic respiratory and allergy problems in residents living in mobile homes.

Employees of mobile day-care centers (66 subjects) reported increased incidence of eye, nose and throat irritation, unnatural thirst, headaches, abnormal tiredness, menstrual disorders, and increased use of analgesics as compared to control workers (Olsen and Dossing, 1982). The mean formaldehyde concentration in these mobile units was 0.29 ppm (0.43 mg/m^3) (range = 0.24 - 0.55 mg/m^3). The exposed workers were exposed in these units for a minimum of 3 months. A control group of 26 subjects in different institutions was exposed to a mean concentration of 0.05 ppm (0.08 mg/m^3) formaldehyde.

Occupants of houses insulated with urea-formaldehyde foam insulation (UFFI) (1726 subjects) were compared with control subjects (720 subjects) for subjective measures of irritation, measures of pulmonary function (FVC, FEV_1, FEF_{25-75}, FEF_{50}), nasal airway resistance, odor threshold for pyridine, nasal cytology, and hypersensitivity skin-patch testing (Broder et al., 1988). The mean length of time of exposure to UFFI was 4.6 years. The mean concentration of formaldehyde in the UFFI-exposed group was 0.043 ppm, compared with 0.035 ppm for the controls. A significant increase in symptoms of eye, nose and throat irritation was observed in subjects from UFFI homes, compared with controls. No other differences from control measurements were observed.

Alexandersson and Hedenstierna (1989) evaluated symptoms of irritation, spirometry, and immunoglobulin levels in 34 wood workers exposed to formaldehyde over a four-year period. Exposure to 0.4 - 0.5 ppm formaldehyde resulted in significant decreases in FVC, FEV_1, and FEF_{25-75}. Removal from exposure for four weeks allowed for normalization of lung function in the non-smokers.

Kriebel et al. (2001) conducted a subchronic epidemiological study of 38 anatomy class students who, on average, were exposed to a geometric mean of 0.70 ± 2.13 ppm formaldehyde for two hours per week over fourteen weeks. After class, eye, nose and throat irritation was significantly elevated compared with pre-laboratory session exposures, with a one unit increase in symptom intensity/ppm formaldehyde. Peak respiratory flow (PEF) was found to decrease by 1%/ppm formaldehyde during the most recent exposure. Changes in PEF and symptom intensity following formaldehyde exposure were most pronounced during the first week of the semester but attenuated with time, suggesting partial acclimatization.

Histology technicians (280 subjects) were shown to have reduced pulmonary function, as measured by FVC, FEV_1, FEF_{25-75}, and FEF_{75-85}, compared with 486 controls (Kilburn et al., 1989). The range of formaldehyde concentrations was 0.2 - 1.9 ppm, volatilized from formalin preservative solution.

Malaka and Kodama (1990) investigated the effects of formaldehyde exposure in plywood workers (93 exposed, 93 controls) exposed for 26.6 years, on average, to 1.13 ppm (range = 0.28 - 3.48 ppm). Fifty-three smokers were present in both exposed and control groups. Exposure assessment was divided into three categories: high (> 5 ppm), low (< 5 ppm), and none (reference group). Subjective irritation and pulmonary function tests were performed on each subject, and chest x-rays were taken of ten randomly selected volunteers from each group. Respiratory symptoms of irritation were found to be significantly increased in exposed individuals, compared with controls. In addition, exposed individuals exhibited significantly reduced FEV_1, FEV_1/FVC, and forced expiratory flow rate at 25% through 75% of FVC ($FEF_{25-75)}$, compared with controls. Forced vital capacity was not significantly reduced. Pulmonary function was not found to be different after a work shift, compared to the same measurement taken before the shift. No differences in chest x-rays were observed between exposed and control workers.

Occupational exposure to formaldehyde concentrations estimated to be 0.025 ppm (0.038 mg/m^3) for greater than six years resulted in complaints by 22 exposed workers of respiratory, gastrointestinal, musculoskeletal, and cardiovascular problems, and in elevated formic acid excretion in the urine (Srivastava et al., 1992). A control group of twenty seven workers unexposed to formaldehyde was used for comparison. A significantly higher incidence of abnormal chest x-rays was also observed in formaldehyde-exposed workers compared with controls.

Chemical plant workers (70 subjects) were exposed to a mean of 0.17 ppm (0.26 mg/m^3) formaldehyde for an unspecified duration (Holmstrom and Wilhelmsson, 1988). Compared with 36 control workers not exposed to formaldehyde, the exposed subjects exhibited a higher frequency of eye, nose, and deep airway discomfort. In addition, the exposed subjects had diminished olfactory ability, delayed mucociliary clearance, and decreased FVC.

Alexandersson et al. (1982) compared the irritant symptoms and pulmonary function of 47 carpentry workers exposed to a mean concentration of formaldehyde of 0.36 ppm (range = 0.04 - 1.25 ppm) with 20 unexposed controls. The average length of employment for the exposed workers was 5.9 years. Symptoms of eye and throat irritation as well as airway obstruction were more common in exposed workers. In addition, a significant reduction in FEV_1, FEV_1/FVC, and MMF was observed in exposed workers compared with controls.

Horvath et al. (1988) compared subjective irritation and pulmonary function in 109 workers exposed to formaldehyde with similar measures in a control group of 254 subjects. The formaldehyde concentrations for the exposed and control groups were

0.69 ppm (1.04 mg/m^3) and 0.05 ppm (0.08 mg/m^3), respectively. Mean formaldehyde concentration in the pre-shift testing facility and the state (Wisconsin) ambient outdoor - formaldehyde level were both 0.04 ppm (0.06 mg/m^3). Duration of formaldehyde exposure was not stated. Subjects were evaluated pre- and post work-shift and compared with control subjects. Significant differences in symptoms of irritation, FEV_1, FEV_1/FVC ratio, FEF_{50}, FEF_{25}, and FEF_{75} were found when comparing exposed subjects' pre- and post work-shift values. However, the pre-workshift values were not different from controls.

The binding of formaldehyde to endogenous proteins creates haptens that can elicit an immune response. Chronic exposure to formaldehyde has been associated with immunological hypersensitivity as measured by elevated circulating IgG and IgE autoantibodies to human serum albumin (Thrasher et al., 1987). In addition, a decrease in the proportion of T-cells was observed, indicating altered immunity. Thrasher et al. (1990) later found that long-term exposure to formaldehyde was associated with autoantibodies, immune activation, and formaldehyde-albumin adducts in patients occupationally exposed, or residents of mobile homes or of homes containing particleboard sub-flooring. The authors suggest that the hypersensitivity induced by formaldehyde may account for a mechanism for asthma and other health complaints associated with formaldehyde exposure.

An epidemiological study of the effects of formaldehyde on 367 textile and shoe manufacturing workers employed for a mean duration of 12 years showed no significant association between formaldehyde exposure, pulmonary function (FVC, FEV_1, and PEF) in normal or asthmatic workers, and occurrence of specific IgE antibodies to formaldehyde (Gorski and Krakowiak, 1991). The concentrations of formaldehyde did not exceed 0.5 ppm (0.75 mg/m^3).

Workers (38 total) exposed for a mean duration of 7.8 years to 0.11 - 2.12 ppm (mean = 0.33 ppm) formaldehyde were studied for their symptomatology, lung function, and total IgG and IgE levels in the serum (Alexandersson and Hedenstierna, 1988). The control group consisted of 18 unexposed individuals. Significant decrements in pulmonary function, FVC ($p < 0.01$) and FEV_1 ($p < 0.05$)) were observed, compared with the controls. Eye, nose, and throat irritation was also reported more frequently by the exposed group. No correlation was found between duration of exposure, or formaldehyde concentration, and the presence of IgE and IgG antibodies.

As described in section 5.1, chronic or repeated exposure to formaldehyde may influence the response of asthmatics to acute or short-term challenges. In the study by Burge et al. (1985) late asthmatic reactions were noted in 3 out of 15 occupationally exposed workers after short-duration exposure to 1.5 – 20.6 ppm formaldehyde. Similarly, among workers with occupational exposure to formaldehyde, asthmatic responses (decreased PEF, FVC, and FEV_1) were reported in 12 workers challenged with 1.67 ppm (2.5 mg/m^3) formaldehyde (Nordman et al., 1985) and in 5 of 28 hemodialysis workers following challenge with formaldehyde vapors (concentration not measured) (Hendrick and Lane, 1977). In contrast, Sheppard et al. (1984) found that in asthmatics not occupationally

exposed to formaldehyde, a 10-minute challenge with 3 ppm formaldehyde coupled with moderate exercise did not induce significant changes in airway resistance or thoracic gas volume. Thus individuals with chronic formaldehyde exposure may be at greater risk for adverse responses to acute exposures. These individuals may have been sensitized immunologically, as in the cases of elevated circulating antibodies, or neurologically, following repeated or chronic exposures to formaldehyde (Sorg et al., 2001a,b).

6.2 Chronic Toxicity to Infants and Children

There are few studies that compare the effects of chronic formaldehyde exposure on children versus adults. Among those that do there is evidence that children are more susceptible to the adverse effects of chronic exposure. Krzyzanowski et al. (1990) assessed chronic pulmonary symptoms and function in 298 children (6-15 years of age) and 613 adults (> 15 years of age) in relation to measured formaldehyde levels in their homes. Information on pulmonary symptoms and doctor-diagnosed asthma and chronic bronchitis was collected by questionnaire. Pulmonary function was assessed as peak expiratory flow rates (PEFR) measured up to four times a day. The prevalence of chronic respiratory symptoms in children was not related to formaldehyde levels measured in tertiles (< 40, 41-60, > 60 ppb). However, doctor-diagnosed asthma and chronic bronchitis were more prevalent in houses with elevated formaldehyde (p for trend < 0.02). This effect was driven by the high disease prevalence observed in homes with kitchen formaldehyde levels >60 ppb, and was especially pronounced among children with concomitant exposure to environmental tobacco smoke (Table 6.1). By comparison, in adults, while the prevalence rates of chronic cough and wheeze were somewhat higher in houses with higher formaldehyde, none of the respiratory symptoms or diseases was significantly related to formaldehyde levels.

Table 6.2.1 Prevalence Rate (per 100) of Diagnosed Bronchitis and Asthma in Children with Formaldehyde (from Krzyzanowski et al., 1990)

	Formaldehyde (ppb)			P value
Bronchitis	**≤ 40 (N)**	**41-60 (N)**	**>60 (N)**	**X^2 trend**
Household mean	3.5 (258)	17.2 (29)	9.1 (11)	<0.02
Main room mean	3.2 (253)	15.6 (32)	9.1 (11)	<0.01
Bedroom mean	3.8 (262)	16.0 (25)	9.1 (11)	<0.04
Subject's bedroom	4.7 (256)	6.7 (30)	11.1 (9)	>0.35
Kitchen	3.5 (255)	0 (22)	28.6 (21)	<0.001
No ETS	4.3 (141)	0 (12)	10.0 (10)	>0.40
ETS	1.9 (106)	0 (10)	45.5 (11)	<0.001
Asthma				
All children	11.7 (256)	4.2 (24)	23.8 (21)	<0.03
No ETS	8.5 (142)	8.3 (12)	0 (10)	>0.50
ETS	15.1 (106)	0 (12)	45.5 (11)	<0.05

In a random effects model, Krzyzanowski et al. (1990) reported that lung function (PEFR) in children, but not adults, was significantly decreased by formaldehyde

(coefficient ± SE: -1.28 ± 0.46 vs 0.09 ± 0.27). Measurements of PEFR in the morning suggested that children with asthma (n = 4) were more severely affected than healthy children (coefficient ± SE: -1.45 ± 0.53 vs 0.09 ± 0.15) (Table 6.2). Compared to children, the effects of formaldehyde on pulmonary function in adults were smaller, transient, limited to morning measurements, and generally most pronounced among smokers exposed to the higher levels of formaldehyde. These studies suggest that children may be more susceptible to the effects of chronic formaldehyde exposure on lung function than are adults.

Table 6.2.2 Relation of PEFR (L/min) to Indoor Formaldehyde
(from Krzyzanowski et al., 1990)

Factor	Child coefficient ± SE	Adult coefficient ± SE
HCHO house mean	-1.28 ± 0.46	0.09 ± 0.27
Morning vs bedtime	-6.10 ± 3.0	-5.90 ± 1.10
HCHO bdrm mean/morning	0.09 ± 0.15	-0.07 ± 0.04
HCHO bdrm mean/morning/asthma	-1.45 ± 0.53	

Among studies of children only, a case-control study by Rumchev et al. (2002) examined risk factors for asthma among young children (6 mo- 3 yr). Cases included children with clinically-diagnosed asthma, and controls were children of the same age group without such a diagnosis. Formaldehyde levels were measured in the homes, once in summer and once in winter. Questionnaires were used to assess potential risk factors for asthma and to collect parental reports of respiratory symptoms characteristic of asthma (cough, shortness of breath, wheeze, runny nose, trouble breathing, and hay fever) in their children. Formaldehyde levels were higher in the homes of children exhibiting asthma symptoms. Estimates of the relative risk for asthma (odds ratios) were adjusted for measured indoor air pollutants, relative humidity, temperature, atopy, family history of asthma, age, gender, socioeconomic status, pets, smoke exposure, air conditioning, and gas appliances. Compared with children exposed to < 8 ppb, children in homes with formaldehyde levels > 49 ppb had a 39% higher risk of asthma ($p < 0.05$) after adjusting for common asthma risk factors.

Franklin et al. (2000) measured exhaled nitric oxide (eNO) levels in 224 children 6-13 years of age as an indicator of inflammation of the lower airways following chronic low-level formaldehyde exposure in the home. While there was no effect of formaldehyde on lung function measured by spirometry, eNO was significantly higher in children from homes with average formaldehyde levels ≥ 50 ppb compared with those from homes with levels ≤ 50 ppb (15.5 ppb eNO vs 8.7; $p = 0.02$).

Garrett et al. (1999) examined the association between formaldehyde levels at home (median 15.8 $\mu g/m^3$; maximum 139 $\mu g/m^3$) and atopy and allergic sensitization in 148 children, 7-14 years of age. The risk of atopy increased by 40% with each 10 $\mu g/m^3$ increase in bedroom formaldehyde. Two measures of allergic sensitization to twelve common environmental allergens, the number of positive skin prick tests and maximum wheal size, both showed linear associations with increasing maximum formaldehyde

exposure levels. After adjusting for parental asthma and allergy, there was no evidence of an association between asthma in the children and formaldehyde levels. However, these data do suggest that formaldehyde levels commonly found in homes can enhance sensitization of children to common aeroallergens.

Of the numerous, primarily occupational, studies in adults, the NOAEL and LOAEL are 32 $\mu g/m^3$ (26 ppb) and 92 $\mu g/m^3$ (75 ppb), respectively, after adjustment for exposure continuity. These values are based on data on nasal and eye irritation observed in Wilhelmsson and Holstrom (1992), and histological lesions in the nasal cavity documented in Edling et al. (1988). However, studies in children, including the Krzyzanowski study above, indicate adverse health impacts in children at concentrations as low as 30 ppb. Wantke et al. (1996) reported that formaldehyde-specific IgE and respiratory symptoms were reduced when children transferred from schools with formaldehyde concentrations of 43 to 75 ppb to schools with concentrations of 23 to 29 ppb. While these human studies are not entirely consistent with each other, and there is potential for confounding in each, nevertheless, taken together, they suggest that children may be more sensitive to formaldehyde toxicity than adults.

6.3 Chronic Toxicity to Experimental Animals

Studies of the effects of chronic formaldehyde exposure in experimental animals tend to focus on lesions in the upper respiratory tract and the hyperplastic or metaplastic changes observed in the respiratory epithelium. Systemic effects, such as changes in body or organ weight, or blood chemistry, appear to be secondary to the effects of the olfactory irritation on feeding behavior. There is also evidence that repeated or long-term exposure to formaldehyde may cause neurologically-based sensitization (Sorg et al., 2001b) and altered expression of stress hormones (Sorg et al., 2001a).

In studies examining respiratory effects, Fischer-344 rats and B6C3F1 mice (120 animals/sex) were exposed to concentrations of 0, 2.0, 5.6, or 14.3 ppm formaldehyde vapor for 6 hours/day, 5 days/week for 24 months (Kerns et al., 1983). The exposure period was followed by up to six months of non-exposure. Interim sacrifices were conducted at 6, 12, 18, 24, 27, and 30 months. Both male and female rats in the 5.6 and 14.3 ppm groups demonstrated decreased body weights over the two-year period. At the 6 month sacrifice, the rats exposed to 14.3 ppm formaldehyde had non-neoplastic lesions of epithelial dysplasia in the nasal septum and turbinates. As the study progressed, epithelial dysplasia, squamous dysplasia, and mucopurulent rhinitis increased in severity and distribution in all exposure groups. In mice, cumulative survival decreased in males from 6 months to the end of the study. Serous rhinitis was detected at 6 months in the 14.3 ppm group of mice. Metaplastic and dysplastic changes were noted at 18 months in most rats in the 14.3 ppm group and in a few mice in the 5.6 ppm exposure group. By 24 months, the majority of mice in the 14.3 ppm group had metaplastic and dysplastic changes associated with serous rhinitis, in contrast to a few mice in the 5.6 ppm group and a few in the 2 ppm group (exact number not given).

Woutersen et al. (1989) exposed male Wistar rats (60 animals/group) 6 hours/day for 5 days/week to 0, 0.1, 1.0 and 10 ppm formaldehyde vapor for 28 months. Compound-related nasal lesions of the respiratory and olfactory epithelium were observed only in the 10 ppm group. In the respiratory epithelium, the lesions consisted of rhinitis, squamous metaplasia and basal cell/pseudoepithelial hyperplasia. In the olfactory region, the lesions included epithelial degeneration and rhinitis. No differences in behavior or mortality were noted among the various groups. However, growth retardation was observed in the 10 ppm group from day 14 onwards. In a parallel study, male Wistar rats were exposed to 0, 0.1, 1.0 and 10 ppm formaldehyde for 3 months followed by a 25-month observation period. Compound-related histopathological changes were found only in the noses of the 10 ppm group and comprised of increased incidences of squamous metaplasia of the respiratory epithelium and rhinitis.

In a chronic exposure study that primarily investigated aspects of nasal tumor development, Monticello et al. (1996) examined nasal cavities of male F-344 rats (0-10 ppm, 90 animals/group; 15 ppm, 147 animals) following exposure to 0, 0.7, 2, 6, 10, and 15 ppm formaldehyde for 6 hours/day, 5 days/week for 24 months. Treatment-related decreases in survival were apparent only in the 15 ppm group. Nasal lesions at the two highest doses included epithelial hypertrophy and hyperplasia, squamous metaplasia, and a mixed inflammatory cell infiltrate. Lesions in the 6 ppm group were minimal to absent and limited to focal squamous metaplasia in the anterior regions of the nasal cavity. No formaldehyde-induced lesions were observed in the 0.7 or 2 ppm groups.

Kamata et al. (1997) exposed 32 male F-344 rats/group to gaseous formaldehyde at 0, 0.3, 2, and 15 ppm 6 hours/day, 5 days/week for up to 28 weeks. A room control, non-exposed group was also included in the study. Five animals per group were randomly selected at the end of the 12, 18, and 24 months, and surviving animals at 28 months were sacrificed for full pathological evaluation. Behavioral effects related to sensory irritation were evident in the 15 ppm group. Significant decreases in food consumption, body weight and survival were also evident in this group. No exposure-related hematological findings were observed. Biochemical and organ weight examination revealed decreased triglyceride levels and absolute liver weights at the highest exposure, but was likely related to reduced food consumption. Abnormal histopathological findings were confined to the nasal cavity. Inflammatory cell infiltration, erosion or edema of the nasal cavity was evident in all groups, including controls. Significantly increased incidence of non-proliferative (squamous cell metaplasia without epithelial cell hyperplasia) and proliferative lesions (epithelial cell hyperplasia with squamous cell metaplasia) were observed in the nasal cavities beginning at 2 ppm. In the 0.3 ppm group, a non-significant increase in proliferative nasal lesions (4/20 animals) were observed in rats that were either sacrificed or died following the 18th month of exposure.

Rusch et al. (1983) exposed groups of 6 male cynomolgus monkeys, 20 male or female rats, and 10 male or female hamsters to 0, 0.2, 1.0, or 3.0 ppm (0, 0.24, 1.2, or 3.7 mg/m^3) formaldehyde vapor for 22 hours/day, 7 days/week for 26 weeks. There was no treatment-related mortality during the study. In monkeys, the most significant findings were hoarseness, congestion and squamous metaplasia of the nasal turbinates in 6/6

monkeys exposed to 2.95 ppm. There were no signs of toxicity in the lower exposure groups. In the rat, squamous metaplasia and basal cell hyperplasia of the nasal epithelia were significantly increased in rats exposed to 2.95 ppm. The same group exhibited decreased body weights and decreased liver weights. In contrast to monkeys and rats, hamsters did not show any signs of response to exposure, even at 2.95 ppm.

Kimbell et al. (1997) exposed male F-344 rats ($\leq$ 6/group) to 0, 0.7, 2, 6, 10, and 15 ppm 6 hr/day, 5 days/week for 6 months. Squamous metaplasia was not observed in any regions of the nasal cavity in any of the control, 0.7, or 2 ppm groups. However, the extent and incidence of squamous metaplasia in the nasal cavity increased with increasing dose beginning at 6 ppm.

In subchronic studies, Wilmer et al. (1989) found that intermittent (8 hours/day, 5 days/week) exposures of rats to 4 ppm formaldehyde for 13 weeks resulted in significant histological changes in the nasal septum and turbinates. In contrast, continuous exposure of rats for 13 weeks to 2 ppm formaldehyde did not produce significant lesions. This study revealed the concentration dependent nature of the nasal lesions caused by formaldehyde exposure. Zwart et al. (1988) exposed male and female Wistar rats (50 animals/group/sex) to 0, 0.3, 1, and 3 ppm formaldehyde vapor for 6 hr/day, 5 days/week for 13 weeks. Compound related histopathological nasal changes varying from epithelial disarrangement to epithelial hyperplasia and squamous metaplasia were found in the 3 ppm group, and were restricted to a small area of the anterior respiratory epithelium. These changes were confirmed by electron microscopy and were not observed in other groups.

Woutersen et al. (1989) exposed rats (20 per group) to 0, 1, 10, or 20 ppm formaldehyde 6 hours/day, 5 days/week for 13 weeks. Rats exposed to 20 ppm displayed retarded growth, yellowing of the fur, and significant histological lesions in the respiratory epithelium. Exposure to 10 ppm did not affect growth, but resulted in significant histological lesions in the respiratory tract. No effects on specific organ weights, blood chemistries, liver glutathione levels, or urinalysis were detected at any level. No significant adverse effects were seen at the 1.0 ppm exposure level.

Appelman et al. (1988) found significant nasal lesions in rats (20 per group; 0, 0.1, 1.0, or 10.0 ppm) exposed to 10 ppm formaldehyde 6 hours/day, 5 days/week for 52 weeks, but exposure to 1.0 ppm or less for this period did not result in nasal histological lesions. However, the rats exposed to formaldehyde displayed decreased body weight in all groups compared with controls.

Apfelbach and Weiler (1991) determined that rats (5 exposed, 10 controls) exposed to 0.25 ppm (0.38 mg/m^3) formaldehyde for 130 days lost the olfactory ability to detect ethyl acetate odor.

Maronpot et al. (1986) exposed groups of 20 mice to 0, 2, 4, 10, 20, or 40 ppm formaldehyde 6 hours/day, 5 days/week, for 13 weeks. Histological lesions in the upper

respiratory epithelium were seen in animals exposed to 10 ppm or greater. Exposure to 40 ppm was lethal to the mice.

A six-month exposure of rats to 0, 0.5, 3, and 15 ppm formaldehyde (3 rats per group) resulted in significantly elevated total lung cytochrome P450 in all formaldehyde-exposed groups (Dallas et al., 1989). The degree of P450 induction was highest after 4 days exposure and decreased slightly over the course of the experiment.

A series of studies have addressed the effects of long-term repeated exposures to formaldehyde on altered functioning of the hypothalamic-pituitary-adrenal (HPA) axis (Sorg et al., 2001a) and on neurobehavioral changes in rats (Sorg et al., 2001b). To study formaldehyde's effects on the HPA, Sorg et al. (2001a) measured corticosterone levels in the trunk blood of male Sprague-Dawley rats 20 or 60 min following acute chamber exposures to air or formaldehyde (0.7 or 2.4 ppm). All groups showed increased corticosterone levels above naive basal levels at 20 min followed by a return to baseline by 60 min, with no differences between treatment groups. A second experiment assessed the effects of repeated formaldehyde exposure (1 h/day, 5 days/week for 2 or 4 weeks) on basal corticosterone levels and those after a final challenge. Basal corticosterone levels were increased above naive values after 2 week exposure to air or 0.7 ppm formaldehyde. By 4 weeks, corticosterone levels in the air group returned to naive values, but remained elevated in the 0.7 ppm formaldehyde group. There were no differences in basal corticosterone levels among either 2.4 ppm exposed groups. After a final air or formaldehyde challenge, the 2 and 4 week air and 0.7 ppm formaldehyde groups had elevated corticosterone levels similar to their acute response, while in the 2 and 4 week 2.4 ppm formaldehyde groups, corticosterone levels were higher than their acute response levels, indicating enhanced reactivity of the HPA axis to subsequent formaldehyde. It thus appears that repeated low-level formaldehyde exposure alters HPA axis functioning and the release of stress hormones. Since glucocorticoids may stimulate or inhibit the synthesis of surfactant-associated proteins in the lung (Liley et al., 1988), the alteration of HPA function may represent another pathway by which formaldehyde affects pulmonary function. For example, the pulmonary surfactants that regulate surface tension in the lungs are in turn regulated by surfactant-associated proteins. Reports of lower airway discomfort associated with chronic formaldehyde exposure may be related to the altered release or activity of these surfactant-associated proteins in the lung.

In another study of the effects of formaldehyde and the hypothalamus-pituitary-adrenal (HPA) axis, Sari et al. (2004) exposed female C3H/He mice to formaldehyde (0, 80, 400, 2000 ppb) by inhalation for 16 h/day, 5 days/week, for 12 weeks. Immunocytochemistry was used to examine corticotropin releasing hormone (CRH)-immunoreactive (ir) neurons in the hypothalamus, and adrenocorticotropin hormone (ACTH)-ir cells in the pituitary. RT-PCR was used to quantify ACTH rnRNA in the pituitary. Two groups of female mice were exposed, one of which comprised control mice with no allergen exposure. The other group was made allergic by injection of ovalbumin and alum prior to exposure to formaldehyde. Animals in the second group were further exposed to aerosolized ovalbumin as a booster four times during the exposure period. In the non-allergic group, formaldehyde caused a dose-dependent increase in the number of CRH-ir

neurons with a similar pattern of increases in ACTHir cells and ACTH mRNA. The allergic mice showed an increase in basal levels of all these markers of HPA activity, and were responsive to the lowest concentration of formaldehyde. Thus at low levels of exposure, allergen and formaldehyde exposure exacerbate each other's effects on the stress response of the HPA.

7. Developmental and Reproductive Toxicity

In humans there are few data on the association of teratogenicity or adverse reproductive effects with formaldehyde exposure. Existing data do not suggest that formaldehyde, by inhalation or oral routes, produces significant teratogenic or reproductive effects (ATSDR, 1999)

A developmental toxicity study on formaldehyde was conducted by Martin (1990). Pregnant rats (25 per group) were exposed to 0, 2, 5, or 10 ppm formaldehyde for 6 hours/day, during days 6-15 of gestation. Although exposure to 10 ppm formaldehyde resulted in reduced food consumption and body weight gain in the maternal rats, no effects on the number, viability or normal development of the fetuses were seen. In addition, Saillenfait et al. (1989) exposed pregnant rats (25 per group) to 0, 5, 10, 20, or 40 ppm formaldehyde from days 6 - 20 of gestation. Maternal weight gain and fetal weight were significantly reduced in the 40 ppm exposure group. No significant fetotoxicity or teratogenic defects were observed at formaldehyde levels that were not also maternally toxic.

Evidence of embryotoxicity was reported by Kitaeva et al. (1990) in embryos of rats that had been exposed to formaldehyde by inhalation 4 h/d, 5 d/wk for 4 months. At 1.5 mg/m^3, but not at 0.5 mg/m^3, there was a significant increase in the proportion of degenerate embryos. By comparison, the bone marrow cells of the mothers appeared to be more sensitive to formaldehyde as shown by significant increases in the numbers of cells with aberrations, and the numbers of chromosomes with aberrations and aneuploidy at both dose levels.

8. Derivation of Reference Exposure Levels

8.1 Formaldehyde Acute Reference Exposure Level

Study	Kulle et al., 1987
Study population	19 nonasthmatic, nonsmoking humans
Exposure method	Whole body to 0.5-3.0 ppm
Exposure continuity	
Exposure duration	3 hr
Critical effects	mild and moderate eye irritation
LOAEL	1 ppm
NOAEL	0.5 ppm
Benchmark concentration	0.44 ppm
Time-adjusted exposure	not applied
Human Equivalent Concentration	not applied
LOAEL uncertainty factor (UF_L)	not applied
Subchronic uncertainty factor (UFs)	not applied
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	1 (default, human study)
Toxicodynamic (UF_{A-d})	1 (default, human study)
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	1 (site of contact; no systemic effects)
Toxicodynamic (UF_{H-d})	10 (asthma exacerbation in children)
Cumulative uncertainty factor	10
Reference Exposure Level	**55 μg/m^3 (44 ppb)**

Acute Reference Exposure Levels are levels at which intermittent one-hour exposures are not expected to result in adverse health effects (see Section 5 of the Technical Support Document).

Kulle et al (1987) was chosen as the critical study for the determination of the acute REL as it used a sensitive endpoint, eye irritation, and it featured human subjects with short-term exposures to a range of formaldehyde concentrations that permitted the use of a benchmark concentration (BMC) approach. As described in the technical support document, OEHHA recommends the use of the BMC approach whenever the available data support it as the BMC method provides a more statistically sound estimate of the point of departure in the REL determination.

The proposed acute REL was based on a BMC_{05} for eye irritation, estimated using log-probit analysis (Crump, 1984). The BMC_{05} is defined as the 95% lower confidence limit of the concentration expected to produce a response rate of 5%. The resulting BMC_{05} from this analysis was 0.44 ppm (0.53 mg/m^3) formaldehyde. The endpoint of eye irritancy appears to be more a function of formaldehyde concentration rather than duration of exposure (Yang et al., 2001), so no time correction factor was applied. An uncertainty factor (UF_{H-k}) of 1 was used since sensory irritation is not expected to involve large toxicokinetic differences among individuals. Although the toxicological endpoint is eye irritation, the REL should protect against all possible adverse effects. The respiratory

irritant effect, with documented potential to exacerbate asthma, is clearly an effect with the potential to differentially impact infants and children. The toxicodynamic component of the intraspecies uncertainty factor $UF_{H\text{-}d}$ is therefore assigned an increased value of 10 to account for potential asthma exacerbation. These considerations are applied equally to the acute, 8-hour and chronic REL.

8.2 Formaldehyde 8-Hour Reference Exposure Level

Study	Wilhelmsson and Holmstrom, 1992
Study population	66 chemical plant workers
Exposure method	Discontinuous occupational exposure
Exposure continuity	8 hr/day, 5 days/week (assumed)
Exposure duration	10 years (average); range 1-36 years
Critical effects	Nasal obstruction and discomfort, lower airway discomfort, and eye irritation.
LOAEL	Mean 0.26 mg/m^3 (range 0.05 – 0.6 mg/m^3) (described as exposed group)
NOAEL	Mean of 0.09 mg/m^3 (described as control group of office workers)
Benchmark concentration	not derived
Time-adjusted exposure	0.09 mg/m^3 (time adjustment not applied)
Human Equivalent Concentration	not applied
LOAEL uncertainty factor (UF_L)	1 (NOAEL observed)
Subchronic uncertainty factor (UFs)	not applied
Interspecies Uncertainty Factor	
Toxicokinetic ($UF_{A\text{-}k}$)	1 (default, human study)
Toxicodynamic ($UF_{A\text{-}d}$)	1 (default, human study)
Intraspecies Uncertainty Factor	
Toxicokinetic ($UF_{H\text{-}k}$)	1 (site of contact; no systemic effects)
Toxicodynamic ($UF_{H\text{-}d}$)	10 (asthma exacerbation in children)
Cumulative uncertainty factor	10
Reference Exposure Level	**9 μg/m^3 (7 ppb)**

The 8-hour Reference Exposure Level is a concentration at or below which adverse noncancer health effects would not be anticipated for repeated 8-hour exposures (see Section 6 in the Technical Support Document).

The 8-hour REL is based on the occupational study by Wilhelmsson and Holmstrom (1992). This study evaluated the effects of formaldehyde on the upper airways of adult human subjects exposed to a mean formaldehyde concentration of 0.26 mg/m^3 during the work day compared with a referent group exposed to 0.09 mg/m^3. The critical effects in this study included nasal obstruction and discomfort, lower airway discomfort, and eye irritation. A NOAEL and a LOAEL may be derived from these data but no other dose-response information was provided. This study included only adults, but there is evidence that children may be more susceptible to long term exposures to formaldehyde than are adults. Thus, in the absence of child-specific data, an intraspecies uncertainty factor of 10 for toxicodynamic variability was applied.

For comparison, the 8-hour REL of 9 μg/m³ is similar to the value of 10 μg/m^3 based on increased pulmonary resistance in guinea pigs following an 8 hr exposure to 0.11 – 1.05 ppm formaldehyde (Swiecichowski et al., 1993). The NOAEL of 0.59 ppm in guinea pigs was adjusted to a Human Equivalent Concentration (HEC) of 0.49 ppm with a regional gas dose ratio (RGDR) of 0.826. Use of the HEC adjustment entails an interspecies uncertainty factor of 6, while an intraspecies uncertainty factor of 10 addresses toxicodynamic variability.

Study	Swiecichowski et al., 1993
Study population	25-35 adult male guinea pigs
Exposure method	Whole body exposure
Exposure continuity	
Exposure duration	8 hr
Critical effects	Increased specific pulmonary resistance
LOAEL	1.0 ppm
NOAEL	0.59 ppm
Benchmark concentration	not derived
Time-adjusted exposure	not applied
Human Equivalent Concentration	0.49 ppm (610 μg/m^3) (0.59 * RGDR 0.826 for pulmonary effects)
LOAEL uncertainty factor (UF_L)	1 (default: NOAEL observed)
Subchronic uncertainty factor (UFs)	not applied
Interspecies Uncertainty Factor	
Toxicokinetic (UF_{A-k})	6 (with HEC adjustment)
Toxicodynamic (UF_{A-d})	1 (with HEC adjustment)
Intraspecies Uncertainty Factor	
Toxicokinetic (UF_{H-k})	1 (no systemic effect)
Toxicodynamic (UF_{H-d})	10 (asthma exacerbation in children)
Cumulative uncertainty factor	60
Reference Exposure Level	**10 μg/m³ (8 ppb)**

Formaldehyde Chronic Reference Exposure Level

Study	Wilhelmsson and Holmstrom, 1992 supported by Edling et al., 1988
Study population	66 human chemical plant workers
Exposure method	Discontinuous occupational exposure
Exposure continuity	8 hr/day, 5 days/week (assumed)
Exposure duration	10 years (average); range 1-36 years
Critical effects	Nasal obstruction and discomfort, lower airway discomfort; histopathological nasal lesions including rhinitis, squamous metaplasia, and dysplasia
LOAEL	Mean 0.26 mg/m^3 (range 0.05 – 0.6 mg/m3) (described as exposed group)
NOAEL	Mean of 0.09 mg/m^3 (described as control group of office workers)
Benchmark concentration	not derived
Time-adjusted exposure	0.09 mg/m^3 for NOAEL group
Human Equivalent Concentration	not applied
LOAEL uncertainty factor (UF_L)	not applied
Subchronic uncertainty factor (UFs)	not applied
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	1 (default, human study)
Toxicodynamic (UF_{A-d})	1 (default, human study)
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	1 (no systemic effects)
Toxicodynamic (UF_{H-d})	10 (asthma exacerbation in children)
Cumulative uncertainty factor	10
Reference Exposure Level	**9 µg/m^3 (7 ppb)**

The chronic Reference Exposure Level is a concentration at which adverse noncancer health effects would not be expected from chronic exposures (see Section 7 in the Technical Support Document).

The study by Wilhelmsson and Holmstrom (1992) was selected for development of the chronic REL as it investigated long-term exposure to formaldehyde relatively free of other confounding exposures. From this study it was possible to determine both a NOAEL and a LOAEL. Since this study included only adults, a combined intraspecies uncertainty factor of 10 for toxicodynamic variability was applied to account for the possibly greater susceptibility of children with long term exposures to formaldehyde.

The susceptibility of young children was examined in a study by Rumchev et al. (2002) that compared children (mean age 25 mo) with a clinical diagnosis of asthma to children without this diagnosis. The LOAEL used (60 µg/m^3) represents the formaldehyde level at which the authors found a statistically elevated risk for asthma-related respiratory symptoms. For this comparison, the NOAEL was taken to be 30 µg/m^3, the lower end of the NOAEL range. Intraspecies uncertainty factors of 3.16 for potential toxicodynamic

variability and 1 for toxicokinetic differences give a cumulative uncertainty factor of 3.16 for an inhalation chronic REL of 10 μg/m^3 (8 ppb), similar to the chronic REL calculated from the critical study.

Study	Rumchev et al., 2002
Study population	88 asthmatic children (mean age 25 mo); 104 nonasthmatic controls (mean age 20 mo)
Exposure method	Ambient in home
Exposure continuity	Continuous assumed
Exposure duration	range 0.5-3 years
Critical effects	Parent-reported asthma-related respiratory symptoms
LOAEL	60 μg/m^3
NOAEL	30 μg/m^3 (lower limit of NOAEL range)
Benchmark concentration	not derived
Time-adjusted exposure	not applied
Human Equivalent Concentration	30 μg/m^3
LOAEL uncertainty factor (UF_L)	1
Subchronic uncertainty factor (UFs)	not applied
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	1 (default, human study)
Toxicodynamic (UF_{A-d})	1 (default, human study)
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	1 (study performed in children)
Toxicodynamic (UF_{H-d})	√10 (inter-individual variation)
Cumulative uncertainty factor	√10
Reference Exposure Level	**10 μg/m^3 (8 ppb)**

The Rumchev study supports an association with exposure to formaldehyde and the observation of asthma-like symptom in children. However, it was not selected for REL development due to the difficulties in distinguishing asthma from other wheezing conditions in the clinical diagnoses in such a young population. There are additional uncertainties associated with the exposure continuity, and the possibility of observational and/or recall bias in the parental reports of respiratory symptoms characteristic of asthma.

For comparison with the chronic REL of 9 μg/m^3 (7 ppb) presented above, Table 8.3.1 below presents a summary of potential formaldehyde RELs based on chronic and subchronic animal studies originally presented in OEHHA (2000). The toxicological endpoint was nasal lesions, consisting principally of rhinitis, squamous metaplasia, and dyplasia of the respiratory epithelium.

The most striking observation is the similarity of potential RELs among the rat chronic studies (exposures ≥ 26 weeks) that contain a NOAEL. The range of RELs from these animal studies, 1.5 – 24.9 ppb, includes the proposed REL (7 ppb) based on a human study. Another related observation is that the NOAEL and LOAEL are similar among all the studies, regardless of exposure duration. The NOAEL and LOAEL are generally in

the range of 1 - 4 ppm and 1 – 10 ppm, respectively, with the exception of the study by Kamata et al. (1997) that may be due to the absence of a dose level between 2 and 0.3 ppm. It is also of interest that the studies of Rusch et al (1983) indicate that monkeys and rats are of about the same sensitivity. In addition, the results of the Rusch studies suggest that, at least for the endpoint of squamous metaplasia, formaldehyde concentration is more important than the total dose since these animals, receiving more continuous exposure, exhibited the same adverse effects seen in studies using more intermittent exposures.

ATSDR has estimated minimum risk levels (MRLs), defined as "an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure" (ATSDR, 1999). For formaldehyde inhalation exposures they describe as "acute" (≤ 14 days), the MRL is 40 ppb based on a LOAEL of 0.4 ppm from a study by Pazdrak et al. (1993), and a 9-fold uncertainty factor (3 for use of a LOAEL; 3 for intraspecies variability). This exposure period is much longer than the acute REL of one hour, but the acute REL represents possibly repeated exposures. The MRL for an "intermediate" exposure period of 15-364 days is 30 ppb based on a NOAEL of 0.98 ppm for clinical signs of nasopharyngeal irritation and lesions in the nasal epithelium in monkeys (Rusch et al., 1983). A chronic MRL (≥ 365 d) of 8 ppb was developed based on damage to nasal epithelium in chemical factory workers (Holmstrom et al., 1989). This number is similar to the chronic REL of 7 ppb reported here. The MRLs are more similar to the chronic RELs developed by OEHHA in that they assume continuous exposure over the specified time period rather than regular but periodic exposures, as assumed for the 8-hour RELs considered above.

8.4 Formaldehyde as a Toxic Air Contaminant

In view of the differential impacts on infants and children identified in Section 6.2, OEHHA recommends that formaldehyde be identified as a toxic air contaminant which may disproportionately impact children pursuant to Health and Safety Code, Section 39669.5(c).

Table 8.3.1. Summary of Chronic and Subchronic Formaldehyde Studies in Experimental Animals

Study	Animal	Duration	Exposure	LOAEL ppm	NOAEL ppm	Time adj	DAF	LOAEL UF	UFak	UFad	UFhk	UFhd	UFsc	Cum UF	REL ppb	REL µg/m3
Woutersen 89	rat	28 mo	6 h 5 d	9.8	1	0.179	0.148	1	1	3.16	1	10	1	30	4.9	6.1
Kerns 83	rat	24 mo	6 h 5 d	2	n/a	0.357	0.296	6	1	3.16	1	10	1	200	1.5	1.8
Monticello 96	rat	24 mo	6 h 5 d	6.01	2.05	0.366	0.304	1	1	3.16	1	10	1	30	10.1	12.6
Kamata 97	rat	24-28 mo	6 h 5 d	2	0.3	0.054	0.044	1	1	3.16	1	10	1	30	1.5	1.8
Appelman 88	rat	52 wk	6 h 5 d	9.4	1	0.179	0.148	1	1	3.16	1	10	1	30	4.9	6.1
Rusch 83	rat	26 wk	22 h 7d	2.95	0.98	0.898	0.746	1	1	3.16	1	10	1	30	24.9	30.8
Kimbell 97	rat	26 wk	6 h 5 d	6	2	0.357	0.296	1	1	3.16	1	10	1	30	9.9	12.3
Wilmer 89	rat	13 wk	8 h 5 d	4	2	0.238	0.198	1	1	3.16	1	10	1	30	6.6	8.2
Woutersen 87	rat	13 wk	6 h 5 d	9.7	1	0.179	0.148	1	1	3.16	1	10	1	30	4.9	6.1
Zwart 88	rat	13 wk	6 h 5 d	2.98	1.01	0.180	0.15	1	1	3.16	1	10	1	30	5.0	6.2
Kerns 83	mouse	24 mo	6 h 5 d	5.6	2	0.357	0.296	1	2	3.16	1	10	1	60	4.9	6.1
Maronpot 86	mouse	13 wk	6 h 5 d	10.1	4.08	0.729	0.605	1	2	3.16	1	10	1	60	10.1	12.5
Rusch 83	monkey	26 wk	22 h 7d	2.95	0.98	0.898	not used	1	2	2	1	10	1	40	22.5	27.8

9.0 References

Akbar-Khanzadeh F, Vaquerano MU, Akbar-Khanzadeh M and Bisesi MS (1994). Formaldehyde exposure, acute pulmonary response, and exposure control options in a gross anatomy laboratory. Am J Ind Med 26(1): 61-75.

Alarie Y (1981). Toxicological evaluation of airborne chemical irritants and allergens using respiratory reflex reactions. . Proceedings of the inhalation toxicology and technology symposium.Ann Arbor Sciences, Inc. 207-231. Kalamazoo, MI, October 23-24, 1980.

Alexandersson R and Hedenstierna G (1988). Respiratory hazards associated with exposure to formaldehyde and solvents in acid-curing paints. Arch Environ Health 43(3): 222-7.

Alexandersson R and Hedenstierna G (1989). Pulmonary function in wood workers exposed to formaldehyde: a prospective study. Arch Environ Health 44(1): 5-11.

Alexandersson R, Hedenstierna G and Kolmodin-Hedman B (1982). Exposure to formaldehyde: effects on pulmonary function. Arch Environ Health 37(5): 279-84.

Amdur MO (1960). The response of guinea pigs to inhalation of formaldehyde and formic acid alone and with a sodium chloride aerosol. Int J Air Pollut 3: 201-20.

Apfelbach R and Weiler E (1991). Sensitivity to odors in Wistar rats is reduced after low-level formaldehyde-gas exposure. Naturwissenschaften 78(5): 221-3.

Appelman LM, Woutersen RA, Zwart A, Falke HE and Feron VJ (1988). One-year inhalation toxicity study of formaldehyde in male rats with a damaged or undamaged nasal mucosa. J Appl Toxicol 8(2): 85-90.

ATSDR. (1999). *Toxicological profile for formaldehyde*. Atlanta, GA: Agency for Toxic Substances and Disease Registry http://www.atsdr.cdc.gov/toxprofiles/tp111.pdf.

Boja JW, Nielsen JA, Foldvary E and Truitt EB, Jr. (1985). Acute low-level formaldehyde behavioural and neurochemical toxicity in the rat. Prog Neuropsychopharmacol Biol Psychiatry 9(5-6): 671-4.

Boysen M, Zadig E, Digernes V, Abeler V and Reith A (1990). Nasal mucosa in workers exposed to formaldehyde: a pilot study. Br J Ind Med 47(2): 116-121.

Broder I, Corey P, Brasher P, Lipa M and Cole P (1988). Comparison of health of occupants and characteristics of houses among control homes and homes insulated with urea formaldehyde foam. III. Health and house variables following remedial work. Environ Res 45(2): 179-203.

Burge PS, Harries MG, Lam WK, O'Brien IM and Patchett PA (1985). Occupational asthma due to formaldehyde. Thorax 40(4): 255-60.

CARB (2005a). Annual Statewide Toxics Summary - Formaldehyde. Sacramento, CA. http://www.arb.ca.gov/adam/toxics/statepages/hchostate.html.

CARB. (2005b). *The California Almanac of Emissions and Air Quality - 2005 Edition*. California Air Resources Board. http://www.arb.ca.gov/aqd/almanac/almanac05/almanac05.htm.

CARB. (2006). *The California Almanac of Emissions and Air Quality - 2006 Edition*. California Air Resources Board. http://www.arb.ca.gov/aqd/almanac/almanac06/almanac2006all.pdf.

Crump KS (1984). A new method for determining allowable daily intakes. Fundam Appl Toxicol 4(5): 854-71.

Dallas CE, Badeaux P, Theiss JC and Fairchild EJ (1989). The influence of inhaled formaldehyde on rat lung cytochrome P450. Environ Res 49(1): 50-9.

Edling C, Hellquist H and Odkvist L (1988). Occupational exposure to formaldehyde and histopathological changes in the nasal mucosa. Br J Ind Med 45(11): 761-5.

Feinman SE (1988). Formaldehyde sensitivity and toxicity. Boca Raton (FL): CRC Press Inc.

Franklin P, Dingle P and Stick S (2000). Raised exhaled nitric oxide in healthy children is associated with domestic formaldehyde levels. Am J Respir Crit Care Med 161(5): 1757-9.

Franks SJ (2005). A mathematical model for the absorption and metabolism of formaldehyde vapour by humans. Toxicology and Applied Pharmacology 206(3): 309-320.

Frigas E, Filley WV and Reed CE (1984). Bronchial challenge with formaldehyde gas: lack of bronchoconstriction in 13 patients suspected of having formaldehyde-induced asthma. Mayo Clin Proc 59(5): 295-9.

Garrett MH, Hooper MA, Hooper BM, Rayment PR and Abramson MJ (1999). Increased risk of allergy in children due to formaldehyde exposure in homes. Allergy 54(4): 330-7.

Gorski P and Krakowiak A (1991). Formaldehyde--induced bronchial asthma--does it really exist? [Abstract]. Pol J Occup Med Environ Health 4(4): 317-20.

Gorski P, Tarkowski M, Krakowiak A and Kiec-Swierczynska M (1992). Neutrophil chemiluminescence following exposure to formaldehyde in healthy subjects and in patients with contact dermatitis. Allergol Immunopathol (Madr) 20(1): 20-3.

Grammer LC, Harris KE, Shaughnessy MA, Sparks P, Ayars GH, Altman LC and Patterson R (1990). Clinical and immunologic evaluation of 37 workers exposed to gaseous formaldehyde. J Allergy Clin Immunol 86(2): 177-81.

Green DJ, Sauder LR, Kulle TJ and Bascom R (1987). Acute response to 3.0 ppm formaldehyde in exercising healthy nonsmokers and asthmatics. Am Rev Respir Dis 135(6): 1261-6.

Harving H, Korsgaard J, Pedersen OF, Molhave L and Dahl R (1990). Pulmonary function and bronchial reactivity in asthmatics during low-level formaldehyde exposure. Lung 168(1): 15-21.

Hendrick DJ and Lane DJ (1977). Occupational formalin asthma. Br J Ind Med 34(1): 11-8.

Holmstrom M and Wilhelmsson B (1988). Respiratory symptoms and pathophysiological effects of occupational exposure to formaldehyde and wood dust. Scand J Work Environ Health 14(5): 306-11.

Holmstrom M, Wilhelmsson B and Hellquist H (1989). Histological changes in the nasal mucosa in rats after long-term exposure to formaldehyde and wood dust. Acta Otolaryngol 108(3-4): 274-83.

Horton AW, Tye R and Stemmer KL (1963). Experimental carcinogenesis of the lung. Inhalation of gaseous formaldehyde or an aerosol of coal tar by C3H mice. J Natl Cancer Inst 30: 31-43.

Horvath EP, Jr., Anderson H, Jr., Pierce WE, Hanrahan L and Wendlick JD (1988). Effects of formaldehyde on the mucous membranes and lungs. A study of an industrial population. Jama 259(5): 701-7.

Kamata E, Nakadate M, Uchida O, Ogawa Y, Suzuki S, Kaneko T, Saito M and Kurokawa Y (1997). Results of a 28-month chronic inhalation toxicity study of formaldehyde in male Fisher-344 rats. J Toxicol Sci 22(3): 239-54.

Kerfoot EJ and Mooney TF (1975). Formaldehyde and paraformaldehyde study in funeral homes. Am Ind Hyg Assoc J 36(7): 533-7.

Kerns WD, Pavkov KL, Donofrio DJ, Gralla EJ and Swenberg JA (1983). Carcinogenicity of formaldehyde in rats and mice after long-term inhalation exposure. Cancer Res 43(9): 4382-92.

Kilburn KH, Warshaw R and Thornton JC (1989). Pulmonary function in histology technicians compared with women from Michigan: effects of chronic low dose formaldehyde on a national sample of women. Br J Ind Med 46(7): 468-72.

Kim CW, Song JS, Ahn YS, Park SH, Park JW, Noh JH and Hong CS (2001). Occupational asthma due to formaldehyde. Yonsei Medical Journal 42(4): 440-445.

Kimbell JS, Gross EA, Richardson RB, Conolly RB and Morgan KT (1997). Correlation of regional formaldehyde flux predictions with the distribution of formaldehyde-induced squamous metaplasia in F344 rat nasal passages. Mutat Res 380(1-2): 143-54.

Kitaeva LV, Kitaev EM and Pimenova MN (1990). [The cytopathic and cytogenetic sequelae of chronic inhalational exposure to formaldehyde on female germ cells and bone marrow cells in rats]. Tsitologiia 32(12): 1212-6.

Krakowiak A, Gorski P, Pazdrak K, Ruta U, Wantke F, Focke M, Hemmer W, Tschabitscher M, Gann M, Tappler P, Gotz M and Jarisch R (1998). Airway response to formaldehyde inhalation in asthmatic subjects with suspected respiratory formaldehyde sensitization. Formaldehyde and phenol exposure during an anatomy dissection course: a possible source of IgE-mediated sensitization? Am J Ind Med 33(3): 274-81.

Kriebel D, Myers D, Cheng M, Woskie S and Cocanour B (2001). Short-term effects of formaldehyde on peak expiratory flow and irritant symptoms. Arch Environ Health 56(1): 11-8.

Krzyzanowski M, Quackenboss JJ and Lebowitz MD (1990). Chronic respiratory effects of indoor formaldehyde exposure. Environ Res 52(2): 117-25.

Kulle TJ, Sauder LR, Hebel JR, Green DJ and Chatham MD (1987). Formaldehyde dose-response in healthy nonsmokers. Japca 37(8): 919-24.

Levine RJ, Andjelkovich DA and Shaw LK (1984). The mortality of Ontario undertakers and a review of formaldehyde-related mortality studies. J Occup Med 26(10): 740-6.

Liley HG, White RT, Benson BJ and Ballard PL (1988). Glucocorticoids both stimulate and inhibit production of pulmonary surfactant protein A in fetal human lung. Proc Natl Acad Sci U S A 85(23): 9096-100.

Liu KS, Huang FY, Hayward SB, Wesolowski J and Sexton K (1991). Irritant effects of formaldehyde exposure in mobile homes. Environ Health Perspect 94: 91-4.

Malaka T and Kodama AM (1990). Respiratory health of plywood workers occupationally exposed to formaldehyde. Arch Environ Health 45(5): 288-94.

Malek FA, Moritz KU and Fanghanel J (2004). Effects of a single inhalative exposure to formaldehyde on the open field behavior of mice. Int J Hyg Environ Health 207(2): 151-8.

Mannino DM, Homa DM, Pertowski CA, Ashizawa A, Nixon LL, Johnson CA, Ball LB, Jack E and Kang DS (1998). Surveillance for asthma--United States, 1960-1995. MMWR CDC Surveill Summ 47(1): 1-27.

Maronpot RR, Miller RA, Clarke WJ, Westerberg RB, Decker JR and Moss OR (1986). Toxicity of formaldehyde vapor in B6C3F1 mice exposed for 13 weeks. Toxicology 41(3): 253-66.

Martin WJ (1990). A teratology study of inhaled formaldehyde in the rat. Reprod Toxicol 4(3): 237-9.

Monteiro-Riviere NA and Popp JA (1986). Ultrastructural evaluation of acute nasal toxicity in the rat respiratory epithelium in response to formaldehyde gas. Fundam Appl Toxicol 6(2): 251-62.

Monticello TM, Swenberg JA, Gross EA, Leininger JR, Kimbell JS, Seilkop S, Starr TB, Gibson JE and Morgan KT (1996). Correlation of regional and nonlinear formaldehyde-induced nasal cancer with proliferating populations of cells. Cancer Res 56(5): 1012-22.

Nagornyi PA, Sudakova Zh A and Shchablenko SM (1979). [General toxic and allergic action of formaldehyde]. Gig Tr Prof Zabol(1): 27-30.

Nielsen GD, Hougaard KS, Larsen ST, Hammer M, Wolkoff P, Clausen PA, Wilkins CK and Alarie Y (1999). Acute airway effects of formaldehyde and ozone in BALB/c mice. Hum Exp Toxicol 18(6): 400-9.

Nordman H, Keskinen H and Tuppurainen M (1985). Formaldehyde asthma--rare or overlooked? J Allergy Clin Immunol 75(1 Pt 1): 91-9.

OEHHA. (2000). *The Air Toxics Hot Spots Program Risk Assessment Guidelines Part III: Technical Support Document for the Determination of Noncancer Chronic Reference Exposure Levels*. Available online at http://www.oehha.ca.gov/air/chronic_rels/pdf/relsP32k.pdf.
http://www.oehha.ca.gov/air/chronic_rels/pdf/relsP32k.pdf.

Olsen JH and Dossing M (1982). Formaldehyde induced symptoms in day care centers. Am Ind Hyg Assoc J 43(5): 366-70.

Pazdrak K, Gorski P, Krakowiak A and Ruta U (1993). Changes in nasal lavage fluid due to formaldehyde inhalation. Int Arch Occup Environ Health 64(7): 515-9.

Porter JA (1975). Letter: Acute respiratory distress following formalin inhalation. Lancet 2(7935): 603-4.

Pross HF, Day JH, Clark RH and Lees RE (1987). Immunologic studies of subjects with asthma exposed to formaldehyde and urea-formaldehyde foam insulation (UFFI) off products. J Allergy Clin Immunol 79(5): 797-810.

Riedel F, Hasenauer E, Barth PJ, Koziorowski A and Rieger CH (1996). Formaldehyde exposure enhances inhalative allergic sensitization in the guinea pig. Allergy 51(2): 94-9.

Ritchie IM and Lehnen RG (1987). Formaldehyde-related health complaints of residents living in mobile and conventional homes. Am J Public Health 77(3): 323-8.

Rumchev KB, Spickett JT, Bulsara MK, Phillips MR and Stick SM (2002). Domestic exposure to formaldehyde significantly increases the risk of asthma in young children. Eur Respir J 20(2): 403-8.

Rusch GM, Clary JJ, Rinehart WE and Bolte HF (1983). A 26-week inhalation toxicity study with formaldehyde in the monkey, rat, and hamster. Toxicol Appl Pharmacol 68(3): 329-43.

Saillenfait AM, Bonnet P and de Ceaurriz J (1989). The effects of maternally inhaled formaldehyde on embryonal and foetal development in rats. Food Chem Toxicol 27(8): 545-8.

Salem H and Cullumbine H (1960). Inhalation toxicities of some aldehydes. Toxicol Appl Pharmacol 2: 183-7.

Sari DK, Kuwahara S, Tsukamoto Y, Hori H, Kunugita N, Arashidani K, Fujimaki H and Sasaki F (2004). Effect of prolonged exposure to low concentrations of formaldehyde on the corticotropin releasing hormone neurons in the hypothalamus and adrenocorticotropic hormone cells in the pituitary gland in female mice. Brain Research 1013(1): 107-116.

Sauder LR, Chatham MD, Green DJ and Kulle TJ (1986). Acute pulmonary response to formaldehyde exposure in healthy nonsmokers. J Occup Med 28(6): 420-4.

Sauder LR, Green DJ, Chatham MD and Kulle TJ (1987). Acute pulmonary response of asthmatics to 3.0 ppm formaldehyde. Toxicol Ind Health 3(4): 569-78.

Schachter EN, Witek TJ, Jr., Brody DJ, Tosun T, Beck GJ and Leaderer BP (1987). A study of respiratory effects from exposure to 2.0 ppm formaldehyde in occupationally exposed workers. Environ Res 44(2): 188-205.

Schachter EN, Witek TJ, Jr., Tosun T, Leaderer BP and Beck GJ (1986). A study of respiratory effects from exposure to 2 ppm formaldehyde in healthy subjects. Arch Environ Health 41(4): 229-39.

Sheppard D, Eschenbacher WL and Epstein J (1984). Lack of bronchomotor response to up to 3 ppm formaldehyde in subjects with asthma. Environ Res 35(1): 133-9.

Skog E (1950). A toxicological investigation of lower aliphatic aldehydes. Acta Pharmacol 6: 299-318.

Solomons K and Cochrane JW (1984). Formaldehyde toxicity. Part I. Occupational exposure and a report of 5 cases. S Afr Med J 66(3): 101-2.

Sorg BA, Bailie TM, Tschirgi ML, Li N and Wu W-R (2001a). Exposure to repeated low-level formaldehyde in rats increases basal corticosterone levels and enhances the corticosterone response to subsequent formaldehyde. Brain Research 898(2): 314-320.

Sorg BA, Tschirgi ML, Swindell S, Chen L and Fang J (2001b). Repeated formaldehyde effects in an animal model for multiple chemical sensitivity. Annals of the New York Academy of Sciences 933(Role of Neural Plasticity in Chemical Intolerance): 57-67.

Srivastava AK, Gupta BN, Bihari V, Gaur JS, Mathur N and Awasthi VK (1992). Clinical studies of employees in a sheet-forming process at a paper mill. Vet Hum Toxicol 34(6): 525-7.

Swiecichowski AL, Long KJ, Miller ML and Leikauf GD (1993). Formaldehyde-induced airway hyperreactivity in vivo and ex vivo in guinea pigs. Environ Res 61(2): 185-99.

Thrasher JD, Broughton A and Madison R (1990). Immune activation and autoantibodies in humans with long-term inhalation exposure to formaldehyde. Arch Environ Health 45(4): 217-23.

Thrasher JD, Wojdani A, Cheung G and Heuser G (1987). Evidence for formaldehyde antibodies and altered cellular immunity in subjects exposed to formaldehyde in mobile homes. Arch Environ Health 42(6): 347-50.

Uba G, Pachorek D, Bernstein J, Garabrant DH, Balmes JR, Wright WE and Amar RB (1989). Prospective study of respiratory effects of formaldehyde among healthy and asthmatic medical students. Am J Ind Med 15(1): 91-101.

Wallenstein G, Rebohle E, Bergmann I, Voigt U and Schneider WD (1978). [Occupational diseases of the respiratory system due to chemical substances with potential allergen effects]. Dtsch Gesundheitsw 33(24): 1119-23.

Wantke F, Demmer CM, Tappler P, Gotz M and Jarisch R (1996). Exposure to gaseous formaldehyde induces IgE-mediated sensitization to formaldehyde in school-children. Clin Exp Allergy 26(3): 276-80.

Wantke F, Focke M, Hemmer W, Bracun R, Wolf-Abdolvahab S, Gotz M, Jarisch R, Gotz M, Tschabitscher M, Gann M and Tappler P (2000). Exposure to formaldehyde and phenol during an anatomy dissecting course: sensitizing potency of formaldehyde in medical students. Allergy 55(1): 84-87.

Weber-Tschopp A, Fischer T and Grandjean E (1977). [Irritating effects of formaldehyde on man (author's transl)]. Int Arch Occup Environ Health 39(4): 207-18.

Wilhelmsson B and Holmstrom M (1992). Possible mechanisms of formaldehyde-induced discomfort in the upper airways. Scand J Work Environ Health 18(6): 403-7.

Wilmer JW, Woutersen RA, Appelman LM, Leeman WR and Feron VJ (1989). Subchronic (13-week) inhalation toxicity study of formaldehyde in male rats: 8-hour intermittent versus 8-hour continuous exposures. Toxicol Lett 47(3): 287-93.

Witek TJ, Jr., Schachter EN, Tosun T, Beck GJ and Leaderer BP (1987). An evaluation of respiratory effects following exposure to 2.0 ppm formaldehyde in asthmatics: lung function, symptoms, and airway reactivity. Arch Environ Health 42(4): 230-7.

Woutersen RA, van Garderen-Hoetmer A, Bruijntjes JP, Zwart A and Feron VJ (1989). Nasal tumours in rats after severe injury to the nasal mucosa and prolonged exposure to 10 ppm formaldehyde. J Appl Toxicol 9(1): 39-46.

Yang X, Zhang YP, Chen D, Chen WG and Wang R (2001). Eye irritation caused by formaldehyde as an indoor air pollution--a controlled human exposure experiment. Biomed Environ Sci 14(3): 229-36.

Zwart A, Woutersen RA, Wilmer JW, Spit BJ and Feron VJ (1988). Cytotoxic and adaptive effects in rat nasal epithelium after 3-day and 13-week exposure to low concentrations of formaldehyde vapour. Toxicology 51(1): 87-99.

Manganese and Compounds Reference Exposure Levels

1. Summary

Acute inhalation of high levels of manganese results in a nonspecific pulmonary edema, while chronic manganese inhalation leads to a characteristic neurotoxicity known as manganism with strong similarities to Parkinson's disease. Manganism is characterized by motor deficits (dystonia, altered gait, fine tremor, generalized rigidity) and may include psychiatric disturbances. At low manganese levels and in the absence of frank manganism, subtle deficits in cognitive and neurobehavioral functions have been reported in both adults and children. Neurodevelopmental deficits have been associated with early life exposure to excessive manganese and include impaired intellectual performance and behavioral disinhibition.

1.1 Manganese Acute REL

An acute REL for manganese was not developed at this time.

1.2 Manganese 8-Hour REL

Reference Exposure Level	**0.05 µg/m^3**
Critical effect(s)	Impairment of neurobehavioral function in humans
Hazard index target(s)	Nervous system

1.3 Manganese Chronic REL

Reference Exposure Level	**0.03 µg/m^3**
Critical effect(s)	Impairment of neurobehavioral function in humans
Hazard index target(s)	Nervous system

2. Physical and Chemical Properties

Table 2.1 Manganese and Manganese Species*

Molecular Formula	*Synonyms*	*Molecular Weight*	*CAS Reg. No.*
Mn	elemental manganese; colloidal manganese; cutaval	54.94 g/mol	7439-96-5
MnO	manganese oxide; manganese monoxide; manganosite	70.94 g/mol	1344-43-0
MnO_2	manganese dioxide; black manganese oxide	86.94 g/mol	1313-13-9
Mn_3O_4	manganese tetroxide; trimanganese tetraoxide; manganomanganic oxide	228.82 g/mol	1317-35-7
$MnCl_2$	manganese chloride; manganese dichloride; manganous chloride	125.84 g/mol	7773-01-5

Description	Lustrous, gray-pink metal (Mn); green (MnO), black (MnO_2) or pink ($MnCl_2$) crystals; brownish-black powder (Mn_3O_4)
Molecular formula	see Table 2.1
Molecular weight	see Table 2.1
Density (in g/cm^3)	7.21-7.4 (Mn – depending on allotropic form); 5.43-5.46 (MnO); 4.88 (Mn_3O_4); 2.977 @ 25°C ($MnCl_2$)
Boiling point	2095°C (Mn); not available (MnO); unknown (Mn_3O_4); 1190°C ($MnCl_2$)
Melting point	1246°C (Mn); 1839°C (MnO); 1567°C (Mn_3O_4); 650°C ($MnCl_2$) (CRC, 2005)
Vapor pressure	1 torr @ 1292°C (Mn); non-volatile at room temperature (Mn_3O_4); not available (MnO; $MnCl_2$)
Solubility	Sol. in dil. acids and aq. solns. of Na- or K-bicarbonate (Mn); sol. in NH_4Cl, insol. in H_2O (MnO); insol. in H_2O, HNO_3, or cold H_2SO_4 (MnO_2 -(Merck, 1976); insol. in H_2O, sol. in HCl (Mn_3O_4); 72.3 g/100 ml H_2O @ 25°C ($MnCl_2$)
Conversion factor	Not applicable (dusts or powders)

3. Occurrence and Major Uses

Metallic manganese is used in the manufacturing of steel, carbon steel, stainless steel, cast iron, and superalloys to increase hardness, stiffness, and strength (HSDB, 2006). Manganese chloride is used in dyeing, disinfecting, batteries, and as a paint drier and dietary supplement. Manganese oxide (MnO) is used in textile printing, ceramics, paints, colored glass, fertilizers, and as food additives. Manganese dioxide is used in batteries and may also be generated from the welding of manganese alloys. Manganese tetroxide may be generated in situations where other oxides of manganese are heated in air (NIOSH, 2005). The 2004 annual statewide emissions of manganese reported in the most recent California Toxics Inventory (CARB, 2005a) were estimated to be 1,055 tons. For 2002, the mean statewide ambient level was 31.5 ng/m^3.

4. Metabolism / Toxicokinetics

Manganese can enter the body by both the oral and inhalation routes. Dermal absorption of manganese is insignificant. Essential manganese is normally absorbed from the intestinal tract as part of the diet. It is estimated that 2 to 5% of ingested manganese is retained in the adult body (Andersen et al., 1999). Retention can be up to 41% in breast-fed infants, and 20% in formula-fed infants (Dorner et al., 1989). Manganese absorption is increased (along with iron absorption) when there is a deficiency of iron in the diet (Davis et al., 1992). Ascorbic acid, calcium and phosphorus also affect manganese utilization (ibid).

As part of the normal manganese homeostatic mechanism, high levels of dietary manganese diminish absorption from the intestinal tract. Manganese appears to be absorbed from the gut largely in the divalent form, with approximately 80% of it subsequently bound in plasma to $ß_1$-globulin and albumin (Foradori et al., 1967). These manganese-protein complexes are efficiently removed from the blood by the liver and returned to the gut in bile for elimination, thus establishing an entero-hepatic circuit for manganese. In the blood, unbound manganese may be converted by ceruloplasmin to the trivalent cation which is then bound by transferrin. Transferrin-manganese complexes are much less efficiently removed by the liver and thus survive first pass elimination to circulate throughout the body (Gibbons et al., 1976). In the brain, transferrin receptors in the capillary beds may mediate uptake in regions with efferents to the nucleus accumbans and the caudate putamen. Other mechanisms also appear to contribute to brain uptake of manganese including a divalent metal transporter (DMT-I), and a less well-defined non-saturable mechanism. From these sites, manganese is thought to move by neuronal transport to the pallidum, thalamic nuclei and substantia nigra; areas involved with motor control and movement (Aschner et al., 2005). While at normal plasma levels, manganese enters the brain mainly across the capillary epithelium, at elevated levels of manganese in the blood, transport across the choroid plexus becomes more prominent (Aschner, 2000).

Manganese exposure via the pulmonary route leads to more rapid absorption with higher efficiency, and with greater transfer to the brain compared with other routes (Drown et al., 1986; Roels et al., 1997). In experiments in rats, Roels *et al*. (1997) used

intratracheal instillation as a surrogate for inhalation for comparison with the oral route (gavage). Intratracheal instillation of $MnCl_2$ (1.22 mg/kg, once weekly for four weeks) raised the steady state manganese levels 68% in blood, 205% in the striatum, 48% in the cortex, and 27% in the cerebellum compared to controls. By gavage, a much higher dose of $MnCl_2$ (24.3 mg/kg) was required to achieve the same blood levels (68%). However, by this route, manganese levels in the striatum and cerebellum were not affected, and levels in the cortex were raised by only 22% (Table 4.1). In animals given a single intratracheal dose of $MnCl_2$ (1.22 mg/kg bw), blood manganese levels peaked within 30 min at 7,050 ng/100 ml. This was followed by a gradual decline but blood levels remained elevated over controls for at least 24 hours. By comparison, the single oral administration of 24.3 mg $MnCl_2$/kg bw resulted in a five-fold lower peak blood level of 1,660 ng/100 ml after one hour, followed by a return to control levels in 12 hours. Thus, compared to ingestion, inhalation of a relatively water soluble form of manganese leads to a rapid increase in blood levels that remain higher for longer, and result in higher brain manganese levels.

Table 4.1 Increase in Tissue Manganese by Route and Chemical Form

	Increase in Tissue Manganese (%)			
Chemical Form and Route	Blood	Striatum	Cortex	Cerebellum
$MnCl_2$ Intratracheal (1.2 mg/kg)	68	205	48	27
$MnCl_2$ Gavage (24.3 mg/kg)	68	0	22	0
MnO_2 Intratracheal (1.2 mg/kg)	41	48	34	31
MnO_2 Gavage (24.3 mg/kg)	0	0	0	0

Using the same exposure protocol with the less soluble MnO_2, intratracheal instillation raised manganese levels 41% in blood, 48% in striatum, 31% in cerebellum, and 34% in cortex. By contrast, neither blood nor brain levels were increased following oral exposure (Table 4.1). As with $MnCl_2$, Mn blood levels following intratracheal MnO_2 reached a higher peak value (1,760 ng Mn/100 ml; 200% increase) than that achieved after gavage (900 ng/100 ml; 27% increase). Blood levels rose more slowly than with $MnCl_2$, starting at 48 – 72 hr after intratracheal instillation and peaking at 168 hr. By gavage, blood levels rose gradually to peak at 144 hr (Roels *et al*., (1997). In these studies, the solubility of the manganese complexes influenced the rate of absorption by either route, but in both cases inhalation resulted in substantially higher blood and brain levels.

In a further demonstration of the dependence of tissue distribution on oxidation state and route of exposure, Reaney *et al.* (2006) exposed rats to 0, 2, or 6 mg/kg Mn(III)-pyrophosphate or Mn(II)Cl_2 intraperitoneally (i.p.) for five weeks. Significantly higher blood manganese levels were seen with Mn(III) vs equimolar Mn(II). A dose-dependent increase in brain manganese was observed, with Mn(III) producing levels that were 25% higher than following Mn(II). This may be related to the higher blood levels of manganese achieved with Mn(III) vs Mn(II) via the i.p. route. Examination of the striatum, globus pallidus, thalamus and cerebral cortex by PIXE (an x-ray fluorescence technique) revealed no differences in the distribution of manganese across these brain

regions. There were, however, differences among regions in response to the concentration and oxidation state of the manganese. In the globus pallidus, the highest cumulative dose (90 mg/kg) of both forms of manganese increased GABA levels compared to controls. By contrast, dopamine levels in globus pallidus at this dose were increased by 60% with Mn(III), but decreased by 40% with Mn(II). The mechanism behind this differential effect is not clear but suggests that manganese oxidation states are important in manganese toxicity.

Drown and colleagues studied the distribution of soluble $^{54}MnCl_2$ and insoluble $^{54}Mn_3O_4$ after instillation into the rat lung (Drown et al., 1986). Initially the soluble form of manganese distributed more rapidly from the lung to the peripheral tissues than did the insoluble form. After two weeks the rates of distribution of the two forms became almost equal. Manganese (^{54}Mn) reached higher concentrations in the liver, kidney and gastrointestinal tissues, but persisted longer in the heart, brain and bone. The manganese was eliminated mainly in bile with very little elimination in urine.

For humans with occupational and/or environmental exposures, the main route of exposure is via inhalation. In both cases the manganese is usually in the form of particulates of various sizes. Manganese deposited in the lung can be absorbed directly into the blood stream, or can migrate (by mucociliary transport) into the upper respiratory tract and then be swallowed for possible absorption in the GI tract. In experimental animals it has been demonstrated that inhaled manganese may be transported via olfactory nerves directly to the brain following absorption from nasal passages (Brenneman et al., 2000; Dorman et al., 2002a; Elder et al., 2006). Neither pulmonary nor gastrointestinal absorption is required for this route of exposure, and the blood-brain barrier is bypassed. Evidence for absorption of particulate manganese oxide from the nose and transport to the brain was provided by Elder *et al*. (2006) in rats. Manganese concentrations in the olfactory bulb increased 3.5-fold following 12 days of intranasal instillation of ultrafine manganese oxide particles (3-8 nm) in both nares. With occlusion of the right nostril and instillation in the left naris, manganese accumulated almost exclusively in the left olfactory bulb. In this experimental paradigm, instillation of either the soluble manganese chloride or the insoluble manganese oxide particles (solubilization rate 1-1.5% per day) in the patent naris resulted in comparable levels of manganese in the ipsilateral side of the olfactory bulb. This, in conjunction with the observation that an increase in manganese in the olfactory bulb was detectable within 30 minutes of the instillation, suggests that particulate rather than dissolved manganese was the form transported to the brain. It is not clear how significant this route of exposure is in humans.

The major route of excretion of manganese is via bile, although a lesser amount is excreted via urine (Davis et al., 1993; ATSDR, 2000). That the liver maintains homeostasis of manganese can be seen by the fact that patients with cirrhosis of the liver accumulate abnormally high levels of manganese in their brains, especially in the globus pallidus (Rose et al., 1999). Similarly, rats that have a liver bypass also show high levels of manganese in the brain, especially in the globus pallidus (Rose *et al*., 1999).

Neonatal humans do not excrete manganese for the first two to three weeks of life. The intestinal barrier to manganese absorption is also immature in premature and neonatal infants (Cawte, 1985).

5. Acute Toxicity of Manganese

Acute inhalation exposure to high levels of manganese as its oxides is associated with pulmonary edema and impaired function (Shiotsuka, 1984). However, a pulmonary inflammatory response is also associated with the inhalation of particulates in general and does not appear to be dependent on the manganese content. The very small body of literature on acute toxicity includes two animal experiments involving acute exposures by inhalation. One is a two-hour exposure of female CD-1 mice to manganese oxide (Mn_3O_4) aerosols (Adkins et al., 1980) that resulted in a NOAEL of 2.9 mg/m^3 based on respiratory effects (edema). The other is a 24 hr exposure of guinea pigs to 22 mg/m^3 MnO_2 (Bergstrom, 1977) that resulted in a NOAEL of 0.9 mg/m^3, corrected for one hour, again for respiratory effects (inflammatory reaction). However, no LOAEL was observed in either study, and no manganese acute inhalation studies were located that demonstrated a dose-response or evaluated other toxicological endpoints.

6. Chronic Toxicity of Manganese

6.1 Chronic Toxicity to Adult Humans

Exposure of humans to manganese by inhalation leads to a suite of neurological effects called “manganism” (Lucchini et al., 1999). Frank manganism is a progressive disease that involves symptoms similar to those of Parkinson’s disease (ATSDR, 2000). Manganism is characterized by altered gait, fine tremor and occasionally psychiatric disturbances. The psychiatric disturbances are seldom seen in Parkinson’s disease, although dementia sometimes occurs late in this disease. Despite their similarities, the symptoms of manganism and Parkinson’s disease differ somewhat (Barbeau, 1984; Calne et al., 1994). Both manganism and Parkinson’s disease involve generalized bradykinesia and widespread rigidity. However, tremor is less frequent and dystonia more frequent in manganism. Manganism is also distinguished by a propensity to fall backward, failure to achieve a sustained therapeutic response to levodopa, and failure to detect a reduction in fluorodopa uptake by positron emission tomography (PET) (Calne *et al*., 1994). In Parkinsonism, the damage appears to be confined to the substantia nigra, whereas in manganism the damage is more widespread, involving other parts of the basal ganglia (Huang et al., 1998).

Manganese accumulates in certain brain structures, especially the extrapyramidal system. Structures rich in dopaminergic neurons show a heightened sensitivity to manganese toxicity. Within these tissues, manganese is found preferentially in mitochondria where it disrupts oxidative phosphorylation and mitochondrial function (Gavin et al., 1999). Cytochrome c, released from damaged mitochondria, leads to apoptosis and loss of neurons (Malecki, 2001). Trivalent manganese can promote the formation of reactive oxygen species (HaMai et al., 2001) that can cause oxidative stress, which in turn has been shown to lead to apoptosis of neurons in the rat brain (Dobson et al., 2003). While

individuals exposed to massive amounts of manganese show frank neurological symptoms as in the Groote Eylandt studies (Kilburn, 1987) and the industrial workers studies (ATSDR, 2000), individuals exposed to lesser amounts of manganese show more subtle neurological deficits in neurobehavioral tasks (Wennberg *et al*., 1992; Lucchini *et al*., 1999).

Adverse effects may occur at exposure levels to manganese that are too low to cause frank manganism. Lucchini and his co-workers studied a group of Italian ferroalloy workers who had been exposed to low levels of manganese dust by inhalation (Lucchini *et al*., 1999). These workers did not exhibit the frank signs of manganism, but they did exhibit subtler neurofunctional changes. The workers were exposed to a "current overall value" of 0.054 mg Mn per m^3 air at the time of the study. Earlier exposures were higher. In order to obtain a measure of cumulative exposure the investigators calculated a "cumulative exposure index" for each worker based on their exposure history in the factory. The cumulative exposure indices correlated positively with blood manganese levels. The workers were subjected to symptom questionnaires and neurobehavioral and neurophysiological testing for the purpose of finding whether neurological effects correlated with cumulative exposure. In multiple regression analyses, positive correlations were found between the log of the cumulative exposure index and the following tests of the Swedish Performance Evaluations System: finger tapping in the dominant ($R = 0.32$, $p = 0.01$) and non-dominant ($R = 0.32$, $p = 0.01$) hands, Symbol Digit ($R = 0.33$, $p = 0.01$) and Digit Span ($R = 0.44$, $p = 0.004$). The moderate but significant correlation coefficients reported in this study suggest that manganese is an important contributor to these effects but likely not the only one. In addition, these results demonstrate that subtle neurological changes are taking place in workers exposed to relatively low levels of manganese in the absence of frank manganism.

Male workers (n = 92, plus 101 matched controls) in an alkaline battery plant in Belgium exposed to manganese dioxide dust were the subjects of a cross-sectional epidemiological study (Roels et al., 1992). Total manganese concentrations, and manganese dust were measured in the workers' breathing zones with personal samplers. Lifetime integrated respirable dust levels (LIRD) ranged from 0.04 to 4.43 mg Mn/m^3 * year, with a geometric mean of 0.793 mg Mn/m^3 * year. The average age of control and exposed groups was 30 years with a mean manganese exposure time of 5.3 years (0.2 to 17.7 years) for the latter group. In exposed workers, the geometric mean levels of blood and urine manganese (corrected for creatinine) were significantly higher ($p < 0.001$) than in controls. The subjects were also evaluated for neurobehavioral function, lung function, and hematological parameters. There were no significant differences in respiratory symptoms between those exposed and controls, and hematological parameters were in the normal range for both groups. In neurobehavioral tests, significant decrements in performance were found in exposed workers on tests for visual reaction time ($p < 0.001$), five measures of eye-hand coordination ($p < 0.005$), and in two of three tests of hand tremor ($p < 0.03$). The LOAEL for this study, based on neurobehavioral effects, corresponds to 0.15 mg respirable manganese dust/m^3 (geometric mean of the LIRD divided by the average exposure time = 0.793 mg Mn/m^3 * year / 5.3 year = 0.15 mg Mn/m^3). This LOAEL was used to calculate the 8-hr and chronic RELs.

Another occupational study of lower exposures was done in Sweden (Wennberg et al., 1992). In this study workers had been exposed for a year or more to manganese dust at mean value concentrations of 0.18 mg/m^3 at one smelter, and 0.41 mg/m^3 at another. They were compared to workers at similar industrial plants without manganese exposure via a suite of neurological tests, including electroencephalogram, brainstem auditory evoked potential, event related auditory evoked potential, and diadochokinesometry (a test of the subject's ability to rotate a handle rapidly). Of these tests, the only one that produced significantly different results in the exposed subjects was the diadochokinesometry. The manganese-exposed workers were unable to rotate the handle as quickly as the control workers. This is interpreted as evidence of a "preclinical" effect of low-level manganese exposure.

A major study of non-industrial human exposures is the study of the natives of Groote Eylandt, a large island off the coast of Australia. The inhabitants of this island are Australian Aborigines. The island is so rich in manganese that the environment has been described as a "manganese ecology" (Kilburn, 1987). The inhabitants are exposed by virtually all routes of exposure, but especially by ingestion of food and water high in manganese. Kilburn studied the natives of Groote Eylandt and compared them to a control group of Australian Aborigines living in another part of Australia. This paper does not quantitate the manganese exposures or body levels of manganese in the study population, and it would be difficult to quantitate exposures in this complex environmental situation. Kilburn reports certain congenital abnormalities, such as deformations of the foot (talipes equinovarus), closed anus (imperforate anus), and anorectal malformations, and neurobehavioral problems, including progressive muscle wasting (amyotrophy) and failure of muscle coordination (ataxia), that apparently occur with greater frequency in the islanders than in the control groups, but these could also be due to genetic factors present in this small population. Indeed all of the problems were seen in just two pedigrees. A likely interpretation would be that the adverse health effects observed reflect gene-environment interactions.

6.2 Chronic Toxicity to Infants and Children

Manganese is an essential nutrient, but it has toxic effects if exposure is excessive or prolonged, especially if exposure is by the inhalation route. Infants and children are expected to be more susceptible than adults to the toxic effects of elevated manganese because they absorb greater amounts of manganese in their gastrointestinal tract, the infant liver has not yet developed the homeostatic mechanisms to maintain safe levels of manganese in the blood and target tissues (especially the brain), and the blood-brain barrier is incompletely formed. Indeed, this susceptibility is evident in a study by Wasserman *et al*. (2006) of 10-year old children (n = 142) in Bangladesh exposed to manganese in drinking water (< 200, 200-499, 500-999, >1,000 µg/l). Comparing the lowest and highest dose groups (< 200 vs > 1,000 µg/l), significant decrements in intellectual function at 9.5-10.5 years of age were revealed in scores on the Wechsler Intelligence Scale for Children-III with increasing daily intake of manganese (full scale, $p < 0.0001$; performance, $p < 0.0001$; verbal, $p < 0.02$). The scores of children with

intermediate manganese exposures were also lower than those of the lowest dose group, but not significantly so. In this study, confounding by co-exposure to arsenic was limited by including only children whose drinking water contained <10 µg As/l. Scores were adjusted for maternal education and intelligence, house type, television, child height and head circumference. Blood levels of manganese, arsenic and lead were also determined and added to the core model. In this case, only blood lead was correlated with decreased intellectual performance. However, in a simultaneous analysis of water manganese, water arsenic, and blood lead, the negative association between manganese water levels and intellectual function test scores remained (Full-Scale ß = -4.56, $p < 0.01$; Performance ß = -3.82, $p < 0.01$).

A number of studies have reported correlations between early life exposure to excessive manganese and symptoms of impaired neurodevelopment as revealed on neurobehavioral tests and in poorer academic performance. In a prospective study of the neurobehavioral effects of *in utero* exposure to manganese, Takser *et al.* (2003) reported an inverse correlation between cord blood manganese at birth and three subscales of psychomotor development (McCarthy scales of children's abilities) measured at three years of age (n = 126): attention (partial r = -0.33, $p < 0.01$), nonverbal memory (partial r = -0.28, $p < 0.01$) and hand skills (partial r = -0.22, $p < 0.05$). The adverse effects of manganese on neurodevelopment in these children persisted after adjustment for gender and maternal education, although the effects of manganese on hand skills were only observed in boys. Similarly, Collip et al. (1983) used a battery of tests, including cognitive and projective tests, psycho-educational evaluation, speech, language and hearing evaluations, and social services evaluations, to identify children who were hyperkinetic and exhibited learning disabilities. In comparison with normal children of the same age, significantly elevated levels of hair manganese (0.434 µg/g; measured at 8 years of age) were reported in children with learning disabilities and hyperactivity compared with normal children (0.268 µg/g) ($p<0.05$). An association between poorer performance in school and elevated hair manganese (1.242 µg/g) has also been observed among children in China compared with children with more normal manganese levels (Zhang et al., 1995).

The uptake of metals into developing teeth provides a record of gestational exposure to manganese. In multiple regression analyses, after controlling for lead, high levels of manganese incorporated into teeth during the 20th week of gestation were positively correlated with behavioral disinhibition at 36 months of age ($R = 0.48$, $p < 0.01$) and, at 54 months, with impulsive errors on the Mirsky Continuous Performance Test ($R = 0.48$, $p < 0.01$) and the Children's Stroop Test ($R = 0.38$, $p < 0.01$). Positive correlations with manganese were also seen in ratings made by both parents and teachers of externalizing and attention problems on the Child Behavior Checklist in the 1st ($R = 0.40 – 0.47$, $p < 0.05$) and 3rd grades ($R = 0.38 – 0.48$, $p < 0.05$), and in the 3rd grade with the teachers' ratings on the Disruptive Behavior Disorders Scale ($R = 0.44$, $p < 0.05$), ADHD ($R = 0.48$, $p <0.01$), and hyperactivity – impulsivity ($R = 0.55$, $p < 0.01$). In contrast, manganese levels in tooth enamel formed in the 62-64th week of gestation (i.e. postnatally) were correlated only with teachers' reports of externalizing behaviors in the 1st ($R= 0.40$, $p < 0.05$) and 3rd grades ($R = 0.57$, $p < 0.01$). It thus appears that high prenatal manganese exposure may adversely affect behaviors expressed postnatally.

There was, however, no correlation between tooth manganese and cognitive ability as measured on the Woodcock-Johnson Psycho-Educational Battery (Ericson et al., 2006).

Subtle neurobehavioral effects were seen in a case report of a 10-year old boy exposed for five years to elevated manganese in the family's drinking water (Woolf et al., 2002). The boy's hair manganese was high (3,091 ppb vs normal reference <260 ppb), as was that of his 16 year-old brother (1,988 ppb). Neuropsychological tests on the 10 year-old revealed intact global cognitive skills but striking deficits in visual and verbal memory (<20th percentile in the Wide Range Assessment of Visual-motor Abilities). No obvious neurobehavioral problems were noted for either the parents or the older sibling.

6.2.1 Potential for Differential Effects in Children

Infants and children may be more susceptible than adults to manganese toxicity for the following reasons:

1. Newborns absorb more manganese from the gastrointestinal tract than do adults.
2. The liver of newborns has not yet developed the ability to maintain safe levels of manganese in the bloodstream and brain tissues by excreting excess manganese in the bile, i.e. homeostasis of manganese has not yet developed.
3. Some infant formulas and foods are high in manganese.
4. The newborn's brain is still developing and the blood-brain barrier is not completely formed.
5. Modeling of the inhalation dosimetry of particles (0.001-10 µm), comparing neonates (3 mo) and adults, in four regions of the respiratory tract (extra-thoracic, tracheo-upper bronchi, bronchiolar, pulmonary), suggests that differences in the dose per unit surface area between neonates and adults are dependent on particle size and respiratory tract region (Ginsberg et al., 2005). In addition, infants and young children experience overall higher deposition of particles than adults.

6.3 Animal Studies of Chronic Toxicity

Animal studies of the toxic effects of chronic manganese exposure have focused on altered neurobehavior and the effects of manganese on the associated brain structures. These studies indicate that differences in age at exposure, route, and chemical form of the metal are critical to the distribution of manganese, and the type and extent of the adverse effects.

The relative sensitivity of neonatal and adult CD rats to manganese-induced neurotoxicity was studied by administering manganese dichloride orally to rats at doses of 0, 25 and 50 mg/kg per day (Dorman et al., 2000). Adults and pups were dosed for 21 consecutive days, and then were evaluated with behavioral tests such as pulse elicited startle response amplitude, and in terms of manganese levels in striatum, hippocampus, hindbrain and cortex. Neonatal rats exposed at the highest level of manganese showed a statistically

significant increase in amplitude of acoustic startle response. They also showed increases in brain levels of manganese. The results suggest that neonates may be at greater risk for manganese-induced neurotoxicity when compared to adults receiving high oral levels of manganese. The authors state that there are known pharmacokinetic processes that may relate to the increase in brain manganese concentration in neonatal rats including increased manganese absorption from the juvenile gastrointestinal tract, an incompletely formed blood-brain barrier, and a virtual absence of excretory mechanisms until weaning.

Neurobehavioral effects may be preceded by changes in brain chemistry. Such changes were studied in four female rhesus monkeys exposed in an inhalation chamber to 30 mg/m^3 respirable manganese dust for five h/d, five d/wk (Bird et al., 1984). After two years the animals were sacrificed and compared to unexposed controls. The exposed monkeys showed decreased dopamine in the caudate and globus pallidus, as well as a 60 to 80% increase in manganese levels in the basal ganglia of the brain. However, the exposed monkeys did not exhibit any of the movement disorders that are characteristic of Parkinson's disease.

The distribution of manganese in primate brain, and its neurobehavioral and cognitive effects in Cynomologus macaques following weekly intravenous injection of $MgSO_4$ (10-15 or 3.26-4.89 mg/kg) for 39 weeks was investigated by Guilarte and associates. Neurobehavior, as rated on a modified Parkinsonian symptoms scale, activity levels measured with an activity monitor, and fine motor skills, assessed as the number of errors while trying to retrieve objects from wells of different sizes, all showed significant decrements ($p < 0.05$) at the end of the experiment compared with baseline (Guilarte et al., 2006a). Over this same period, stereotypical or compulsive-like behaviors, such as licking/biting fingers and grooming significantly increased in frequency with manganese exposure ($p < 0.01$) (Schneider et al., 2006). Imaging studies were performed at 128 days and 157 days after the start of manganese exposure, and included T-1 weighted magnetic resonance imaging (MRI), magnetic resonance spectroscopy (H-MRS) and positron emission tomography (PET). As assessed by PET, manganese decreased the ability of amphetamine to stimulate dopamine release in the striatum, apparently without the loss of dopaminergic terminals. The authors speculate that the inhibition of dopamine release may alter the excitability of nigrostriatal dopaminergic neurons and/or may alter dopamine compartmentalization. The former case may contribute to the behavioral symptoms while, in the latter case, the probability of dopamine oxidation and consequent neuronal damage may be increased (Guilarte et al., 2006a). Neuronal loss or dysfunction in these monkeys was suggested by a change in brain metabolites with chronic manganese exposure. Specifically, significant decreases in the N-acetylaspartate:creatinine ratio in parietal cortex ($p = 0.028$), and a near significant ($p = 0.055$) decrease in the white matter were observed.

6.4 Dietary Exposure to Manganese

Newborns and infants may be exposed to more manganese in their diets than are adults. Infant formulas based on cow's milk have about 16 times more manganese than human milk (Dorner et al., 1989). Soy based formulas have even higher levels of manganese – about 40 times the manganese of human milk (Tran et al., 2002a; Tran et al., 2002b).

Formula usage can lead to significantly elevated body burdens of manganese. For example, the hair manganese in normal infants at birth was reported to be 0.19 µg/g hair and, in breast-fed infants, increased to 0.330 µg/g at four months of age. By comparison, hair manganese levels in infants on a formula diet reached 0.965 µg/g at six weeks of age, and 0.685 µg/g at four months (Collipp et al., 1983). In addition, infants can have a less varied diet than adults and may consume more of certain foods that are high in manganese (e.g., sweet potatoes, 2.6 mg/cup; spinach, 1.8 mg/cup; oatmeal, 1.4 mg/cup; (NWU, 2006)).

6.5 Nutritional Requirement

Manganese is an essential nutrient involved in the formation of bone, and in amino acid, cholesterol, and carbohydrate metabolism (FNB, 2004). It is required in a number of metalloenzymes, including arginase, glutamine synthetase, phosphoenolpyruvate decarboxylase, and superoxide dismutase (FNB, 2004). Levels of manganese in adult tissues are maintained at stable levels by homeostatic mechanisms that involve regulation of both uptake and excretion (ATSDR, 2000). Manganese homeostasis is not maintained in newborn infants, and it is not clear how long it takes for it to develop (FNB, 2004); homeostasis in mice takes 17 to 18 days to become effective (Fechter, 1999). Rat pups born to manganese-exposed mothers (dosed with 2000 ppm Mn in drinking water throughout pregnancy and for 11 days of lactation) have seven times the manganese (whole body) as controls (Kostial et al., 2005). By weaning (11 days after birth) the manganese concentration in both groups is virtually the same, indicating that in rat pups manganese homeostasis may begin shortly after birth and become effective by weaning (Kostial *et al*., 2005).

Adequate intakes (AI) of manganese have been established by the Food and Nutrition Board of the Institute of Medicine (FNB, 2004). They are given in Table 6.5.1 below. This table also contains tolerable upper intake levels (UL) for manganese consumption. It is of note that in many cases the UL is not very far above the AI level. For children one to three years of age the UL is less than twice the AI.

The AI for infants 0 to 6 months was set based on the amount of manganese in human milk and the average amount of milk consumed. There are no reports of nursing infants showing any symptoms of manganese deficiency (FNB, 2004). The AI for infants 7 to 12 months of age is based on the manganese content of a typical diet including human milk and other foods. This AI is much higher than the one for infants 0 to 6 months because the manganese content of other foods is generally much higher than the manganese content of human milk (FNB, 2004).

Table 6.5.1 Adequate Intakes and Tolerable Upper Intake Levels for Manganese for Different Age Groups

Group	Adequate Intake (AI) (mg/day)	Tolerable Upper Intake Level (UL) (mg/day)
Infants, 0-6 months	0.003	"not possible to establish"
Infants, 7-12 months	0.6	"not possible to establish"
Children, 1-3 years	1.2	2
Children, 4-8 years	1.5	3
Boys, 9-13 years	1.9	6
Boys, 14-18 years	2.2	9
Girls, 9-13 years	1.6	6
Girls, 14-18 years	1.6	9
Men, 19 to >70 years	2.3	11
Women, 19 to >70 years	1.8	11
Pregnant women, 14-18 yrs	2	9
Pregnant women, 19-50 yrs	2	11
Lactating mothers, 14-18 years	2.6	9
Lactating mothers, 19-50 years	2.6	11

7. Developmental and Reproductive Toxicity

While data are scarce on the developmental effects of perinatal manganese exposure in humans, rats exposed to supplemental manganese (50, 250, 500 µg/day) beginning at birth show decreased dopamine in the striatum and poorer performance on behavioral tests (Tran et al., 2002b). In children on long-term parenteral nutrition resulting in blood manganese levels of 615-1840 nmol/l (vs reference range of 72-210 nmol/l), elevated manganese levels have been seen in globus pallidus and subthalamic nuclei (Fell et al., 1996), suggesting an enhanced potential for neurological damage. This is consistent with the decrements in intellectual function in children exposed to manganese in drinking water reported by Wasserman et al. (2006).

The effects of manganese on reproduction in humans have been reported in epidemiological studies of workers with occupational exposure to manganese. The results have been mixed with Gennart et al. (1992) reporting no effect on fertility among workers exposed to a median manganese dust level of 0.71 mg/m^3, while those exposed to 0.07-8.61 mg/m^3 (geometric mean 0.94 mg/m^3) in a study by Lauwerys et al. (1985) sired a statistically significant lower number of children during the period of paternal exposure. However, workers in the Gennart et al. study were exposed to the relatively insoluble manganese oxide and had mean urine manganese levels of 0.82 µg/g creatinine. By comparison, the workers in the study by Lauwerys et al. were exposed to the more soluble manganese salts in addition to the oxide, and had mean urinary manganese levels of 4.37 µg/g creatinine. Thus the differences in the effects of manganese on reproduction

reported in these two studies may be due to the significant differences in manganese exposures.

Adverse changes in reproductive parameters and behaviors have been seen in studies of rodents exposed to high levels of manganese. In immature female rats (23 days old), manganese (1-25 µg $MnCl_2$) introduced into the third ventricle of the brain significantly and dose-dependently stimulated the release of leutinizing hormone (LH). This effect was apparently at the level of the hypothalamus as pretreatment with the LH releasing hormone (LHRH) receptor antagonist, acycline, prior to manganese exposure blocked the release of LH (Pine et al., 2005). These authors further reported that serum LH, follicle stimulating hormone, and estradiol were all elevated by 29 days of age in rats that had received $MnCl_2$ by gavage starting on postnatal day 12. In these animals manganese altered the timing of reproductive events resulting in a significantly ($p<0.001$) earlier onset of puberty as measured by vaginal opening.

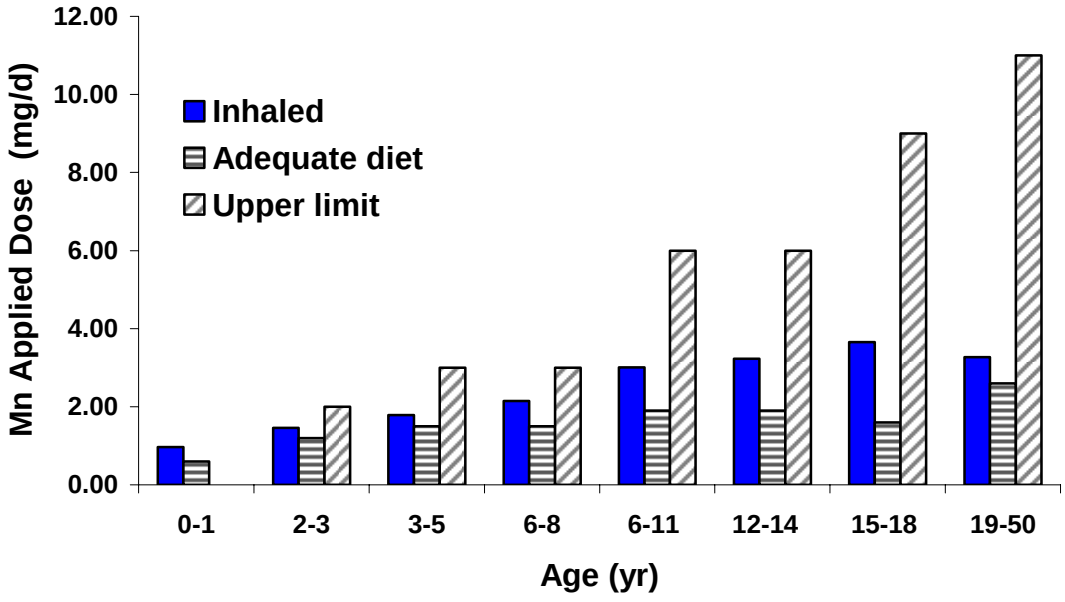
In adult male rats, exposure to 1,000 ppm manganese sulfate in drinking water for 12 weeks significantly suppressed sexual performance compared with controls as measured by prolonged ejaculatory latencies ($p < 0.001$), and increased post-ejaculatory intervals ($p < 0.05$). Displays of aggressive behaviors (lateralizations, boxing bouts and fights with stud males) were also reduced ($p < 0.001$). The extent to which the altered behaviors represent neurological effects versus effects on testes and androgen production is not clear. However, among females mated to the manganese-treated males, the total number of resorptions was significantly increased ($p < 0.025$), suggesting a testicular effect. This is supported by a significant ($p < 0.001$) reduction in absolute and relative testes weights, and absolute seminal vesicle weights among manganese-exposed males (Bataineh et al., 1998). An effect of manganese on male reproductive organs was also investigated in mice following 43 days of oral manganese acetate (7.5 – 30 mg/kg/d) (Ponnapakkam et al., 2003). Unlike the study with rats above, there was no significant change in testicular weight or pathology with manganese exposure in the mice. Nor was there evidence of abnormal mating behavior. However, epididymal weights were significantly lower ($p < 0.05$) and there was a significant ($p < 0.001$) dose-dependent decrease in sperm number and motility.

The available data suggest that manganese is a reproductive toxicant in animals (both males and females) albeit at relatively high doses. Neurobehavioral toxicity manifests at levels encountered in the environment (Wasserman *et al*., 2006). Whether this decrement in intellectual function represents a true developmental effect with permanent consequences is not clear.

8. Derivation of Reference Exposure Levels

The determination of safe exposure levels to manganese is complicated by its status as an essential nutrient. However, as described above, inhalation of manganese results in a qualitatively and quantitatively different exposure compared to oral intake, with inhalation resulting in more rapid uptake and higher blood and brain levels. While dietary manganese levels moderate intestinal absorption of manganese, there appears to be no affect of dietary manganese on the pharmacokinetics of inhaled manganese (Dorman et al., 2001; Dorman et al., 2002b). To provide perspective on potential manganese exposure from inhalation relative to the suggested upper limits for age-dependent dietary intake, Figure 8.1 below shows the amount of manganese children of various ages and lactating mothers (19-50 yr old) would inhale if they were exposed to the manganese levels reported in the Roels *et al* (1992) occupational study (0.215 mg/m^3) compared with the recommended adequate intakes and upper limits listed in Table 6.5.1. The study by Roels is used in the development of the RELs below, and the value of 0.215 mg/m^3 was selected from that study as it represents a real-world mean level of exposure that is associated with neurotoxicity in adults. The figure presents applied doses, not the actual amount absorbed since it is not known if humans absorb manganese more efficiently from the lungs than from the gut, as was shown to be the case for rats (Roels *et al*., 1997). However, the graph suggests that for infants and children receiving adequate dietary manganese, additional exposure to manganese by inhalation presents a greater risk for manganese overdose than it does for adults at a given air concentration. Further, evidence of neurodevelopmental toxicity in animals and humans underscores increased sensitivity in children relative to adults. This enhanced risk among infants and children will be addressed in the development of the RELs below.

Figure 8.1 Manganese Dose from Inhalation and Diet by Age



8.1 Manganese Acute Reference Exposure Level

Acute Reference Exposure Levels (RELs) are levels at which intermittent one-hour exposures are not expected to result in adverse health effects (see Section 5 of the Technical Support Document (TSD)). Pulmonary damage and inflammation are the principal endpoints associated with acute inhalation exposure to manganese. However, at present the database is insufficient to allow the development of an acute REL for manganese based on inhalation studies. No studies were located that reported dose-response data for acute inhalation exposures, nor was it possible to determine both LOAELs and NOAELs from the available data.

8.2 Manganese 8-Hour Reference Exposure Level

Study	Roels *et al.*, 1992
Study population	102 workers in a battery plant
Exposure method	Inhalation of workplace air
Exposure continuity	
Exposure duration	8 hr/day, 0.2-17.7 yr (mean 5.3 yr)
Critical effects	Impaired neurobehavior: visual reaction time, eye-hand coordination, hand steadiness
LOAEL	0.15 mg/m^3
NOAEL	not observed
Benchmark concentration	not derived
Time-adjusted exposure	0.107 mg/m^3 (0.15 mg/m^3 x 5/7)
LOAEL uncertainty factor (UF_L)	6 (default, no NOAEL, mild effect)
Subchronic uncertainty factor (UFs)	√10 (default 8-12% of lifetime)
Interspecies Uncertainty Factor	
Toxicokinetic (UF_{A-k})	1 (default: human study)
Toxicodynamic (UF_{A-d})	1 (default: human study)
Intraspecies Uncertainty Factor	
Toxicokinetic (UF_{H-k})	10 (greater lung deposition in children)
Toxicodynamic (UF_{H-d})	10 (greater susceptibility of children to neurotoxicity)
Cumulative uncertainty factor	2000
Reference Exposure Level	**0.05 µg/m^3**

The 8-hour Reference Exposure Level is a concentration at or below which adverse noncancer health effects would not be anticipated for repeated 8-hour exposures (see Section 6 in the TSD).

The proposed 8-hr REL for manganese is 0.05 µg/m^3 based on impairment of neurobehavioral function in humans in the occupational study of Roels et al. (1992) described in Section 6.1. This study yielded an average occupational exposure for the exposed group (LOAEL, no NOAEL observed) of 0.15 mg Mn/m^3 corresponding to a time-adjusted concentration of 0.107 mg/m^3 (based on an 8 hour TWA occupational exposure for 5 days/week).

A cumulative UF of 2,000 was used, comprising a 6 for the LOAEL uncertainty factor associated with mild effects (see Section 4.4.5 of the TSD), √10 for subchronic to chronic conversion (average exposure duration = 5.3 yr; Section 4.4.6 of the TSD), and 10 each for intraspecies toxicokinetic (UF_{H-k}) and toxicodynamic (UF_{H-d}) uncertainty. This REL is based on healthy adult male workers with adjustments for the potentially greater susceptibility of children. The intraspecies UF_{H-k} of 10 was chosen in part to reflect the 3-4-fold greater deposition of inhaled particulates in the 1-10 µm size range in the lungs of neonates relative to adults exposed to similar particulate levels in ambient air (Ginsberg et al., 2005). In addition, based on studies with neonatal and adult rats (Dorman et al., 2005), neonates accumulate higher levels of manganese in the brain more quickly than do adults with similar exposures.

A UF_{H-d} of 10 is used to address the expectation that the still-developing brain of newborn and infant children is more sensitive to the effects of manganese and that injuries to the nervous system during development are anticipated to have lasting effects.

8.3 Manganese Chronic Reference Exposure Level

Study	Roels et al., 1992
Study population	102 workers in a battery plant
Exposure method	Inhalation of workplace air
Exposure continuity	
Exposure duration	8 hr/day, 0.2-17.7 yr (mean 5.3 yr)
Critical effects	Impaired neurobehavior: visual reaction time, eye-hand coordination, hand steadiness
LOAEL	0.15 mg/m^3
NOAEL	not observed
Benchmark concentration	not derived
Time-adjusted exposure	0.054 mg/m^3 (0.15 mg/m^3 x 10/20 x 5/7)
LOAEL uncertainty factor (UF_L)	6 (default, no NOAEL, mild effect)
Subchronic uncertainty factor (UFs)	√10 (default 8-12% of lifetime)
Interspecies Uncertainty Factor	
Toxicokinetic (UF_{A-k})	1 (default: human study)
Toxicodynamic (UF_{A-d})	1 (default: human study)
Intraspecies Uncertainty Factor	
Toxicokinetic (UF_{H-k})	10 (greater lung deposition in children)
Toxicodynamic (UF_{H-d})	10 (greater susceptibility of children to neurotoxicity)
Cumulative uncertainty factor	2000
Reference Exposure Level	**0.03 µg/m^3**

The chronic Reference Exposure Level is a concentration at which adverse noncancer health effects would not be expected from chronic exposures (see Section 7 in the Technical Support Document).

The proposed chronic REL for manganese is 0.03 μg/m^3, based on impairment of neurobehavioral function in humans in the occupational study of Roels et al. (1992). This study yielded an average occupational exposure for the exposed group (LOAEL, no NOAEL observed) of 0.15 mg Mn/m^3 corresponding to a time-adjusted concentration of 0.054 mg/m^3 (based on an 8 hour TWA occupational exposure to 10 m^3 manganese contaminated air per day out of 20 m^3 total air inhaled per day over 5 days/week).

A cumulative UF of 2,000 was used, comprising a 6 for the LOAEL uncertainty factor associated with mild effects (see Section 4.4.5 of the TSD), 3 for subchronic to chronic conversion (average exposure duration = 5.3 yr; Section 4.4.6 of the TSD), and 10 each for intraspecies toxicokinetic (UF_{H-k}) and toxicodynamic (UF_{H-d}) uncertainty. This REL is based on healthy adult male workers with adjustments for the potentially greater susceptibility of children. The intraspecies UF_{H-k} of 10 was chosen in part to reflect the 3-4-fold greater deposition of inhaled particulates in the 1-10 μm range in the lungs of neonates relative to adults exposed to similar particulate levels in ambient air (Ginsberg et al., 2005). In addition, based on studies with neonatal and adult rats (Dorman et al., 2005), neonates accumulate higher levels of manganese in the brain more quickly than do adults with similar exposures.

A UF_{H-d} of 10 is used to address the expectation that the still-developing brain of newborn and infant children is more sensitive to the effects of manganese and that injuries to the nervous system during development are anticipated to have lasting effects. This REL was developed with specific consideration of the potentially greater susceptibility of children to manganese neurotoxicity. For comparison, the RfC for chronic manganese inhalation developed by the US EPA is 0.05 μg/m^3 (U.S.EPA, 1993) also based on Roels et al. (1992).

8.4 Manganese as a Toxic Air Contaminant

In view of the potential for high exposure coupled with a lower ability to regulate manganese, and enhanced neurodevelopmental susceptibility leading to differential impacts in infants and children identified in Section 6.2.1, OEHHA recommends that manganese be identified as a toxic air contaminant which may disproportionately impact children pursuant to Health and Safety Code, Section 39669.5(c).

9. References

Adkins B, Jr., Luginbuhl GH and Gardner DE (1980). Acute exposure of laboratory mice to manganese oxide. Am Ind Hyg Assoc J 41(7): 494-500.

Andersen ME, Gearhart JM and Clewell HJ, 3rd (1999). Pharmacokinetic data needs to support risk assessments for inhaled and ingested manganese. Neurotoxicology 20(2-3): 161-71.

Aschner M (2000). Manganese: brain transport and emerging research needs. Environ Health Perspect 108 Suppl 3: 429-32.

Aschner M, Erikson KM and Dorman DC (2005). Manganese dosimetry: species differences and implications for neurotoxicity. Crit Rev Toxicol 35(1): 1-32.

ATSDR. (2000). *Toxicological profile for manganese*. U.S. Department of Health and Human Services.

Barbeau A (1984). Manganese and extrapyramidal disorders (a critical review and tribute to Dr. George C. Cotzias). Neurotoxicology 5(1): 13-35.

Bataineh H, Al-Hamood MH and Elbetieha AM (1998). Assessment of aggression, sexual behavior and fertility in adult male rat following long-term ingestion of four industrial metals salts. Hum Exp Toxicol 17(10): 570-6.

Bergstrom R (1977). Acute pulmonary toxicity of manganese dioxide. Scand J Work Environ Health 3 Suppl 1: 1-41.

Bird ED, Anton AH and Bullock B (1984). The effect of manganese inhalation on basal ganglia dopamine concentrations in rhesus monkey. Neurotoxicology 5(1): 59-65.

Brenneman KA, Wong BA, Buccellato MA, Costa ER, Gross EA and Dorman DC (2000). Direct olfactory transport of inhaled manganese ((54)MnCl(2)) to the rat brain: toxicokinetic investigations in a unilateral nasal occlusion model. Toxicol Appl Pharmacol 169(3): 238-48.

Calne DB, Chu NS, Huang CC, Lu CS and Olanow W (1994). Manganism and idiopathic parkinsonism: similarities and differences. Neurology 44(9): 1583-6.

CARB. (2005a). *California Toxics Inventory for 2004*. California Air Resources Board. http://www.arb.ca.gov/toxics/cti/cti.htm.

Cawte J (1985). Psychiatric sequelae of manganese exposure in the adult, foetal and neonatal nervous systems. Aust N Z J Psychiatry 19(3): 211-7.

Collipp PJ, Chen SY and Maitinsky S (1983). Manganese in infant formulas and learning disability. Ann Nutr Metab 27(6): 488-94.

CRC (2005). CRC Handbook of Chemistry and Physics. Boca Raton (FL): CRC Press.

Davis CD, Malecki EA and Greger JL (1992). Interactions among dietary manganese, heme iron, and nonheme iron in women. Am J Clin Nutr 56(5): 926-32.

Davis CD, Zech L and Greger JL (1993). Manganese metabolism in rats: an improved methodology for assessing gut endogenous losses. Proc Soc Exp Biol Med 202(1): 103-8.

Dobson AW, Weber S, Dorman DC, Lash LK, Erikson KM and Aschner M (2003). Oxidative stress is induced in the rat brain following repeated inhalation exposure to manganese sulfate. Biol Trace Elem Res 93(1-3): 113-26.

Dorman DC, Brenneman KA, McElveen AM, Lynch SE, Roberts KC and Wong BA (2002a). Olfactory transport: a direct route of delivery of inhaled manganese phosphate to the rat brain. J Toxicol Environ Health A 65(20): 1493-511.

Dorman DC, McElveen AM, Marshall MW, Parkinson CU, James RA, Struve MF and Wong BA (2005). Tissue manganese concentrations in lactating rats and their offspring following combined in utero and lactation exposure to inhaled manganese sulfate. Toxicol Sci 84(1): 12-21.

Dorman DC, Struve MF, James RA, McManus BE, Marshall MW and Wong BA (2001). Influence of dietary manganese on the pharmacokinetics of inhaled manganese sulfate in male CD rats. Toxicol Sci 60(2): 242-51.

Dorman DC, Struve MF, Vitarella D, Byerly FL, Goetz J and Miller R (2000). Neurotoxicity of manganese chloride in neonatal and adult CD rats following subchronic (21-day) high-dose oral exposure. J Appl Toxicol 20(3): 179-87.

Dorman DC, Struve MF and Wong BA (2002b). Brain manganese concentrations in rats following manganese tetroxide inhalation are unaffected by dietary manganese intake. Neurotoxicology 23(2): 185-95.

Dorner K, Dziadzka S, Hohn A, Sievers E, Oldigs HD, Schulz-Lell G and Schaub J (1989). Longitudinal manganese and copper balances in young infants and preterm infants fed on breast-milk and adapted cow's milk formulas. Br J Nutr 61(3): 559-72.

Drown DB, Oberg SG and Sharma RP (1986). Pulmonary clearance of soluble and insoluble forms of manganese. J Toxicol Environ Health 17(2-3): 201-12.

Elder A, Gelein R, Silva V, Feikert T, Opanashuk L, Carter J, Potter R, Maynard A, Ito Y, Finkelstein J and Oberdorster G (2006). Translocation of inhaled ultrafine manganese oxide particles to the central nervous system. Environ Health Perspect 114(8): 1172-8.

Ericson JE, Crinella FM, Clarke-Stewart KA, Allhusen VD, Chan T and Robertson RT (2006). Prenatal manganese levels linked to childhood behavioral disinhibition. Neurotoxicol Teratol.

Fechter LD (1999). Distribution of manganese in development. Neurotoxicology 20(2-3): 197-201.

Fell JM, Reynolds AP, Meadows N, Khan K, Long SG, Quaghebeur G, Taylor WJ and Milla PJ (1996). Manganese toxicity in children receiving long-term parenteral nutrition. Lancet 347(9010): 1218-21.

FNB (2004). Dietary Reference Intake Tables: Elements Table. Food and Nutrition Board, Institutes of Medicine (IOM, 2004). http://www.iom.edu/Object.File/Master/7/294/Webtableminerals.pdf.

Foradori AC, Bertinchamps A, Gulibon JM and Cotzias GC (1967). The discrimination between magnesium and manganese by serum proteins. J Gen Physiol 50(9): 2255-66.

Gavin CE, Gunter KK and Gunter TE (1999). Manganese and calcium transport in mitochondria: implications for manganese toxicity. Neurotoxicology 20(2-3): 445-53.

Gennart JP, Buchet JP, Roels H, Ghyselen P, Ceulemans E and Lauwerys R (1992). Fertility of male workers exposed to cadmium, lead, or manganese. Am J Epidemiol 135(11): 1208-19.

Gibbons RA, Dixon SN, Hallis K, Russell AM, Sansom BF and Symonds HW (1976). Manganese metabolism in cows and goats. Biochim Biophys Acta 444(1): 1-10.

Ginsberg GL, Foos BP and Firestone MP (2005). Review and analysis of inhalation dosimetry methods for application to children's risk assessment. J Toxicol Environ Health A 68(8): 573-615.

Guilarte TR, Chen MK, McGlothan JL, Verina T, Wong DF, Zhou Y, Alexander M, Rohde CA, Syversen T, Decamp E, Koser AJ, Fritz S, Gonczi H, Anderson DW and Schneider JS (2006a). Nigrostriatal dopamine system dysfunction and subtle motor deficits in manganese-exposed non-human primates. Exp Neurol 202(2): 381-90.

HaMai D, Campbell A and Bondy SC (2001). Modulation of oxidative events by multivalent manganese complexes in brain tissue. Free Radic Biol Med 31(6): 763-8.

HSDB. (2006). *Manganese compounds*. National Library of Medicine. http://toxnet.nlm.nih.gov/cgi-bin/sis/search/f?./temp/~YuJ7Vy:2.

Huang CC, Chu NS, Lu CS, Chen RS and Calne DB (1998). Long-term progression in chronic manganism: ten years of follow-up. Neurology 50(3): 698-700.

Kilburn CJ (1987). Manganese, malformations and motor disorders: findings in a manganese-exposed population. Neurotoxicology 8(3): 421-9.

Kostial K, Blanusa M and Piasek M (2005). Regulation of manganese accumulation in perinatally exposed rat pups. J Appl Toxicol 25(2): 89-93.

Lauwerys R, Roels H, Genet P, Toussaint G, Bouckaert A and De Cooman S (1985). Fertility of male workers exposed to mercury vapor or to manganese dust: a questionnaire study. Am J Ind Med 7(2): 171-6.

Lucchini R, Apostoli P, Perrone C, Placidi D, Albini E, Migliorati P, Mergler D, Sassine MP, Palmi S and Alessio L (1999). Long-term exposure to "low levels" of manganese oxides and neurofunctional changes in ferroalloy workers. Neurotoxicology 20(2-3): 287-97.

Malecki EA (2001). Manganese toxicity is associated with mitochondrial dysfunction and DNA fragmentation in rat primary striatal neurons. Brain Res Bull 55(2): 225-8.

Merck (1976). The Merck Index. Rahway, NJ: Merck and Co.

NIOSH. (2005). *NIOSH Pocket Guide to Chemical Hazards; Manganese tetroxide (as Mn)*. Centers for Disease Control. http://www.cdc.gov/niosh/npg/npgd0381.html

NWU. (2006). Nutrition Fact Sheet: Manganese. Retrieved 8-17-07, from http://www.feinberg.northwestern.edu/nutrition/factsheets/manganese.html.

Pine M, Lee B, Dearth R, Hiney JK and Dees WL (2005). Manganese acts centrally to stimulate luteinizing hormone secretion: a potential influence on female pubertal development. Toxicol Sci 85(2): 880-5.

Ponnapakkam TP, Bailey KS, Graves KA and Iszard MB (2003). Assessment of male reproductive system in the CD-1 mice following oral manganese exposure. Reprod Toxicol 17(5): 547-51.

Reaney SH, Bench G and Smith DR (2006). Brain Accumulation and Toxicity of Mn(II) and Mn(III) Exposures. Toxicol Sci 93(1): 114-124.

Roels H, Meiers G, Delos M, Ortega I, Lauwerys R, Buchet JP and Lison D (1997). Influence of the route of administration and the chemical form (MnCl2, MnO2) on the absorption and cerebral distribution of manganese in rats. Arch Toxicol 71(4): 223-30.

Roels HA, Ghyselen P, Buchet JP, Ceulemans E and Lauwerys RR (1992). Assessment of the permissible exposure level to manganese in workers exposed to manganese dioxide dust. Br J Ind Med 49(1): 25-34.

Rose C, Butterworth RF, Zayed J, Normandin L, Todd K, Michalak A, Spahr L, Huet PM and Pomier-Layrargues G (1999). Manganese deposition in basal ganglia structures results from both portal-systemic shunting and liver dysfunction. Gastroenterology 117(3): 640-4.

Schneider JS, Decamp E, Fritz S, Schultz A, Emberger H and Guilarte TR (2006). Effects of chronic manganese exposure on cognitive and motor functioning in nonhuman primates. Journal of Neurochemistry 96(Suppl. 1): 89.

Shiotsuka R. (1984). Inhalation toxicity of manganese dioxide and magnesium oxide-manganese dioxide mixture. NTIS ADA-148868. Inhalation Toxicology Facility, Medical Dept., Brookhaven National Laboratory, Uptown, NY. Fort Detrick, MD

Takser L, Mergler D, Hellier G, Sahuquillo J and Huel G (2003). Manganese, monoamine metabolite levels at birth, and child psychomotor development. Neurotoxicology (Amsterdam) 24(4-5): 667-674.

Tran TT, Chowanadisai W, Crinella FM, Chicz-DeMet A and Lonnerdal B (2002a). Effect of high dietary manganese intake of neonatal rats on tissue mineral accumulation, striatal dopamine levels, and neurodevelopmental status. Neurotoxicology 23(4-5): 635-43.

Tran TT, Chowanadisai W, Lonnerdal B, Le L, Parker M, Chicz-Demet A and Crinella FM (2002b). Effects of neonatal dietary manganese exposure on brain dopamine levels and neurocognitive functions. Neurotoxicology 23(4-5): 645-51.

U.S.EPA. (1993). Manganese (CASRN 7439-96-5) Reference concentration for chronic inhalation exposure (RfC). http://www.epa.gov/IRIS/subst/0373.htm Retrieved 3/28/06, from http://www.epa.gov/IRIS/subst/0373.htm.

Wennberg A, Hagman M and Johansson L (1992). Preclinical neurophysiological signs of parkinsonism in occupational manganese exposure. Neurotoxicology 13(1): 271-4.

Woolf A, Wright R, Amarasiriwardena C and Bellinger D (2002). A child with chronic manganese exposure from drinking water. Environmental Health Perspectives 110(6): 613-616.

Zhang G, Liu D and He P (1995). [Effects of manganese on learning abilities in school children]. Zhonghua Yu Fang Yi Xue Za Zhi 29(3): 156-8.

Mercury Reference Exposure Levels

(Hg^0 Elemental; Quicksilver)

CAS 7439-97-6

1. Summary

Elemental mercury exposures adversely affect several organ systems, with the effects of acute, high level inhalation exposures first appearing in the lungs as pulmonary dysfunction, possibly followed by respiratory failure leading to death. At lower levels of exposure, the kidneys and brain, especially the developing brain, are more sensitive targets. Short term maternal exposure to mercury vapor during pregnancy may result in long lasting neurobehavioral effects in the offspring, an effect upon which the acute REL is based. Chronic, low level exposures also adversely affect the central nervous system and manifest as motor deficits (tremors, unsteady gate, performance decrements), mood changes (irritability, nervousness), poor concentration, short-term memory deficits, tremulous speech, blurred vision, paresthesia, and decreased nerve conduction. Renal and cardiovascular functions are also impaired with long term exposure. This REL focuses on inhalation exposures. There is a large body of literature on methylmercury poisoning as well as the toxicology of ingested mercury. Much of the latter is reviewed in OEHHA's documentation of the Public Health Goal for drinking water (OEHHA, 1999)

1.1 Mercury Acute REL

Reference Exposure Level	**0.6 µg Hg/m³ (0.07 ppb Hg^0)**
Critical effect(s)	CNS disturbances in offspring
Hazard Index target(s)	Nervous system

1.2 Mercury 8-Hour REL

Reference Exposure Level	**0.06 µg Hg/m³ (0.007 ppb Hg^0)**
Critical effect(s)	Impairment of neurobehavioral functions in humans
Hazard Index target(s)	Nervous system

1.3 Mercury Chronic REL

Reference Exposure Level	**0.03 µg Hg/m³ (0.004 ppb Hg^0)**
Critical effect(s)	Impairment of neurobehavioral functions in humans
Hazard Index target(s)	Nervous system

2. Physical & Chemical Properties - Elemental Mercury

Description	Silver-white, heavy, mobile, odorless, liquid metal
Molecular formula	Hg^0
Molecular weight	200.59 g/mol
Density	13.53 g/cm^3 @ 25° C
Boiling point	356.73° C
Melting point	-38.7° C
Vapor pressure	2 x 10^{-3} mm Hg @ 25° C
Solubility	soluble in nitric acid, to some extent in lipids, and up to 0.28 µmol in water @ 25° C
Odor threshold	odorless
Conversion factor	1 ppm in air = 8.34 mg/m^3 @ 25° C

3. Occurrence and Major Uses

Mercury and mercury-containing compounds are widely used in diverse applications. Thermometers, barometers and thermostats take advantage of mercury's uniform temperature-dependent volume expansion over a broad temperature range. It is used in mercury arc and fluorescent lamps, as a catalyst in oxidation of organic compounds, in the extraction of gold and silver from ores, and as a cathode in electrolysis. It is also used in pulp and paper manufacturing, as a component of batteries, in dental amalgams, and in the manufacture of switching devices such as oscillators, the manufacture of chlorine and caustic soda, as a lubricant, and as a laboratory reagent. To a lesser extent mercury has been used as a grain fumigant, in pharmaceuticals, agricultural chemicals, and as a preservative (ACGIH, 1986).

The annual statewide emissions of mercury from mobile, stationary and natural sources reported in the California Toxics Inventory for 2004 were estimated to be 18 tons (CARB, 2005a). Statewide ambient levels of mercury in 2002 were 1.7 ng/m^3 (CARB, 2005b). Mercury emitted in the metallic form is slowly oxidized in the atmosphere to the ionic mercurous and mercuric (+1 and +2) forms, which are much more soluble in water. These forms dissolve in raindrops and are deposited onto land and water. Much of this precipitation enters sediment of streams or other water bodies, where it is converted to methylmercury and can be accumulated by fish. Thus human exposure to air-borne mercury may be direct, via inhalation, and indirect, through a diet containing contaminated fish. For the purposes of evaluating a Reference Exposure Level, however, we focus on studies of inhalation exposure to mercury.

4. Metabolism / Toxicokinetics

Inhalation exposure to mercury is usually to vapors of the elemental form. However, combustion processes may also emit mercury salts (chlorides and oxides). Thus inhalation

exposure to these forms also occurs. Exposure to the inorganic forms of mercury, the mercurous and mercuric salts, also occurs via the oral route. However, absorption from the intestinal tract is much less efficient (2-38%) than from the lungs (70-80%) (ATSDR, 1999). To protect against oral exposure to inorganic mercury via drinking water, OEHHA (1999) has developed a public health goal (PHG) of 0.0012 mg/L (1.2 ppb) as a level of exposure expected to be protective of public health. The difference between the PHG and the REL values reported in this document in part reflects differences in the toxicokinetics by the different routes of exposure. For inhalation exposure to mercury vapor, modeling based on human and experimental animal studies suggests that approximately 80% of inhaled mercury is deposited in the respiratory tract, of which about 70% is rapidly absorbed into the blood with a half-time of around 1 min. The remainder is absorbed more slowly with half-times of 8 hr to 5 days (Leggett et al., 2001). Absorption is markedly decreased if the breathing is done only through the mouth (Teisinger and Fiserova-Bergerova, 1965). It is not clear whether this difference is related to the direct uptake of mercury from nasal passages but mercury is known to be transported via olfactory nerves directly to the brain (Tjalve and Henriksson, 1999). In the blood, elemental mercury (Hg^0) may be oxidized by catalase and peroxidase to the more toxic inorganic forms. Cellular membranes and the blood-brain barrier are readily permeable to Hg^0, but much less so to the inorganic forms. Residual Hg^0 in the blood may enter target cells and be oxidized to the mercuric form intracellularly, effectively trapping it in the cells. The biological half-life of mercury in the human head is reported to be 21 days, and 64 days in the kidney (Hursh et al., 1976). Mercury is eliminated in urine, feces and exhaled air.

Mercury exerts its toxicity through several mechanisms mainly related to the high affinity of the mercuric ion for sulfhydryl groups. By binding to non-protein sulfhydryls such as glutathione and N-acetyl cysteine, mercury alters intracellular thiol status, thus promoting oxidative stress and lipid peroxidation. Mercury interacts with the mitochondrial electron transport chain resulting in increased H_2O_2. There is a concomitant depletion of mitochondrial glutathione, depolarization of the inner mitochondrial membrane, and increased susceptibility of the mitochondrial membrane to peroxidation. Mitochondrial function is thus impaired and oxidative stress increased (Lund et al., 1993). In addition to mercury's pro-oxidant effects, the binding of mercury by sulfhydryl-containing proteins disrupts a broad range of critical cellular functions such as microtubule polymerization (Yole et al., 2007), DNA transcription (Rodgers et al., 2001), glutamine synthesis (Allen et al., 2001), and calcium homeostasis (Yole et al., 2007). These effects may lead to cell dysfunction and death, an effect that is exacerbated by mercury's ability to promote auto-immune responses (Rowley and Monestier, 2005). Indeed, among genetically susceptible individuals, much of the renal pathology associated with mercury exposure has been attributed to auto-antibodies to renal proteins (Hua et al., 1993). Disruption of cellular processes during development can have severe and long-lasting effects. This is especially true during the growth and organization of the central nervous system as it is critically dependent on cell division and neuronal migration. These processes in turn depend on microtubule polymerization which is powerfully inhibited by both mercuric and methylmercury.

5. Acute Toxicity of Mercury

5.1 Acute Toxicity to Adult Humans

The respiratory tract is the first organ system affected in cases of acute inhalation poisonings (Levin et al., 1988). Acute exposure to Hg^0 can lead to shortness of breath within 24 hours and a rapidly deteriorating course leading to death due to respiratory failure (Kanluen and Gottlieb, 1991; Asano et al., 2000). In a case report, Kanluen and Gottlieb (1991) observed four individuals from a private home where silver was being smelted from dental amalgam containing an unknown amount of Hg^0. All individuals died 9-23 days post-exposure from respiratory distress despite treatment with dimercaprol, a mercury chelator. Autopsy revealed acute lung injury characterized by necrotizing bronchiolitis with edema, emphysema, and obliteration of alveolar spaces with extensive interstitial fibrosis. The concentrations of mercury to which the individuals were exposed and the duration of exposure are not known.

Central nervous system (CNS) effects such as tremors or increased excitability are sometimes seen in cases of acute accidental exposures (Netterstrom et al., 1996). Long-term effects from a single exposure to Hg^0 were reported in 6 male workers exposed to an estimated concentration of 44 mg Hg/m^3 for a period of several hours (McFarland and Reigel, 1978). Long-term CNS effects included nervousness, irritability, lack of ambition, and loss of sexual drive for several years. Shortness of breath also persisted for years in all cases. Acute inhalation exposure to Hg^0 vapors from broken thermometers resulted in generalized skin eruptions in 15 individuals (Nakayama et al., 1983). The doses and durations of exposure were not estimated.

A similar symptomatology was reported by Sexton et al. (1978) following the spillage of 100-300 ml of elemental mercury in two mobile homes that exposed 11 people to mercury vapor for one to two months. Following one to two weeks of exposure, the most intensely exposed residents, three teenage girls, reported the onset of anorexia, painful mouth, abdominal cramps, mild diarrhea, bleeding gingiva, irritated eyes, insomnia, difficulty concentrating and general restlessness. Prior to the girls' hospitalization, changes in academic performance, handwriting and personality were noted by the girls' teachers. A similar constellation of symptoms including intention tremor was subsequently observed in the other eight exposed residents. Skin rashes of varying severity were also seen among five of the residents. Blood mercury levels ranged from 183 to 620 ng/ml (normal is < 5 ng/ml). The highest air mercury level measured in one of the vacated and sealed trailers was 1.0 mg/m^3 five months after the initial spill. Neurological exams at two to four months following termination of exposure were normal for eight of the residents. However, at four months, two of the intensely exposed girls still showed neurological abnormalities as manifested in difficulties copying simple diagrams, and abnormal electroencephalograms.

Predisposing Conditions for Mercury Toxicity

Medical: Persons with preexisting nervous system disorders or kidney diseases might be at increased risk of mercury toxicity. Also at higher risk are persons previously sensitized to mercury (Lerch and Bircher, 2004), and those with genetic susceptibililty to mercury-induced

hypersensitivity (Warfvinge et al., 1995). Developing organisms (fetuses and infants) are especially susceptible to the neurotoxicity of mercury (USEPA, 1997).

Other: People who consume significant amounts of fish from areas with advisories for daily fish intake due to mercury contamination may be more susceptible to the chronic toxicity of airborne mercury due to existing body burden.

5.2 Acute Toxicity to Infants and Children

The data regarding the toxic effects of acute exposure of children to Hg^0 are largely limited to case reports with little or no information on actual exposure levels. In children who inhale high levels of toxic Hg^0 vapors, pulmonary dysfunction is the primary cause of mortality. For example, autopsy of a 4-month-old child who died following acute exposure to Hg^0 vapors revealed pulmonary and general edema, nephrotic degeneration, ventricular dilation, and a greyish, necrotic appearance in the digestive mucosa (Campbell, 1948). In another case study, severe interstitial pneumonitis, erosion of the bronchial epithelium, membrane lesions of the alveoli and alveolar ducts, and significantly elevated Hg in the kidneys and liver were documented by Matthes et al. (1958) following the deaths of three children aged 4, 20, and 30 months from acute Hg^0 vapor exposure in the home. Cases of CNS disturbances, including irritability, insomnia, malaise, anorexia, fatigue, ataxia, and headache have been reported in children exposed to vapor from spilled elemental mercury in their homes (Florentine and Sanfilippo, 1991).

5.3 Acute Toxicity to Experimental Animals

As reported for humans, acute inhalation exposure of experimental animals to high levels of mercury is associated with pulmonary toxicity. However, the effects of mercury inhalation following short term exposure have also been examined in the context of neurotoxicity, notably neurobehavioral effects, and mercury deposition and distribution in the nervous system, as well as pathological changes in various organs.

Pathological changes in lung tissues similar to those reported in humans (edema, fibrosis, and necrosis of alveolar epithelium and hyaline membranes) were observed by Livardjani et al. (1991) in rats exposed to 26 mg (3.1 ppm) Hg/m^3 for 1 hr, or 27 mg (3.2 ppm) for 2 hr. A dose-dependence of lung pathology and mortality was reported. No mortality was observed during the subsequent 15 d following the 1 hr exposure, while 50% mortality and more severe lesions were seen during the first 5 d following the 2 hr exposure.

In a study of pulmonary effects of mercury inhalation, as well as the possible role of metallothionein (MT), Yoshida et al. (1999) exposed both MT-null and wild-type mice to 6.6 - 7.5 mg/m^3 (0.79 - 0.90 ppm) mercury vapor for 4 hr on 3 consecutive days. Examination of the lungs 24 hours after exposure revealed severe congestion, atelectasis (incomplete expansion of the lung), and mild hemorrhage of the alveoli in MT-null mice, along with 60% mortality. Among wild-type mice, these pulmonary effects were much less severe, pulmonary MT expression was markedly increased, and no lethality was observed. Mercury

was found bound to MT in the lungs of wild-type, but not in MT-null mice. MT thus appears to ameliorate the effects of mercury inhalation.

The neurobehavioral manifestations in the offspring of mice with maternal exposure to mercury vapor during pregnancy suggest damage to motor control and learning centers. In the study upon which the acute REL derivation is based, Danielsson et al. (1993) exposed pregnant rats (12 per group) by inhalation to 1.8 mg/m³ (0.22 ppm) of Hg^0 vapor for 1 hour/day (0.07 mg/kg/d) or 3 hours/day (0.2 mg/kg/d) during gestational days 11-14 and 17-20. The dose level was selected to avoid maternal toxicity. Tests of motor activity (locomotion, rearing, rearing time, total activity) in the offspring at 3 months of age revealed significant dose-dependent deficits compared to controls ($p < 0.01$). When tested at 14 months of age, the hypoactivity seen at 3 months was no longer apparent and, in the 0.2 mg/kg/d dose group, was replaced with significant hyperactivity (Table 5.3.1).

Table 5.3.1 Effects of Prenatal Metallic Mercury on Motor Activity

			3 months			**14 months**	
		Control	**0.07**	**0.2**	**Control**	**0.07**	**0.2**
Activity	**Day**	(SEM)	mg/kg/d	mg/kg/d	(SEM)	mg/kg/d	mg/kg/d
Locomotion	1	2785 (135)	2141 (104)*	2212 (135)*	1862 (119)	1289 (167)	1767 (127)
	2	2069 (127)	1432 (119)*	1385 (143)*	1194 (111)	1218 (104)	1512 (119)
	3	1719 (175)	1663 (191)	1090 (135)*	1162 (111)	915 (135)	1369 (119)
Rearing	1	404 (25)	321 (25)*	338 (25)*	204 (22)	143 (20)	210 (27)
	2	312 (29)	190 (20)*	161 (25)*	87 (22)	110 (28)	123 (22)
	3	247 (29)	238 (18)	157 (32)*	84 (18)	98 (25)	106 (18)
Rearing time	1	431 (19)	243 (20)*	232 (22)*	159 (21)	78 (24)	167 (26)
	2	269 (21)	138 (23)*	160 (24)*	66 (19)	99 (24)	114 (23)
	3	212 (21)	179 (23)	138 (21)*	87 (17)	76 (22)	138 (24)
Total activity	1	4854 (271)	3836 (318)*	3979 (302)*	3565 (302)	2435 (223)*	3151 (271)
	2	3804 (223)	2737 (239)*	2817 (350)*	2308 (255)	2324 (302)	3151 (334)*
	3	3183 (318)	3183 (350)	2132 (318)*	2228 (255)	2069 (271)	2546 (2711)

*p<0.01 Data estimated from Danielsson et al. (1993) Figure 1.

Significant learning deficits (swim maze performance) were observed in the 0.2 mg/kg/d-exposed, but not the lower-exposure rats tested at 15 months of age ($p < 0.05$) (Table 5.3.2). The brain concentrations of mercury in the 0.2 mg/kg/d dose group (0.012 mg/kg) were 2.5-fold higher than in the 0.07 mg/kg/d dose group (0.005 mg/kg), and 12-fold higher than in the control group (0.001 mg/kg).

Table 5.3.2 Prenatal Metallic Mercury and Learning Deficits

			7 months			**15 months**	
		Control	**0.07**	**0.2**	**Control**	**0.07**	**0.2**
Morris maze	**Day**		mg/kg/d	mg/kg/d		mg/kg/d	mg/kg/d
	1	53	48	46	42	40	29
	2	30	41	26	29	21	13*

*p<0.01 Data estimated from Danielsson et al. (1993) Figure 3.

These data indicate adverse effects of mercury exposure on the developing brain, but it is not clear at what nervous tissue levels effects first manifest.

To evaluate mercury deposition in neurons at low exposure levels, Pamphlett and Coote (1998) exposed female BALB/c mice to mercury vapor at 25 $\mu g/m^3$ (0.003 ppm) for 2-20 hr, or to 500 $\mu g/m^3$ (0.06 ppm) for 5-240 min. At 25 $\mu g/m^3$, mercury was first found in the perikarya of scattered large motor neurons in the lateral anterior horn of the spinal cord after 12 hr of exposure. Exposure at this level for 16 and 20 hr resulted in labeling of most of the large neurons of this area. By comparison, mercury was found in renal tubular epithelium after only 2 hr of exposure. Mice that survived longer than 6 weeks showed no mercury in the renal epithelia while mercury persisted in the brainstem motor neurons up to 30 weeks. At the higher dose of 500 $\mu g/m^3$, mercury labeling of spinal motor neurons was seen after only 30 min. The doses that resulted in mercury uptake into mouse motor neurons in these experiments are similar to those that workers in mercury-using occupations may receive in the course of a few hours. While the toxicological significance of the observed mercury labeling was not addressed in these mice, the accumulation of mercury in the motor neurons is consistent with the behavioral alterations reported above.

The effects of short term, high level exposure to mercury are not limited to pulmonary and nervous tissues. Severe cellular degeneration and necrosis were observed in the kidneys, brain, colon, and heart tissue of 2 rabbits exposed for 4 hours to 29.7 mg Hg/m^3 (3.6 ppm) (Ashe et al., 1953). Exposure of rabbits to 31.3 mg Hg/m^3 (3.8 ppm) for 1 hour resulted in moderate pathological changes (unspecified), but no necrosis, in the brain and kidney. In contrast, heart and lung tissues showed mild pathologic changes (Ashe et al., 1953). Increased duration (6 hours/day for 5 days) of exposure at this concentration was lethal.

6. Chronic Toxicity of Mercury

6.1 Chronic Toxicity to Adult Humans

This section briefly summarizes a large body of literature on mercury toxicity, emphasizing studies of inhalation exposure useful in the development of the 8-hr and chronic reference exposure levels. The reader is referred to OEHHA (1999) for more information on measuring toxicity by the oral route of exposure. The effects of chronic exposure to mercury vapor have been known for centuries and are most pronounced in the central nervous system. Toxic effects include tremors (mild or severe), unsteady gate, irritability, poor concentration, short-term memory deficits, tremulous speech, blurred vision, performance decrements, paresthesia, and decreased nerve conduction (Smith et al., 1970; Langolf et al., 1978; Fawer et al., 1983; Piikivi et al., 1984; Albers et al., 1988; Kishi et al., 1993). While some motor system disturbances can be reversed upon cessation of exposure, memory deficits may be permanent (Kishi et al., 1993). Studies have shown effects such as tremor and decreased cognitive skills in workers exposed to approximately 25 $\mu g/m^3$ (0.003 ppm) mercury vapor (Piikivi et al., 1984; Piikivi and Hanninen, 1989; Piikivi and Toulonen, 1989) (see discussion below).

The kidney is also a sensitive target organ of mercury toxicity. Effects such as proteinuria, proximal tubular and glomerular changes, albuminuria, glomerulosclerosis, and increased urinary N-acetyl-β-glucosaminidase have been seen in workers exposed to approximately 25-60 μg/m^3 (0.003 - 0.007 ppm) mercury vapor (Roels et al., 1982; Bernard et al., 1987; Barregard et al., 1988; Piikivi and Ruokonen, 1989).

Chronic exposure to mercury vapors has also resulted in cardiovascular effects such as increased heart rate and blood pressure (Piikivi, 1989; Fagala and Wigg, 1992; Taueg et al., 1992); and in leukocytosis and neutrophilia (Fagala and Wigg, 1992).

A number of other studies with similar exposure levels also found adverse psychological and neurological effects in exposed versus unexposed individuals. Fawer et al. *(1983)* measured intention tremor with an accelerometer attached to the third metacarpal of the right hand in 26 male workers (mean age of 44 years) exposed to low concentrations of mercury vapor. The men worked either in a chloralkali plant (n = 12), a fluorescent tube manufacturing plant (n = 7), or in acetaldehyde production (n = 7). Twenty-five control subjects came from different parts of the same plants and were not occupationally exposed to mercury. The average exposure as measured by personal air sampling was 0.026 mg/m^3 (0.003 ppm) and the average duration of exposure was 15 years. The measurements of intention tremor were significantly higher in exposed workers than in controls ($p = 0.011$). Using the average exposure as a LOAEL and adjusting for occupational ventilation rates and workweek, the resultant LOAEL is 0.009 mg/m^3 (0.001 ppm),

Piikivi and Tolonen (1989) studied the effects of long-term exposure to mercury vapor on electroencephalograms (EEGs) of 41 chloralkali workers exposed for a mean of 15.6 years as compared to 41 matched controls. EEGs were analyzed both qualitatively and quantitatively. In the qualitative analysis, EEGs were interpreted visually with classification of normality and abnormality based on a previously established scale that separated focal, generalized and paroxysmal disturbances into four classes (normal, or mildly, moderately, or severely disturbed). Exposed workers, who had blood mercury levels of 11.6 μg/L, tended to have an increased number of EEG abnormalities and brain activity was found to be significantly lower than matched controls ($p < 0.001$). The abnormalities were most prominent in the parietal cortex, but absent in the frontal cortex. The authors used a conversion factor calculated by Roels et al. (1989) to extrapolate from blood mercury levels of 12 μg/L to an air concentration of 25 μg/m^3 (0.003 ppm).

Another study by Piikivi (1989) examined subjective and objective symptoms of autonomic dysfunction in the same 41 chloralkali workers described above. The exposed workers had mean blood levels of 11.6 μg/L corresponding to a TWA exposure of 25 μg Hg/m^3 in air (Roels et al., 1987). The workers were tested for pulse rate variation in normal and deep breathing, the Valsalva maneuver, vertical tilt, and blood pressure responses during standing and isometric work. The only significant difference in subjective symptoms was an increased reporting of palpitations in exposed workers. The objective tests demonstrated an increase in pulse rate variations at 30 μg Hg/m^3 (0.006 ppm; extrapolated from blood levels based on methods of Roels et al. (1987)), which is indicative of autonomic reflex dysfunction.

Piikivi and Hanninen (1989) studied subjective symptoms and psychological performance on a computer-administered test battery in 60 chloralkali workers exposed to approximately 25 $\mu g/m^3$ mercury vapor for a mean of 13.7 years. The subjective symptoms, evaluated by questionnaire, included the frequency or intensity of memory disturbancies, difficulties concentrating, sleep disorders, and hand tremors. In addition a mood scale was used to evaluate tension, depression, anger, fatigue, and confusion. The psychomotor tests included finger tapping, eye-hand coordination, symbol digit substitution, pattern comparison, and a continuous performance test. Memory and learning effects were captured on tests of associate learning, associate memory, pattern memory, and serial digit learning. A statistically significant increase in subjective symptoms of sleep disturbance, and memory disturbance was noted in the exposed workers ($p < 0.001$), as were increased anger, fatigue and confusion ($p < 0.01$). There were no differences in objective measures of memory, learning, or motor abilities, with the exception of poorer eye-hand coordination ($p < 0.001$).

A study by Ngim et al. (1992) assessed neurobehavioral performance in a cross-sectional study of 98 dentists exposed to a TWA concentration of 14 $\mu g\ Hg/m^3$ (range 0.7 to 42 $\mu g/m^3$) compared to 54 controls with no history of occupational exposure to mercury. Exposed dentists were matched to the control group for age, amount of fish consumption, and number of amalgam fillings. Air concentrations were measured with personal sampling badges over typical working hours (8-10 hours/day) and converted to a TWA. Blood samples were also taken (average 9.8 $\mu g/L$). The average concentration in air was estimated at 23 $\mu g\ Hg/m^3$ when the methods of Roels et al. (1987) were used. The average duration in this study of dentists was only 5.5 years, shorter than the above studies. The performance of the dentists was significantly worse than controls on a number of neurobehavioral tests measuring motor speed (finger tapping), visual scanning, visuomotor coordination and concentration, visual memory, and visuomotor coordination speed ($p < 0.05$). These neurobehavioral changes are consistent with central and peripheral neurotoxicity commonly observed in cases of chronic mercury toxicity.

Liang et al. (1993) investigated workers in a fluorescent lamp factory with a computer-administered neurobehavioral evaluation system and a mood-inventory profile. The cohort consisted of 88 individuals (19 females and 69 males) exposed for at least 2 years prior to the study. Exposure was monitored with area samplers and ranged from 8 to 85 $\mu g\ Hg/m^3$ across worksites. The average level of exposure was estimated at 33 $\mu g\ Hg/m^3$ and the average duration of exposure was estimated at 15.8 years. The exposed cohort performed significantly worse than the controls on tests of finger tapping, mental arithmetic, two digit searches, switching attention, and visual reaction time ($p < 0.05$-0.01). The effects on performance persisted after controlling for chronological age as a confounding factor.

6.2 Chronic Toxicity to Infants and Children

A number of case studies indicate that long-term exposure to Hg^0 in children is associated with severe arterial hypertension, acrodynia, seizures, tachycardia, anxiety, irritability and general malaise (Sexton et al., 1978; Torres et al., 2000). These symptoms are consistent with the brain and kidneys as the principle target organs for Hg^0. By comparison, for methylmercury (MeHg), the brain is the most toxicologically relevant organ. An extensive

literature supports the association between chronic MeHg exposure and neurological and developmental deficits in children (Choi, 1989; Harada, 1995; Grandjean et al., 1999). Unlike inorganic mercury, both Hg^0 and MeHg easily cross cell membranes, the blood brain barrier, and the placenta (Ask et al., 2002). Intracellular oxidation of Hg^0 and the slower demethylation of MeHg both lead to the mercuric ion that binds cellular macromolecules, trapping it within the cell and contributing to the toxicity associated with exposures to the respective forms. While the complete mechanisms of toxicity for the two forms are not well understood and are likely not identical, there are important similarities. Methylmercury and the mercuric ion formed from Hg^0 avidly bind to protein sulfhydryls and may inactivate enzymes. Disruption of protein synthesis has been reported after exposure to either Hg^0 or MeHg, although the former is the more powerful inhibitor (NAS, 2000). The neurotoxic effects observed in adult rats following *in utero* exposure to Hg^0, MeHg, or both, are reportedly similar with MeHg potentiating the effects of Hg^0 (Fredriksson et al., 1996). Given the high susceptibility of children to MeHg and the apparent similarities in mechanisms with Hg^0, children are expected to be more susceptible to Hg^0 toxicity as well.

There is a considerable body of evidence from human poisoning episodes that mercury exposure in utero and postnatally results in developmental neurotoxicity (McKeown-Eyssen et al., 1983; Grandjean et al., 1994; Harada, 1995; Grandjean et al., 1997). Thus, infants and children are susceptible subpopulations for adverse health effects from mercury exposure. These effects fall into several general categories: 1) effects on neurological status (Castoldi et al., 2001); 2) age at which developmental milestones are achieved (Marsh et al., 1979); 3) infant and preschool development (Kjellstrom et al., 1986; Kjellstrom et al., 1989); 4) childhood development (age 6 and above) (Grandjean et al., 1997); and 5) sensory or neurophysiological effects (Murata et al., 1999). These studies and others are extensively reviewed by the U.S. EPA (2000) and the NAS (2000)

Whereas MeHg and elemental mercury readily cross the blood-brain barrier and the placental barrier, the mercuric ion (Hg2+) does not readily cross these barriers. However, in fetuses and neonates mercuric species concentrate more in the brain because the blood-brain barrier is incompletely formed. Methylmercury and elemental mercury are lipophilic and are distributed throughout the body. In adults mercuric species accumulate more in the kidney. However, in neonates mercuric species do not concentrate in the kidneys but are more widely distributed to other tissues (NAS, 2000). It is possible that the increased distribution of mercuric species to the brain in fetuses and neonates accounts for some of the sensitivity of the brain to mercury during these developmental periods. The sensitivity of the fetal brain might also be due to the high proportion of dividing and differentiating cells during neuronal development in the fetal and neonatal periods. These dividing cells may be more sensitive to damaging effects of mercury-protein complexes. Furthermore, neurodevelopment is a "one-way street". Disruption along the route results in permanent deficits. Methylmercury can also alter the relative levels of thyroid hormones to which the fetus is exposed and upon which normal neurodevelopment depends.

In addition to prenatal and postnatal dietary exposure, neonates may receive added postnatal dietary exposure to mercuric species and MeHg from breast milk (Drexler and Schaller, 1998; Sundberg et al., 1999). Animal data suggest that suckling rats retain a higher percentage of

ingested organic mercury than do adults, with much higher concentrations in the brain (Kostial et al., 1978). School children can be accidentally exposed to elemental mercury which is a curiosity and an attractive nuisance (George et al., 1996; Lowry et al., 1999). Younger children may also be exposed when elemental mercury is spilled on floors and carpets where they are more active.

6.3 Chronic Toxicity to Experimental Animals

Studies of the effects of mercury in experimental animals generally employ mercury levels in excess of those to which humans are exposed in most settings, thus limiting their ability to model the consequences of long-term, low level exposures. To address this issue, and to test for a role of metallothionein (MT) in mitigating mercury's effects ,Yoshida et al. (2004) exposed wild type and MT-null mice to mercury vapor at 0.06 mg/m^3 (0.007 ppm), 8 hr/day for 23 weeks. Neurobehavioral effects in open field and passive avoidance tests were evaluated at 12 and 23 weeks, and brain levels of mercury were determined. Mercury levels in the brains of mice were 0.66 and 0.97 µg/g tissue for MT-null and wild type, respectively. For comparison, the authors cite human brain mercury levels ranging from 0.3 µg/g in dental personnel to 33 µg/g in retired mercury miners. Mercury-exposed mice showed enhanced motor activity that was statistically significant for both strains at 12 weeks ($p < 0.01$), and for the MT-null mice at 23 weeks ($p < 0.05$). In a learning task (passive avoidance of an electric shock), there were no significant differences between controls and either strain of mouse at 12 weeks of exposure. However, after 23 weeks of exposure, MT-null, but not wild type mice, showed significantly less avoidance than controls ($p < 0.05$) suggesting impaired long-term memory. These data suggest that long-term mercury exposure that results in brain levels of mercury comparable to those seen in occupationally-exposed humans, causes changes in neurobehavior, an effect that is exacerbated by low levels of MT. For comparison, Fawer et al (1983) reported increased intention tremor in human workers exposed to an average of 0.003 ppm for an average of 15 years (Section 6.1).

There is a substantial body of work delineating the neurotoxic effects of MeHg exposure on animals exposed in utero. A comparison between mercury vapor and MeHg, separately and in concert, was conducted in rats. Fredriksson et al. (1996) exposed pregnant rats to MeHg by gavage (2 mg/kg/d during days 6-9 of gestation), and metallic mercury (Hg^0) vapor by inhalation (1.8 mg/m^3 (0.22 ppm) for 1.5 h per day during gestation days 14-19), or both. Controls received the combined vehicles for each of the two treatments. The dose by inhalation was approximately 0.1 mg Hg^0/kg/day. No differences were observed among groups in clinical observations and developmental markers up to weaning. Tests of behavioral function, performed at 4-5 months of age, included spontaneous motor activity, spatial learning in a circular bath, and instrumental maze learning for food reward. Offspring of dams exposed to Hg^0 showed hyperactivity over all three measures of motor activity: locomotion, rearing and total activity. This effect was enhanced in the animals of the MeHg + Hg^0 group. Compared to either the control or MeHg groups in the swim maze test, rats in the MeHg + Hg^0 and Hg^0 groups took longer to reach a submerged platform whose location they had learned the previous day. Similarly, both the MeHg + Hg^0 and Hg^0 groups showed more ambulations and rearings in the activity test prior to the learning trial in the enclosed radial arm maze. During the learning trial, these same animals showed longer latencies and made more errors in acquiring the food reward. Generally, the results indicated that prenatal

exposure to Hg^0 caused alterations to both spontaneous and learned behaviours, suggesting some deficit in adaptive functions. In these experiments, exposure to MeHg was not observed to alter these functions but rather appeared to potentiate the effects of Hg^0.

The similarities in the effects of MeHg and Hg^0 imply similar targets in the brain, which appears to be the case. Pregnant squirrel monkeys were exposed to mercury vapor (0.5 or 1 mg/m^3 (0.06 or 0.12 ppm)) for 4 or 7 hours per day starting in the fifth to the seventh week of gestation and generally ending between 18 and 23 weeks of gestational age (Warfvinge, 2000). The concentration of mercury was found to be higher in maternal (0.80-2.58 µg/g tissue) than in offspring (0.20-0.70 µg/g) brains, but with similar cerebellar distributions. In this study, mercury was localized mainly to Purkinje cells and Bergmann glial cells; similar to the distribution seen after MeHg exposure. The nuclei affected in these and other studies are part of the motor system.

In rats exposed to mercury vapor at ~1 mg/m^3 (0.12 ppm) for 6 h/d, 3 d/wk for 5 wk (low dose), or 24 h/d, 6 d/wk for 5 wk (high dose), an exposure duration-dependent loss of Purkinje cells and proliferation of Bergmann glial cells was observed (Hua et al., 1995). Whereas mercury accumulated to a higher degree in kidney compared to brain, the mercury level in kidney only increased 17% (90 to 105 µg/g tissue) from low to high doses, while that of the brain increased 608% (0.71 to 5.03 µg/g). These neuropathological changes were observed at the same mercury doses as this group reported previously for kidney autoimmune disease (Hua et al., 1993). The brain is a more sensitive target for mercury toxicity in part due to its greater ability to concentrate the metal.

7. Developmental and Reproductive Toxicity

Occupational exposure to mercury vapor has been associated with reproductive problems in a number of epidemiological studies. In a study of 418 dental assistants, Rowland et al. (1994) reported that the fecundability of the women with high exposure to dental amalgams was 63% (95% CI 42-96%) of that reported for the dental assistants with no amalgam exposure. Similarly, in a Chinese study by Yang et al. (2002), there was a significantly higher prevalence of abdominal pain (OR 1.47, 95% CI 1.03: 2.11) and dysmenorrhea (OR 1.66, 95% CI 1.07; 2.59) among female factory workers exposed to ambient mercury vapor (0.001-0.200 mg/m^3) compared with factory workers without mercury exposure. In another study of female factory workers exposed to mercury vapors, the frequency of adverse birth outcomes, especially congenital anomalies, was higher among those exposed to mercury levels at or substantially lower than 0.6 mg/m^3 (Elghany et al., 1997).

The adverse effects of elemental mercury exposure have also been demonstrated in animal models. In rats, elemental mercury readily crosses the placental barrier and accumulates in the fetus following inhalation (Morgan et al., 2002). Pregnant rats exposed by inhalation to 1.8 mg/m^3 of metallic mercury for 1 hour or 3 hours/day during gestation (days 11 through 14 plus days 17 through 20) bore pups that displayed significant dose-dependent deficits in behavioral measurements 3-7 months after birth compared to unexposed controls (Danielsson et al., 1993). Behaviors measured included spontaneous motor activity, performance of a spatial learning task, and habituation to the automated test chamber. The pups also showed

dose-dependent, increased mercury levels in their brains, livers, and kidneys 2-3 days after birth.

Morgan et al. (2002) exposed pregnant rats for 2 hr per day to 1, 2, 4, or 8 mg/m^3 mercury vapor during gestation days (GD) 6-15, and found a dose-dependent distribution of mercury to all maternal and fetal tissues. Adverse effects on resorptions, postnatal litter size and neonatal body weights were only observed at the highest mercury dose, which was also maternally toxic. It is of interest to note that following cessation of maternal exposure on GD 15, the mass of the fetal brain and its content of mercury both increased 10-fold. Thus the fetal brain continued to accumulate mercury eliminated from maternal tissues. This suggests that the period of fetal exposure is longer than that of maternal exposure, and may affect more neurodevelopmental stages than the timing of the maternal exposure would suggest.

Mercury and mercury compounds, including inorganic forms, are listed under California Proposition 65 (Cal/EPA, Safe Drinking Water and Toxic Enforcement Act of 1986) as developmental toxins. It should be noted that there is substantial evidence in humans of the developmental toxicity of methylmercury exposure. However, this REL summary is meant to be applied to elemental and inorganic mercury, and thus we are not describing methylmercury toxicity in depth in this document.

8. Derivation of Reference Exposure Levels

8.1 Mercury Acute Reference Exposure Level

Study	Danielsson et al., 1993
Study population	groups of 12 pregnant rats
Exposure method	inhalation of metallic mercury vapors
Exposure continuity	
Exposure duration	1 hour per day
Critical effects	CNS disturbances in offspring
LOAEL	1.8 mg/m³
NOAEL	not observed
Benchmark concentration	not derived
Time-adjusted exposure	
Human Equivalent Concentration	n/a
LOAEL uncertainty factor (UF_L)	10 (default; severe effect, no NOAEL)
Subchronic uncertainty factor (UFs)	
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	√10 (default, animal study)
Toxicodynamic (UF_{A-d})	10 (greater human vs rat susceptibility)
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	√10 (default: critical study in young)
Toxicodynamic (UF_{H-d})	√10 (default: critical study in young)
Cumulative uncertainty factor	3000
Reference Exposure Level	**0.6 µg Hg/m³ (0.07 ppb Hg^0)**

Acute Reference Exposure Levels are levels at which intermittent one-hour exposures are not expected to result in adverse health effects (see Section 5 of the Technical Support Document (TSD)).

In the absence of acute inhalation studies in humans, the study by Danielsson et al. (1993) was selected as the critical study since it used a sensitive endpoint, neurotoxicity, in a highly susceptible, developmental stage. Maternal rats were exposed by inhalation to 1.8 mg/m³ of metallic mercury vapor for 1 hour/day or 3 hours/day during gestation. The offspring displayed significant dose-dependent deficits in behavior 3-7 months after birth compared to controls. The default uncertainty factor of 10 is applied for the use of a LOAEL for moderate to severe effects in the absence of a NOAEL.

A default interspecies uncertainty factor of √10 for toxicokinetic (UF_{A-k}) variability was used, while a larger interspecies UF_{A-d} of 10 for toxicodynamic differences was used to reflect the potentially greater developmental susceptibility of humans versus rats. This is based, in part, on Lewandowski et al. (2003) who used a parallelogram approach to analyze in vivo and in vitro data on the responses of rats, mice, and humans to MeHg. Their analysis suggests that humans may be up to 10-fold more sensitive to MeHg than are rats. Application of Lewandowski's analysis assumes that the human and rat responses to

elemental mercury are comparable with those to MeHg. The study by Fredriksson et al. (1996) (above) supports this assumption for neurobehavioral effects. A greater susceptibility of humans to adverse neurobehavioral effects following early-life exposures compared with experimental animals has also been seen with other metals, especially lead. For example, Schwartz (1994) reported no evidence for a threshold for neurobehavioral effects in children with blood lead levels (BLL) of 1 µg/dL compared with BLLs of less than 15 µg/dL in primates (Gilbert and Rice, 1987) and less than 20 µg/dL in rats (Cory-Slechta et al., 1985).

Since the critical study involved early life exposures, the default intraspecies toxicodynamic uncertainty factor (UF_{H-d}) of $\sqrt{10}$ was employed to account for individual variability. The intraspecies toxicokinetic uncertainty factor of $\sqrt{10}$ reflects the absence of data in young humans, but also the lack of reason to expect major age differences, at least in the short-term kinetics. The resulting acute REL was 0.6 $\mu g/m^3$ (0.07 ppb).

This REL is developed for metallic mercury vapor but would be expected to be protective for inhalation of mercury salts. Although mercury salts have no significant vapor pressure under normal atmospheric conditions, they are of concern as hazards if aerosolized or produced during combustion. Animals exposed to mercury vapor inhalation had ten-fold higher brain mercury levels than animals exposed to a similar amount of injected inorganic mercury (mercuric nitrate) (Berlin et al., 1969); however the relationship between kinetics of mercury vapor and mercuric salts has not been extensively studied and may be complex, and dependent on the route, level and timing of exposure.

8.2 Mercury 8-Hour Reference Exposure Level

Study	Piikivi and Hanninen (1989); Fawer et al. (1983); Piikivi and Tolonen (1989); Piikivi (1989); Ngim et al. (1992)
Study population	Humans
Exposure method	Inhalation of workplace air
Exposure continuity	8 hours per day, 5 days/week
Exposure duration	13.7 to 15.6 year
Critical effects	Neurotoxicity as measured by: intention tremor; memory and sleep disturbances; decreased performance on neurobehavioral tests (finger tapping, visual scan, visuomotor coordination, visual memory); decreased EEG activity
LOAEL	25 μg/m^3 (3 ppb)
NOAEL	not observed
Benchmark concentration	not derived
Time-adjusted exposure	18 μg/m^3 for LOAEL group
LOAEL uncertainty factor (UF_L)	10 (default, severe effect, no NOAEL)
Subchronic uncertainty factor (UFs)	1
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	1 (default: human study)
Toxicodynamic (UF_{A-d})	1 (default: human study)
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	√10 (default for inter-individual variability)
Toxicodynamic (UF_{H-d})	10 (greater susceptibility of children and their developing nervous systems)
Cumulative uncertainty factor	300
Reference Exposure Level	**0.06 μg Hg/m^3 (0.007 ppb Hg^0)**

The 8-hour Reference Exposure Level is a concentration at or below which adverse noncancer health effects would not be anticipated for repeated 8-hour exposures (see Section 6 of the Technical Support Document).

The half life of elimination of mercury in humans following a single inhalation exposure of 14-24 min. was 21 days from the head, 64 days from the kidney, and 58 days from the body as a whole (Hursh et al., 1976). Urinary elimination among workers occupationally exposed for several years had an elimination half life of 55 days (Sallsten et al., 1994). Thus, since mercury is only slowly eliminated, the interval between daily 8-hr exposures is not long enough for the elimination of significant amounts of the metal and it will accumulate in the body with repeated exposure. In view of this bioaccumulative property of mercury exposure in humans, it was considered necessary to use the same study and derivation (in terms of 24-hour uptake) for the 8-hour REL as for the chronic REL described below. However, the exposure duration adjustment used in this case reflects a repeated exposure of 8 hours per day with an activity-related air intake of 10 m^3 per day (i.e. half that assumed for a 24-hour period for the chronic REL). This adjustment reflects the expectation that activity levels, and hence breathing rates, will be higher during the exposure period than during the remaining 16

hours. The increased breathing rate enhances mercury inhalation during the 8 hour exposure period.

The studies chosen for determination of the 8-hr REL examined neurotoxicity in humans as a sensitive endpoint following long-term exposures. They all point to a LOAEL of approximately 25 $\mu g/m^3$ (3 ppb) with a time-adjusted value of 18 $\mu g/m^3$ (25 x 5/7). In the absence of a NOAEL, we applied an uncertainty factor of 10, the default with neurotoxicity considered a moderate to potentially severe effect. The critical study was conducted in humans and was not a subchronic study so no interspecies or subchronic uncertainty factors were applied. To allow for interindividual variability and to specifically account for greater susceptibility among children, an overall intraspecies uncertainty factor of 30 was applied with a toxicokinetic factor (H-k) of $\sqrt{10}$ to reflect interindividual variability, and a toxicodynamic factor of 10 that reflects the higher susceptibility of the developing nervous system. The cummulative uncertainty is 300, and the resultant 8-hour REL is thus 0.06 μg Hg/ m^3 (0.007 ppb Hg °).

8.3 Mercury Chronic Reference Exposure Level

Study	Piikivi and Hanninen (1989); Fawer et al. (1983); Piikivi and Tolonen (1989); Piikivi (1989); Ngim et al. (1992)
Study population	Humans
Exposure method	Inhalation of workplace air
Exposure continuity	8 hours per day (10 m^3/workday), 5 days/week
Exposure duration	13.7 to 15.6 year
Critical effects	Neurotoxicity as measured by: intention tremor; memory and sleep disturbances; decreased performance on neurobehavioral tests (finger tapping, visual scan, visuomotor coordination, visual memory); decreased EEG activity
LOAEL	25 μg/m^3 (3 ppb)
NOAEL	not observed
Benchmark concentration	not derived
Time-adjusted exposure	8.9 μg/m^3 for LOAEL group
LOAEL uncertainty factor (UF_L)	10 (default, severe effect, no NOAEL)
Subchronic uncertainty factor (UFs)	1
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	1 (default: human study)
Toxicodynamic (UF_{A-d})	1 (default: human study)
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	√10 (default for inter-individual variability)
Toxicodynamic (UF_{H-d})	10 (greater susceptibility of children and their developing nervous systems)
Cumulative uncertainty factor	300
Reference Exposure Level	**0.03 μg Hg/m^3 (0.004 ppb Hg^0)**

The chronic Reference Exposure Level is a concentration at which adverse noncancer health effects would not be expected from chronic exposures (see Section 7 in the Technical Support Document).

To calculate the chronic REL, studies were chosen that examined a sensitive endpoint (neurotoxicity) in humans following long-term exposures. They all point to a LOAEL of approximately 0.025 mg/m^3 (3 ppb). When adjusted for worker ventilation and workweek exposure, the LOAEL becomes 0.009 mg/m^3. In the absence of a NOAEL, we applied an uncertainty factor of 10, the default with neurotoxicity considered a moderate to potentially severe effect. The critical study was conducted in humans and was not a subchronic study so no interspecies or subchronic uncertainty factors were applied. To allow for interindividual variability and to specifically account for greater susceptibility among children, an overall intraspecies uncertainty factor of 30 was applied with a toxicokinetic factor (H-k) of √10 to reflect interindividual variability, and a toxicodynamic factor of 10 that reflects the higher susceptibility of the developing nervous system. The cummulative uncertainty is 300, and the resultant chronic REL is thus 0.03 μg Hg/ m^3 (0.004 ppb Hg °).

The U.S.EPA (1995) based its RfC of 0.3 μg/m^3 (0.04 ppb) on the same study but used an intraspecies uncertainty factor of 3, a LOAEL uncertainty factor of 3 and included a Modifying Factor (MF) of 3 for database deficiencies (lack of developmental and reproductive toxicity data). This modifying factor was not used by OEHHA since allowance was made via the $UF_{H\text{-}d}$ for the known sensitivity of children to the neurodevelopmental impacts of mercury.

It is noteworthy that none of the above studies discussed in sufficient detail a dose-response relationship between mercury vapor inhalation and the toxic effects measured. Because none of the studies mention a level below which toxic effects were not seen (a NOAEL), the extrapolation from a LOAEL to a NOAEL should be regarded with caution. Secondly, one study (Ngim et al., 1992) demonstrated neurotoxic effects from mercury inhalation at an exposure level slightly above the other studies, but for a shorter duration. It is possible that mercury could cause neurotoxic effects after a shorter exposure period than that reported in the study used in derivation of the chronic REL.

As mentioned above, OEHHA (1999) has developed a PHG for inorganic mercury in drinking water of 0.0012 mg Hg/L (1.2 ppb) as a level of exposure expected to be protective of public health. This value was based on data from a 1993 study by the National Toxicology Program that supported a NOAEL of 0.16 mg Hg/kg-day for renal toxicity in rats with chronic oral exposure. Application of the cumulative uncertainty factor of 1,000 (10 for use of a subchronic study, and 10 each for inter- and intraspecies variability) used in the PHG derivation, gives an oral REL of 0.16 μg Hg/kg-day. This value is several-fold higher than the chronic REL developed above for inhalation of elemental mercury, and reflects the greater ease with which elemental mercury (vs. inorganic mercury) penetrates membranes, especially when exposure is via inhalation versus the oral route.

8.4 Mercury as a Toxic Air Contaminant that Disproportionately Impacts Children

In view of the differential impacts on infants and children identified in Section 6.2.1, and the possibility of direct (inhalation) and indirect exposure (through a diet containing aquatic animals contaminated with methylmercury), OEHHA recommends that elemental mercury be identified as a toxic air contaminant (TAC) which disproportionately impacts children under Health and Safety Code, Section 39699.5.

9. References

ACGIH (1986). Documentation of the Threshold Limit Values and Biological Exposure Indices. Cincinnati (OH): American Conference of Governmental Industrial Hygenists.

Albers JW, Kallenbach LR, Fine LJ, Langolf GD, Wolfe RA, Donofrio PD, Alessi AG, Stolp-Smith KA and Bromberg MB (1988). Neurological abnormalities associated with remote occupational elemental mercury exposure. Ann Neurol 24(5): 651-9.

Allen JW, Mutkus LA and Aschner M (2001). Mercuric chloride, but not methylmercury, inhibits glutamine synthetase activity in primary cultures of cortical astrocytes. Brain Res 891(1-2): 148-57.

Asano S, Eto K, Kurisaki E, Gunji H, Hiraiwa K, Sato M, Sato H, Hasuike M, Hagiwara N and Wakasa H (2000). Acute inorganic mercury vapor inhalation poisoning. Pathol Int 50(3): 169-74.

Ashe W, Largent E, Dutra F, Hubbard D and Blackstone M (1953). Behavior of mercury in the animal organism following inhalation. Ind. Hyg. Occup. Med. 17: 19-43.

Ask K, Akesson A, Berglund M and Vahter M (2002). Inorganic mercury and methylmercury in placentas of Swedish women. Environ Health Perspect 110(5): 523-6.

ATSDR. (1999). Toxicological Profile for Mercury. U.S. Department for Human Health Services. Atlanta, GA

Barregard L, Hultberg B, Schutz A and Sallsten G (1988). Enzymuria in workers exposed to inorganic mercury. Int Arch Occup Environ Health 61(1-2): 65-9.

Berlin M, Fazackerley J and Nordberg G (1969). The uptake of mercury in the brains of mammals exposed to mercury vapor and to mercuric salts. Arch Environ Health 18(5): 719-29.

Bernard AM, Roels HR, Foidart JM and Lauwerys RL (1987). Search for anti-laminin antibodies in the serum of workers exposed to cadmium, mercury vapour or lead. Int Arch Occup Environ Health 59(3): 303-9.

Campbell J (1948). Acute mercurial poisoning by inhalation of metallic vapour in an infant. Can Med Assoc J 58: 72-75.

CARB (2005a). Annual Statewide Toxics Summary - mercury http://www.arb.ca.gov/aqd/toxics/statepages/hgstate.html. Sacramento, CA.

CARB. (2005b). *California Toxics Inventory for 2004*. California Air Resources Board. http://www.arb.ca.gov/toxics/cti/cti.htm.

Castoldi AF, Coccini T, Ceccatelli S and Manzo L (2001). Neurotoxicity and molecular effects of methylmercury. Brain Res Bull 55(2): 197-203.

Choi BH (1989). The effects of methylmercury on the developing brain. Prog Neurobiol 32(6): 447-70.

Cory-Slechta DA, Weiss B and Cox C (1985). Performance and exposure indices of rats exposed to low concentrations of lead. Toxicol Appl Pharmacol 78(2): 291-9.

Danielsson BR, Fredriksson A, Dahlgren L, Gardlund AT, Olsson L, Dencker L and Archer T (1993). Behavioural effects of prenatal metallic mercury inhalation exposure in rats. Neurotoxicol Teratol 15(6): 391-6.

Drexler H and Schaller KH (1998). The mercury concentration in breast milk resulting from amalgam fillings and dietary habits. Environ Res 77(2): 124-9.

Elghany NA, Stopford W, Bunn WB and Fleming LE (1997). Occupational exposure to inorganic mercury vapour and reproductive outcomes. Occup Med (Lond) 47(6): 333-6.

Fagala GE and Wigg CL (1992). Psychiatric manifestations of mercury poisoning. J Am Acad Child Adolesc Psychiatry 31(2): 306-11.

Fawer RF, de Ribaupierre Y, Guillemin MP, Berode M and Lob M (1983). Measurement of hand tremor induced by industrial exposure to metallic mercury. Br J Ind Med 40(2): 204-8.

Florentine MJ and Sanfilippo DJ, 2nd (1991). Elemental mercury poisoning. Clin Pharm 10(3): 213-21.

Fredriksson A, Dencker L, Archer T and Danielsson BR (1996). Prenatal coexposure to metallic mercury vapour and methylmercury produce interactive behavioural changes in adult rats. Neurotoxicol Teratol 18(2): 129-34.

George L, Scott FE, Cole D, Siracusa L, Buffett C, Hunter W and Zinkewich R (1996). The mercury emergency and Hamilton school children: a follow-up analysis. Can J Public Health 87(4): 224-6.

Gilbert SG and Rice DC (1987). Low-level lifetime lead exposure produces behavioral toxicity (spatial discrimination reversal) in adult monkeys. Toxicol Appl Pharmacol 91(3): 484-90.

Grandjean P, Weihe P and Nielsen JB (1994). Methylmercury: significance of intrauterine and postnatal exposures. Clin Chem 40(7 Pt 2): 1395-400.

Grandjean P, Weihe P, White RF, Debes F, Araki S, Yokoyama K, Murata K, Sorensen N, Dahl R and Jorgensen PJ (1997). Cognitive deficit in 7-year-old children with prenatal exposure to methylmercury. Neurotoxicol Teratol 19(6): 417-28.

Grandjean P, White RF, Nielsen A, Cleary D and de Oliveira Santos EC (1999). Methylmercury neurotoxicity in Amazonian children downstream from gold mining. Environ Health Perspect 107(7): 587-91.

Harada M (1995). Minamata disease: methylmercury poisoning in Japan caused by environmental pollution. Crit Rev Toxicol 25(1): 1-24.

Hua J, Brun A and Berlin M (1995). Pathological changes in the Brown Norway rat cerebellum after mercury vapour exposure. Toxicology 104(1-3): 83-90.

Hua J, Pelletier L, Berlin M and Druet P (1993). Autoimmune glomerulonephritis induced by mercury vapour exposure in the Brown Norway rat. Toxicology 79(2): 119-29.

Hursh JB, Cherian MG, Clarkson TW, Vostal JJ and Mallie RV (1976). Clearance of mercury (HG-197, HG-203) vapor inhaled by human subjects. Arch Environ Health 31(6): 302-9.

Kanluen S and Gottlieb CA (1991). A clinical pathologic study of four adult cases of acute mercury inhalation toxicity. Arch Pathol Lab Med 115(1): 56-60.

Kishi R, Doi R, Fukuchi Y, Satoh H, Satoh T, Ono A, Moriwaka F, Tashiro K and Takahata N (1993). Subjective symptoms and neurobehavioral performances of ex-mercury miners at an average of 18 years after the cessation of chronic exposure to mercury vapor. Mercury Workers Study Group. Environ Res 62(2): 289-302.

Kjellstrom T, Kennedy P, Wallis S, Stewart A, Friberg L, Lind B, Wutherspoon T and Mantell C. (1989). Physical and mental development of children with prenatal exposure to mercury from fish. Stage 2: Interviews and psychological tests at age 6. Report 3642. National Swedish Environmental Protection Board. Solna, Sweden

Kjellstrom T, Kennedy S, Wallis S and Mantell C. (1986). Physical and mental development of children with prenatal exposure to mercury from fish. Stage I: Preliminary tests at age 4. Report #3080. National Swedish Environmental Protection Board. Solna, Sweden

Kostial K, Kello D, Jugo S, Rabar I and Maljkovic T (1978). Influence of age on metal metabolism and toxicity. Environ Health Perspect 25: 81-6.

Langolf GD, Chaffin DB, Henderson R and Whittle HP (1978). Evaluation of workers exposed to elemental mercury using quantitative tests of tremor and neuromuscular functions. Am Ind Hyg Assoc J 39(12): 976-84.

Leggett RW, Munro NB and Eckerman KF (2001). Proposed revision of the ICRP model for inhaled mercury vapor. Health Phys 81(4): 450-5.

Lerch M and Bircher AJ (2004). Systemically induced allergic exanthem from mercury. Contact Dermatitis 50(6): 349-53.

Levin M, Jacobs J and Polos PG (1988). Acute mercury poisoning and mercurial pneumonitis from gold ore purification. Chest 94(3): 554-6.

Lewandowski TA, Ponce RA, Charleston JS, Hong S and Faustman EM (2003). Effect of methylmercury on midbrain cell proliferation during organogenesis: potential cross-species differences and implications for risk assessment. Toxicol Sci 75(1): 124-33.

Liang YX, Sun RK, Sun Y, Chen ZQ and Li LH (1993). Psychological effects of low exposure to mercury vapor: application of a computer-administered neurobehavioral evaluation system. Environ Res 60(2): 320-7.

Livardjani F, Ledig M, Kopp P, Dahlet M, Leroy M and Jaeger A (1991). Lung and blood superoxide dismutase activity in mercury vapor exposed rats: effect of N-acetylcysteine treatment. Toxicology 66(3): 289-95.

Lowry LK, Rountree PP, Levin JL, Collins S and Anger WK (1999). The Texarkana mercury incident. Tex Med 95(10): 65-70.

Lund BO, Miller DM and Woods JS (1993). Studies on Hg(II)-induced H2O2 formation and oxidative stress in vivo and in vitro in rat kidney mitochondria. Biochem Pharmacol 45(10): 2017-24.

Marsh DO, Myers GJ, Clarkson TW, Amin-Zaki L, Tikriti S and Majeed MA (1979). Fetal methylmercury poisoning: clinical and toxicological data on 29 cases. Ann Neurol 7(4): 348-53.

Matthes FT, Kirschner R, Yow MD and Brennan JC (1958). Acute poisoning associated with inhalation of mercury vapor; report of four cases. Pediatrics 22(4 Part 1): 675-88.

McFarland RB and Reigel H (1978). Chronic mercury poisoning from a single brief exposure. J Occup Med 20(8): 532-4.

McKeown-Eyssen GE, Ruedy J and Neims A (1983). Methyl mercury exposure in northern Quebec. II. Neurologic findings in children. Am J Epidemiol 118(4): 470-9.

Morgan DL, Chanda SM, Price HC, Fernando R, Liu J, Brambila E, O'Connor RW, Beliles RP and Barone S, Jr. (2002). Disposition of inhaled mercury vapor in pregnant rats: maternal toxicity and effects on developmental outcome. Toxicol Sci 66(2): 261-73.

Murata K, Weihe P, Renzoni A, Debes F, Vasconcelos R, Zino F, Araki S, Jorgensen PJ, White RF and Grandjean P (1999). Delayed evoked potentials in children exposed to methylmercury from seafood. Neurotoxicol Teratol 21(4): 343-8.

Nakayama H, Niki F, Shono M and Hada S (1983). Mercury exanthem. Contact Dermatitis 9(5): 411-7.

NAS. (2000). Toxicological Effects of Methyl Mercury. National Academy of Sciences. Washington D.C.

Netterstrom B, Guldager B and Heeboll J (1996). Acute mercury intoxication examined with coordination ability and tremor. Neurotoxicol Teratol 18(4): 505-9.

Ngim CH, Foo SC, Boey KW and Jeyaratnam J (1992). Chronic neurobehavioural effects of elemental mercury in dentists. Br J Ind Med 49(11): 782-90.

OEHHA. (1999). Public Health Goal for Inorganic Mercury in Drinking Water. Office of Environmental Health Hazard Assessment. California Environmental Protection Agency. Scramento, CA

Pamphlett R and Coote P (1998). Entry of low doses of mercury vapor into the nervous system. Neurotoxicology 19(1): 39-47.

Piikivi L (1989). Cardiovascular reflexes and low long-term exposure to mercury vapour. Int Arch Occup Environ Health 61(6): 391-5.

Piikivi L and Hanninen H (1989). Subjective symptoms and psychological performance of chlorine-alkali workers. Scand J Work Environ Health 15(1): 69-74.

Piikivi L, Hanninen H, Martelin T and Mantere P (1984). Psychological performance and long-term exposure to mercury vapors. Scand J Work Environ Health 10(1): 35-41.

Piikivi L and Ruokonen A (1989). Renal function and long-term low mercury vapor exposure. Arch Environ Health 44(3): 146-9.

Piikivi L and Tolonen U (1989). EEG findings in chlor-alkali workers subjected to low long term exposure to mercury vapour. Br J Ind Med 46(6): 370-5.

Rodgers JS, Hocker JR, Hanas RJ, Nwosu EC and Hanas JS (2001). Mercuric ion inhibition of eukaryotic transcription factor binding to DNA. Biochem Pharmacol 61(12): 1543-50.

Roels H, Lauwerys R, Buchet JP, Bernard A, Barthels A, Oversteyns M and Gaussin J (1982). Comparison of renal function and psychomotor performance in workers exposed to elemental mercury. Int Arch Occup Environ Health 50(1): 77-93.

Rowland AS, Baird DD, Weinberg CR, Shore DL, Shy CM and Wilcox AJ (1994). The effect of occupational exposure to mercury vapour on the fertility of female dental assistants. Occup Environ Med 51(1): 28-34.

Rowley B and Monestier M (2005). Mechanisms of heavy metal-induced autoimmunity. Mol Immunol 42(7): 833-8.

Sallsten G, Barregard L and Schutz A (1994). Clearance half life of mercury in urine after the cessation of long term occupational exposure: influence of a chelating agent (DMPS) on excretion of mercury in urine. Occup Environ Med 51(5): 337-42.

Schwartz J (1994). Low-level lead exposure and children's IQ: a meta-analysis and search for a threshold. Environ Res 65(1): 42-55.

Sexton DJ, Powell KE, Liddle J, Smrek A, Smith JC and Clarkson TW (1978). A nonoccupational outbreak of inorganic mercury vapor poisoning. Arch Environ Health 33(4): 186-91.

Smith RG, Vorwald AJ, Patil LS and Mooney TF, Jr. (1970). Effects of exposure to mercury in the manufacture of chlorine. Am Ind Hyg Assoc J 31(6): 687-700.

Sundberg J, Jonsson S, Karlsson MO and Oskarsson A (1999). Lactational exposure and neonatal kinetics of methylmercury and inorganic mercury in mice. Toxicol Appl Pharmacol 154(2): 160-9.

Taueg C, Sanfilippo DJ, Rowens B, Szejda J and Hesse JL (1992). Acute and chronic poisoning from residential exposures to elemental mercury--Michigan, 1989-1990. J Toxicol Clin Toxicol 30(1): 63-7.

Teisinger J and Fiserova-Bergerova V (1965). Pulmonary Retention And Excretion Of Mercury Vapors In Man. Ind Med Surg 34: 580-4.

Tjalve H and Henriksson J (1999). Uptake of metals in the brain via olfactory pathways. Neurotoxicology 20(2-3): 181-95.

Torres AD, Rai AN and Hardiek ML (2000). Mercury intoxication and arterial hypertension: report of two patients and review of the literature. Pediatrics 105(3): E34.

U.S.EPA. (2000). Reference Dose for Mercury. External Review Draft. NCEA-S-0930. National Center for Environmental Assessment.

USEPA. (1995). *Mercury, elemental Reference concentration for chronic inhalation exposure (RfC)* http://www.epa.gov/iris/subst/0370.htm.

USEPA. (1997). Mercury Study Report to Congress. Health Effects of Mercury and Mercury Compounds (Vol V.). Office of Air Quality Planning and Standards; Office of Research and Development.

Warfvinge K (2000). Mercury distribution in the neonatal and adult cerebellum after mercury vapor exposure of pregnant squirrel monkeys. Environ Res 83(2): 93-101.

Warfvinge K, Hansson H and Hultman P (1995). Systemic autoimmunity due to mercury vapor exposure in genetically susceptible mice: dose-response studies. Toxicol Appl Pharmacol 132(2): 299-309.

Yang JM, Chen QY and Jiang XZ (2002). Effects of metallic mercury on the perimenstrual symptoms and menstrual outcomes of exposed workers. Am J Ind Med 42(5): 403-9.

Yole M, Wickstrom M and Blakley B (2007). Cell death and cytotoxic effects in YAC-1 lymphoma cells following exposure to various forms of mercury. Toxicology 231(1): 40-57.

Yoshida M, Satoh M, Shimada A, Yasutake A, Sumi Y and Tohyama C (1999). Pulmonary toxicity caused by acute exposure to mercury vapor is enhanced in metallothionein-null mice. Life Sci 64(20): 1861-7.

Yoshida M, Watanabe C, Satoh M, Yasutake A, Sawada M, Ohtsuka Y, Akama Y and Tohyama C (2004). Susceptibility of metallothionein-null mice to the behavioral alterations caused by exposure to mercury vapor at human-relevant concentration. Toxicol Sci 80(1): 69-73.

Appendix E. Application of Toxicokinetic Modeling and Analysis of Toxicokinetic Differences by Age at Exposure.

E.1 Applications of Toxicokinetic Analysis and PBPK Modeling 2

E.2 Published Summaries of Age-Dependent Toxicokinetics 4

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E.1 Applications of Toxicokinetic Analysis and PBPK Modeling

Physiologically based pharmacokinetic (PBPK) models consist of a series of equations representing bodily compartments (e.g., liver, lung, highly perfused tissues, less perfused tissues), fluid flows, and biotransformation reactions that represent real biological tissues and physiological processes in the body. The models simulate the time course of absorption, distribution, metabolism, and excretion (ADME) of chemicals that enter the body.

PBPK models may also provide a scientific methodology for determining duration adjustments, and for making interspecies extrapolations, while evaluating additional uncertainty related to interspecies differences and intraspecies variability. PBPK modeling can be used to support route-to-route extrapolation, as in the situation where it is necessary to predict the toxicity of a substance from an inhaled dose from the results of an experiment in which a test species was exposed by the oral route in order to develop an inhalation REL**.**

A range of modeling approaches can be used to characterize exposures and resulting delivered doses to target tissues. The dose of the parent compound or of a toxic metabolite at a target tissue, rather than the applied dose, may provide a better basis for determining a NOAEL or point of departure (POD) in a benchmark dose assessment, especially where toxicokinetic features such as saturation of metabolism complicate and obscure the underlying toxicodynamic dose-response relationship. The relevance of a specific modeling approach depends on the physical and chemical characteristics of the material (e.g., stable or reactive gases, particulate matter, lipophilic or water-soluble compounds), the method and route of exposure or delivery, and the toxicities under consideration (e.g., contact site or systemic toxic effects) (U.S.EPA, 1994a; Andersen and Jarabek, 2001; Overton et al., 2001; U.S.EPA, 2004). All of these approaches attempt to improve the understanding of the dose-response relationship by describing and estimating the dose delivered to the relevant areas of the body, and can provide a reduction in uncertainty and an improved scientific basis for the risk value.

In the ideal case, where sufficient data are available, OEHHA will apply PBPK modeling to the dose-response assessment, instead of the default application of the pharmacokinetic portions of the intraspecies and interspecies uncertainty factors, and in preference to the default human equivalent concentration (HEC) procedure for applying interspecies dosimetric adjustments, as described in section 4 of this document. However, it must be recognized that in most cases sufficient data are not available to allow PBPK modeling to be used in developing a REL. Even when pharmacokinetic models for a compound and route of interest are identified it may not always be advisable to rely on these, for example, when independent data separate from those used to calibrate a model are not available to check that model's predictive validity.

OEHHA has explored PBPK modeling to evaluate the adequacy of default uncertainty factors, in particular the previously applied default of 10 for intraspecies variability, i.e. interindividual variability in the human population. We have used PBPK modeling to gain insight into the range of interindividual variability, focusing on the differences among infants, children and adults. Such information is useful in determining whether risk assessment procedures are sufficiently protective of infants and children. We also review available studies that have examined kinetic differences at age of exposure using information on pharmaceuticals. (For ethical reasons

studies of kinetics in children are largely confined to pharmaceuticals, where the subjects may receive some benefit from the exposure to the drug.) These studies demonstrate differences in clearance of chemicals by age, which in several cases exceed the previously used default factor of √10 for toxicokinetic variability in the human population.

The purposes of this appendix are:

1. To document published literature, and present our investigations using modeling approaches, which inform the selection of a default value for the intraspecies toxicokinetic uncertainty factor (UF_{H-k}) which is reasonably protective of members of the general population, specifically including infants and children.

2. To explore the use of toxicokinetic models for interspecies extrapolation, when sufficient data are available to use this approach as an alternative to the existing HEC adjustment for dosimetry (US EPA, 1994) and/or the application of an uncertainty factor (UF_{A-k}) to allow for the uncertainty in interspecies extrapolation of toxicokinetics.

3. To explore and present various toxicokinetic models as examples which may be useful in REL development in those cases where sufficient data are available to use this approach rather than merely applying assumed (default) uncertainty factors. Detailed results and model codes are presented to facilitate the application of these examples.

E.2 Published Summaries of Age-Dependent Toxicokinetics

OEHHA has reviewed published pharmacokinetic analyses which may be of interest in illustrating the applicability of these methods to specific problems in risk assessment identified in the main part of this document, and in particular to the question of how different the kinetics of toxicants may be in infants and children relative to adults (e.g., Renwick and Lazarus, 1998; Dorne et al., 2001). This is a subset of the larger question of how extensive is the inter-individual variability in kinetics for the human population as a whole, but one which is of particular concern in relation to the mandate under SB 25 to determine whether existing risk assessment practices (which have previously focused primarily on effects in adults) are sufficiently protective of the young. The objectives of this literature review were both to identify examples of successful analyses relevant to noncancer risk assessment, and secondly to assess whether a sufficient number and range of examples have been studied to inform the selection of uncertainty factor values in the general case where compound-specific and age-specific information or kinetic models are not available.

E.2.1 Age-dependent Toxicokinetic Parameters.

The following tables show published values, excerpted from kinetic studies of pharmaceuticals, of a variety of kinetic parameters where age-dependent differences have been observed. The examples in the literature of analyses of the effects of age on disposition of chemicals deal with drugs; ethical concerns generally rule out clinical studies of the effect of toxic pollutants or industrial chemicals on juvenile subjects. But the pharmacokinetics of drugs are studied as part of the requirements for registration by the US FDA (and similar regulatory authorities in other countries). In addition, the use of drugs in pediatrics has resulted in information on their disposition in younger patients. These data provide a foundation for evaluating chemical disposition by age at exposure for airborne toxicants as well as drugs, since the metabolic pathways responsible for activation and clearance of these toxicants are in general the same as those responsible for handling drugs. Some discussion of these data and age-specific characteristics of the underlying processes of absorption, distribution, metabolism and excretion appears in Section 3.1 of the main document. The principal pharmacokinetic terms used are: clearance (CL) the quantity of blood from which the chemical has been removed or cleared per unit body weight or surface area per unit time; the half-life ($T_{1/2}$) of the chemical in the blood or the time required to reduce the chemical blood concentration by half as a result of excretion, metabolism etc.; the area under the chemical blood concentration times time curve (AUC), a measure of the duration of internal dosimetry; and the maximum chemical concentration in the blood (Cmax), a measure of the intensity of exposure. Depending on the mode of action (MOA) either duration or intensity may be more closely related to the toxic effects observed. Similar metrics may also apply to key metabolites.

TABLE E.2.1. COMPOUNDS SHOWING REDUCED ELIMINATION IN INFANTSAND/OR CHILDREN[1].

Compound	Parameter	Age	Value
Morphine	CL (mL/kg-min)	<7 d 7d – 2 mo 2 – 6 mo	8.7 ± 5.8 11.9 ± 5.1 28.0 ± 8.9
Paracetamol	CL (L/kg-hr)	< 10 d 1-12 mo	0.15 0.37
Pipecuronium	CL (mL/kg-min)	6.8 mo 4.6 yr Adult (42 yr)	1.5 2.3 2.5
Desacetylcefotaxime	$T_{1/2}$ (hr)	Neonate Infant Adult	9.4 2.1 1.6
Ganciclovir	CL (mL/kg-min)	2-50 d Adult	3.4 4.2
Alfentanil	CL (mL/kg-min)	Newborn Newborn Adult	3.2 1.5-1.7 6.0
Trichloroethanol (from chloral hydrate)	$T_{1/2}$ (hr)	Neonate Adult	35 8
Trichloroethanol glucuronide	$T_{1/2}$ (hr)	Neonate Adult	30 7
Digoxin	CL renal (mL/1.73 m^2-min)	1 week 3 mo 12 mo	32 ± 7 66 ± 30 88 ± 43

[1] Adapted from Renwick and Lazarus (1998): CL = Clearance; $T_{1/2}$ = Half life.

TABLE E.2.2. CYP1A2 MEDIATED METABOLIC PARTIAL CLEARANCES IN HEALTHY VOLUNTEERS

Drug	CYP1A2 Pathway	Number of subjects	Weighted Mean mL/kg-min	Weighted SD	CV
p.o. administration					
Caffeine	1-N-Demethylation	5	0.24	0.07	29.2
Caffeine	3-N-Demethylation	5	1.84	1.08	58.7
Caffeine	7-N-Demethylation	5	0.08	0.02	25.0
Theophylline	1-N-Demethylation	13	0.21	0.11	52.4
Theophylline	3-N-Demethylation	13	0.16	0.10	62.5
Theobromine	1-N-Demethylation	23	0.20	0.09	42.5
Paraxanthine	7-N-Demethylation	6	0.89	0.26	29.2
i.v. administration					
Theophylline	1-N-Demethylation	22	0.16	0.06	37.4
Theophylline	3-N-Demethylation	6	0.19	0.06	31.1
R-Warfarin	6-Hydroxylation	6	0.26 mL/min	0.15	59.1

[1] Adapted from Dorne et al. (2001): p.o. = oral; i.v = intravenous; SD = standard deviation; CV = coefficient of variation. Weighted SD = standard deviation weighted by coefficient of variation

TABLE E.2.3. INTER-INDIVIDUAL VARIATION IN TOXICOKINETICS OF CAFFEINE IN HEALTHY VOLUNTEERS[1].

Toxicokinetic Parameter	Number of subjects	Weighted mean	Weighted SD	CV
p.o. administration				
CL mL/kg-min	163	1.20	0.43	35.7
CL mL/min	10	142	79.1	55.7
AUC/dose ng/mL-hr	15	17,200	9,490	55.2
Cmax/dose ng/mL	67	1,780	435	24.1
i.v. administration				
CL mL/kg-min	20	1.97	0.92	46.8
AUC/dose ng/mL-hr	8	14,050	5,760	41.0

[1] Adapted from (Dorne et al., 2001). P.o. = oral; i.v. = intravenous; CL = Clearance; AUC = area under the blood concentration x time curve; Cmax = maximum blood concentration; SD = Standard Deviation; CV = Coefficient of Variation.

TABLE E.2.4. TOXICOKINETICS OF CAFFEINE: COMPARISONS BETWEEN HEALTHY ADULTS AND DIFFERENT SUBGROUPS.[1]

Toxicokinetic Parameter	Number of subjects	Weighted mean	Weighted SD	CV	Ratio S/H	Ratio CV
Smokers						
CL mL/kg-min p.o.	38	2.62	0.93	35.5	0.46	0.99
Cmax/dose ng/mL	6	1,750	610	34.9	0.98	1.43
Pregnant women						
CL mL/kg-min p.o. 36 wk	6	0.72	0.38	52.8	1.67	1.48
CL mL/kg-min p.o. 38 wk	8	0.39	0.18	46.2	3.08	1.29
Cmax/dose ng/mL	8	2,018	1,460	72.3	1.13	2.95
Elderly						
CL mL/kg-min i.v.	18	1.43	0.50	35.2	1.96	0.75
AUC/dose ng/mL-hr p.o.	8	12,400	5,920	47.9	0.78	0.90
Cmax/dose ng/mL	8	370.4	64.5	17.4	0.21	0.71
Children						
CL mL/kg-min p.o.	3	1.79	0.57	31.8	0.67	0.89
Infants						
CL mL/kg-min p.o.	4	1.00	1.04	104	1.20	2.91
Neonates						
CL mL/kg-min p.o.	5	0.127	0.023	18.1	9.45	0.51
CL mL/kg-min i.v.	31	0.14	0.06	42.2	13.9	0.90
Cmax/dose ng/mL	16	1280	1000	7.8	0.72	0.32
Liver disease						
CL mL/kg-min p.o.	81	0.62	0.61	98.9	1.96	2.77
CL mL/kg-min i.v.	45	1.00	0.48	48.3	1.96	1.03
Cmax/dose ng/mL	27	1700	283	16.6	0.96	0.68
Renal disease						
CL mL/kg-min i.v.	5	0.78	0.35	44.6	2.53	0.95

[1] Adapted from (Dorne et al., 2001): p.o. = oral; CL = Clearance; Cmax = maximum blood concentration; AUC = area under the blood concentration x time curve; SD = standard deviation; CV = coefficient of variation; Ratio S/H = ratio between subgroup and healthy volunteers; Ratio CV= ratio between the variability of the subgroup and the healthy volunteers..

TABLE E.2.5. INTERINDIVIDUAL VARIATION IN TOXICOKINETICS OF THEOPHYLLINE IN HEALTHY VOLUNTEERS[1]

Toxicokinetic Parameter	Number of subjects	Weighted mean	Weighted SD	CV
p.o. administration				
CL mL/kg-min	106	0.60	0.38	41.4
AUC/dose ng/mL-hr	22	24,300	5,790	23.8
Cmax/dose ng/mL	32	4,600	842	18.2
i.v. administration				
CL mL/kg-min	100	1.00	0.29	29.2
AUC/dose ng/mL-hr	14	51,900	9,840	19.0

[1] Adapted from (Dorne et al., 2001): p.o = oral; i.v. = intravenous; CL = clearance; AUC = area under the blood concentration x time curve; Cmax = maximum blood concentration; SD = standard deviation; CV = coefficient of variation.

TABLE E.2.6. TOXICOKINETICS OF THEOPHYLLINE: COMPARISONS BETWEEN HEALTHY ADULTS AND DIFFERENT SUBGROUPS

Toxicokinetic Parameter	Number of subjects	Weighted mean	Weighted SD	CV	Ratio S/H	Ratio CV
Smokers						
CL mL/kg-min p.o.	15	1.15	0.30	25.9	0.79	0.63
AUC/dose ng/mL-hr p.o.	6	12,200	4,850	39.8	0.50	1.67
CL mL/kg-min i.v.	8	0.72	0.17	23.6	1.39	0.81
AUC/dose ng/mL-hr i.v.	14	32,900	10,300	31.3	1.58	1.65
Pregnant women						
CL mL/kg-min p.o.	14	0.83	0.22	25.8	1.20	0.88
Elderly non-smokers						
CL mL/kg-min p.o.	19	0.73	0.11	15.0	1.24	0.36
CL mL/kg-min i.v.	41	0.72	0.32	45.2	1.39	1.55
Cmax/dose ng/mL	19	2,700	408	14.3	0.59	0.79
Children						
CL mL/kg-min p.o.	3	1.79	0.57	31.8	0.67	0.89
Infants						
CL mL/kg-min p.o.	33	1.00	0.58	58.1	0.90	1.40
Cmax ng/mL	20	2,610	990	37.9	0.57	2.08
CL mL/kg-min i.v.	43	0.46	0.17	36.1	2.16	1.24
Neonates						
CL mL/kg-min i.v.	220	0.35	0.11	31.1	2.87	0.94

TABLE E.2.6. TOXICOKINETICS OF THEOPHYLLINE: COMPARISONS BETWEEN HEALTHY ADULTS AND DIFFERENT SUBGROUPS

Toxicokinetic Parameter	Number of subjects	Weighted mean	Weighted SD	CV	Ratio S/H	Ratio CV
Liver disease						
CL mL/kg-min p.o.	35	0.38	0.16	42.7	2.36	1.03
CL mL/kg-min i.v.	68	0.52	0.40	78.4	1.94	2.69
Renal disease						
CL mL/kg-min i.v.	31	0.97	0.33	34.3	1.03	1.18

[1] Adapted from (Dorne et al., 2001): p.o = oral; i.v. intravenous; CL = clearance; AUC = area under the blood concentration x time curve; Cmax = the maximum blood concentration; SD -= standard deviation; CV = coefficient of variation; Ratio S/H = ratio between subgroup and healthy volunteers; Ratio CV = ratio between the variability of the subgroup and the healthy volunteers.

TABLE E.2.7. INTERINDIVIDUAL VARIATION IN TOXICOKINETICS OF THEOBROMINE AND PARAXANTHINE IN HEALTHY VOLUNTEERS AFTER ORAL ADMINISTRATION[1]

Toxicokinetic Parameter	Number of subjects	Weighted mean	Weighted SD	CV
Theobromine				
CL mL/kg-min	45	1.02	0.33	42.8
AUC/dose ng/mL-hr	6	12,738	5,474	43.0
Cmax/dose ng/mL	3	1,478	378	21.4
Paraxanthine				
CL mL/kg-min	6	1.71	0.30	17.6

[1] Adapted from (Dorne et al., 2001): SD = standard deviation; CV = coefficient of variation; CL = Clearance; AUC = area under the blood concentration x time curve; Cmax = maximum blood concentration.

TABLE E.2.8. PATHWAY-SPECIFIC TOXICOKINETIC UNCERTAINTY FACTORS FOR CHILDREN AFTER ORAL EXPOSURE AND NEONATES AFTER INTRAVENOUS EXPOSURE[1].

Pathway	Nc	Ns	N	LN 95%	LN97.5%	LN99%
Children						
CYP1A2	1	12	195	1.4	1.6	1.8
CYP2C19	1	1	25	5.4	6.9	9.0
CYP2D6	1	2	173	22	31	45
CYP3A4	3	3	16	1.4	1.6	1.8
Hydrolysis	3	3	43	1.5	1.7	2.0
Glucuronidation	5	13	131	1.3	1.4	1.5
Glycine conjugation	1	1	20	1.5	1.6	1.8
NAT	1	1	25	2.0	2.2	2.5
NAT	1	1	25	2.2	2.3	2.4
Renal excretion	6	9	126	1.2	1.3	1.5
Neonates						
CYP1A2	2	7	251	11	12	14
CYP3A4	2	5	35	8.1	9.7	12
Glucuronidation	4	14	94	8.6	10	12
Glycine conjugation	2	1	10	25	26	28
Renal excretion	7	33	656	2.8	3.0	3.4

[1] Adapted from (Dorne et al., 2005). Nc = number of compounds; Ns = number of studies; N = number of subjects; LN = pathway related uncertainty factors for upper percentiles of the lognormal distributions. These potential uncertainty factors would be equated with the UF_{H-k} described in the main document. In this case the pharmacokinetic component of the interindividual variability is presented as upper percentiles of lognormal distributions of fitted data by metabolic pathway. It illustrates that a given percentile may not give an adequate level of protection depending upon the pathway critical to the toxic effect.

The studies summarized above in addition to those discussed in the text of the main document indicate that the uncertainty sub-factor to account for toxicokinetic variability in the human population is not sufficient to protect neonates and possibly infants and children. For example, in Table E2.8 above Dorne *et al.* (2005) analyze data on kinetic variability in neonates and healthy adults for five metabolic pathways (CYP1A2, CYP3A4, glucuronidation, glycine conjugation, and renal excretion). In all cases except renal excretion, uncertainty factors derived to cover 95 percent of the population, based on lognormal distributions of the study data, exceeded the default value of 3.16. The 95% values ranged from 2.8 to 25. If a more health protective criterion of 99% coverage is adopted, the range of factors would be 3.4 to 28. Even older children showed a significant lack of coverage at the 95% level with the CYP2C19 and CYP2D6 pathways with factors of 5.4 and 22, respectively, albeit with limited data. While not listed in Table E2.8, Dorne *et al*. (2005) note that limited data for CYP2D6 in two neonates showed internal doses 19- and 33-fold higher than in healthy adults. Taken together with the data in older children this may indicate a general greater susceptibility of infants and children to toxicants using the CYP2D6 pathway.

E.2.2 Published PBPK Models of Inter-individual Variability

The following section describes and reviews a selection of specific published models that have been used to address the sources and extent of inter-individual variability (between variously sensitive subpopulations of adults and between adults and children).

Pelekis et al. (2001) used a physiological model to derive adult and child pharmacokinetic uncertainty factors for selected volatile organic compounds (VOCs). The chemicals modeled were dichloromethane (DCM), tetrachloroethylene (PCE), toluene (TOL), m-xylene (XYL), styrene (ST), carbon tetrachloride (CATE), chloroform (CHLO), and trichloroethylene (TCE). Adult models of low (50 kg) and high (90 kg) body weight were compared with a 10 kg-based child model. Fat contents varied from 51 percent for the 90 kg adult model to 17 percent for the 10 kg child. Ventilation:perfusion ratios varied from 0.76 (50 kg) to 1.38 (10 kg). Fractional liver flows (of cardiac output) ranged from 0.11 (50 kg) to 0.34 (90 kg). All PBPK models were flow-limited with exposure by inhalation, arterial circulation to Fat, Slowly Perfused, Rapidly Perfused and Liver model compartments, metabolism in the Liver, and combination of compartment outputs in venous blood. The arterial and venous bloods were not explicitly modeled. Also no VOC metabolites were specifically modeled. A range of physiological parameters (blood:air and tissue:blood) were used for each body model and the eight VOC chemicals based on literature values.

Simulations involved exposure to one ppm VOC and estimation of arterial and venous blood concentrations (CA, CV), and tissue concentrations (Ci) after 30 days continuous exposure. A comparison of the two adult models (Adult high body weight and fat content versus Adult low body weight and fat content) shows relatively few significant departures from unity for the dose metrics estimated. CATE ratios ranged from 2.85 (C rapidly perfused) to 1.71 (C_{liver}). DCM ranged from 0.29 (C_{liver}) to 1.04 ($C_{arterial\ blood}$). Comparisons of the Adult high/Child average from the PBPK model show some larger differences. For the C_{liver} dose metric the PBPK models predicted the following Adult/Child values: ST (0.033), XYL (0.037), TCE (0.061), DCM (0.092), CHLO (0.11). These model predictions would indicate up to a 30-fold higher concentration of the VOC chemicals in child liver than in adult liver via the inhalation route.

This is a useful approach, involving important environmental toxicants and a relevant exposure route. However, it is limited since the models and dose metrics employed address only the parent compounds. Relevant toxic effects may in fact be more closely related to the tissue dosimetry of metabolites, which were not specifically modeled. In addition, the use of a single child body weight is probably insufficient to assess the full range of physiological variability throughout development, particularly in the neonatal period. It is worth noting, however, that the higher concentrations of the VOCs in a child's liver might be expected to result in higher peak concentrations of metabolites of those compounds in the liver, and possibly also in other tissues.

Jonsson and Johanson (2001) used a PBPK model of DCM to study the influence of metabolic polymorphism on cancer risk estimates. A flow-limited PBPK model was comprised of lung, perirenal fat, subcutaneous fat, working muscle, resting muscle, rapidly perfused tissue, and liver. Exposure was by inhalation; metabolism by glutathione *S*- transferase T1 (GSTT1) and mixed function oxidases (MFO) occurred in lung and liver. The model was fitted to published toxicokinetic data on 27 male volunteers exposed to 250-1000 ppm DCM. Excess cancer risk resulting from lifelong exposures to 1-1000 ppm DCM was estimated using Bayesian and Monte Carlo methods. The relevant dose metric used was DNA-protein cross-links (DPX) in liver, which was derived from the amount of DCM metabolized via the GSTT1 pathway. Data on the frequencies of the three GSTT1 genotypes (0/0, +/0, +/+) in the Swedish population were used in the analysis. The results indicated large inter-individual variability in estimated risk, even within the two metabolizing groups (+/0, +/+). The mean risk in +/+ individuals was 50 –71 percent higher than for the general population. The results also indicate that the 3.16 factor for PK human variability may not be adequately protective for noncancer endpoints. The authors estimated that five percent of the individuals in the Swedish population would not be covered by a factor of 2.7-3.3 away from the mean (calculated from the 95 percent upper confidence limit in Table 7 of Jonsson and Johanson. One percent of individuals would not be covered by a 4.2-7.1 factor (from 99 percent upper confidence interval (UCL) in Table 7 of the published paper) and 0.1 percent by a 7.3-14.5 factor (99.9 percent UCL in Table 7 of the published paper).

These investigators noted that:

> "These results support the cautionary point of Renwick and Lazarus (1998) that an intraspecies uncertainty factor higher than 3.16 should be considered for substances that, like DCM, have pronounced bioactivation polymorphism and therefore a flatter distribution than expected from unimodal log-normal distribution."

They also note that the most sensitive individuals possess a combination of high GSTT1 activity and low metabolic capacity for the competing MFO pathway, which is likely mediated by CYP2E1. CYP2E1 is highly inducible, a factor that would contribute to inter-individual variability. While this paper addresses risk of DCM exposures in adults, the conclusions may apply even more strongly to infants and young children where inhalation may result in greater exposures per unit body weight and metabolic systems, particularly the MFO enzymes, are still under varying stages of development.

Ginsberg et al. (2004b) used PBPK modeling to evaluate the difference between neonates and adults in the pharmacokinetic handling of theophylline and caffeine. Both chemicals are largely metabolized by CYP1A2: caffeine to theophylline, theobromine, and paraxanthine; and

theophylline to 3-methylxanthine, 1-methyluric acid, and 1,3-dimethyluric acid. In neonates theophylline is also "back" methylated to caffeine. Caffeine is cleared much more slowly in neonates than in adults (0.15 vs. 1.57 mL/kg-min, respectively); theophylline is also cleared somewhat more slowly in neonates (0.35 vs. 0.86 mL/kg-min, respectively). The PBPK models, which used biochemical parameters scaled up from *in vitro* data, were able to simulate the large differences in half-life and clearance rates between adults and neonates for these chemicals. This included the faster clearance of theophylline versus caffeine in neonates. It was concluded that the extra "back" methylation path in neonates, while relatively small in percentage terms (i.e., percent of theophylline metabolite excreted in urine), could largely account for the differences seen between adults and neonates. The results emphasize the importance of different metabolic pathways operating in neonates and infants during development.

Price et al. (2003) used age-specific regressions for physiological parameters in a PBPK model for inhaled furan. The model contained compartments for brain, slowly perfused tissues, fat, liver, and the remainder of the body. The ages modeled were six, ten, 14 years and adult. It was assumed that furan was a rapidly metabolized VOC in all age-specific models in that the rate of metabolism was limited by blood flow to the liver. In 36-hour simulations involving a 30-hour exposure to 1 μg/L furan, the authors observed up to 50% higher concentrations of furan in the blood and of furan metabolites in the liver of children compared with adults. These are relatively small differences. Younger ages, which show larger differences in metabolic enzyme profiles and other kinetic factors, were not modeled. It is also questionable whether or not metabolism is truly flow-limited at the younger ages.

Gentry et al. (2003) evaluated the impact of pharmacokinetic differences on tissue dosimetry during pregnancy and lactation with a PBPK modeling approach. Six chemicals representing a variety of physiochemical properties were selected for study: isopropanol, vinyl chloride, methylene chloride, tetrachloroethylene, nicotine and TCDD. These chemicals not only provided differences in volatility, lipophilicity, and water solubility, but also different pharmacokinetic features including metabolic production of stable or reactive metabolites in the liver and competing pathways of metabolism. Model predicted changes in dosimetry during pregnancy were largely the result of the development of metabolic pathways in the fetus or changes in the tissue composition in the mother and fetus. For example, the fetal activity of alcohol dehydrogenase (ADH) was undetectable prior to three months gestation but rose to 0.23 of the adult value at birth. Generally, predicted blood concentrations were lower in the neonate during lactation than in the fetus during gestation. This decrease was relatively slight for TCDD but four orders of magnitude for vinyl chloride. Predicted fetal/neonatal exposures versus maternal exposures ranged from two fold greater (TCDD) to several orders of magnitude lower (isopropanol). The results of this study are in general agreement with reports on pharmaceuticals indicating that the greatest child/adult pharmacokinetic differences are seen in the perinatal period (Renwick et al., 2000; Ginsberg et al., 2002).

Pelekis et al. (2003) estimated intraspecies adult and child pharmacokinetic uncertainty factors using a probabilistic framework applied to a PBPK model of dichloromethane. A number of variates were included as distributions in the analysis including: age, body weight, inhalation rate, activity level, liver weight, fat weight, blood volume and blood flow to the liver and biochemical parameters. The authors found that the tissue dose ratios ($UF_{H\text{-}TK}$, the ratio of the 95th percentile to the 50th percentile) varied only between 1.88 and 1.98 within the population

depending on age and tissue. Many of the assumptions employed in this study are open to question, particularly the assumption that both Phase I and Phase II metabolic elimination paths are ten times greater in adults than in infants on a body weight basis. First order elimination by Phase II metabolism usually scales to the –0.3 power of body weight, which gives an adult: infant difference closer to two-fold than ten-fold on a body weight basis. Without specific data on metabolic elimination of DCM in infants and children a health protective assumption should be used.

Sarangapani et al. (2003) used a PBPK model to evaluate the impact of age- and gender-specific lung morphology and ventilation rate on the inhalation dosimetry of model toxicants. The toxicants were selected to represent category one (irreversibly reactive; ozone), category two (nonreactive water soluble; isopropanol) and category three (nonreactive water insoluble; styrene, vinyl chloride, perchloroethylene) gases. Ten PBPK models were run for males and females from 1 month of age to 75 years. Model structure was similar to Sarangapani et al. (2002) but simplified to three main respiratory tract compartments of extra thoracic (ET), tracheobronchial (TB), and pulmonary (PU) with the ET and TB each divided into three subcompartments from airway lumen to circulating blood. In addition to different anatomical and physiological values for the age and gender models, biochemical parameters were also varied with age (e.g., relative activity of CYP2E1 26.1% at 1 month to 90% at 15 yr; and alcohol dehydrogenase (ADH) 24.9% at 1 month to 83.6% at 25 yr). Dose metrics evaluated included parent and metabolite concentrations in blood, liver and lung. According to the author's analysis, only two chemicals showed higher dose metrics in children than in adults (25 yr model). For the isopropanol model with CYP2E1 and ADH metabolism, the blood concentration of the metabolite acetone was 8-fold higher in 1 month male and 11-fold higher in 1 month female than in respective 25 yr models. Ozone PU extraction per unit surface area was 8.6- to 12.5-fold higher in 1 month male and female models than in respective 25 yr models. The results of this study are in general agreement with other PBPK studies of children. "The age of greatest concern is clearly the perinatal period. The most important factor appears to be the potential for decreased clearance of toxic chemicals in the perinatal period due to immature metabolic enzyme systems, although this same factor can also reduce risk from the reactive metabolites during the same period." Although this model is simpler in structure than the Sarangapani et al. (2002), it is less well described and it has been difficult to verify the predictions for styrene, isopropanol and ozone. In our hands the ozone model gave the closest agreement of child/adult values of 13.1 and 19.4 for PU Cmax in one month/25 yr males and females, respectively.

Clewell et al. (2004) evaluated age- and gender-specific differences in tissue dosimetry with a predictive PBPK life-stage model. The model was implemented for six environmental chemicals with various physicochemical and biochemical properties and modes of toxic action. Isopropanol was studied by oral, dermal and inhalation routes of exposure with blood concentrations of parent and acetone metabolite as dose metrics of interest. The other chemicals studied were vinyl chloride, dichloromethane, tetrachloroethylene, TCDD, and nicotine. Each of these was evaluated by the oral route with dose metrics of blood concentrations of parent and either concentration of metabolite in blood or rate of parent metabolism/kg of liver volume. The dose metrics at external exposure levels of 1 ppb (inhalation) and/or 1 μg/kg-d were estimated continuously, as well as at specific ages of 1, 3, and 6 months, and 1, 5, 10, 15, 25, 50, and 75 years. The results were summarized in age-group ranges of birth to 6 months, 6 months to 5

years, 5 to 25 years, and 25 to 75 years. In general, predictions of average pharmacokinetic dose metrics for a chemical across the life stages were within two-fold, although larger transient variations were predicted, especially during the neonatal period. For the sole chemical investigated by the inhalation route, isopropanol, the highest dose ratio relative to 25 year old was 2.0 for the parent and 3.9 for the metabolite, both in the birth to 6 months of age grouping. The respective ratios for oral (drinking water) and dermal isopropanol exposures were equal or lower than those for the inhalation route for all groups up to 25 years of age. The authors concluded that the most important age-dependent pharmacokinetic factor was the potential for decreased clearance of a toxic chemical in the perinatal period due to the immaturity of xenobiotic metabolism. They note that this same factor may also reduce the production of reactive metabolites. A limitation of this study is that only one compound was evaluated by inhalation. Vinyl chloride, dichloromethane, and tetrachloroethylene could also have been evaluated by the inhalation route.

A preliminary conclusion based on this limited modeling was that a PK UF of 10 would account for inter-individual differences including infants and children for this set of compounds. This is larger than the standard assumption that an uncertainty factor of √10 is sufficient to account for inter-individual differences in human pharmacokinetics.

E.3 OEHHA Studies using PBPK Modeling to Assess Interindividual and Interspecies Differences:

Pilot study of ethylbenzene, vinyl chloride, toluene, styrene/styrene oxide, naphthalene/naphthalene oxides and ten aliphatic aldehydes.

As noted previously, OEHHA has an interest in applying PBPK modeling, when data permit, to replace the pharmacokinetic portion of the intraspecies safety factor. The approach used in applying PBPK modeling to assessing children's environmental health risks has been similar to that of Pelekis et al. (2001) noted above. We have used a case study approach using published PBPK models of selected environmental toxicants, adjusted anatomical and physiological parameters to simulate infant and child ages from newborn to 18 years, and compared these with adult models. In these models we have scaled metabolic parameters as a function of body weight. In addition to modeling age-related differences in human pharmacokinetics, the models were run with age-appropriate parameter values for rats in order to explore interspecies comparisons and, specifically, the extent to which age-related differences in the rat resemble those anticipated in humans. A low and high concentration was modeled for each chemical, and tissue doses were compared between rodent and human models for several of the chemicals.

Where possible we have focused on dose metrics involving toxicologically relevant metabolites. The chemicals selected for this pilot study were: ethylbenzene, vinyl chloride, toluene, styrene/styrene oxide, naphthalene/naphthalene oxides, and formaldehyde. There are PBPK models available for these chemicals for both the rat and human. Several aliphatic aldehydes have been measured in ambient air monitoring studies (Uebori and Imamura, 2004). We modeled the straight chain aliphatic aldehydes from acetaldehyde to decanal (R_nCHO, n = 1-9). The model output in these investigations is the animal to human ratios for blood concentrations. PBPK estimates are bound to be highly chemical dependent and strongly influenced by the metric chosen, blood/air and fat/blood partition coefficients, fractional tissue flows, metabolic parameters, and other factors.

Initial findings by this approach were given at the Children's Environmental Health Symposium (Brown, 2001). Of the seven chemicals studied with oral and inhalation exposures (vinyl chloride, DCM, TCE, chloroform, arsenic, butadiene, and naphthalene) three chemicals showed greater internal doses in children compared to adults: DCM, TCE, and butadiene, all via the inhalation route. A preliminary conclusion based on this limited modeling was that a UF_{H-k} of 10 would account for inter-individual differences including infants and children for this set of compounds.

In follow up work we have attempted to standardize the modeling approach for different chemicals as much as possible and focus on inhalation exposures only. For example, we have employed several of the age specific regressions for model parameters suggested by Price et al. (2003). Also in a few cases we have used more elaborate lung modeling, for example as proposed by Sarangapani et al. (2002) for styrene and styrene oxide, as opposed to the simpler lung modeling of Evelo et al. (1993) for butadiene. Two or three similar child models were used with differing fractional tissue flows more heavily weighted towards rapidly perfused tissues than in adults. A summary of the results obtained using this modified approach is given in Table Table E.3.13. Child/adult values around two are due solely to scaling and indicate little difference. In Table E.3.13 chloroform and furan exhibited little difference under the modeling conditions employed. The other chemicals showed child/adult differences for various metrics ranging from about three to 120. They appeared to be in increasing order as follows: naphthalene/naphthalene oxide; PCE; styrene/styrene oxide; vinyl chloride; MTBE; TCE; BaP; DCM; and butadiene.

It should be emphasized that this analysis focuses on those metrics that show increases in child/adult values and the highest of these across the age-specific models simulated, since we are trying to test whether the traditional UF_H is adequate across all chemicals. In a few cases, metrics showed lower values in children than in adults, i.e. child/adult values < 1. These metrics have not been included in the tables below.

E.3.1 Materials and Methods

Prior to our simulation study, we evaluated the purpose, structure, mathematical representation, parameter estimation (calibration), computer implementation and predictive validity of PBPK models to be used in health risk assessment.

E.3.1.1 Mathematical representation

Model structures were chosen to represent the category of gas (1, 2 or 3) traditionally used in dosimetric adjustments across species. The type of PBPK model used by OEHHA is dependent on the physicochemical characteristics and toxicokinetic properties of the agent in question. Broadly speaking, gaseous agents fall into one of three categories, based on solubility or reactivity with tissues, which affects how deep into the respiratory tract (RT) the chemicals penetrate, and where toxicity occurs (local or systemic).

- Category 1 gases interact mainly at the site of contact: either the nasal or respiratory tracts (RT) as portals of entry.

- Category 2 gases have effects both locally, on the RT, and systemically.
- Category 3 gases mainly have remote systemic effects.

E.3.1.2 Parameter estimation (calibration)

Initial comparisons were limited to rat/human data and in the absence of parameter values, scaled for adults and immature animals/children. Immature rats and human children were modeled following the recommendations of Clewell et al. (2004) and Price et al. (2003), respectively. Metabolic parameters (Vmaxs) were scaled to the ¾ power of body weight. Note that known differences in cytochrome P450 and Phase II enzymes (beyond those described by body weight scaling), which are broadest when comparing the neonate with an adult, are not included in this modeling (see discussion above of Sarangapani et al. 2003 where metabolic differences during development are incorporated into PBPK modeling for CYP2E1 and ADH mediated chemicals). All simulations were for resting animals with alveolar ventilation equaling cardiac output.

E.3.1.3 Computer implementation

Each model was constructed from published code or equations and transcribed into Berkeley Madonna code and model performance was tested for accuracy. Model simulations were conducted using Berkeley Madonna software (www.berkeleymadonna.com, version 8.0.1).

E.3.1.4 Predictive validity

For agents in Category 1, OEHHA has examined a 4-compartment RT model of the type described by Sarangapani et al. (2004) that is similar to a 3-compartment default model of the RT recommended by Hanna et al. (2001), with uptake defined by regional mass transfer coefficients. Depending on the agent being studied, for some Category 1 gases, OEHHA explored nasal models as described by Frederick et al. (1998) and Georgieva et al. (2003).

E.3.1.4.1 Category 1: nasal model for formaldehyde

- A version of a published rat nasal model for formaldehyde was adjusted to accommodate human conditions (Georgieva et al., 2003). This is a nose only model with no body. The nasal region is divided into two parts, essentially anterior and posterior, and each compartment consists of about 25 layers from air to bone. This is a diffusion-limited model using average flux values determined by computational fluid dynamics (CFD) methods (Georgieva et al., 2003). The endpoint is DPX (DNA-protein cross-links pmol/mg DNA), but HCHO tissue concentrations (pM) and DPX-AUC (pmol min/mg DNA) are also available. Diffusivity parameters are for the hydrated form of formaldehyde, methylene glycol. DPX values with this whole nose model for the rat are about one-fourth those which focus on flux hot spots within the nasal region.

In order to extend the adult model to immature rats and children we assumed:

(1) that the mucosal nasal surface was directly proportional to body weight;

(2) that saturable metabolism Vmax scaled with the ¾ power of body weight;

(3) that the first order rates of binding, loss, and DPX loss scaled with the –0.25 power of body weight; and

(4) that the average flux vs. air flow rate could be interpolated from the tables and figures in Kimbell et al. (2001b). The following relations were used to determine the formaldehyde average flux in units of pmol/mm^2/hr/ppm HCOH (y in the equations below):

Human: $y = 5.0 \times IF^{1.7281}$, where IF = inspiratory flow rate in L/min;
Rat: $y = 0.7 \times IF^{1.05}$, where IF is in mL/min

IF is 2 x minute volume, and hence a function of body weight (BW).

MODEL STRUCTURE: Georgieva et al. (2003) (rat model)

- Rat and human data sets/parameter values (Georgieva et al, 2003) were obtained by interpolation of data for average flux versus air flow rate (Kimbell et al., 2001a; 2001b).for neonatal and immature rats and human children, scaled with $BW^{0.75}$. First order rates were scaled with $BW^{0.25}$ (Clewell et al., 2003a).

E.3.1.4.2 Models for Category 2 gases

For Category 2 gases, OEHHA has examined RT-PBPK models of the type described by Sarangapani et al., (2004). These models include both RT compartments and body compartments for remote distribution and metabolism as recommended by Hanna (2001). These are complex hybrid diffusion-limited, flow-limited, "Respiratory Tract" models consisting of a 16 compartment lung (upper RT, conducting airways, terminal bronchioles, and alveoli; each times lumen, mucus, epithelial cell, and blood exchange sub-compartments) and a five compartment body (liver, fat, muscle, vessel rich group, and blood). The models predict the concentrations of both the parent and a metabolite (usually an oxide).

The model structure (Sarangapani et al., 2004) was used with rat and human data sets/parameter values for styrene and styrene oxide obtained from Sarangapani et al. (2002) and Csanady et al. (2003). Human and rat parameters for naphthalene and naphthalene oxides were obtained from Sarangapani et al. (2002) and Willems et al. (2001)

E.3.1.4.3 Models for Category 3 gases

For Category 3 gases, with mainly remote effects, OEHHA has explored either a one-compartment or, alternatively, a two-compartment lung model as described by Evelo et al. (1993), consisting of a high-perfusion alveolar exchange compartment and a low-perfusion bronchial compartment. During our exploratory analysis, we discovered that in some instances flow-limited model components may be augmented or replaced with diffusion-limited components based on physicochemical/kinetic properties and improved model performance (e.g., dioxin).

A simple flow-limited model was used, with compartments for liver, fat, muscle, and lung where the lung is divided into bronchiolar and alveolar sub-compartments (Evelo et al., 1993). Model

parameters were derived from quantitative structure parameter relations (QSPR) or published models/data. Rat body weight was 0.25 kg, and human 70 kg. While metabolic parameters were available for the aliphatic series of aldehydes in both humans and rats, chemical parameters were not available and had to be estimated.

Model predictions are based on chemical property estimation methods for partition coefficients (Lyman, 1982; Paterson and Mackay, 1989; Haddad et al., 2000). The metabolic parameters of the straight chain aliphatic aldehydes (Vmax, Km) were from Mitchell and Petersen (1989) for rats and Kelson et al. (1997) for humans.

For ethylbenzene, the model structure (Evelo et al., 1993) was used with rat flow parameters from Tardif et al. (1997), and with human parameters scaled from rat according to $BW^{0.75}$ (Haddad et al., 2001). Metabolic parameters were scaled from adult rat and human (Sams et al., 2004); rat metabolic parameters were scaled with $BW^{0.75}$ (Clewell et al., 2003a)

For vinyl chloride the same model was used with human and rat metabolic parameters scaled to $BW^{0.75}$ (Chen and Blancato, 1989) and with rat parameters from Clewell et al. (2003a). For toluene, human and rat parameters were obtained from Tardif et al. (1995), with other rat parameters from Chen and Blancato (1989)

The model (Evelo et al., 1993) was applied to the aliphatic aldehyde group (Ethanal – Decanal) using human and rat parameters from Haddad et al. (2001), Paterson and Mackay (1989), Mitchell and Petersen (1989), and Kelson et al. (1997).

Values of chronic and acute reference exposure levels for the six test chemicals ranged between four and five orders of magnitude (3.0 $\mu g/m^3$ for formaldehyde to 1.8 x 10^{+5} $\mu g/m^3$ for vinyl chloride). The chemicals were simulated at 8-hour exposures ranging from 1 $\mu g/m^3$ to 10 mg/m^3. Within this range, the models exhibited linearity of response. For the remainder of the study, we simulated low-level exposures of 1 $\mu g/m^3$ for 8 hours within a 24-hour observation period. The internal dose metrics we examined were Cmax (parent and metabolite peak concentration in the blood), AUC (parent and metabolite concentration in blood at the end of the exposure period), and AMET (amount of parent compound metabolized/kg body weight /day in tissue). For ethylbenzene, vinyl chloride, toluene, styrene, naphthalene and formaldehyde, we examined the ratio of human to rat chemical concentration or amount of metabolite among adults. We also calculated a dosimetric adjustment factor (DAF), which is simply the reciprocal of the human/rat ratios, tabulated below, which can be used to derive a human equivalent concentration (HEC), i.e., animal exposure concentration (mg/m^3) x DAF = HEC. We also compared young humans and animals for simulations for the same set of chemicals. Since the human ages and rat body weights do not correspond exactly in terms of developmental stage, chemical concentrations and metabolite amounts are compared for the youngest and averaged over all. The average human to rat values for the two human parameter sets were then averaged as well.

E.3.2 Results

E.3.2.1 Ethylbenzene, Vinyl Chloride, Toluene, Styrene, Naphthalene, Formaldehyde

E.3.2.1.1 Interspecies comparisons for adults

The dose predictions for Cmax, AUC and AMET resulting from an exposure to 1 $\mu g/m^3$ and 10 mg/m^3 for 8 h during a 24-hour exposure time, are shown in Table E.3.1 and Table E.3.2, respectively. For the most part, the model predictions are quite linear in this exposure range. Models with differing sets of metabolic parameters for a particular chemical predict different amounts of the chemical metabolite in tissue compartments, e.g., styrene oxide. For example, the model for styrene and styrene oxide (SO) shows much larger values for SO concentration metrics with the metabolic parameter set from Csanady et al. (2003) than with the parameter set of Sarangapani et al. (2002). With the exception of toluene (about four-fold) the human/animal maximum values were less than two-fold for the dose metrics examined for low and high exposure levels.

TABLE E.3.1. PBPK MODEL PREDICTIONS FOR SELECTED CHEMICALS: LOW END OF RANGE (1 μg/m^3 x 8 hr/d, 24 hr SIMULATIONS)

Chemical Species	Cmax blood p*M*	AUC blood p*M*hr/d	Amount metabolized[2] pmol/kg-d	Model basis and source of metabolic parameters
Ethylbenzene[1] Human	55.9	560	870	Scaled from rat (Haddad et al., 2001)
Ethylbenzene Rat	38.2	290	900	Tardiff et al. (1997)
Ethylbenzene **Human/rat**	1.46	1.93	0.97	
Vinyl Chloride Human	15.4	126.3	106.45	Chen & Blancato (1989)
Vinyl Chloride Rat	21.9	172.4	519.36	Chen & Blancato (1989)
Vinyl Chloride **Human/Rat**	0.70	0.73	0.20	
Toluene Human	32.5	274.2	365.7	Tardif et al. (1995)
Toluene Rat	7.3	62.0	736.0	Tardif et al. (1995); Chen & Blancato (1989)
Toluene **Human/Rat**	4.45	4.42	0.50	
Styrene(ST)/Styrene Oxide (SO) Human	ST = 0.15 SO = 5.1	ST = 72.2 SO = 2.4	STp450 = 1.9 SOeh = 1.75 SOgst = 0.053	Sarangapani et al. (2002)
Styrene/SO Rat	ST = 0.38 SO = 0.065	ST = 181.2 SO = 0.031	STp450 = 22.6 SOeh = 9.32 SOgst = 9.24	Sarangapani et al. (2002)
Styrene/SO Human	ST = 0.15 SO = 0.024	ST = 73.8 SO = 11.3	STp450 = 1.77 SOeh = 0.82 SOgst = 0.29	Metabolic parameters (Sarangapani et al., 2002; Csanady et al., 2003)
Styrene/SO Rat	ST = 0.42 SO = 0.021	ST = 200.1 SO = 10.3	STp450 = 14.6 SOeh = 10.4 SOgst = 1.36	Metabolic parameters (Sarangapani et al., 2002; Csanady et al., 2003)
Styrene/SO **Human/Rat Mean**	ST = 0.38 SO = 39.8	ST = 0.38 SO = 39.2	STp450 = 0.10	

TABLE E.3.1. PBPK MODEL PREDICTIONS FOR SELECTED CHEMICALS: LOW END OF RANGE (1 μg/m^3 x 8 hr/d, 24 hr SIMULATIONS)

Chemical Species	Cmax blood p*M*	AUC blood p*M*hr/d	Amount metabolized[2] pmol/kg-d	Model basis and source of metabolic parameters
Naphthalene (NAP)/Naphthalene Oxide (NPO) Human	NAP = 0.24 NPO = 0.0026	NAP = 117.5 NO = 1.29	NAPp450 = 0.012 NPOeh = 0.12 NPOgst = 1.55	Sarangapani et al. (2002); Willems et al. (2001)
Naphthalene/NPO Rat	NAP = 0.24 NPO = 0.0085	NAP = 115.3 NPO = 4.07	NAPp450 = 0.68 NPOeh = 1.24 NPOgst = 9.86	Sarangapani et al. (2002); Willems et al. (2001)
Naphthalene/NPO **Human/Rat**	NAP = 1.0 NPO = 0.31	NAP = 1.0 NPO = 0.32	NAPp450 = 0.02	

Chemical Species	Nasal Cmax p*M*	Nasal DPXmax pmol/mg DNA (/mm^2 nasal surface area)	Nasal AUCDPX pmol min/mg DNA/d	Model basis and source of metabolic parameters
Formaldehyde Human surface area (SA) = 21411 mm^2	2800	1.4E-3 (6.5E-8)	0.72	Georgieva et al. (2003); (Kimbell et al., 2001a) (Kimbell et al., 2001b)
Formaldehyde Rat SA = 1777 mm^2	1600	2.1E-3 (1.2E-6)	1.92	Georgieva et al. (2003); Kimbell et al. (2001b); Kimbell et al. (2001a)
Formaldehyde **Human/Rat**	1.75	0.67	0.38	

[1] Ethylbenzene simulations were 48 hr.
[2] p450 = cytochrome p450 epoxidation reaction, eh = epoxide hydrolase, gst = glutathione *S*-transferase.

TABLE E.3.2. PBPK MODEL PREDICTIONS FOR SELECTED CHEMICALS: HIGHEND OF RANGE (10 mg/m^3 x 8 hr/d, 24 hr SIMULATIONS)

Chemical Species	**Cmax blood n*M***	**AUC blood n*M*hr/d**	**Amount metabolized [1] nmol/kg-d**	**Model basis**
Ethylbenzene Human	290	2690	4690	Scaled from rat (Haddad et al., 2001)
Ethylbenzene, Rat	430	3240	9480	Tardif et al. (1997)
Ethylbenzene **Human/Rat**	0.67	0.83	0.49	
Vinyl Chloride Human	0.15	1260	1060	Chen & Blancato (1989)
Vinyl Chloride, Rat	0.10	812	4874	Chen & Blancato (1989)
Vinyl Chloride **Human/Rat**	1.5	1.6	0.22	
Toluene, Human	0.31	2570	3640	Tardif et al. (1995)
Toluene, Rat	0.073	620	7360	Tardif et al. (1995); (Chen and Blancato, 1989)
Toluene, **Human/Rat**	4.24	4.14	0.36	
Styrene/SO, Human	ST = 1.49 SO = 0.050	ST = 12.0 SO = 0.41	STp450 = 18.6 SOeh = 17.1 SOgst = 0.53	Sarangapani et al. (2002)
Styrene/SO, Rat	ST = 3.8 SO = 0.64	ST = 30.0 SO = 5.2	STp450 = 227 SOeh = 93.4 SOgst = 92.4	Sarangapani et al. (2002)
Styrene/SO, Human	ST = 1.53 SO =0.24	ST = 12.3 SO = 1.88	STp450 = 17.7 SOeh = 8.1 SOgst = 2.9	Metabolic parameters (Sarangapani et al., 2002; Csanady et al., 2003)
Styrene/SO, Rat	ST = 4.2 SO = 0.22	ST = 33.3 SO = 1.67	STp450 = 144 SOeh = 104 SOgst = 13.6	Metabolic Parameters (Sarangapani et al. 2002; Csanady et al., 2003)
ST/SO **Human/Rat, Mean**	ST = 0.38 SO = 0.35	ST = 0.39 SO = 0.33		

TABLE E.3.2. PBPK MODEL PREDICTIONS FOR SELECTED CHEMICALS: HIGHEND OF RANGE (10 mg/m^3 x 8 hr/d, 24 hr SIMULATIONS)

Chemical Species	**Cmax blood n*M***	**AUC blood n*M*hr/d**	**Amount metabolized [1] nmol/kg-d**	**Model basis**
Naphthalene/NPO, Human	NAP = 2.41 NPO = 0.026	NAP = 19.7 NPO = 0.22	NAPp450 = 1.18 NPOeh = 1.21 NPOgst =15.4	Sarangapani et al. (2002); Willems et al. (2001)
Naphthalene/NPO, Rat	NAP = 2.36 NPO = 0.085	NAP = 19.2 NO = 0.68	NAPp450 = 6.92 NPOeh = 12.5 NPOgst = 98.6	Sarangapani et al. (2002); Willems et al. (2001)
Naphthalene/NPO, **Human/Rat**	NAP = 1.0 NPO = 0.3	NAP = 1.0 NPO = 0.3		

Chemical Species	**Nasal Cmax μ*M***	**Nasal DPXmax pmol/mg DNA**	**Nasal AUC DPX nmol min/mg DNA/d**	**Model basis and source of metabolic parameters**
Formaldehyde Human SA = 21411 mm^2	29	6.66	7.65	Georgieva et al. (2003); Kimbell et al. (2001a) Kimbell et al. (2001b)
Formaldehyde Rat SA = 1777 mm^2	16	9.67	19.24	Georgieva et al. (2003); Kimbell et al. (2001a) Kimbell et al. (2001b)
Formaldehyde **Human/Rat**	1.8	0.7	0.4	

[1] p450 = cytochrome p450 epoxidation reaction, eh = epoxide hydrolase, gst = glutathione *S*-transferase; DPX = DNA-protein cross-links.

E.3.2.1.2 Intraspecies comparisons for young humans and animals

In Table E.3.3, the results of PBPK model predictions of low-level exposure to ethylbenzene for human children with two sets of metabolic parameters are presented. Sams et al. (2004) investigated the enzyme kinetics of the initial hydroxylation of ethylbenzene to form 1-phenylethanol. Human liver microsomes were obtained from TCS Cellworks. The production of 1-phenylphenol with the human microsomes exhibited biphasic kinetics with a high affinity, low Km, component (mean Km = 8 μM; Vmax = 689 pmol/min/mg protein; n = 6 livers) and a low affinity, high Km, component (Km = 391 μM; Vmax = 3039 pmol/min/mg protein; n = 6). Experiments with inhibitors and recombinant CYP isoforms indicated that CYP2E1 was the major form of the high affinity component and that CYP1A2 was very likely involved in the low affinity component. Haddad et al. (2001) investigated PBPK modeling of chemical mixtures including ethylbenzene. The biochemical parameters were based on studies in rats: VmaxC = 6.39 mg/hr/kg bw; Km = 1.04 mg/L. For human PBPK models the Vmax was scaled, i.e., Vmax = VmaxC x BW^0.75 = mg/hr.

With the parameters from Sams et al. (2004) the concentration metrics are higher and the metabolism (AMET) is lower than with the values from Haddad et al. (2001). While the differences appear large it should be appreciated that the Sams values are based on analysis of isolated microsomes in vitro. Extrapolating these values to a whole body PBPK model probably involves greater uncertainty than extrapolating from rat to human. Table E.3.4 gives the corresponding values for the immature rat. Also presented in this table are the human/rat ratios for children and adults. Since the human ages and rat body weights do not correspond exactly in terms of developmental stage, they are compared for the youngest and averaged over all. If the average immature values for human/rat for the two parameter sets are used with the blood Cmax metric, the corresponding dosimetric adjustment factor (DAF) for ethylbenzene would be 0.21. If only the neonate values are used, the DAF would be 0.22.

TABLE E.3.3. PBPK MODEL PREDICTIONS FOR ETHYLBENZENE WITH HUMAN AGE-SPECIFIC REGRESSIONS AND ALTERNATIVE METABOLIC PARAMETERS (1 μg/m^3 x 8 hr/d, 24-48 hr SIMULATIONS)

Age Group	Cmax blood p*M*	AUC blood p*M*hr/d	Amount Metabolized pmol/kg-d	Model basis
Ethyl Benzene, Human Age 1 yr	100	1300	13	Metabolic parameters scaled from adult (Sams et al., 2004)
Age 3 yr	110	1450	11	
Age 5 yr	120	1620	10	
Age 10 yr	120	1580	8.2	
Age 14 yr	120	1420	6.3	
Age 18 yr	110	1510	5.9	
Adult	110	1750	7.2	
Ethyl Benzene, Human Age 1 yr	55.9	570	370	Parameters scaled to BW$^{0.75}$(Haddad et al., 2001)
Age 3 yr	58.6	570	370	
Age 5 yr	62.2	660	475	
Age 10 yr	53.9	550	500	
Age 14 yr	48.6	470	390	
Age 18 yr	35.0	330	380	
Adult	55.9	560	870	
Rat Mature	38.2	290	900	

TABLE E.3.4. PBPK MODEL PREDICTIONS FOR ETHYLBENZENE WITH AGE-SPECIFIC PARAMETERS FROM CLEWELL ET AL**. 2003 MODELING OF NEONATAL RAT (1 μg/m^3 x 8 hr/d, 24-48 hr SIMULATIONS)**

Age Group	Cmax blood p*M*	AUC blood p*M*hr/d	Amount metabolized pmol/kg-d	Model basis
Ethylbenzene, Rat Neonate BW = 0.0075 kg	17.0	130	450	Scaled BW$^{0.75}$ (Haddad et al., 2001: Clewell et al., 2003a)
BW = 0.015 kg	17.0	135	450	
BW = 0.03 kg	17.0	138	440	
BW = 0.06 kg	17.2	140	430	
BW = 0.12 kg	17.1	140	420	
BW = 0.20 kg	17.4	145	420	
Human neonate/Rat neonate	5.88	10.0	0.029	Parameters (Sams et al., 2004)
Human neonate/Rat neonate	3.29	4.38	0.82	Parameters (Haddad et al., 2001)
Human/Rat Immature Mean	6.61	9.19	0.018	Parameters (Sams et al., 2004)
Human/Rat Immature Mean	3.06	3.61	0.97	Parameters (Haddad et al., 2001)
Mean DAF Immature	0.21	0.16	6.79 (G_{mean})	

Note: Human neonate/Rat neonate = 100pM/17.0pM = 5.88 (Sams Cmax); Human/Rat Immature Mean = (5.88+6.47+7.06+6.98+7.01+6.32)/6 = 6.61 (Sams Cmax); Gmean = geometric mean; DAF = dosimetric adjustment factor; human/rat values in this table were calculated using human values from Table E3.3. Mean DAF based on immature values i.e. 1/((6.61 + 3.06)/2).

Table E.3.5 gives PBPK simulation values for toluene for both immature rats and human children. As above, the individual human/rat ratios are given for neonates and the mean is based on all immature ages (i.e., all except adult) simulated. The mean DAFs are given at the bottom of the table. In this case the DAFs are close to unity for both concentration based metrics. Similarly Table E.3.6 gives the corresponding values for vinyl chloride. In this case the mean DAF based on blood concentration (Cmax) and average immature values was 1.19. The human/rat ratios for the three chemicals with similar model structures (Table E.3.5 to Table E.3.8) are quite similar with blood Cmax and AUC based DAFs averaging 1.62, 0.96, and 1.17, respectively for children. For adults the concentration-based ratios were very similar, averaging 1.12 for ethylbenzene and 1.47 for vinyl chloride. For toluene, the adult ratios differed substantially: 3.1 for Cmax and 0.31 for AUC.

TABLE E.3.5. PBPK MODEL PREDICTIONS FOR TOLUENE WITH AGE-SPECIFIC REGRESSIONS (1 μg/m^3 X 8 hr/d, 24 hr SIMULATIONS)

Age Group	Cmax blood p*M*	AUC blood p*M*hr/d	Amount metabolized pmol/kg-d	Model basis
Toluene, Human: Age 1 yr	83.2	771	551	Metabolic parameters scaled to BW$^{0.75}$ (Haddad et al., 2001)
Age 3 yr	85.6	825	637	
Age 5 yr	90.0	899	754	
Age 10 yr	61.3	580	684	
Age 15 yr	52.9	472	486	
Age 18 yr	51.7	483	440	
Adult	30.0	255	365	
Toluene, Rat Neonate, 0.0075 kg	108.7	873	33201	Parameters scaled to BW$^{0.75}$ (Haddad et al., 2001)
BW = 0.015 kg	86.1	688	16409	
BW = 0.03 kg	72.6	579	8149	
BW = 0.06 kg	65.0	516	4058	
BW = 0.12 kg	58.7	478	2024	
BW = 0.20 kg	52.8	457	1206	
BW = 0.25 Adult	92.4	80.2	375	
HumanNeonate/Rat Neonate	0.76	0.88	0.016	
Human/Rat ImmatureMean	0.97	1.13	0.15	
Mean DAF	1.03	0.88	6.7	

Note: Human neonate/Rat neonate = 83.2 pM/108.7 pM = 0.76 (Cmax); Human/Rat Immature Mean = (0.76+0.99+1.24+0.94+0.90+0.98)/6 = 0.97 (Cmax); DAF = dosimetric adjustment factor. Mean DAF = 1/0.97 = 1.03 (Cmax).

TABLE E.3.6. PBPK MODEL PREDICTIONS FOR VINYL CHLORIDE WITH AGE-SPECIFIC REGRESSIONS (1 μg/m^3 x 8 hr/d, 24 hr SIMULATIONS)

Age Group	Cmax blood p*M*	AUC blood p*M*hr/d	Amount Metabolized pmol/kg-d	Model basis
Vinyl Chloride, Human, Age 1 yr	16.5	137.2	101.3	Metabolic parameters scaled as BW$^{0.75}$(Chen & Blancato 1989)
Age 3 yr	17.0	138.7	108.2	
Age 5 yr	17.4	140.0	116.6	
Age 10 yr	16.3	132.3	137.2	
Age 15 yr	16.0	131.9	102.5	
Age 18 yr	16.5	133.9	87.7	
Adult	14.4	117.6	101.0	
Vinyl Chloride, Rat Neonate, 0.0075 kg	18.9	149.6	424.4	Parameters scaled to BW$^{0.75}$ (Clewell et al., 2003a; Chen & Blancato 1989)
BW = 0.015 kg	19.0	150.3	421.3	
BW = 0.03 kg	19.2	151.9	414.7	
BW = 0.06 kg	19.7	155.5	397.8	
BW = 0.12 kg	20.7	162.9	363.8	
BW = 0.20 kg	21.7	172.2	321.5	
BW = 0.25 adult	21.6	169.4	511.4	
Human/Rat Neonate	0.89	0.92	0.24	
Human/Rat Immature Mean	0.84	0.87	0.28	
Mean DAF	1.19	1.15	3.6	

Note: Human neonate/Rat neonate = 16.5 pM/18.9 pM = 0.87 (Cmax); Human/Rat Immature Mean = (0.87+0.89+0.91+0.83+0.77+0.76)/6 = 0.84 (Cmax); DAF = dosimetric adjustment factor. Mean DAF = 1/0.84 = 1.19 (Cmax).

In Table E.3.7 are summarized the results obtained with the respiratory tract (RT) model with naphthalene. This model predicts concentrations of both parent (NP) and oxidative metabolite naphthalene oxide (NPO). The predicted values for the latter are shown in parentheses. Also included is an average lung concentration of the naphthalene oxides. In this model the isomeric naphthalene oxides are grouped together for simplicity. For the usual concentration metrics of Cmax and AUC in the blood the DAFs range from 8 to 14 for parent and oxide metabolite in the child and 8 to 6, respectively in the adult. For the predicted lung oxide concentration the DAF is 0.17 for the child and 0.07 for the adult.

TABLE E.3.7. PBPK MODEL PREDICTIONS FOR NAPHTHALENE/NAPHTHALENE OXIDES (NPO) WITH AGE-SPECIFIC REGRESSIONS (NAPHTHALENE 1 μg/m^3 x 8 hr/d, 24 hr SIMULATIONS)

Age Group	Cmax blood p*M* NP (NPO)	AUC blood p*M*hr/d NP (NPO)	Amount Naphthalene Metabolized pmol/kg-d	Avg. NPO Conc. in Lung p*M**
Human, Age 1 yr	0.22 (0.0032)	1.83 (0.027)	1.5	0.057
Age 3 yr	0.22 (0.003)	1.83 (0.025)	1.6	0.062
Age 5 yr	0.22 (0.0033)	1.83 (0.026)	1.8	0.064
Age 10 yr	0.19 (0.0031)	1.5 (0.025)	5.2	0.065
Age 15 yr	0.18 (0.0026)	1.48 (0.022)	3.8	0.07
Age 18 yr	0.18 (0.0026)	1.49 (0.021)	3.3	0.07
Adult	0.18 (0.0019)	1.49 (0.016)	4.6	0.073
Rat Neonate, 0.0075 kg	1.7 (0.3)	13.8 (2.3)	1.97	0.07
BW = 0.015 kg	1.7 (0.16)	13.7 (1.3)	2.26	0.037
BW = 0.03 kg	1.7 (0.08)	13.5 (0.65)	2.7	0.020
BW = 0.06 kg	1.68 (0.04)	13.2 (0.33)	3.17	0.011
BW = 0.12 kg	1.7 (0.023)	13.3 (0.18)	3.75	0.0072
BW = 0.20 kg	1.67 (0.016)	13.3 (0.12)	4.3	0.0050
BW = 0.25 kg (adult)	1.5 (0.012)	12.2 (0.095)	4.56	0.0048
Human/Rat Neonate	0.13 (0.011)	0.13 (0.012)	0.76	0.81
Human/Rat Immature Mean	0.10 (0.07)	0.12 (0.072)	0.92	5.88
Mean DAF Immature	10 (14.3)	8.3 (13.9)	1.1	0.17
Human/Rat Adult	0.12 (0.16)	0.12 (0.17)	1.01	15.2
DAF Adult	8.3 (6.2)	8.3 (5.9)	0.99	0.066

Note: (*)Average of upper respiratory tract and terminal bronchiole model compartments Cmax for naphthalene oxides; NP = naphthalene; NPO = oxidative metabolite; models based on Sarangapani et al. (2002); Willems et al. (2001); and Clewell et al. (2003a). Human neonate/Rat neonate = 0.22pM/1.7pM = 0.13 (NP Cmax); Human/Rat Immature Mean = (0.13+0.13+0.13+0.11+0.11+0.11)/6 = 0.103 (NP Cmax); DAF = dosimetric adjustment factor. Mean DAF = 1/0.10 = 10 (NP Cmax). HEC = DAF x Animal Exposure Concentration

The predicted values obtained with styrene exposure in a similar RT model are shown in Table E.3.8. For children the average DAF (based on the immature values i.e. all values except adult) for the concentration-based metrics was 0.42 ((0.41 + 0.42) /2) for the parent compound (ST) and 0.18 ((0.17 + 0.20)/2) for the oxide metabolite (SO). For the adult these values were 1.07 and 0.18, respectively. To recap if we were to calculate the human equivalent concentration (HEC) based on these values we might consider multiplying an immature rat exposure concentration by 0.42 or an adult rat value by 1.07 if the toxic effect were due to the parent compound (i.e., HEC = DAF*Animal Exposure Concentration).

TABLE E.3.8. PBPK MODEL PREDICTIONS FOR STYRENE/ STYRENE OXIDE WITH AGE-SPECIFIC REGRESSIONS (1 μg/m^3 X 8 hr/d, 24 hr SIMULATIONS)

Age group	**Cmax blood p*M* ST** (SO)	**AUC blood p*M*hr/d ST** (SO)	**Amount of Styrene Metabolized pmol/kg-d**	**Average SO Conc. in Lung p*M****
Human Age 1 yr	0.27 (0.0027)	2.23 (0.022)	1.34	1E-5
Age 3 yr	0.28 (0.0032)	2.25 (0.026)	1.34	1E-5
Age 5 yr	0.28 (0.0037)	2.30 (0.030)	1.34	8E-6
Age 10 yr	0.27 (0.012)	2.22 (0.094)	1.94	9E-6
Age 15 yr	0.27 (0.012)	2.18 (0.095)	1.53	8E-6
Age 18 yr	0.27 (0.026)	2.20 (0.095)	1.39	8E-6
Adult	0.15 (0.024)	1.23 (0.18)	1.77	2.4E-5
Rat Neonate, 0.0075 kg	0.09 (3.7E-4)	0.73 (0.003)	6.5	8.7E-3
BW = 0.015 kg	0.097 (5.4E-4)	0.76 (0.004)	8.1	7.5E-3
BW = 0.03 kg	0.10 (0.0084)	0.83 (0.0067)	10.0	7.0E-3
BW = 0.06 kg	0.12 (0.0014)	0.93 (0.011)	13.8	6.5E-3
BW = 0.12 kg	0.14 (0.0024)	1.08 (0.018)	19.0	7.0E-3
BW = 0.20 kg	0.16 (0.0036)	1.26 (0.029)	25.0	7.5E-3
BW = 0.25 kg (adult)	0.16 (0.0041)	1.32 (0.033)	28.0	7.5E-3
Human/Rat Neonate	3.0 (7.3)	3.05 (7.3)	0.21	0.0011
Human/Rat Immature Mean	2.42 (5.74)	2.40 (5.0)	0.11	0.0012
Child Mean DAF	0.41 (0.17)	0.42 (0.2)	9.1	833
Adult DAF	1.07 (0.17)	1.07 (0.18)	15.8	3.12

*Average styrene oxide concentration of upper respiratory tract and terminal bronchiole model compartments; Cmax = maximum blood concentration for styrene (ST) and styrene oxide (SO); AUC = blood concentration x time for styrene and styrene oxide; models based on Sarangapani et al. (2002); and Clewell et al. (2003a). Human neonate/Rat neonate = 0.27 pM/0.09 pM = 3.00 (ST Cmax); Human/Rat Immature Mean = (3.00+2.87+2.80+2.25+1.93+1.69)/6 = 2.42 (ST Cmax); DAF = dosimetric adjustment factor. Mean DAF = 1/2.42 = 0.41 (ST Cmax). Human Equivalent Concentration (HEC) = DAF x Animal Concentration.

TABLE E.3.9. PBPK MODEL PREDICTIONS FOR FORMALDEHYDE WITH AGE-SPECIFIC PARAMETERS FROM CLEWELL *et al.*, 2003a: MODELING OF NEONATAL AND IMMATURE RAT (1 μg/m^3 x 8 hr/d, 24 hr SIMULATIONS)

Age Group	Nasal Cmax p*M*	Nasal DPXmax pmol/mg DNA	Nasal AUCDPX pmol min/mg DNA-d	Model basis
Rat				
Neonate, BW = 0.0075 kg	53	3.2 x 10-5	0.033	Scaled BW0.75 and first order rates BW-0.25 Georgieva et al. (2003); Clewell et al. (2003a)
BW = 0.015 kg	110	7.8 x 10^{-5}	0.080	
BW = 0.03 kg	220	1.8 x 10^{-4}	0.184	
BW = 0.06 kg	430	4.1 x 10^{-4}	0.406	
BW = 0.12 kg	820	9.4 x 10^{-4}	0.872	
BW = 0.20 kg	1320	1.8 x 10^{-3}	1.57	
Adult: BW = 0.25 kg	1600	2.1 x 10^{-3}	1.92	

Note: Cmax = maximum concentration; DPXmax = maximum DNA-protein crosslinks concentration; AUCDPX = the area under the DPX x time curve per day.

TABLE E.3.10. PBPK MODEL PREDICTIONS FOR FORMALDEHYDE WITH AGE-SPECIFIC PARAMETERS: MODELING OF HUMAN CHILDREN (1 μg/m3 x 8 hr/d, 24 hr SIMULATIONS).

Age Group	Nasal Cmax p*M*	Nasal DPXmax pmol/mg DNA	Nasal AUCDPX pmol min/mg DNA-d	Model basis
Human 3 month Neonate, BW = 5.7 kg	150	6.2×10^{-5}	0.035	Scaled $BW^{0.75}$ and first order rates $BW^{-0.25}$ (Georgieva et al., 2003; Clewell *et al*. 2003a)
1 yr, BW = 10.1 kg	390	1.7×10^{-4}	0.094	
3 yr, BW = 14.6 kg	860	3.9×10^{-4}	0.215	
5 yr, BW = 19.4 kg	1400	6.5×10^{-4}	0.348	
10 yr, BW = 32.6 kg	1700	8.4×10^{-4}	0.437	
15 yr, BW = 54.5 kg	2360	1.2×10^{-3}	0.603	
18 yr, BW = 63.1 kg	2700	1.4×10^{-3}	0.682	
Human/Rat Neonate	2.83	1.94	1.10	
DAF Neonate	0.35	0.52	0.91	
Human/Rat Immature Mean	2.90	1.47	0.80	
DAF Immature Mean	0.34	0.68	1.25	
Adult, BW = 70 kg	2700	1.4×10^{-3}	0.684	
Human/Rat Adult	1.69	0.67	0.36	
DAF Adult	0.59	1.49	2.78	

Note: Cmax = maximum concentration; DPXmax = maximum DNA-protein crosslinks concentration; AUCDPX = the area under the DPX x time curve per day. Human neonate/Rat neonate = 150 pM/53 pM = 2.83 (Cmax); Human/Rat Immature Mean = (2.83+3.54+3.91+3.26+2.07+1.79)/6 = 2.90 (Cmax); DAF = dosimetric adjustment factor. Mean DAF = 1/2.78 = 0.36 (Cmax). Human Equivalent Concentration (HEC) = DAF x Animal Concentration.

E.3.2.1.3 Summary of HEC factors for Adults and Children/pups

TABLE E.3.11. DAFs SUMMARY BASED ON PBPK MODELING OF INTERNAL DOSIMETRY

Chemical Species	**Cmax blood (range)**	**AUC blood (range)**	**Amount Metabolized /kg-d**	**Other**
Ethyl Benzene Child Average	0.21	0.16	6.79	
Ethyl Benzene Adult	0.52	0.34	11.37	
Naphthalene/NPO Child Average.	(8-14)	(8-14)	1.1	0.17 Cmax NPO lung
Naphthalene/NPO Adult	(6-8)	(6-8)	0.99	0.065 Cmax NPO lung
Toluene Child Average.	1.03	0.88	6.7	
Toluene Adult	3.1	0.31	2.0	
Vinyl Chloride (VCl) Child Average.	1.19	1.15	3.6	
VCl Adult	1.50	1.44	5.1	
Styrene Child Average	0.41	0.42	9.1	833 (child/rat pup)
SO Child Average	0.17	0.2		
Styrene Adult Average	1.07	1.07	15.8	3.12 (human/rat)
SO Adult Average	0.17	0.18		
Child Gmean	1.94	1.63	6.1	
Adult Gmean	1.85	1.30	3.9	
	Nasal Cmax	**Nasal DPXmax**	**Nasal AUCDPX**	
Formaldehyde Child Mean	0.34	0.68	1.25	
Formaldehyde Adult	0.59	1.49	2.78	

Note: Note: Cmax = maximum concentration; DPXmax = maximum DNA-protein crosslinks concentration; AUCDPX = the area under the DPX x time curve per day. Human Equivalent Concentration (HEC) = DAF x Animal Exposure Concentration.

Table E.3.11 provides a summary of Table E.3.3 - Table E.3.10. For the five test compounds that provide blood concentration metrics (Cmax, AUC), the child DAFs have geometric means of 1.94 and 1.63, respectively. Adult values were only slightly lower at 1.85 and 1.30, respectively. The results of the formaldehyde nasal model, which differs significantly in

structure from the previous five chemicals, are presented in Table E.3.9 and Table E.3.10. For the child the DAFs for nasal tissue Cmax and DPXmax were 0.34 and 0.68. The value for the AUC DPX was 1.25. For the adult these DAFs were higher at 0.59, 1.49, and 2.78, respectively. The predicted formaldehyde DAFs are also given at the bottom of Table E.3.11 with separate column headings.

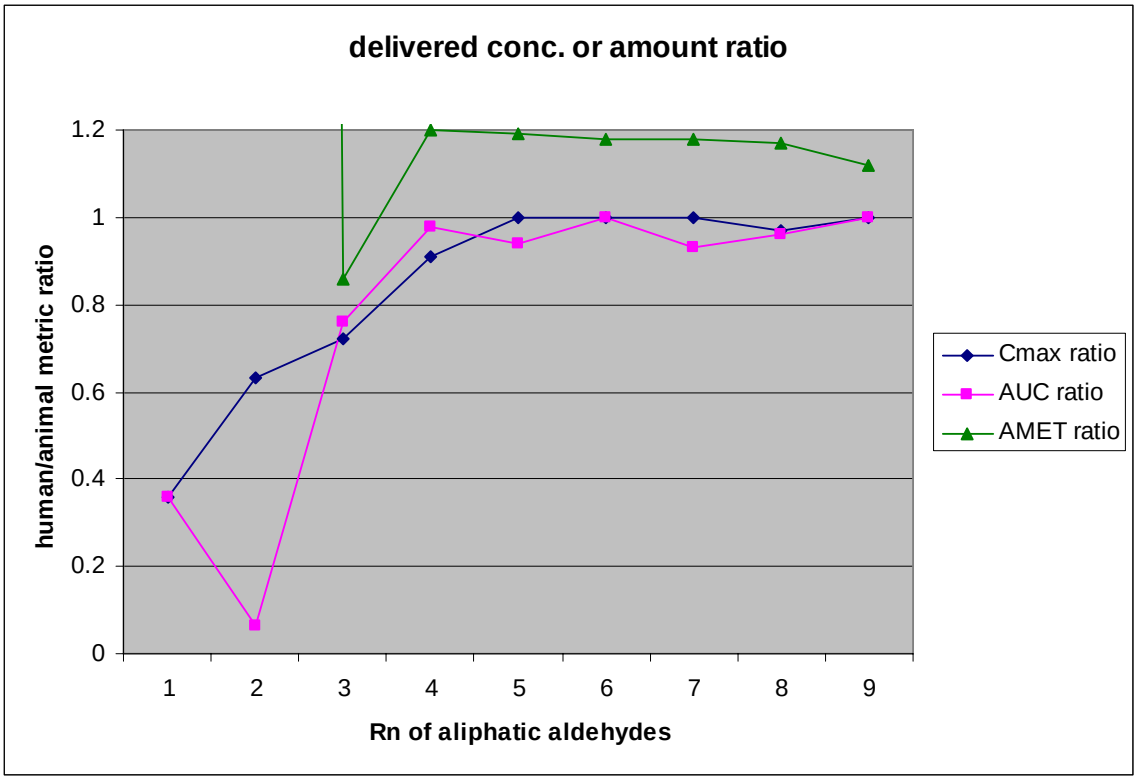
E.3.2.2 Aliphatic Aldehydes

The body of Table E.3.12 gives the ratio of Human/Rat metric values (unitless). The reciprocals of the mean (bottom) represent a factor (i.e., the DAF) by which to multiply the respective animal toxicity criteria in order to calculate the HEC. The models were formulated for adults only. There appears to be a clear difference between the shorter chain length, water-soluble aldehydes and the longer chain length, fat-soluble aldehydes. This difference is reflected in the metabolic parameters where both acetaldehyde and propionaldehyde have two saturable metabolic paths: a high-capacity, low-affinity and a low-capacity, high-affinity, as opposed to the single saturable path for the fatty aldehydes. Overall the HEC factors for the aliphatic aldehydes appear similar to the other compounds studied in adults with blood concentration ratios for each metric averaging 1.3 vs. 1.3 to 1.85 for the geometric means of the models for the five test compounds which give similar metrics. If the values for acetaldehyde and propionaldehyde are removed from the mean, the Cmax HEC factor is reduced to 1.06. This PBPK series approach may also be applicable to the straight chain aliphatic hydrocarbons and acids.

TABLE E.3.12. HUMAN/RAT PBPK MODEL PREDICTIONS FOR ALIPHATIC ALDEHYDES: (1 μg/m^3 x 8 hr/d, 24 hr SIMULATIONS)

Chemical Species	Cmax blood	AUC blood	Amount Metabolized	Model Basis
Acetaldehyde	0.36	0.36	11.4	Haddad et al. (2000); Paterson & MacKay (1989); Mitchell & Petersen (1989); Kelson et al. (1997)
Propionaldehyde	0.63	0.065	24.1	
Butyraldehyde	0.72	0.76	0.86	
Pentanal	0.91	0.98	1.20	
Hexanal	1.0	0.94	1.19	
Heptanal	1.0	1.0	1.18	
Octanal	1.0	0.93	1.18	
Nonanal	0.97	0.96	1.17	
Decanal	1.0	1.0	1.12	
Mean	0.84	0.78	4.82	
DAF	1.18	1.28	0.21	

FIGURE E.3-1 HUMAN/ANIMAL METRIC RATIOS FOR ALIPHATIC ALDEHYDES.



E.3.3 Discussion

The rat neonatal PBPK values in Tables E.3.4 to E.3.8 and Table E.3.10 are derived from the Clewell et al. (2003a) paper on neonatal perchlorate dosimetry. The values range from body weights of 0.0075 kg to 0.1985 kg. Except for fat and slowly perfused compartments, which vary inversely with each other and body weight, the tissues are a fixed percentage of body weight. Blood flows are also a fixed percentage of cardiac output, which itself is a fixed percent of body weight (14 L/hr/kg). This scheme differs from that of Price et al. (2003) and their age-specific regressions for human neonates and children. In the latter paper fractional blood flows, specifically those for liver, vary by much more than do tissue volumes. The rat values may vary more with respect to developmental age than indicated by Clewell et al. (2003a). These physiological differences may have influenced the results in Table E.3.5 and the human/rat comparisons.

In general the DAFs based on PBPK model-predicted blood concentration for adults seem lower and those for children seem higher than those produced by the current HEC methodology, which is not chemical specific but based on ventilation rates and lung surface area. Thus if we credit the chemical specific PBPK approach, the current methodology may underestimate the HEC for children and overestimate it for adults. However, these interim conclusions are based on a very limited number of chemicals and on many assumptions. HECs based on internal dosimetry PBPK estimates are bound to be highly chemical dependent and strongly influenced by the dose

metric chosen, blood/air and fat/blood partition coefficients, fractional tissue flows, metabolic parameters, and other factors.

This report also estimated values for immature rats where the examples are given in the tables, as well as for adult rat and human child, to assist in derivation of a DAF. It is anticipated that future laboratory studies will more often involve immature animals to assess accurately the toxicity of environmental agents throughout the postnatal development period.

TABLE E.3.13. SUMMARY OF INFANTS' AND CHILDREN'S PBPK MODELING BY OEHHA WITH SELECTED ENVIRONMENTAL TOXICANTS BY INHALATION

Chemical and Exposure	Tissue and Dose Metric	Age at Maximum Child/Adult	Child/Adult Maximum	Basis for the Model
Chloroform 1 ppm x 24 hr	Liver AMET	Newborn	2.3	Corley et al. (1990); Price et al. (2003)
	Kidney AMET	Newborn	2.3	
TCE 1 ppm x 24 hr	Lung $Cmax_{CH}$	Newborn	9.6	Abbas & Fisher (1997); Fisher et al. (1998); Price et al. (2003)
	Venous blood AUC_{TCE}	3 yr	10.6	
	AUC_{CH}	Newborn	12.5	
	Lung AUC_{CH}	1.5 mo	12.9	
	Liver AUC_{TCA}	3 yr	10.3	
	Liver AMET	Newborn	15.7	
Vinyl chloride 1 ppm x 24 hr	Liver Risk M	Newborn	10.2	Clewell et al. (1995); Price et al. (2003)
	Liver Risk G	Newborn	11.0	
Dichloromethane 1 ppm x 24 hr	Liver MFO AMET	Newborn	2.1	OSHA (1997); Price et al. (2003)
	Lung MFO AMET	Newborn	2.1	
	Liver GST AMET	Newborn	43.3	
	Lung GST AMET	Newborn	11.6	
DCM 1 ppm x 24 hr respiratory tract model	Liver MFO AMET	Newborn	3.0	OSHA (1997); Sarangapani et al., (2002); Price et al, (2003)
	Lung MFO AMET	Newborn	60.7	
	MFO AMET total	Newborn	3.0	
	Liver GST AMET	Newborn	42.7	
	Lung GST AMET	Newborn	65.9	
	GST AMET total	Newborn	56.6	
Styrene/styrene (ST/SO) oxide 1 ppm x 24 hr	Lung MFOAMET/L tissue-d	Newborn	9.3	Sarangapani et al., (2002; Price et al., (2003)
	Lung EH AMET/Ltissue-d	Newborn	9.2	
	Lung GST AMET/Ltissue –d	Newborn	4.7	
ST/SO 1 ppm x 24 hr respiratory tract model	Lung MFOAMET/L tissue-d	0.5 mo	2.7	Sarangapani et al. (2002); Price et al. (2003)
	Lung EH AMET/Ltissue-d	0.5 mo	2.6	
	Lung GST AMET/Ltissue–d	0.5 mo	2.7	
ST/SO 50 ppm x 2 hr respiratory tract model, Csanady et al. biochemical parameters	Lung MFO AMET/Ltissue-d	0.5 mo	2.7	Sarangapani et al. (2002); Csanady et al. (2003); Price et al. (2003)
	Lung EH AMET/Ltissue-d	0.5 mo	2.8	
	Lung GST AMET/Ltissue–d	0.5 mo	2.9	
	Liver + Lung AMET/Ltissue-d	Newborn	3.5	
ST/SO 50 ppm x 2 hr respiratory tract model, Sarangapani et al. biochemical parameters	Lung MFOAMET/L tissue-d	0.5 mo	2.5	Sarangapani et al. (2002); Price et al. (2003)
	Lung EH AMET/Ltissue-d	0.5 mo	3.0	
	Lung GST AMET/Ltissue–d	Newborn	6.0	
	Liver + Lung AMET/Ltissue-d	Newborn	6.5	

TABLE E.3.13. SUMMARY OF INFANTS' AND CHILDREN'S PBPK MODELING BY OEHHA WITH SELECTED ENVIRONMENTAL TOXICANTS BY INHALATION

Chemical and Exposure	Tissue and Dose Metric	Age at Maximum Child/Adult	Child/Adult Maximum	Basis for the Model
ST/SO 1 ppm x 24 hr respiratory tract model of Csanady et al.	Lung MFOAMET/Ltissue-d	0.5 mo	7.3	Csanady et al. (2003); Price et al. (2003)
	Venous Blood SO Cmax	3.5 mo to 1 yr	5.1	
	Lung alveoli SO Cmax	3.5 mo	4.3	
	Lung alveoli AUC_{SO}	Newborn to 5 yr	4.2	
ET/EO 1 ppm x 24 hr PBPK model of Csanady et al.	Clearance of ET and EO by liver, Venous blood Cmax, AUC EO in liver, blood, Hb and DNA adducts	Newborn (AMET EO μmol/kg-d)	32.1	Csanady et al. (2000); Price et al. (2003)
Butadiene (BD) 1 ppm x 24 hr BD/BMO/DEB model	Liver + Lung DEB AMET μmol/kg-d	Newborn	7.1	Kohn & Melnick (1993); Johanson & Filser (1993); Price et al. (2003)
	Venous blood AUC_{BMO} μM hr	Newborn	71	
	Venous blood AUC_{DEB} μM hr	Newborn	16.2	
	Liver AUC_{DEB} μM hr	Newborn	20.7	
	Lung AUC_{BMO} μM hr	Newborn	32.8	
	Lung AUC_{DEB} μM hr	Newborn	17.2	
BD/BMO 1 ppm x 24 hr respiratory tract model	Lung BMO→DEB AMET μmol/Llung-d	Newborn	33.8	Sarangapani et al. 2002; Kohn & Melnick (1993); Price et al. (2003)
	Liver BMO→DEB AMET μmol/Lliver-d	Newborn	19.2	
BD/BMO 1 ppm x 24 hr respiratory tract model	Lung alveoli BMO→DEB AMET μmol/Lalveoli-d	Newborn	120	Sarangapani et al. (2002); Kohn & Melnick (1993); Price et al. (2003)
	Lung bronchi BMO→DEB AMET μmol/Lbronchi-d	Newborn	33.8	
MTBE 1 ppm x 24 hr; 10 ppm x 8 hr VPs 0.8, 1.25	Blood, brain Cmaxs μM, AUCs μM hr, AMET μmol/kg-d	3-8 yr	1.2 to 12.4 highly dependent on VP	(Licata et al., 2001); Price et al. 2003; Evelo et al. (1993)
PCE 1ppm x 24 hr; 10 ppm x 8 hr VP = 1	AUC^{PCE} blood, liver, brain, AMET, AUC^{TCA}, TCAurine/kg-d	Newborn	1.1 to 4.6	Gearhart et al. (1993); Loizou (2001); Price et al. (2003)
Furan 1 ppm x 24 hr 0-13yr + adult Flow-limited liver metabolism	Liver AMET μmol/kg-d Brain AUC μMhr	13 yr	2.2	Price et al. (2003)
Carbon tetrachloride 1 ppm x 24 hr, 10 ppm x 8 hr	Liver AMET μmol/kg-d, Blood or liver AUC μMhr, blood or liver Cmax	Newborn	1.6	Thrall et al. (2000); Price et al. (2003)

TABLE E.3.13. SUMMARY OF INFANTS' AND CHILDREN'S PBPK MODELING BY OEHHA WITH SELECTED ENVIRONMENTAL TOXICANTS BY INHALATION

Chemical and Exposure	Tissue and Dose Metric	Age at Maximum Child/Adult	Child/Adult Maximum	Basis for the Model
Toluene 1 ppm x 24 hr	LiverAMET μmol/kg-d, Blood or liver AUC μMhr, blood or liver Cmax	5 yr	3.6	Tardiff et al. (1995); Price et al. (2003)
Xylene 1 ppm x 24 hr	LiverAMET μmol/kg-d, Blood or liver AUC μMhr, blood or liver Cmax	5 yr	4.5	Tardif et al. (1995); Price et al. (2003)
Toluene/Xylene mixed model with competitive inhibition, 10/10, 1/10, 10/1 ppm x 8 hr	Liver AMET μmol/kg-d, Blood or liver AUC μMhr, blood or liver Cmax	5 yr	5.2	Tardif et al. (1995); Price et al. (2003)
Benzo[a]pyrene vapor 10 ppb x 24 hr; Hybrid diffusion-limited-lung flow-limited-body model	Lung alveoli, bronchi. Liver AMET μmol/kg-d. AUC^{BaP} μM min; V_{max}s scaled from uninduced and 3-MC induced rats	Newborn	4.3 to 31.9 uninduced 3.7 to 26.1 induced	Wiersma and Roth (1983); Gerde et al. (1991); Moir et al. (1998); Price et al. (2003); and others
Benzo[a]pyrene particle 1 μg/m3 x 24 hr; hybrid model as above	Lung alveoli, bronchi. Liver AMET μmol/kg-d. AUC^{BaP} μM min; V_{max}s scaled from uninduced and 3-MC induced rats	Newborn to 1 yr	9.7 to 18.6 uninduced 10.8 to 22.0 induced	As above and Sun et al. (1982): Sun et al. (1984): ICRP (1994); Gerde et al. (2001); Ramiesh et al. (2001)
NAP/NO 1 ppm x 24 hr respiratory tract model	Lung AMET μmol/$L_{alveoli}$-d	Newborn	2.4	Sweeney et al. (1996); Willems et al. (2001); Price et al. (2003)
	$AMET^{NO}$ GST μmol/kg-d	Newborn	3.1	

Notes: AMET = amount metabolized; Cmax = maximum concentration in blood or tissue; CH = chloral hydrate; TCA = trichloroacetic acid; AUC = area under the concentration x time curve; Risk M = μmol metabolites DNA bound/L liver/d; Risk G = μmol metabolites conjugated with glutathione/L liver/d; MFO = mixed function oxidase (P450) pathway; EH = epoxide hydrolase pathway; GST = glutathione sulfotransferase pathway; BMO = butadiene monoxide; DEB = diepoxybutane; AMET DEB amount of BMO oxidized to DEB. Model based on [Kohn & Melnick (1993)], Evelo et al.. (1993), Sarangapani et al. (2002), Jonsson, (2001). Exposure for 24 hr, simulations 48 hr. respiratory tract model = model with diffusion limited lung (upper airways, conducting airways, transitional bronchioles, and alveoli) and flow limited body (fat muscle, vessel rich group and liver) based on Sarangapani et al.. (2002) with BD/BMO parameters from [Kohn and Melnick (1993)]. VP = ventilation:perfusion ratio (alveolar ventilation/cardiac output). MTBE = methyl *tert*-butyl ether; PCE = tetrachloroethylene; TCA = trichloroacetic acid.

E.3.4 Uncertainty Factor for Variability within the Human Population

- Traditional application and previously published analyses.

A 10-fold uncertainty factor (UF_H) has traditionally been used by risk assessors to account for variability within the human population. As understanding of the sources of interindividual variability has evolved, this uncertainty factor has been regarded as consisting of two components, both with a value of √10, attributed to differences in toxicokinetics and toxicodynamics, respectively. The overall uncertainty factor is intended to account for the greater susceptibility to chemical toxicity of various sensitive subpopulations, including infants and children. Intraspecies variability in toxicokinetics can be better quantified now because of better data and advances in modeling techniques.

A high degree of inter-individual variability (2-to-30-fold) in response to chemical exposure has been reported (Weil, 1972; Krasovskii, 1976). Hattis has shown that human variability in response to some medications may range over more than 3 orders of magnitude (>1,000-fold) (Hattis, 1996a; 1996b). Similar inter-individual variability has been shown in airway responsiveness and lung volume among normal and asthmatic subjects (O'Connor et al., 1987; Bylin et al., 1995). In a study of asthmatic subjects, Horstman (1986) found that there was a 7-fold distribution in the range of sulfur dioxide concentrations required to produce bronchoconstriction. Thus, it is reasonable to conclude that asthmatics may be at least seven times as sensitive to the effects of sulfur dioxide as normal individuals. The inter-individual variability has been recently modeled, indicating a distribution that ranges from 1 to >20 with a value of 10 for the 85th percentile (Gillis et al., 1997). Thus, based on this analysis, the use of a 10-fold uncertainty factor might not be protective of approximately 15% of the population. Further research into the considerations, circumstances, subpopulations, and endpoints of greater susceptibility is needed.

OEHHA has, like U.S.EPA (1994a), generally applied a 10-fold uncertainty factor to address the greater susceptibility of sensitive individuals. In accordance with U.S.EPA guidelines, when an exposure level is estimated from a study that includes the assessment of a sensitive human sub-population, an intraspecies factor of 1 is used (U.S.EPA, 1994a). Since the true degree of variability of response in the human population is unknown, the effectiveness of this method in providing protection to nearly all individuals is uncertain.

As noted by Dourson and Stara (1983), the steepness of the dose-response relationship affects the adequacy of the uncertainty factor for sensitive individuals. They summarized the range of dose response slopes reported by Weil (1972), indicating that, based on studies of acute lethality, a 10-fold factor was health-protective in most cases (Weil, 1972). However, in our experience, dose response curves for acute lethality exposures are generally steeper than those for non-lethal acute or chronic exposures (Table E.14).

TABLE E.3.14. COMPARISON OF SLOPES OF MILD AND LETHAL EFFECTS[A].

Chemical	Mild Effects[b]	Lethality[c]
Acrolein (irritation)	3.3	14.4
Ammonia (irritation)	6.9	14.3
Vinyl chloride (CNS effects)	7.5	31.9

[a] Log-normal dose-response slope values are the mean of up to 5 studies.
[b] Human data for mild effects include: (Hine et al., 1961; Lester et al., 1963; MacEwen et al., 1970; Verberk, 1977).
[c] Animal LC_{50} studies include: (Silver and McGrath, 1948; Champeix and Catilina, 1967; Philippin et al., 1970; Prodan et al., 1975; Appelman et al., 1982; Kapeghian et al., 1982; U.S.EPA, 1992a; 1992b)

Because the true variability is unknown, there may be a portion of the population for whom the chronic RELs will not be protective. It is OEHHA's intent that the levels will protect the general population including those in the high end of susceptibility. As information defining susceptible individuals becomes available, it is our intent to adjust the methodology as necessary to protect such individuals.

E.3.5 Adequacy of the $UF_{H\text{-}k}$ for younger ages – newer analyses.

Dorne et al. (2001) evaluated the validity of the $10^{0.5}$ (3.16) human toxicokinetic subfactor in relation to CYP1A2 metabolism using published data on clearance (CL), AUC and peak plasma concentrations (Cmax) for caffeine, theophylline, theobromine, paraxanthine, and R-warfarin in human volunteers. After oral dosing, the variation (coefficient of variation, CV) in metabolic clearance in healthy adults of the first four compounds ranged from 25 to 63 percent (mean = 42 percent) in nine studies of 70 subjects. For i.v. dosing the variability of theophylline and R-warfarin ranged from 31 to 59 percent (mean = 43 percent) in four studies of 34 subjects. The authors concluded that in the case of kinetics of compounds metabolized by CYP1A2 "essentially the whole of the healthy adult population would be covered by the 3.16 kinetic default for both steady state (CL and AUC) and acute exposures (Cmax) assuming a normal distribution, while between <0.01 to 1.8% would be outside the default factor of 3.16 assuming a log-normal distribution". The authors identified population subgroups for which the default UF of 3.16 would be less protective. These included about one-half of pregnant women at term (based on caffeine at 38 weeks gestation), neonates (99-100 percent not covered), 13 percent of infants, but only 0.1 percent of children, who would have internal doses falling outside the default. It should be noted that these conclusions are based on a relatively few drugs administered orally or parenterally.

Ginsberg et al. (2002) also evaluated child/adult pharmacokinetic differences by analyzing the therapeutic drug literature. The authors identified about 100 chemicals with some pharmacokinetic (PK) data in children and a subset of 45 of these was selected for further study. Of the 45 chemicals, eight were excreted unchanged in urine, 18 had some form of CYP metabolism, six were unclassified, six were subject to glucuronidation, two to alcohol dehydrogenase, two to sulfation and one to glutathione conjugation. The subjects were classified as premature neonates (≤ 1 week, 7 chemicals), full-term neonates (≤ 1 week, 19 chemicals),

newborns (1 week-2 months, 14 chemicals), early infants (2-6 months, 7 chemicals), toddlers (6 mo-2 yr, 14 chemicals), preadolescents (2-12 yr, 26 chemicals), adolescents (12-18 yr, 7 chemicals) and adults (42 chemicals). The kinetic parameters evaluated (number of chemicals) were AUC (9), clearance (27), Cmax (5), half-life ($t_{1/2}$, 41), and volume of distribution (Vd, 25).

Multiple regression analysis was used to evaluate relationships between age groups and the log mean PK parameter value across chemicals. In general, for many chemicals, early life stages (premature and full-term neonates, newborns 1 week to 2 months) appeared to be different from adults in terms of clearance, $t_{1/2}$, and Vd. For 40 chemicals with half-life data, the analysis showed that half-lives in premature neonates were about four-fold longer than in adults ($P < 0.001$) and about two-fold longer in full term neonates to two months of age ($P < 0.001$). For 27 chemicals with clearance data, premature to two months of age infants showed significantly lower clearance ($P<0.01$) and six months to 12-year-old children significantly higher clearance ($P<0.0001$) than adults. For the CYP1A2 substrates caffeine and theophylline, neonates to infants two months of age showed about four to nine-fold longer half-lives than adults while older age groups six months-12 years had significantly shorter half-lives than adults. A similar pattern was observed with the CYP3A substrates (e.g., alfentanil, carbamazepine, fentanyl, lignocaine).

The overall study results indicate that premature and full-term neonates tend to have three to nine times longer half-lives than adults for the drugs studied. Like the previous work of Renwick et al. (2000) and Dorne et al. (2001) noted above, the drugs studied were administered orally or parenterally and not via inhalation. While some of the same metabolic pathways are no doubt involved, it is difficult to make direct extrapolations from drugs to environmental toxicants. The authors note that three of the included chemicals, chloral hydrate, dichloroacetic acid and trichloroacetic acid, are major metabolites of trichloroethylene (TCE) and tetrachloroethylene (PCE), both important environmental contaminants.

Dorne et al. (2005a) estimated intraspecies pharmacokinetic uncertainty factors based on analysis of a database on human variability in phase I metabolism (CYP1A2, CYP2A6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4, hydrolysis, alcohol dehydrogenase (ADH)), phase II metabolism (N-acetyltransferases (NAT), glucuronidation, glycine conjugation, sulfation) and renal excretion. The authors derived pathway-specific UF_{H-k}s covering 95%, 97.5%, and 99% of the population of healthy adults, and other subgroups. For healthy adults exposed to toxicants metabolized by monomorphic pathways (CYP1A2, CYP2A6, CYP2E1, CYP3A4, ADH, hydrolysis, glucuronidation, sulfation, glycine conjugation) the UF_{H-k} of 3.16 was adequate to cover more than 99% of the population. However, for toxicants subject to polymorphic pathways, particularly CYP2C19 (99% - UF of 52) and CYP2D6 (99% - UF of 26) poor metabolizers and NAT slow acetylators (99% - UF of 5.2), these subpopulations were not adequately covered by a 3.16 UF_{H-k}. Children and neonates were among the subgroups analyzed. Children exposed to toxicants metabolized by CYP2C19 (99% - UF of 9.0) and CYP2D6 (99% - UF of 45) were not adequately protected by a UF of 3.16. Neonates were not adequately protected by the CYP1A2 (99% - UF of 14), CYP3A4 (99% - UF of 12), glucuronidation (99% - UF of 12), and glycine conjugation (99% - UF of 28) pathways and only marginally by the renal excretion path (99% - UF of 3.4). All of the compounds in the database evaluated were administered by the oral or intravenous routes. In addition, the UFs are estimated from internal dose metrics (AUCs or Cmaxs) for the parent compounds assuming that it is the toxicant of

concern. This may not be the case with many environmental toxicants of concern. The authors argue for the use of pathway-specific UFs in risk assessment instead of defaults. This may be feasible in some instances where metabolism, modes of action, and potential polymorphisms are well understood. However, there will still be a need for adequately protective defaults for sensitive subgroups when this is not the case. In view of the results of the authors' analysis it is apparent the UF_{H-k} of 3.16 is not adequately protective for infants and many children.

PBPK models can give useful predictions of how the body handles a particular chemical and its metabolites. The models address issues of internal body or tissue dosimetry, route to route extrapolation, and, in some cases, interspecies extrapolation. To date relatively few published models for various environmental pollutants address infant and child exposure in a systematic fashion. This is parallel to the bulk of toxicity testing in animals which is usually initiated in young adult animals.

Pelekis et al. (2001) used a physiological model to derive adult and child pharmacokinetic UFs for selected volatile organic compounds (VOCs). The chemicals modeled were dichloromethane (DCM), tetrachloroethylene (PCE), toluene (TOL), m-xylene (XYL), styrene (ST), carbon tetrachloride (CATE), chloroform (CHLO), and trichloroethylene (TCE). Adult models of low (50 kg) and high (90 kg) body weight were compared with a 10 kg-based child model. Fat contents varied from 51 percent for the 90 kg adult model to 17 percent for the 10 kg child. Ventilation:perfusion ratios varied from 0.76 (50 kg) to 1.38 (10 kg). Fractional liver flows (of cardiac output) ranged from 0.11 (50 kg) to 0.34 (90 kg). All PBPK models were flow-limited with exposure by inhalation, arterial circulation to Fat, Slowly Perfused, Rapidly Perfused and Liver model compartments, metabolism in the Liver, and combination of compartment outputs in venous blood. The arterial and venous bloods were not explicitly modeled, nor were VOC metabolites specifically modeled. A range of physiological parameters (blood:air and tissue:blood) was used for each body model and the eight VOC chemicals based on literature values.

Simulations involved exposure to one ppm VOC and estimation of arterial and venous blood concentrations (CA, CV), and tissue concentrations (Ci) after 30 days continuous exposure. A comparison of the two adult models (Adult high/Adult low) shows relatively few significant departures from unity for the dose metrics estimated. CATE ratios ranged from 2.85 ($C_{rapidly\ perfused}$) to 1.71 (C_{liver}). DCM ranged from 0.29 (C_{liver}) to 1.04 ($C_{arterial}$ blood). Comparisons of the Adult high/Child average from the PBPK model show some larger differences. For the C_{liver} dose metric the PBPK models predicted the following Adult/Child values: ST (0.033), XYL (0.037), TCE (0.061), DCM (0.092), CHLO (0.11). These model predictions would indicate up to a 30-fold higher concentration of the VOC chemicals in child liver than in adult liver via the inhalation route.

While this is a useful approach involving important environmental toxicants and a relevant exposure route, the models and dose metrics employed address only the parent compounds where relevant toxic effects may be more closely related to the tissue dosimetry of metabolites, which were not specifically modeled. The use of a single child body weight is insufficient to assess the full range of physiological variability throughout development, particularly in the neonatal period. It is worth noting, however, that the higher concentrations of the VOCs in a child's liver

might be expected to result in higher peak concentrations of metabolites of those compounds in the liver.

E.3.6 Adequacy of the $UF_{H\text{-}k}$ for Younger Ages – Indications from PBPK Modeling

The results of limited PBPK modeling with age-specific parameters and a range of about 20 chemicals are summarized in Table E3.13. For a variety of dose metrics for parent chemicals and metabolites it appears that a $UF_{H\text{-}k}$ of $\sqrt{10}$ may be inadequate for one or more of the age-group models evaluated. Most frequently the newborn models showed the greatest child/adult ratios. It is important to note that the large majority of the studies and PBPK modeling exercises described above involve relatively short-term exposures that represent environmental, occupational, or therapeutic scenarios. Extreme situations of short-term high exposures or very long-term low level exposures were not simulated. Also considerable variation in child breathing rates was not modeled in a systematic fashion. Despite these limitations the results are considered indicative of the types of exposures of greatest concern with respect to infants and children.

E.4 Toxicokinetic Model Parameters for Individual Chemicals

This section provides a sampling of the parameters used in the PBPK modeling (Table E.3.13). Not all the chemical or all the age-specific parameters are given but the early age groups (ages 0-6 yr) have been emphasized.

TABLE E.4.1. PBPK MODEL PARAMETERS FOR FURAN: 0-6 YEARS OF AGE

Tissue/Compartment	Volume, Vi L	Flow, Qi, L/hr	Partition, Pi tissue/blood	Metabolism
0-6 yr Model				
Fat, f	(1)	0.053*Qtot	33.39	
Liver l	(2)	0.0795*Qtot	4.69	Cart*Ql
Muscle, m	(3)	0.03*Qtot	3.24	
Brain, brain	(4)	(8)	8.82	
Lung, Vlu	(5)	Qtot	4.69	
Lung Alveoli, Valv	0.9*Vlu	0.93*Qtot	4.69	
Lung bronchi,Vbr	0.1*Vlu	0.07*Qtot	4.69	
Other body	BW-(Vf + Vl + Vlu + Vbrain + Vm)	Qtot – (Qf + Ql + Qm + Qbrain)	4.69	
Alveolar ventilation, Qalv		0.8*Qtot		
Cardiac Output, Qtot		(6)		
Blood:Air, Pb			2.47	
Body weight, BW	(7)			

Age (yr)-specific regressions : (1) Vf = (0.0162*Age^5 – 1.9784*Age^4 + 51.963*Age^3 – 459.38*Age^2 +1566.8*Age + 1004.2)/1000; (2)Vl = (0.0072*Age^5 – 0.3975*Age^4 + 7.9052*Age^3 – 65.624*Age^2 + 262.02*Age + 157.52)/1000; (3) Vm = (-0.0623*Age^5 + 2.3433*Age^4 – 26.559*Age^3 + 144.75*Age^2 + 339.84*Age + 1648.2)/1000; (4) Vbrain = (1E4*((Age + 0.213)/(6.030 + 6.895*Age)))/1000; (5) Vlu = (-0.0346*Age^4 + 1.5069*Age^3 – 20.31*Age^2 + 123.99*Age + 59.213)/1000; (6) Qtot = 0.012*Age^3 – 1.2144*Age^2 + 40.324*Age + 44.414; (7) BW = (-1.9*Age^4 + 72.8*Age^3 – 813.1*Age^2 + 5535.6*Age + 4453.7)/1000; (8) Qbrain = -0.0024*Age^4 + 0.1305*Age^3 – 2.4822*Age^2 + 18.025*Age + 15.197. For 7-10 yr model Qf = 0.05*Qtot; Ql = 0.118*Qtot; Qm = 0.045*Qtot; Qbrain = 0.159*Qtot; For 11-18yr model Qf = 0.044*Qtot; Ql = 0.136*Qtot; Qm = 0.068*Qtot; Qbrain = 0.116*Qtot. For adult Qf = 0.052*Qtot; Ql = 0.26*Qtot; Qm = 0.1648Qtot; Qbrain = 0.1148Qtot. (Price, 2003)

TABLE E.4.2. PBPK MODEL PARAMETERS FOR MTBE: 0-6 YEARS OF AGE

Tissue/Compartment	Volume, Vi L	Flow, Qi, L/hr	Partition, Pi tissue/blood	Metabolism
0-6 yr Model				
Fat, f	(1)	0.053*Qtot	4.79	
Liver, l	(2)	0.0795*Qtot	0.723	Vmax1, Km1 Vmax2, Km2
Muscle, m	(3)	0.03*Qtot	1.181	
VRG, vrg	(4)	0.674*Qtot	0.723	
Lung, Vlu	(5)	Qtot	0.723	
Kidneys, kid		0.164*Qtot		
Lung Alveoli, Valv	0.9*Vlu	0.93*Qtot	0.723	
Lung bronchi,Vbr	0.1*Vlu	0.07*Qtot	0.723	
Alveolar ventilation, Qalv		0.8*Qtot		
Cardiac Output, Qtot		(6)		
Blood:Air, Pb			17.7	
Body weight, BW	(7)			

Age (yr)-specific regressions : (1) Vf = (0.0162*Age^5 – 1.9784*Age^4 + 51.963*Age^3 – 459.38*Age^2 +1566.8*Age + 1004.2)/1000; (2)Vl = (0.0072*Age^5 – 0.3975*Age^4 + 7.9052*Age^3 – 65.624*Age^2 + 262.02*Age + 157.52)/1000; (3) Vm = (-0.0623*Age^5 + 2.3433*Age^4 – 26.559*Age^3 + 144.75*Age^2 + 339.84*Age + 1648.2)/1000; (4) Vkid = (9.373E-4*Age^5 – 0.0569*Age^4 + 1.1729*Age^3 – 10.34*Age^2 + 44.604*Age + 28.291)/1000; (5) Vlu = (-0.0346*Age^4 + 1.5069*Age^3 – 20.31*Age^2 + 123.99*Age + 59.213)/1000; (6) Qtot = 0.012*Age^3 – 1.2144*Age^2 + 40.324*Age + 44.414; (7) BW = (-1.9*Age^4 + 72.8*Age^3 – 813.1*Age^2 + 5535.6*Age + 4453.7)/1000; For 7-10 yr model Qf = 0.05*Qtot; Ql = 0.118*Qtot; Qm = 0.045*Qtot; Qkid = 0.12*Qtot; For 11-18yr model Qf = 0.044*Qtot; Ql = 0.136*Qtot; Qm = 0.068*Qtot; Qkid = 0.136*Qtot. For adult Qf = 0.052*Qtot; Ql = 0.26*Qtot; Qm = 0.1648Qtot; Qkid = 0.26Qtot. Vmax1 = 3.38E-5*$BW^{0.75}$; Vmax2 = 6.2E-6*$BW^{0.75}$ mol/hr; Km1 = 6.17E-5M; Km2 = 3.8E-6M.

TABLE E.4.3. PBPK MODEL PARAMETERS FOR PCE: 0-6 YEARS OF AGE

Tissue/Compartment	Volume, Vi L	Flow, Qi, L/hr	Partition, Pi tissue/blood	Metabolism
Fat 1, f1	(1) 0.8*Vf	0.615*0.053*Qtot	125.2	
Fat 2, f2	(1) 0.2*Vf	0.385*0.053*Qtot	125.2	
Liver l	(2)	0.0795*Qtot	5.28	Vmax1, Km1,K2
Muscle, m	(3)	0.03*Qtot	6.11	
VRG, vrg	BW – (Vf+Vl+Vm+Vlu)	0.674*Qtot	5.06	
Lung, Vlu	(4)	Qtot	5.06	
Lung Alveoli, Valv	0.9*Vlu	0.93*Qtot	5.06	
Lung bronchi,Vbr	0.1*Vlu	0.07*Qtot	5.06	
Alveolar ventilation, Qalv		Qtot		
Cardiac Output, Qtot		(5)		
Blood:Air, Pb			11.58	
Body weight, BW	(6)			

Age (yr)-specific regressions : (1) Vf = (0.0162*Age^5 – 1.9784*Age^4 + 51.963*Age^3 – 459.38*Age^2 +1566.8*Age + 1004.2)/1000; (2)Vl = (0.0072*Age^5 – 0.3975*Age^4 + 7.9052*Age^3 – 65.624*Age^2 + 262.02*Age + 157.52)/1000; (3) Vm = (-0.0623*Age^5 + 2.3433*Age^4 – 26.559*Age^3 + 144.75*Age^2 + 339.84*Age + 1648.2)/1000; (4) Vlu = (-0.0346*Age^4 + 1.5069*Age^3 – 20.31*Age^2 + 123.99*Age + 59.213)/1000; (5) Qtot = 0.012*Age^3 – 1.2144*Age^2 + 40.324*Age + 44.414; (6) BW = (-1.9*Age^4 + 72.8*Age^3 – 813.1*Age^2 + 5535.6*Age + 4453.7)/1000; For 7-10 yr model Qf = 0.05*Qtot; Ql = 0.118*Qtot; Qm = 0.045*Qtot; Qkid = 0.12*Qtot; For 11-18yr model Qf = 0.044*Qtot; Ql = 0.136*Qtot; Qm = 0.068*Qtot; Qkid = 0.136*Qtot. For adult Qf = 0.052*Qtot; Ql = 0.26*Qtot; Qm = 0.1648Qtot; Qkid = 0.26Qtot. Vmax1 = 1.69E-6*$BW^{0.75}$mol/hr; Km1 = 4.6E-5M; K2 = 2.0*$BW^{-0.25}$.

TABLE E.4.4. PBPK MODEL PARAMETERS FOR BAP: 0-6 YEARS OF AGE

Tissue/Compartment	Volume, Vi L	Flow, Qi, L/hr	Partition, Pi tissue/blood	Metabolism
Fat , f	(1)	0.053*Qtot	294.7	
Liver, l	(2)	0.0795*Qtot	7.0	Vmax1, Km1, Vmaxlu Kmlu
Muscle, m	(3)	0.03*Qtot	4.0	
KVRG, kvrg	BW – (Vf+Vl+Vm+Vlu)	Qtot – (Qf + Ql + Qm)	4.0	
Lung, Vlu	(4)	Qtot		
Lung Alveoli, Valv	0.9*Vlu	0.93*Qtot	1.3	
Lung bronchi,Vbr	0.1*Vlu	0.07*Qtot	2.3	
Alveolar ventilation, Qalv		Qtot		
Cardiac Output, Qtot		(5)		
Blood:Air, Pb			10	
Body weight, BW	(6)			

Age (yr)-specific regressions : (1) Vf = (0.0162*Age^5 – 1.9784*Age^4 + 51.963*Age^3 – 459.38*Age^2 +1566.8*Age + 1004.2)/1000; (2)Vl = (0.0072*Age^5 – 0.3975*Age^4 + 7.9052*Age^3 – 65.624*Age^2 + 262.02*Age + 157.52)/1000; (3) Vm = (-0.0623*Age^5 + 2.3433*Age^4 – 26.559*Age^3 + 144.75*Age^2 + 339.84*Age + 1648.2)/1000; (4) Vlu = (-0.0346*Age^4 + 1.5069*Age^3 – 20.31*Age^2 + 123.99*Age + 59.213)/1000; (5) Qtot = 0.012*Age^3 – 1.2144*Age^2 + 40.324*Age + 44.414; (6) BW = (-1.9*Age^4 + 72.8*Age^3 – 813.1*Age^2 + 5535.6*Age + 4453.7)/1000; For 7-10 yr model Qf = 0.05*Qtot; Ql = 0.118*Qtot; Qm = 0.045*Qtot; Qkid = 0.12*Qtot; For 11-18yr model Qf = 0.044*Qtot; Ql = 0.136*Qtot; Qm = 0.068*Qtot; Qkid = 0.136*Qtot. For adult Qf = 0.052*Qtot; Ql = 0.26*Qtot; Qm = 0.1648Qtot; Qkid = 0.26Qtot. Vmax1 = 1.7E-9*$(BW/0.25)^{0.75}$mol/hr; Km1 = 5.5E-6M; Vmaxlu = 1.2E-11*$(BW/0.25)^{0.75}$ mol/hr, Kmlu = 2.2E-7M .

TABLE E.4.5. PBPK-RT MODEL PARAMETERS FOR NAP/NO: 0-5 YEARS OF AGE

Tissue/Compartment	Volume, Vi L	Flow, Qi, L/hr	Partition, Pi tissue/blood NAP/NO	Metabolism
Fat , f	(1)	0.053*Qtot	160/22.9	
Liver, l	(2)	0.0795*Qtot	7.0/7.0	Vmax1, Km1, Vmax2, Km2, Km2ih,Vmax3, Km3GSH, Km3NO
Muscle, m	(3)	0.03*Qtot	4.0/4.0	
VRG, vrg	BW – (Vf+Vl+Vm +Vlu+Vblood)	Qtot – (Qf + Ql + Qm)	4.0/4.0	
Vblood, blood	0.075*BW			
Lung, Vlu	(4)	Qtot		Vmaxlu, Kmlu, Vmax2lu, Km2, Km2ih, Vmax3, Km3GSH, Km3NO
Lung URT, Vua	0.0026*Vlu	0.0025*Qtot		
Lung CA,Vca	0.018*Vlu	0.0075*Qtot		
Lung TB,Vtb	0.043*Vlu	0.0067*Qtot		
Lung PU, Vpu	0.9378Vlu	0.983*Qtot		
Alveolar ventilation, Qalv		0.82*Qtot		
Cardiac Output, Qtot		(5)		
Blood:Air, Pb			571/571	
Body weight, BW	(6)			

Age (yr)-specific regressions : (1) Vf = 0.0162*Age^5 – 1.9784*Age^4 + 51.963*Age^3 – 459.38*Age^2 +1566.8*Age + 1004.2; (2)Vl = 0.0072*Age^5 – 0.3975*Age^4 + 7.9052*Age^3 – 65.624*Age^2 + 262.02*Age + 157.52; (3) Vm = -0.0623*Age^5 + 2.3433*Age^4 – 26.559*Age^3 + 144.75*Age^2 + 339.84*Age + 1648.2; (4) Vlu = -0.0346*Age^4 + 1.5069*Age^3 – 20.31*Age^2 + 123.99*Age + 59.213; (5) Qtot = (0.012*Age^3 – 1.2144*Age^2 + 40.324*Age + 44.414)*(1000/60); (6) BW = -1.9*Age^4 + 72.8*Age^3 – 813.1*Age^2 + 5535.6*Age + 4453.7; For 7-10 yr model Qf = 0.05*Qtot; Ql = 0.118*Qtot; Qm = 0.045*Qtot; Qkid = 0.12*Qtot; For 11-18yr model Qf = 0.044*Qtot; Ql = 0.136*Qtot; Qm = 0.068*Qtot; Qkid = 0.136*Qtot. For adult Qf = 0.052*Qtot; Ql = 0.26*Qtot; Qm = 0.1648Qtot; Qkid = 0.26Qtot. Vmax1 (P450) = 2.46E-2*MPl*Vl/(BW/250)$^{0.25}$μmol/min; Km1 = 0.003mM; Vmaxlu (P450) = 2.45E-3*MPlu*Vlu/(BW/250)$^{0.25}$ μmol/min, Kmlu = 0.006mM . Vmax2l (Epoxide Hydrolase) = 4.0E-3*MPl*Vl/(BW/250)$^{0.25}$ μmol/min, Km2 = 0.001 mM, Km2ih = 2.0E-4 mM, Vmax2lu = 9.0E-3*MPlu*Vlu/(BW/250)$^{0.25}$ μmol/min, Km2lu = 0.001 mM, Km2luih = 2E-4 mM. Vmax3 (GST) = 0.5*CPl*Vl/(BW/250)$^{0.25}$ μmol/min, Km3(GSH) = 3.3 mM, Km3(NO) = 0.05 mM, Vmax3lu = 0.4*CPlu*Vlu/(BW/250)$^{0.25}$ μmol/min. MPl = 14.5 mg/mL, MPlu = 3.0 mg/mL, CPl = 58 mg/mL, CPlu = 54 mg/mL tissue.

TABLE E.4.6. PBPK-RT MODEL PARAMETERS FOR BD/BMO: 0-5 YEARS OF AGE

Tissue/Compartment	Volume, Vi L	Flow, Qi, L/hr	Partition, Pi tissue/blood BD/BMO	Metabolism
Fat , f	(1)	0.053*Qtot	118.2/1.808	
Liver, l	(2)	0.0795*Qtot	5.49/0.654	Vmax1, Km1, Vmax2, Km2, Km2ih,VmaxG, KmG_{GSH}, KmG_{BMO} Vmax3, Km3
Muscle, m	(3)	0.03*Qtot	5.26/0.653	
VRG, vrg	BW – (Vf+Vl+Vm +Vlu+Vblood)	Qtot – (Qf + Ql + Qm)	5.34/0.635	
Vblood, blood	0.075*BW			
Lung, Vlu	(4)	Qtot		Vmaxlu, Kmlu, K1, K2, Vmax3lu
Lung URT, Vua	0.0026*Vlu	0.0025*Qtot		
Lung CA,Vca	0.018*Vlu	0.0075*Qtot		
Lung TB,Vtb	0.043*Vlu	0.0067*Qtot		
Lung PU, Vpu	0.9378Vlu	0.983*Qtot		
Alveolar ventilation, Qalv		0.82*Qtot		
Cardiac Output, Qtot		(5)		
Blood:Air, Pb			1.5/60	
Body weight, BW	(6)			

Age (yr)-specific regressions : (1) Vf = 0.0162*Age^5 – 1.9784*Age^4 + 51.963*Age^3 – 459.38*Age^2 +1566.8*Age + 1004.2; (2)Vl = 0.0072*Age^5 – 0.3975*Age^4 + 7.9052*Age^3 – 65.624*Age^2 + 262.02*Age + 157.52; (3) Vm = -0.0623*Age^5 + 2.3433*Age^4 – 26.559*Age^3 + 144.75*Age^2 + 339.84*Age + 1648.2; (4) Vlu = -0.0346*Age^4 + 1.5069*Age^3 – 20.31*Age^2 + 123.99*Age + 59.213; (5) Qtot = (0.012*Age^3 – 1.2144*Age^2 + 40.324*Age + 44.414)8(1000/60); (6) BW = -1.9*Age^4 + 72.8*Age^3 – 813.1*Age^2 + 5535.6*Age + 4453.7; For 7-10 yr model Qf = 0.05*Qtot; Ql = 0.118*Qtot; Qm = 0.045*Qtot; Qkid = 0.12*Qtot; For 11-18yr model Qf = 0.044*Qtot; Ql = 0.136*Qtot; Qm = 0.068*Qtot; Qkid = 0.136*Qtot. For adult Qf = 0.052*Qtot; Ql = 0.26*Qtot; Qm = 0.1648Qtot; Qkid = 0.26Qtot. Vmax1 (P450) = 7.08E-2*MPl*Vl*$(7E4/BW)^{0.25}$ /60 µmol/min; Km1 = 0.00514mM; Vmaxlu (P450) = 9.09E-3*MPlu*Vlu*$(7E4/BW)^{0.25}$ /60 µmol/min, Kmlu = 0.002mM. Vmax2 (Epoxide Hydrolase) = 1.1*MPl*Vl*$(7E4/BW)^{0.25}$/60 µmol/min, Km2 = 0.58 mM, Km2ih = 0.116 mM, K1 = 0.1914*Vlu*Mplu*$(7E4/BW)^{-0.25}$/60 µmol/min. VmaxGl (GST) = 2.71*CPl*Vl*$(7E4/BW)^{0.25}$/60 µmol/min, Km3G(GSH) = 0.1 mM, KmG(BMO) = 10.4 mM, K2 (GST) =0.1536*Vlu*Cplu*$(7E4/BW)^{-0.25}$/60 µmol/min. Vmax3(P450) = 14.8*Vl*MPl*$(7E4/BW)^{0.25}$/60 µmol/min , Vmax3lu = 1.7*Vlu*Cplu*$(7E4/BW)^{0.25}$/60 µmol/min. MPl = 14.5 mg/mL, MPlu = 3.0 mg/mL, CPl = 58 mg/mL, CPlu = 54 mg/mL tissue.

TABLE E.4.7. PBPK MODEL PARAMETERS FOR BD/BMO/DEB: 0-5 YEARS OF AGE

Tissue/ Compartment	Volume, Vi L	Flow, Qi, L/hr	Partition, Pi tissue/blood BD/BMO/DEB	Metabolism
Fat , f	(1)	0.053*Qtot	118.2/1.808/0.715	
Liver, l	(2)	0.0795*Qtot	5.49/0.6545/0.7	Vmaxl, Km,Vmaxl1 Km1, Km1ih, Vmaxl2, $Km2_{GSH}$, $Km2_{BMO}$ Vmaxl3, Km3, Km3ih, Ke
Muscle, m	(3)	0.03*Qtot	5.26/0.6533/0.697	
VRG, kvrg	BW – (Vf+Vl+Vm+Vlu)	Qtot – (Qf + Ql + Qm)	5.34/0.6348/0.6	
Lung, Vlu	(4)	Qtot	4.02/0.4725/0.6	Vmaxlu, Kmlu, K1, K2,Ke
Lung Alveoli, Valv	0.9*Vlu	0.93*Qtot		Vmax3pu
Lung bronchi,Vbr	0.1*Vlu	0.07*Qtot		Vmax3br
Alveolar ventilation, Qalv		0.82*Qtot		
Cardiac Output, Qtot		(5)		
Blood:Air, Pb			1.5/60/300	
Body weight, BW	(6)			

Age (yr)-specific regressions : (1) Vf = (0.0162*Age^5 – 1.9784*Age^4 + 51.963*Age^3 – 459.38*Age^2 +1566.8*Age + 1004.2)/1000; (2)Vl = (0.0072*Age^5 – 0.3975*Age^4 + 7.9052*Age^3 – 65.624*Age^2 + 262.02*Age + 157.52)/1000; (3) Vm = (-0.0623*Age^5 + 2.3433*Age^4 – 26.559*Age^3 + 144.75*Age^2 + 339.84*Age + 1648.2)/1000; (4) Vlu = (-0.0346*Age^4 + 1.5069*Age^3 – 20.31*Age^2 + 123.99*Age + 59.213)/1000; (5) Qtot = 0.012*Age^3 – 1.2144*Age^2 + 40.324*Age + 44.414; (6) BW = (-1.9*Age^4 + 72.8*Age^3 – 813.1*Age^2 + 5535.6*Age + 4453.7)/1000; For 7-10 yr model Qf = 0.05*Qtot; Ql = 0.118*Qtot; Qm = 0.045*Qtot; Qkid = 0.12*Qtot; For 11-18yr model Qf = 0.044*Qtot; Ql = 0.136*Qtot; Qm = 0.068*Qtot; Qkid = 0.136*Qtot. For adult Qf = 0.052*Qtot; Ql = 0.26*Qtot; Qm = 0.1648Qtot; Qkid = 0.26Qtot. Vmax1 (P450) = 7.08E-8*$(70/BW)^{0.25}$mol/hr/mg MPl/Ltissue; Km1 = 5.14E-6M; Vmaxlu = 9.0E-9*$(70/BW)^{0.25}$ mol/hr/mg MPlu/Ltissue, Kmlu = 2.0E-6M . Vmaxl1(EH) = 1.1E-6*$(70/BW)^{0.25}$ mol/hr/mg MPl/Ltissue, Km1 = 5.8E-4 M, Km1ih = 1.16E-4 M, K1 = 0.1914*$(70/BW)^{-0.25}$ mol/hr/mg MPlu/Ltissue. Vmaxl2 = 2.71E-6*$(70/BW)^{0.25}$ mol/hr/mg CPl/Ltissue, $Km2_{GST}$ = 1.04E-2M. $Km2_{BMO}$ = 1.0E-4M, K2 = 0.1536*$(70/BW)^{-0.25}$ mol/hr/mg CPlu/Ltissue. Vmaxl3 (P450) = 1.48E-5*$(70/BW)^{0.25}$ mol/hr, Km3 = 1.56E-5M, Km3ih = 3.12E-6M, Vmax3pu = 1.7E-6*$(70/BW)^{0.25}$ mol/hr, Vmax3br = 2.0E-7*$(70/BW)^{0.25}$ mol/hr. Ke(DEB elimination) = 0.6*$(70/BW)^{-0.25}$ /hr

TABLE E.4.8. PBPK-RT MODEL PARAMETERS FOR STYRENE/SO: 0-5 YEARS OF AGE

Tissue/ Compartment	Volume, Vi L	Flow, Qi, L/hr	Partition, Pi tissue/blood ST/SO	Metabolism
Fat , f	(1)	0.053*Qtot	93.8/6.1	
Liver, l	(2)	0.0795*Qtot	2.71/2.6	Vmax1, Km, Vmax2, Km2,VmaxG, KmG_{GSH}, KmG_{SO}
Muscle, m	(3)	0.0304*Qtot	1.96/1.5	
VRG, vrg	BW – (Vf+Vl+Vm+ Vlu+Vblood)	Qtot – (Qf + Ql + Qm)	2.60/0.6	
Vblood, blood	0.075*BW			
Lung, Vlu	(4)	Qtot		
Lung URT, Vua	0.0026*Vlu	0.0025*Qtot		Vmaxua, Kmlu, Vmaxua2, Kmlu2, VmaxGua, KmG_{GSH}, Km_{GSO}
Lung CA,Vca	0.018*Vlu	0.0075*Qtot		
Lung TB,Vtb	0.043*Vlu	0.0067*Qtot		Vmaxtb, Kmlu, Vmaxtb2, Kmlu2, VmaxGtb, KmG_{GSH}, Km_{GSO}
Lung PU, Vpu	0.9378Vlu	0.983*Qtot		
Alveolar ventilation, Qalv		0.82*Qtot		
Cardiac Output, Qtot		(5)		
Blood:Air, Pb			48/2000	
Body weight, BW	(6)			

Age (yr)-specific regressions : (1) Vf = 0.0162*Age^5 – 1.9784*Age^4 + 51.963*Age^3 – 459.38*Age^2 +1566.8*Age + 1004.2; (2)Vl = 0.0072*Age^5 – 0.3975*Age^4 + 7.9052*Age^3 – 65.624*Age^2 + 262.02*Age + 157.52; (3) Vm = -0.0623*Age^5 + 2.3433*Age^4 – 26.559*Age^3 + 144.75*Age^2 + 339.84*Age + 1648.2; (4) Vlu = -0.0346*Age^4 + 1.5069*Age^3 – 20.31*Age^2 + 123.99*Age + 59.213; (5) Qtot = (0.012*Age^3 – 1.2144*Age^2 + 40.324*Age + 44.414)8(1000/60); (6) BW = -1.9*Age^4 + 72.8*Age^3 – 813.1*Age^2 + 5535.6*Age + 4453.7; For 7-10 yr model Qf = 0.05*Qtot; Ql = 0.118*Qtot; Qm = 0.045*Qtot; Qkid = 0.12*Qtot; For 11-18yr model Qf = 0.044*Qtot; Ql = 0.136*Qtot; Qm = 0.068*Qtot; Qkid = 0.136*Qtot. For adult Qf = 0.052*Qtot; Ql = 0.26*Qtot; Qm = 0.1648Qtot; Qkid = 0.26Qtot. Vmax1 (P450) = $0.033*(7E4/BW)^{0.25}$ µmol/min/mLtissue; Kmlu = 0.01mM; Vmaxua = Vmaxtb (P450) = $4.17E\text{-}5*(7E4/BW)^{0.25}$ µmol/min/mLtissue, Kmlu = 0.0175mM. Vmax2 (Epoxide Hydrolase) = $0.075*(7E4/BW)^{0.25}$ µmol/min/mLtissue, Km2 = 0.01 mM. Vmaxua2 = Vmaxtb2 = $0.0112*(7E4/BW)^{0.25}$ µmol/min/mLtissue, Kmlu2 = 0.0156 mM. VmaxGl (GST) = $0.467*(7E4/BW)^{0.25}$ µmol/min/mLtissue, KmG_{GSH} = 0.1 mM, KmG_{SO} = 2.5 mM, VmaxGua = VmaxGtb = $1.36*(7E4/BW)^{0.25}$ µmol/min/mLtissue.

TABLE E.4.9. PBPK MODEL PARAMETERS FOR VINYL CHLORIDE: 0-5 YEARS OF AGE

Tissue/Compartment	Volume, Vi L	Flow, Qi, L/hr	Partition, Pi tissue/blood	Metabolism
Fat , f	(1)	0.053*Qtot	20.7	
Liver, l	(2)	0.0795*Qtot	1.45	Vmax1, Km1, Vmax2 Km2
Muscle, m	(3)	0.03*Qtot	0.83	
VRG, kvrg	BW – (Vf+Vl+Vm +Vlu)	Qtot – (Qf + Ql + Qm)	1.45	
Lung, Vlu	(4)	Qtot		
Lung alveoli, Valv	0.9*Vlu	0.93*Qtot	1.45	
Lung bronchi,Vbr	0.1*Vlu	0.07*Qtot	1.45	
Alveolar ventilation, Qalv		0.82*Qtot		
Cardiac Output, Qtot		(5)		
Blood:Air, Pb			1.16	
Body weight, BW	(6)			

Age (yr)-specific regressions : (1) Vf = (0.0162*Age^5 – 1.9784*Age^4 + 51.963*Age^3 – 459.38*Age^2 +1566.8*Age + 1004.2)/1000; (2)Vl = (0.0072*Age^5 – 0.3975*Age^4 + 7.9052*Age^3 – 65.624*Age^2 + 262.02*Age + 157.52)/1000; (3) Vm = (-0.0623*Age^5 + 2.3433*Age^4 – 26.559*Age^3 + 144.75*Age^2 + 339.84*Age + 1648.2)/1000; (4) Vlu = (-0.0346*Age^4 + 1.5069*Age^3 – 20.31*Age^2 + 123.99*Age + 59.213)/1000; (5) Qtot = 0.012*Age^3 – 1.2144*Age^2 + 40.324*Age + 44.414; (6) BW = (-1.9*Age^4 + 72.8*Age^3 – 813.1*Age^2 + 5535.6*Age + 4453.7)/1000; For 7-10 yr model Qf = 0.05*Qtot; Ql = 0.118*Qtot; Qm = 0.045*Qtot; Qkid = 0.12*Qtot; For 11-18yr model Qf = 0.044*Qtot; Ql = 0.136*Qtot; Qm = 0.068*Qtot; Qkid = 0.136*Qtot. For adult Qf = 0.052*Qtot; Ql = 0.26*Qtot; Qm = 0.1648Qtot; Qkid = 0.26Qtot. Vmax1 = $4.0*BW^{0.75}$mg/hr; Km1 = 1.0 mg/L; Vmax2 = $0.1*BW^{0.75}$ mg/hr, Km2 = 10 mg/L .

TABLE E.4.10. PBPK MODEL PARAMETERS FOR TCE AND METABOLITES: 0-5 YEARS OF AGE

Tissue/Compartment	Volume, Vi L	Flow, Qi, L/hr	Partition, Pi tissue/blood TCE/CH/TCA/ TCOH/TCOG	Metabolism
Fat, f	(1)	0.053*Qtot	36.38/	
Liver, l	(2)	0.0795*Qtot	1.73/1.42/1.18/ 1.30/0.56	Vmax1, Km1 PTCA, PTCOH, KTCA, Vmax2,Km2
Muscle, m	(3)	0.03*Qtot	2.36/	
VRG, vrg	(4)	0.674*Qtot	1.73/	
Lung, Vlu	(5)	Qtot	2.61/1.65/0.54/ 0.78/1.06	
Kidneys, kid	(6)	0.164*Qtot	2.07/0.98/0.74/ 1.02/1.44	
Lung Alveoli, Valv	0.9*Vlu	0.93*Qtot	2.61/1.65/0.54/ 0.78/1.06	
Lung bronchi,Vbr	0.1*Vlu	0.07*Qtot	2.61/1.65/0.54/ 0.78/1.06	
Body (metabolite submodels)			/1.35/0.88/1.11/ 1.11	
Alveolar ventilation, Qalv		0.8*Qtot		
Cardiac Output, Qtot		(7)		
Blood:Air, Pb			15.91/	
Body weight, BW	(8)			

Age (yr)-specific regressions : (1) Vf = (0.0162*Age^5 – 1.9784*Age^4 + 51.963*Age^3 – 459.38*Age^2 +1566.8*Age + 1004.2)/1000; (2)Vl = (0.0072*Age^5 – 0.3975*Age^4 + 7.9052*Age^3 – 65.624*Age^2 + 262.02*Age + 157.52)/1000; (3) Vm = (-0.0623*Age^5 + 2.3433*Age^4 – 26.559*Age^3 + 144.75*Age^2 + 339.84*Age + 1648.2)/1000; (4) Vkid = (9.373E-4*Age^5 – 0.0569*Age^4 + 1.1729*Age^3 – 10.34*Age^2 + 44.604*Age + 28.291)/1000; (5) Vlu = (-0.0346*Age^4 + 1.5069*Age^3 – 20.31*Age^2 + 123.99*Age + 59.213)/1000; (6)Vkid = (9.373E-4*Age^5 – 0.0569*Age^4 + 1.1729*Age^3 – 10.34*Age^2 + 44.604*Age + 28.291)/1000 (7) Qtot = 0.012*Age^3 – 1.2144*Age^2 + 40.324*Age + 44.414; (8) BW = (-1.9*Age^4 + 72.8*Age^3 – 813.1*Age^2 + 5535.6*Age + 4453.7)/1000; For 7-10 yr model Qf = 0.05*Qtot; Ql = 0.118*Qtot; Qm = 0.045*Qtot; Qkid = 0.12*Qtot; For 11-18yr model Qf = 0.044*Qtot; Ql = 0.136*Qtot; Qm = 0.068*Qtot; Qkid = 0.136*Qtot. For adult Qf = 0.052*Qtot; Ql = 0.26*Qtot; Qm = 0.1648Qtot; Qkid = 0.26Qtot. Vmax1 (TCE→CH) = 2.49E-4*$BW^{0.75}$ mol/hr, Km1 = 3.51E-5M; Vmax2 (TCOH→TCOG) = 1.11E-4*$BW^{0.75}$ mol/hr, Km2 = 1.06E-4M. PTCA(CH→TCA) = 115*BW /hr; PTCOH (CH→TCOH) = 309*BW /hr; KTCA (TCOH→TCA) = 10 /hr. Urinary excretion rates /hr: KU_{TCA} = 1.55*BW; KU_{TCOH} = 1.14*BW; KU_{TCOG} = 32.8*BW. CH = chloral hydrate; TCA = trichloroacetic acid; TCOH = trichloroethanol; TCOG = trichloroethanol glucuronide.

TABLE E.4.11. PBPK MODEL PARAMETERS FOR DCM: 0-5 YEARS OF AGE

Tissue/Compartment	Volume, Vi L	Flow, Qi, L/hr	Partition, Pi tissue/blood	Metabolism
Fat, f	(1)	0.053*Qtot	7.239	
Liver, l	(2)	0.0795*Qtot	0.824	Vmax1, Km, Kfl
Muscle, m	(3)	0.03*Qtot	1.09	
VRG, vrg	(4)	0.674*Qtot	0.788	
Lung, Vlu	(5)	Qtot	0.552	
Lung Alveoli, Valv	0.9*Vlu	0.93*Qtot	0.552	Vmaxpu, Km, Kfpu
Lung bronchi,Vbr	0.1*Vlu	0.07*Qtot	0.552	Vmaxbr, Km, Kfbr
Alveolar ventilation, Qalv		0.8*Qtot		
Cardiac Output, Qtot		(6)		
Blood:Air, Pb			9.09	
Body weight, BW	(7)			

Age (yr)-specific regressions : (1) Vf = (0.0162*Age^5 – 1.9784*Age^4 + 51.963*Age^3 – 459.38*Age^2 +1566.8*Age + 1004.2)/1000; (2)Vl = (0.0072*Age^5 – 0.3975*Age^4 + 7.9052*Age^3 – 65.624*Age^2 + 262.02*Age + 157.52)/1000; (3) Vm = (-0.0623*Age^5 + 2.3433*Age^4 – 26.559*Age^3 + 144.75*Age^2 + 339.84*Age + 1648.2)/1000; (4) Vkid = (9.373E-4*Age^5 – 0.0569*Age^4 + 1.1729*Age^3 – 10.34*Age^2 + 44.604*Age + 28.291)/1000; (5) Vlu = (-0.0346*Age^4 + 1.5069*Age^3 – 20.31*Age^2 + 123.99*Age + 59.213)/1000; (6) Qtot = 0.012*Age^3 – 1.2144*Age^2 + 40.324*Age + 44.414; (7) BW = (-1.9*Age^4 + 72.8*Age^3 – 813.1*Age^2 + 5535.6*Age + 4453.7)/1000; For 7-10 yr model Qf = 0.05*Qtot; Ql = 0.118*Qtot; Qm = 0.045*Qtot; Qkid = 0.12*Qtot; For 11-18yr model Qf = 0.044*Qtot; Ql = 0.136*Qtot; Qm = 0.068*Qtot. For adult Qf = 0.052*Qtot; Ql = 0.26*Qtot; Qm = 0.1648Qtot; Qkid = 0.26Qtot. Vmaxl (P450) = 8.58E-5*$BW^{0.7}$ mol/hr; Vmaxpu = 0.9*1.46E-3*Vmaxl; Vmaxbr = 0.1*1.46E-3*Vmaxl, Km = 8.7E-6M; Kfl (GST) = 1.26*$BW^{-0.3}$, Kfpu = 0.9*0.242*Kfl, Kfbr = 0.1*0.242*Kfl

TABLE E.4.12. PBPK MODEL PARAMETERS FOR ETHYLENE/EO: 0-6 YEARS OF AGE

Tissue/Compartment	Volume, Vi L	Flow, Qi, L/hr	Partition, Pi tissue/blood ET/EO	Metabolism
Fat , f	(1)	0.053*Qtot	8.73/0.70	
Liver, l	(2)	0.0795*Qtot	2.05/0.89	ClrET ClrEO L/hr
Muscle, m	(3)	0.0304*Qtot	2.95/1.08	
VRG, vrg	BW – (Vf+Vl+Vm +Vlu+Vblood)	Qtot – (Qf + Ql + Qm)	2.18/1.03	
Vlubld, lung blood	0.0079*BW			
Vart, arterial blood	0.0178*BW			
Vven, venous blood	0.0533*BW			
Alveolar ventilation, Qalv		0.82*Qtot		
Cardiac Output, Qtot		(4)		
Blood:Air, Pb			0.22/61	
Body weight, BW	(5)			

Age (yr)-specific regressions : (1) Vf =(0.0162*Age^5 – 1.9784*Age^4 + 51.963*Age^3 – 459.38*Age^2 +1566.8*Age + 1004.2)/1000; (2)Vl = (0.0072*Age^5 – 0.3975*Age^4 + 7.9052*Age^3 – 65.624*Age^2 + 262.02*Age + 157.52)/1000; (3) Vm = (-0.0623*Age^5 + 2.3433*Age^4 – 26.559*Age^3 + 144.75*Age^2 + 339.84*Age + 1648.2)/1000; (4) Qtot = 0.012*Age^3 – 1.2144*Age^2 + 40.324*Age + 44.414; (5) BW = (-1.9*Age^4 + 72.8*Age^3 – 813.1*Age^2 + 5535.6*Age + 4453.7)/1000; For 7-10 yr model Qf = 0.05*Qtot; Ql = 0.118*Qtot; Qm = 0.045*Qtot; For 11-18yr model Qf = 0.044*Qtot; Ql = 0.136*Qtot; Qm = 0.068*Qtot; Qkid = 0.136*Qtot. For adult Qf = 0.052*Qtot; Ql = 0.26*Qtot; Qm = 0.1648Qtot; Qkid = 0.26Qtot. Metabolic clearance by liver: ClrET (P450) = $74.9*(70/BW)^{0.25}$ L/hr; ClrEO (EH+GST) = $1.53*(70/BW)^{0.25}$ L/hr. (Csanady et al., 2000; Price et al., 2003)

TABLE E.4.13. PBPK-RT MODEL PARAMETERS FOR STYRENE/SO ADULT

Tissue/Compartment	Volume, Vi L	Flow, Qi, L/hr	Partition, Pi tissue/blood ST/SO	Metabolism
Fat , f	0.19*BW	0.05*Qtot	93.8/6.1	
Liver, l	0.026*BW	0.26*Qtot	2.71/2.6	Vmaxl1, Kml1, Vmaxl2, Kml2eh, Kml2appVmaxl3, $Kml3_{GSH}$, $Kml3_{SO}$ Kdl
Muscle, m	0.541*BW	0.25*Qtot	1.96/1.5	
VRG, vrg	BW – (Vf+Vl+Vm +Vlu+Vblood)	Qtot – (Qf + Ql + Qm)	2.60/2.6	
Vlubld, lung blood	0.0079*BW			
Vart, arterial blood	0.0178*BW			
Vven, venous blood	0.0533*BW			
Lung tissue, Vlu	0.0076*BW			
Vluc, conducting zone, fs = 0.1	fs*Vlu			Vmaxlu1, Kmlu1, Vmaxlu2, Kmlu2, Vmaxlu3, $Kmlu3_{GSH}$, Kmlu3, $Kdlu_{SO}$
Vlua, alveolar zone	(1-fs)*Vlu			Vmaxlu1, Kmlu1, Vmaxlu2, Kmlu2, Vmaxlu3, $Kmlu3_{GSH}$, Kmlu3, $Kdlu_{SO}$
Alveolar ventilation, Qalv , L/hr		300		
Cardiac Output, Qtot , L/hr		372		
Blood:Air, Pb			70/2370	
Body weight, BW kg	70			

Vmaxl = 0.002 mmol/hr/mL tissue, Kml1 = 0.01 mM; Vmaxl2 = 0.0045, Kml2eh = 0.001, Kml2app = 0.01; Vmaxl3 = 0.028, Kml3G = 0.1, Kml3so = 2.5,Kdl = 0.2; Vmaxlu1 = 2.5E-6, Kmlu1 = 0.0175; Vmaxlu2 = 6.73E-4, Kmlu2 = 0.0156; Vmaxlu3 = 0.082, Kmlu3 = 0.082; Kmlu3G = 0.1, Kmlu3so = 2.5, Kdlu = 2.0. (Csanady et al., 2003)

TABLE E.4.14. PBPK MODEL PARAMETERS FOR CARBON TETRACHLORIDE: 0-6 YEARS OF AGE

Tissue/Compartment	Volume, Vi L	Flow, Qi, L/hr	Partition, Pi tissue/blood	Metabolism
Fat , f	(1)	0.053*Qtot	79.4	
Liver, l	(2)	0.0795*Qtot	3.14	Vmax1, Km
Muscle, m	(3)	0.03*Qtot	1.00	
VRG, vrg	BW – (Vf+Vl+Vm+Vlu)	Qtot – (Qf + Ql + Qm)	1.00	
Lung, Vlu	(4)	Qtot		
Lung alveoli, Valv	0.9*Vlu	0.93*Qtot	1.00	
Lung bronchi,Vbr	0.1*Vlu	0.07*Qtot	1.00	
Alveolar ventilation, Qalv		Qtot		
Cardiac Output, Qtot		(5)		
Blood:Air, Pb			4.52	
Body weight, BW	(6)			

Age (yr)-specific regressions : (1) Vf = (0.0162*Age^5 – 1.9784*Age^4 + 51.963*Age^3 – 459.38*Age^2 +1566.8*Age + 1004.2)/1000; (2)Vl = (0.0072*Age^5 – 0.3975*Age^4 + 7.9052*Age^3 – 65.624*Age^2 + 262.02*Age + 157.52)/1000; (3) Vm = (-0.0623*Age^5 + 2.3433*Age^4 – 26.559*Age^3 + 144.75*Age^2 + 339.84*Age + 1648.2)/1000; (4) Vlu = (-0.0346*Age^4 + 1.5069*Age^3 – 20.31*Age^2 + 123.99*Age + 59.213)/1000; (5) Qtot = 0.012*Age^3 – 1.2144*Age^2 + 40.324*Age + 44.414; (6) BW = (-1.9*Age^4 + 72.8*Age^3 – 813.1*Age^2 + 5535.6*Age + 4453.7)/1000; For 7-10 yr model Qf = 0.05*Qtot; Ql = 0.118*Qtot; Qm = 0.045*Qtot; Qkid = 0.12*Qtot; For 11-18yr model Qf = 0.044*Qtot; Ql = 0.136*Qtot; Qm = 0.068*Qtot; Qkid = 0.136*Qtot. For adult Qf = 0.052*Qtot; Ql = 0.26*Qtot; Qm = 0.1648Qtot; Qkid = 0.26Qtot. Vmax1 = 1.35E-7*$BW^{0.75}$mol/hr; Km = 5.68E-5 mol/L. 23.0 mg MP/mL liver tissue. (Thrall et al., 2000; Price et al., 2003)

TABLE E.4.15: PBPK MODEL PARAMETERS FOR TOLUENE 0-6 YEARS OF AGE

Tissue/Compartment	Volume, Vi L	Flow, Qi, L/hr	Partition, Pi tissue/blood	Metabolism
Fat , f	(1)	0.053*Qtot	65.8	
Liver, l	(2)	0.0795*Qtot	2.98	Vmax1, Km, Ki
Muscle, m	(3)	0.03*Qtot	1.37	
VRG, vrg	BW – (Vf+Vl+Vm+Vlu)	Qtot – (Qf + Ql + Qm)	2.66	
Lung, Vlu	(4)	Qtot		
Lung alveoli, Valv	0.9*Vlu	0.93*Qtot	2.66	
Lung bronchi,Vbr	0.1*Vlu	0.07*Qtot	2.66	
Alveolar ventilation, Qalv		Qtot		
Cardiac Output, Qtot		(5)		
Blood:Air, Pb			15.6	
Body weight, BW	(6)			

Age (yr)-specific regressions : (1) Vf = (0.0162*Age^5 – 1.9784*Age^4 + 51.963*Age^3 – 459.38*Age^2 +1566.8*Age + 1004.2)/1000; (2)Vl = (0.0072*Age^5 – 0.3975*Age^4 + 7.9052*Age^3 – 65.624*Age^2 + 262.02*Age + 157.52)/1000; (3) Vm = (-0.0623*Age^5 + 2.3433*Age^4 – 26.559*Age^3 + 144.75*Age^2 + 339.84*Age + 1648.2)/1000; (4) Vlu = (-0.0346*Age^4 + 1.5069*Age^3 – 20.31*Age^2 + 123.99*Age + 59.213)/1000; (5) Qtot = 0.012*Age^3 – 1.2144*Age^2 + 40.324*Age + 44.414; (6) BW = (-1.9*Age^4 + 72.8*Age^3 – 813.1*Age^2 + 5535.6*Age + 4453.7)/1000; For 7-10 yr model Qf = 0.05*Qtot; Ql = 0.118*Qtot; Qm = 0.045*Qtot; Qkid = 0.12*Qtot; For 11-18yr model Qf = 0.044*Qtot; Ql = 0.136*Qtot; Qm = 0.068*Qtot; Qkid = 0.136*Qtot. For adult Qf = 0.052*Qtot; Ql = 0.26*Qtot; Qm = 0.1648Qtot; Qkid = 0.26Qtot. Vmax1 = 5.2E-5*BW*(70/BW)^0.25 mol/hr; Km = 5.97E-6 M, Ki = 3.8E-6 M. (Tardif et al., 1995; Price et al., 2003)

TABLE E.4.16. PBPK MODEL PARAMETERS FOR XYLENE: 0-6 YEARS OF AGE

Tissue/Compartment	Volume, Vi L	Flow, Qi, L/hr	Partition, Pi tissue/blood	Metabolism
Fat , f	(1)	0.053*Qtot	77.8	
Liver, l	(2)	0.0795*Qtot	3.02	Vmax1, Km, Ki
Muscle, m	(3)	0.03*Qtot	3.00	
VRG, vrg	BW – (Vf+Vl+Vm+ Vlu)	Qtot – (Qf + Ql + Qm)	4.42	
Lung, Vlu	(4)	Qtot		
Lung alveoli, Valv	0.9*Vlu	0.93*Qtot	4.42	
Lung bronchi,Vbr	0.1*Vlu	0.07*Qtot	4.42	
Alveolar ventilation, Qalv		Qtot		
Cardiac Output, Qtot		(5)		
Blood:Air, Pb			26.4	
Body weight, BW	(6)			

Age (yr)-specific regressions: (1) Vf = (0.0162*Age^5 – 1.9784*Age^4 + 51.963*Age^3 – 459.38*Age^2 +1566.8*Age + 1004.2)/1000; (2)Vl = (0.0072*Age^5 – 0.3975*Age^4 + 7.9052*Age^3 – 65.624*Age^2 + 262.02*Age + 157.52)/1000; (3) Vm = (-0.0623*Age^5 + 2.3433*Age^4 – 26.559*Age^3 + 144.75*Age^2 + 339.84*Age + 1648.2)/1000; (4) Vlu = (-0.0346*Age^4 + 1.5069*Age^3 – 20.31*Age^2 + 123.99*Age + 59.213)/1000; (5) Qtot = 0.012*Age^3 – 1.2144*Age^2 + 40.324*Age + 44.414; (6) BW = (-1.9*Age^4 + 72.8*Age^3 – 813.1*Age^2 + 5535.6*Age + 4453.7)/1000; For 7-10 yr model Qf = 0.05*Qtot; Ql = 0.118*Qtot; Qm = 0.045*Qtot; Qkid = 0.12*Qtot; For 11-18yr model Qf = 0.044*Qtot; Ql = 0.136*Qtot; Qm = 0.068*Qtot; Qkid = 0.136*Qtot. For adult Qf = 0.052*Qtot; Ql = 0.26*Qtot; Qm = 0.1648Qtot; Qkid = 0.26Qtot. Vmax1 = 7.9E-5*BW*(70/BW)^0.25 mol/hr; Km = 1.88E-6 M, Ki = 5.6E-6. (Tardif et al., 1995; Price et al., 2003)

E.5 Toxicokinetics: Berkeley Madonna Model Codes

This section provides PBPK model code for a selection of the chemicals studied. The models follow a standard format although the order is not critical for Berkeley Madonna (A = mass, Q = flow rate, V = volume, P = partition coefficient, Cv = concentration leaving the tissue, f= fat, l = liver, m = muscle (vessel poor tissues), vrg = vessel rich group of tissues, lu = lung, br = bronchi, pu = alveoli, BW = body weight = volume at 1 kg/L, Amet = amount metabolized)

E.5.1 Model Code for Furan 0-5 yr child

```
METHOD Stiff

STARTTIME = 0
STOPTIME= 48
DT = 0.001
{furan moles}
init Af = 0
init Al = 0
init Am = 0
init Avrg = 0
init Abr = 0
init Apu = 0
init Abrain = 0
{moles furan metabolized}
init Ametl = 0
init Ametlg = 0
Init AUCbrain = 0
{tissue flows L/hr}
Qtot = 0.012*Age^3 - 1.2144*Age^2 + 40.324*Age + 44.414
Qalv = 0.8*Qtot
Qf = 0.053*Qtot
Ql = 0.0795*Qtot
Qm = 0.03*Qtot
Qvrg = Qtot - (Qf + Ql + Qm + Qbrain)
Qpu = 0.93*Qtot
Qbr = 0.07*Qtot
Qbrain = -0.0024*Age^4 + 0.1305*Age^3 - 2.4822*Age^2 + 18.025*Age + 15.197
{tissue volumes L}
Vf = (0.0162*Age^5 - 1.9784*Age^4 + 51.963*Age^3 - 459.38*Age^2 + 1566.8*Age + 1004.2)/1000
Vl = (0.0072*Age^5 - 0.3975*Age^4 + 7.9052*Age^3 - 65.624*Age^2 + 262.02*Age + 157.52)/1000
Vm = (-0.0623*Age^5 + 2.3433*Age^4 - 26.559*Age^3 + 144.75*Age^2 + 339.84*Age + 1648.2)/1000
Vvrg = BW - ( Vf + Vl + Vlu + Vbrain + Vm)
Vlu = (-0.0346*Age^4 + 1.5069*Age^3 - 20.31*Age^2 + 123.99*Age + 59.213)/1000
Vpu = 0.90*Vlu
```

```
Vbr = 0.10*Vlu
Vbrain = (1E4*((Age + 0.213)/(6.030 + 6.895*Age)))/1000
BW = (-1.9*Age^4 + 72.8*Age^3 - 813.1*Age^2 + 5535.6*Age + 4453.7)/1000
Age = 3.0
{blood/air and tissue/blood partition coefficients}
Pb = 2.47
Pl = 4.69
Pf = 33.39
Pm = 3.24
Pbrain = 8.82
Pvrg = 4.69
Ppu = 4.69
Pbr = 4.69
{metabolic parameters, E }
E = 1.0
{exposure in ppm converted to moles/L}
Cair = IF TIME <= 24 THEN 1*(1E-6/25.45) ELSE 0
{calculated concentrations of furan}
Cart = (Qpu*Cvpu + Qbr*Cvbr)/Qtot
Cvf = Af/(Vf*Pf)
Cvbrain = Abrain/(Vbrain*Pbrain)
Cvl = Al/(Vl*Pl)
Cvm = Am/(Vm*Pm)
Cvvrg = Avrg/(Vvrg*Pvrg)
Cvpu = Apu/(Vpu*Ppu)
Cvbr = Abr/(Vbr*Pbr)
Cvtot = (Ql*Cvl + Qf*Cvf + Qm*Cvm + Qvrg*Cvvrg + Qbrain*Cvbrain)/Qpu
Cvipu = (Qalv*Cair + Qpu*Cvtot)/((Qalv/Pb) + Qpu)
Cexh = Cvipu/Pb
{differential equations for furan uptake and metabolism}
d/dt(Abrain) = Qbrain*(Cart - Cvbrain)
d/dt(Apu) = Qpu*(Cvipu - Cvpu)
d/dt(Abr) = Qbr*(Cart - Cvbr)
d/dt(Al) = Ql*(Cart - Cvl)  - Cart*Ql*E
d/dt(Af) = Qf*(Cart - Cvf)
d/dt(Am) = Qm*(Cart - Cvm)
d/dt(Avrg) = Qvrg*(Cart - Cvvrg)
{amount of furan metabolized in the liver and AUC in brain}
d/dt(Ametl)  = Cart*Ql*E
d/dt(Ametlg) = Cart*Ql*E/BW
d/dt(AUCbrain) = Cvbrain
```

E.5.2 Model Code for MTBE 0-6 Yr Child

METHOD Stiff

```
STARTTIME = 0
STOPTIME= 48
DT = 0.001
{mtbe moles}
init Af = 0
init Al = 0
init Am = 0
init Avrg = 0
init Akid = 0
init Abr = 0
init Apu = 0
{moles mtbe metabolized}
init Amet1 = 0
init Amet2 = 0
{area under the venous blood concn x time curve, mtbe}
init AUCvtot = 0
init AUCvl = 0
init AUCvpu = 0
init AUCvbr = 0
init AUCvkid = 0
init AUCvvrg = 0
{tissue flows L/hr}
Qtot = 0.012*Age^3 - 1.2144*Age^2 + 40.324*Age + 44.414
Qalv = 0.8*Qtot
Qf = 0.053*Qtot
Ql = 0.0795*Qtot
Qm = 0.03*Qtot
Qkid = 0.164*Qtot
Qvrg = 0.674*Qtot
Qpu = 0.93*Qtot
Qbr = 0.07*Qtot
{tissue volumes L}
BW = (-1.9*Age^4 + 72.8*Age^3 - 813.1*Age^2 + 5535.6*Age + 4453.7)/1000
Vf = (0.0162*Age^5 - 1.9784*Age^4 + 51.963*Age^3 - 459.38*Age^2 + 1566.8*Age + 1004.2)/1000
Vl = (0.0072*Age^5 - 0.3975*Age^4 + 7.9052*Age^3 - 65.624*Age^2 + 262.02*Age + 157.52)/1000
Vm = (-0.0623*Age^5 + 2.3433*Age^4 - 26.559*Age^3 + 144.75*Age^2 + 339.84*Age + 1648.2)/1000
Vvrg = BW - (Vf + Vl + Vkid + Vm + Vlu)
Vkid = (9.737E-4*Age^5 - 0.0561*Age^4 + 1.1729*Age^3 - 10.34*Age^2 + 44.604*Age + 28.291)/1000
Vlu = (-0.0346*Age^4 + 1.5069*Age^3 - 20.31*Age^2 + 123.99*Age + 59.213)/1000
Vpu = 0.9*Vlu
Vbr = 0.1*Vlu
```

```
{blood/air and tissue/blood partition coefficients, mtbe}
Pb = 17.7
Pl = 0.723
Pf = 4.79
Pm = 1.181
Pkid = 0.723
Pvrg = 0.723
Ppu = 0.723
Pbr = 0.723
{mtbe metabolic parameters, mol/hr, mol/L}
Vmax1 = 3.38E-5*BW^0.75
Vmax2 = 6.2E-6*BW^0.75
Km1 = 6.17E-5
Km2 = 3.8E-6
{exposure in ppm converted to moles}
Cair = IF TIME <= 24 THEN 1*(1E-6/24.45) ELSE 0
Age = 0.0
{calculated concentrations of mtbe}
Cart = (Qpu*Cvpu + Qbr*Cvbr)/Qtot
Cvf = Af/(Vf*Pf)
Cvl = Al/(Vl*Pl)
Cvkid = Akid/(Vkid*Pkid)
Cvm = Am/(Vm*Pm)
Cvvrg = Avrg/(Vvrg*Pvrg)
Cvpu = Apu/(Vpu*Ppu)
Cvbr = Abr/(Vbr*Pbr)
Cvtot = (Ql*Cvl + Qf*Cvf + Qm*Cvm + Qvrg*Cvvrg + Qkid*Cvkid)/Qpu
Cvipu = (Qalv*Cair + Qpu*Cvtot)/((Qalv/Pb) + Qpu)
Cexh = Cvipu/Pb
{differential equations for mtbe uptake and metabolism}
d/dt(Apu) = Qpu*(Cvipu - Cvpu)
d/dt(Abr) = Qbr*(Cart - Cvbr)
d/dt(Al) = Ql*(Cart - Cvl) - Vmax1*Cvl/(Km1 + Cvl) - Vmax2*Cvl/(Km2 + Cvl)
d/dt(Af) = Qf*(Cart - Cvf)
d/dt(Akid) = Qkid*(Cart - Cvkid)
d/dt(Am) = Qm*(Cart - Cvm)
d/dt(Avrg) = Qvrg*(Cart - Cvvrg)
{amount of mtbe metabolized in liver by high and low affinity pathways}
d/dt(Amet1)  = Vmax1*(Al/Vl)/(Km1 + (Al/Vl))
d/dt(Amet2) = Vmax2*(Al/Vl)/(Km2 + (Al/Vl))
{AUCs for mtbe}
d/dt(AUCvtot) = Cvtot
d/dt(AUCvl) = Cvl
d/dt(AUCvpu) = Cvpu
```

```
d/dt(AUCvbr) = Cvbr
d/dt(AUCvkid) = Cvkid
d/dt(AUCvvrg) = Cvvrg
```

E.5.3 Model Code for PCE 0-6 yr Child

METHOD Stiff

```
STARTTIME = 0
STOPTIME= 240
DT = 0.001
{PCE moles}
init Af1 = 0
init Af2 = 0
init Al = 0
init Am = 0
init Abrain = 0
init Akid = 0
init Avrg = 0
init Abr = 0
init Apu = 0
init TCA = 0
init TCAurine = 0
{moles PCE metabolized}
init Amet1 = 0
{area under the venous blood concn x time curve, pce, TCA}
init AUCvtot = 0
init AUCvl = 0
init AUCTCA = 0
init AUCvbrain = 0
{tissue flows L/hr}
Qtot = 0.012*Age^3 - 1.2144*Age^2 + 40.324*Age + 44.414
Qalv = K*Qtot
K = 0.8
Qf1 = 0.043*Qtot
Qf2 = 0.01*Qtot
Ql = 0.0795*Qtot
Qm = 0.03*Qtot
Qkid = 0.08*Qtot
Qbrain = -0.0024*Age^4 + 0.1305*Age^3 - 2.4822*Age^2 + 18.025*Age + 15.197
Qvrg = Qtot - (Qf1 + Qf2 + Ql + Qm + Qkid + Qbrain)
Qpu = 0.93*Qtot
Qbr = 0.07*Qtot
```

```
{tissue volumes L}
BW = (-1.9*Age^4 + 72.8*Age^3 - 813.1*Age^2 + 5535.6*Age +4453.7)/1000
Vf = (0.0165*Age^5 - 1.9784*Age^4 + 51.963*Age^3 - 459.38*Age^2 + 1566.8*Age + 1004.2)/1000
Vf1 = 0.8*Vf
Vf2 = 0.2*Vf
Vl = (0.0072*Age^5 - 0.3975*Age^4 + 7.9052*Age^3 - 65.624*Age^2 + 262.02*Age + 157.52)/1000
Vm = (-0.0623*Age^5 + 2.3433*Age^4 - 26.559*Age^3 + 144.75*Age^2 + 339.84*Age + 1648.2)/1000
Vbrain = (1E4*(Age + 0.213)/(6.030 + 6.895*Age))/1000
Vkid = (9.737E-4*Age^5 - 0.0561*Age^4 + 1.1729*Age^3 - 10.34*Age^2 + 44.604*Age + 28.291)/1000
Vvrg = BW - (Vf + Vl + Vm + Vkid + Vbrain + Vlu)
Vlu = (-0.0346*Age^4 + 1.5069*Age^3 - 20.31*Age^2 + 123.99*Age + 59.213)/1000
Vpu = 0.9*Vlu
Vbr = 0.1*Vlu
{blood/air and tissue/blood partition coefficients, PCE}
Pb = 11.6
Pl = 5.27
Pf1 = 125.0
Pf2 = 125.0
Pbrain = 125.0
Pkid = 5.05
Pm = 6.1
Pvrg = 5.27
Ppu = 5.27
Pbr = 5.27
{PCE metabolic parameters, mol/hr, mol/L}
Vmax1 = 1.69E-6*BW^0.75
Km1= 4.6E-5
KeC = 0.05
Ke = KeC/BW^0.25
Ku = 0.5
{exposure in ppm converted to moles}
Cair = IF TIME <= 24 THEN 1*(1E-6/24.45) ELSE 0
Age = 0
{calculated concentrations of PCE}
Cart = (Qpu*Cvpu + Qbr*Cvbr)/Qtot
Cvf1 = Af1/(Vf1*Pf1)
Cvf2 = Af2/(Vf2*Pf2)
Cvl = Al/(Vl*Pl)
Cvbrain = Abrain/(Vbrain*Pbrain)
Cvkid = Akid/(Vkid*Pkid)
Cvm = Am/(Vm*Pm)
Cvvrg = Avrg/(Vvrg*Pvrg)
Cvpu = Apu/(Vpu*Ppu)
Cvbr = Abr/(Vbr*Pbr)
```

```
Cvtot = (Ql*Cvl + Qf1*Cvf1 + Qm*Cvm + Qvrg*Cvvrg + Qf2*Cvf2 + Qbrain*Cvbrain +
Qkid*Cvkid)/Qpu
Cvipu = (Qalv*Cair + Qpu*Cvtot)/((Qalv/Pb) + Qpu)
Cexh = Cvipu/Pb
Ctca = TCA/(BW*0.1)
{differential equations for pce uptake and metabolism}
d/dt(Apu) = Qpu*(Cvipu - Cvpu)
d/dt(Abr) = Qbr*(Cart - Cvbr)
d/dt(Al) = Ql*(Cart - Cvl) - Vmax1*Cvl/(Km1 + Cvl)
d/dt(Af1) = Qf1*(Cart - Cvf1)
d/dt(Af2) = Qf2*(Cart - Cvf2)
d/dt(Akid) = Qkid*(Cart - Cvkid)
d/dt(Abrain) = Qbrain*(Cart - Cvbrain)
d/dt(Am) = Qm*(Cart - Cvm)
d/dt(Avrg) = Qvrg*(Cart - Cvvrg)
d/dt(TCA) = 0.15*Vmax1*Cvl/(Km1 + Cvl)  - Ke*TCA - Ku*TCA
d/dt(TCAurine) = TCA*Ku
{amount of PCE metabolized in liver }
d/dt(Amet1)  = Vmax1*(Al/Vl)/(Km1 + (Al/Vl))
init Ametg = 0
d/dt(Ametg) = Amet1/BW
{AUCs for PCE}
d/dt(AUCvtot) = Cvtot
d/dt(AUCvl) = Cvl
d/dt(AUCTCA) = Ctca
d/dt(AUCvbrain) = Cvbrain
```

E.5.4 Model Code for BaP vapor 0-6 yr Child

METHOD Stiff

```
STARTTIME = 0
STOPTIME= 2880
DT = 0.001
{Alveolar compartments, moles}
init AAP = 0
init AAVA = 0
init AAV1 = 0
limit AAV1 >= 0
init AAV2 = 0
limit AAV2 >= 0
init AAVE = 0
limit AAVE >= 0
```

```
init AAVB = 0
limit AAVE >= 0
init Ameta1 = 0
init Ameta2 = 0
init LNth = 0
init AUCCalv = 0
{Bronchiolar compartments, moles}
init ABP = 0
init ABM = 0
init ABL1 = 0
init ABL2 = 0
init ABL3 = 0
init ABBL = 0
init Ametb1 = 0
init Ametb2 = 0
init Ametb3 = 0
init AUCCbron = 0
{Venous and arterial blood, moles}
init Aven = 0
init Aart = 0
{Body compartments, input, output, moles}
init Af = 0
init Am = 0
init Akvrg = 0
init Aliv = 0
init Aurine = 0
init Aet  = 0
init Ametliv = 0
init AUCCliv = 0
{Model parameters, constants}
Vf = (0.0165*Age^5 - 1.9784*Age^4 + 51.963*Age^3 - 459.38*Age^2 + 1566.8*Age + 1004.2)/1000
Vm = (-0.0623*Age^5 + 2.3433*Age^4 - 26.559*Age^3 + 144.75*Age^2 + 339.84*Age + 1648.2)/1000
Vkvrg = BW - (Vf + Vm + Vliv + Vlu + Vart + Vven)
Vliv = (0.0072*Age^5 - 0.3975*Age^4 + 7.9052*Age^3 - 65.624*Age^2 + 262.02*Age + 157.52)/1000
Vlu = (-0.0346*Age^4 + 1.5069*Age^3 - 20.31*Age^2 + 123.99*Age + 59.213)/1000
Valv = 0.9*Vlu
Vbron = 0.1*Vlu
Vart = BW*0.05/3
Vven = BW*0.05*2/3
Ka = 1.0E-3
Kb  = 100
Kbln = 6.9E-6
Kaln = 6.9E-7
Kln = 1.16E-5
```

```
DL = 2.14E-11
SF = 1.04
Vmaxlu = 1.2E-11*(BW/.25)^0.75
Vmaxliv = 1.7E-9*(BW/.25)^0.75
Kmliv = 5.5E-6
Kmlu = 2.2E-7
Pf = 294.7
Pm = 4.0
Pkvrg = 4.0
Pliv = 7.0
Pb =10
Palv = 1.3
Pbron = 2.3
BW = (-1.9*Age^4 + 72.8*Age^3 - 813.1*Age^2 + 5535.6*Age + 4453.7)/1000
Qtot = (0.012*Age^3 - 1.2144*Age^2 + 40.324*Age + 44.414)/60
Qvent = ((17.874*Age) + 39.785)/60
Qalv = 0.93*Qtot
Qbron = 0.07*Qtot
Qf = 0.053*Qtot
Qm = 0.03*Qtot
Qkvrg = Qtot - (Qf + Qliv + Qm)
Qliv = 0.0795*Qtot
MPliv = 5.8E4
MPlu = 3E3
{Concentrations, mol/L, ppm}
Cairex = Exposure
Exposure = IF TIME < 1440 THEN 1E-2*(1E-6/25.45) ELSE 0 {ppm to mol/L}
Age = 0
Cair = AAVA/(0.5*Vlu)
Calv = (AAV1+AAV2+AAVE)/Valv
Cbron = (ABL1+ABL2+ABL3)/Vbron
Cart = Aart/Vart
Cven = Aven/Vven
Cliv = Aliv/Vliv
{differential equations, alveoli moles, L, min}
d/dt(AAVA) = Qvent*(AAV1/Valv) - Qvent*(AAVA/0.5) + Cairex*Qvent
d/dt(AAP) = - AAP*0.9*4.8E-4 - AAP*0.1*4.8E-4
d/dt(AAV1) = AAP*0.9*4.8E-4 - Ka*((AAV1/(Valv*0.25*0.9)) - (AAVE/(Valv*0.75))) + Qvent*(AAVA/0.5)
- Qvent*(AAV1/Valv) - SF*Vmaxlu*MPlu*AAV1/(Kmlu + (AAV1/(Valv*0.9*0.25)))
d/dt(AAV2) =  AAP*0.1*4.8E-4
- Ka*((AAV2/(Valv*0.25*0.1)) - (AAVE/(Valv*0.75))) - SF*Vmaxlu*MPlu*AAV2/(Kmlu +
(AAV2/(Valv*0.1*0.25)))
d/dt(AAVE) = Ka*((AAV1/(Valv*0.25*0.9)) - (AAVE/(Valv*0.75))) + Ka*((AAV2/(Valv*0.25*0.1)) -
(AAVE/(Valv*0.75))) - Ka*((AAVE/(Valv*0.75)) - (AAVB/(Vven*Palv))) + Kln*LNth - Kaln*AAVE
```

d/dt(AAVB) = Ka*((AAVE/(Valv*0.75)) - (AAVB/(Vven*Palv))) + (Aven/Vven)*Qalv - AAVB*Qalv/(Vven*Palv)

d/dt(Ameta1) = SF*Vmaxlu*MPlu*AAV1/(Kmlu + (AAV1/(Valv*0.9*0.25)))

d/dt(Ameta2) = SF*Vmaxlu*MPlu*AAV2/(Kmlu + (AAV2/(Valv*0.1*0.25)))

d/dt(LNth) = Kbln*ABL1 + Kbln*ABL2 + Kbln*ABL3 + Kaln*AAVE - Kln*LNth

d/dt(AUCCalv) = Calv

{differential equations, bronchi}

d/dt(ABP) = - ABP*4.8E-4

d/dt(ABM) = ABP*4.8E-4 - Ka*Kb*((ABM/0.06) - (ABL1/(Vbron*0.333)))

d/dt(ABL1) = Ka*Kb*((ABM/0.06) - (ABL1/(Vbron*0.333))) - Ka*(ABL1/(Vbron*0.333) - ABL2/(Vbron*0.333)) + DL*Kb*(ABL1/(Vbron*0.333) - ABL3/(Vbron*0.333)) - SF*Vmaxlu*MPlu*ABL1/(Kmlu + (ABL1/(Vbron*0.333))) - Kbln*ABL1 + Kln*LNth

d/dt(ABL2) = Ka*(ABL1/(Vbron*0.333) - ABL2/(Vbron*0.333)) + DL*Kb*(ABL1/(Vbron*0.333) - ABL3/(Vbron*0.333)) - Ka*Kb*(ABL2/(Vbron*0.333) - ABL3/(Vbron*0.333)) - SF*Vmaxlu*MPlu*ABL2/(Kmlu + (ABL2/(Vbron*0.333))) - Kbln*ABL2 + Kln*LNth

d/dt(ABL3) = Ka*Kb*(ABL2/(Vbron*0.333) - ABL3/(Vbron*0.333)) - Ka*ABL3/(Vbron*0.333) - SF*Vmaxlu*MPlu*ABL3/(Kmlu + (ABL3/(Vbron*0.333))) - Kbln*ABL3 + Kln*LNth

d/dt(ABBL) = Ka*ABL3/(Vbron*0.333) + Qbron*(Aven/Vven) - ABBL*Qbron/(Vven*Pbron)

d/dt(Ametb1) = SF*Vmaxlu*MPlu*ABL1/(Kmlu + (ABL1/(Vbron*0.333)))

d/dt(Ametb2) = SF*Vmaxlu*MPlu*ABL2/(Kmlu + (ABL2/(Vbron*0.333)))

d/dt(Ametb3) = SF*Vmaxlu*MPlu*ABL3/(Kmlu + (ABL3/(Vbron*0.333)))

d/dt(AUCCbron) = Cbron

{differential equations, body}

d/dt(Aart) = AAVB*Qalv/(Vven*Palv) + ABBL*Qbron/(Vven*Pbron) - (Aart/Vart)*(Qf + Qm + Qkvrg + Qliv)

d/dt(Aven) = Af*Qf/(Vf*Pf) + Am*Qm/(Vm*Pm) + Akvrg*Qkvrg/(Vkvrg*Pkvrg) + Aliv*Qliv/(Vliv*Pliv) - (Aven/Vven)*Qalv -(Aven/Vven)*Qbron

d/dt(Af) = Cart*Qf - Af*Qf/(Vf*Pf)

d/dt(Am) = Cart*Qm - Am*Qm/(Vm*Pm)

d/dt(Akvrg) = Cart*Qkvrg - Akvrg*Qkvrg/(Vkvrg*Pkvrg) - Akvrg*0.2

d/dt(Aliv) = Cart*Qliv - Aliv*Qliv/(Vliv*Pliv) - SF*Vmaxliv*MPliv*Aliv/(Kmliv + (Aliv/Vliv)) + Aet*0.01

d/dt(Aet) = - Aet*0.01

d/dt(Aurine) = Akvrg*0.2

d/dt(Ametliv) = SF*Vmaxliv*MPliv*Aliv/(Kmliv + (Aliv/Vliv))

d/dt(AUCCliv) = Cliv

E.5.5 Model Code for NAP/NO RT 0-6 yr Child

METHOD Stiff

STARTTIME = 0

STOPTIME= 2880

DT = 0.0001

{Naphthalene (NAP) in upper respiratory tract compartment (URT) umoles}

```
init ANURTa = 0
init ANURTmuc = 0
init ANURTepi = 0
Limit ANURTepi >= 0
init ANURTex = 0
Limit ANURTex >= 0
init AMETua = 0
{NAP oxide (NO) in upper respiratory compartment (URT) umoles}
init ABURTa = 0
init ABURTmuc = 0
init ABURTepi = 0
init GSHua = 1.0*VURTepi
init ABURTex = 0
Limit ABURTex >= 0
init ABMET2ua = 0
init ABMETGua = 0
{NAP in conducting airways compartment (CA), umoles}
init ANCAa = 0
init ANCAmuc = 0
init ANCAepi = 0
init ANCAex = 0
Limit ANCAex >= 0
init AMETca = 0
{NO in conducting airways compartment (CA), umoles}
init ABCAa = 0
init ABCAmuc = 0
init ABCAepi = 0
init ABCAex = 0
Limit ABCAex >= 0
init GSHca = 1.0*VCA
init ABMET2ca = 0
init ABMETGca = 0
{NAP in transitional bronchioles compartment (TB), umoles}
init ANTBa = 0
init ANTBmuc = 0
init ANTBepi = 0
init ANTBex = 0
Limit ANTBex >= 0
init AMETtb = 0
{NO in transitional bronchioles compartment (TB), umoles}
init ABTBa = 0
init ABTBmuc = 0
init ABTBepi = 0
init GSHtb = 1.0*VTBepi
```

```
init ABTBex = 0
Limit ABTBex >= 0
init ABMET2tb = 0
init ABMETGtb = 0
{NAP in pulmonary compartment (PU), umoles}
init ANPUa = 0
init ANPUmuc = 0
init ANPUepi = 0
init ANPUex = 0
Limit ANPUex >= 0
init ANex = 0
Limit ANex >= 0
init AMETpu = 0
{NO in pulmonary compartment (PU), umoles}
init ABPUa = 0
init ABPUmuc = 0
init ABPUepi = 0
init ABPUex = 0
init ABex = 0
Limit ABex >= 0
init GSHpu = 1.0*VPU
init ABMET2pu = 0
init ABMETGpu = 0
{model equations}
Q = RPM*TVOL
Cairin = exposure/(24.36*1E3)
VURTepi = SAURT*WUA
VURTmuc = SAURT*WSMua
VURTex = SAURT*WUAs
VCAmuc = SACA*WSMca
VCAepi = SACA*WCA
VCAex = SACA*WCAs
VTBmuc = SATB*WSMtb
VTBepi = SATB*WTA
VTBex = SATB*WTAs
VPUmuc = SAPU*WSMpu
VPUepi = SAPU*WPA
VPUex = SAPU*WTAs
Vlu = 59.213 + 123.99*Age - 20.31*Age^2 + 1.5069*Age^3 - 0.0346*Age^4
VURT = 0.0026*Vlu
VCA = 0.018*Vlu
VTB = 0.043*Vlu
VPU = 0.937*Vlu
{calculated concentrations of NAP umol/mL}
```

Curtepil = (ANURTepi/VURTepi)
Ccaepil = (ANCAepi/VCAepi)
Ctbepil = (ANTBepi/VTBepi)
Cpuepil = (ANPUepi/VPUepi)
Cvurtex = (ANURTex/(VURTex*Pvrg))
Cvcaex = (ANCAex/(VCAex*Pvrg))
Cvtbex = (ANTBex/(VTBex*Pvrg))
Cvpuex = (ANPUex/(VPUex*Pvrg))
Cvex = (ANURTex+ANCAex+ANTBex+ANPUex)/((VURTex+VCAex+VTBex+VPUex)*Pvrg)
{calculated concentrations of NO umol/mL}
CBurtepil = (ABURTepi/VURTepi)
CBcaepil = (ABCAepi/VCAepi)
CBtbepil = (ABTBepi/VTBepi)
CBpuepil = (ABPUepi/VPUepi)
CBvurtex = (ABURTex/(VURTex*PBvrg))
CBvcaex = (ABCAex/(VCAex*PBvrg))
CBvtbex = (ABTBex/(VTBex*PBvrg))
CBvpuex = (ABPUex/(VPUex*PBvrg))
CBvex = (ABURTex+ABCAex+ABTBex+ABPUex)/((VURTex+VCAex+VTBex+VPUex)*PBvrg)
{concentrations of GSH, mM}
CGSHuab = 2.5
CGSHua = GSHua/VURT
CGSHcab = 2.0
CGSHca = GSHca/VCA
CGSHtbb = 1.0
CGSHtb = GSHtb/VTB
CGSHpub = 1.0
CGSHpu = GSHpu/VPU
init inhaleddose = 0
d/dt(Inhaleddose) = Cairin*Qalv
Exposure = IF TIME <= 1440 THEN 1 ELSE 0
ExposureB = IF TIME <= 1440 THEN 0 ELSE 0
Age = 3.0
{upper respiratory tract constants}
PMA = 30 {mucus:air partition coeff}
KOURT = 198.0 {mass transfer coeffs., cm/min}
KTRURT = 1.92
KBOURT = 0.192
KOCA = 18.1
KTRCA = 1.92
KBOCA = 0.192
KOTB = 15.8
KTRTB = 1.92
KBOTB = 0.192

KOPU = 15.8
KTRPU = 1.92
KBOPU = 0.192
KMUC = 0.001 {diffusion constants, cm2/min}
KSQM = 0.0002
KG = 6.0
SAURT = VURT/WUA {surface areas, cm2}
SACA = VCA/WCA
SATB = VTB/WTA
SAPU = VPU/WPA
VURTa = 0.00035*TLC {luminal volumes, cm3}
VCAa = 0.0105*TLC
VTBa = 0.042*TLC
VPUa = 0.944*TLC
TLC = 236.5 + 282*Age - 4.775*Age^2 + 0.285*Age^3 {mL}
RPM = 53.5*(BW/1E3)^-0.26 {breaths/min}
TVOL = 35.45 + 33.56*Age - 1.47*Age^2 + 0.0793*Age^3 {tidal volume mL/breath}
{thicknesses (W) of upper airways epithelium (UA), submucosa (UAs);mucus (SM); conducting airways epi (CA), submucosa (CAs); transitional airways epi (TA), submucosa (TAs); and pulmonary airways epi (PA), cm}
WUA = 0.005
WSMua = 0.001
WSMca = 0.0005
WSMtb = 0.0002
WSMpu = 0.0001
WCA = 0.0025
WTA = 0.001
WPA = 0.0005
WUAs = 0.01
WCAs = 0.005
WTAs = 0.002
Qua = 0.0025*Qtot {blood flow to the URT region}
Qca = 0.0075*Qtot {blood flow to the CA}
Qta = 0.0067*Qtot {blood flow to the TA}
{metabolic constants umol/min, umol/mL, based on Sweeny et al. 1996, Willems et al. 2001 rat values scaled to larger BWs, 2 = EH, G = conj}
Vmaxua = 2.45E-3*3.0*VURTepi/(BW/250)^0.25
Vmaxca = 2.45E-3*3.0*VCAepi/(BW/250)^0.25
Vmaxtb = 2.45E-3*3.0*VTBepi/(BW/250)^0.25
Vmaxpu = 2.45E-3*3.0*VPUepi/(BW/250)^0.25
Vmaxl = 2.46E-2*14.5*Vl/(BW/250)^0.25
Km = 0.003 {umol/mL}
Kmlu = 0.006
Vmaxl2 = 4.0E-3*14.5*Vl/(BW/250)^0.25 {EH}
Vmax2ua = 9.0E-3*3.0*VURTepi/(BW/250)^0.25

Vmax2ca = 9.0E-3*3.0*VCAepi/(BW/250)^0.25

Vmax2tb = 9.0E-3*3.0*VTBepi/(BW/250)^0.25

Vmax2pu = 9.0E-3*3.0*VPUepi/(BW/250)^0.25

Km2lu = 0.001

Km2 = 0.001

Km2ih = 2E-4

Kec = 400

init Kgshl = 0.003*Vl {GSH /min}

d/dt(Kgshl) = (2.4E-4*((CGSHlb + 2.0)/ (CGSHl + 2.0)) - 0.005*0.003)/58

Kgshua = 0.003*VURT

Kgshca = 0.003*VCA

Kgshtb = 0.003*VTB

Kgshpu = 0.003*VPU

Kge = 2.5E-3

VmaxGl = 0.5*58*Vl/(BW/250)^0.25 {umol/min/liver, GST}

VmaxGua = 0.4*54.0*VURTepi/(BW/250)^0.25

VmaxGca = 0.4*54.0*VCAepi/(BW/250)^0.25

VmaxGtb = 0.4*54.0*VTBepi/(BW/250)^0.25

VmaxGpu = 0.4*54.0*VPUepi/(BW/250)^0.25

KmG1 = 3.3 {GSH}

KmG2 = 0.05 {NO}

MPl = 14.5 {mg microsomal protein /mL tissue}

MPlu = 3.0 {mg microsomal protein/mL tissue}

CPl = 58 {mg cytosolic protein/mL tissue}

CPlu = 54 {mg cytosolic protein/mL tissue}

KNOH = 0.25 {naphthol fomation}

{differential equations for NAP in URT compartment, URT}

d/dt(ANURTa) = Q*(Cairin - (ANURTa/VURTA)) - KOURT*SAURT*((ANURTa/VURTA)-(ANURTmuc/(PMA*VURTmuc)))

d/dt(ANURTmuc) = KOURT*SAURT*((ANURTa/VURTa) - (ANURTmuc/(PMA*VURTmuc))) - KTRURT*SAURT*((ANURTmuc/(VURTmuc*PMA)) - (ANURTepi/(VURTepi*Pvrg)))

d/dt(ANURTepi) = KTRURT*SAURT*((ANURTmuc/(VURTmuc*PMA)) - (ANURTepi/(VURTepi*Pvrg))) - KBOURT*SAURT*((ANURTepi/(VURTepi*Pvrg)) - (ANURTex/(VURTex*Pvrg))) - Vmaxua*(ANURTepi/(VURTepi*Pvrg))/(Kmlu + (ANURTepi/(VURTepi*Pvrg)))

d/dt(ANURTex) = KBOURT*SAURT*((ANURTepi/VURTepi) - (ANURTex/VURTex)) + Qua*(Cart - (ANURTex/(VURTex*Pvrg)))

d/dt(AMETua) = Vmaxua*(ANURTepi/(VURTepi*Pvrg))/(Kmlu + (ANURTepi/(VURTepi*Pvrg)))

d/dt(GSHua) = Kgshua*(CGSHuab - (GSHua/VURTepi)) - Kge*GSHua - VmaxGua*(ABURTepi/(VURTepi*PBvrg))*CGSHua/(KmG1*(ABURTepi/(VURTepi*PBvrg)) + KmG2*CGSHua + CGSHua*(ABURTepi/(VURTepi*PBvrg)))

{differential equations for NO in URT compartment, URT}

d/dt(ABURTa) = Q*(CBairin - (ABURTa/VURTa)) - KOURT*SAURT*((ABURTa/VURTa)-(ABURTmuc/(PMA*VURTmuc)))

d/dt(ABURTmuc) = KOURT*SAURT*((ABURTa/VURTa) - (ABURTmuc/(PMA*VURTmuc))) - KTRURT*SAURT*((ABURTmuc/(VURTmuc*PMA)) - (ABURTepi/(VURTepi*PBvrg)))

```
d/dt(ABURTepi) = KTRURT*SAURT*((ABURTmuc/(VURTmuc*PMA)) -
(ABURTepi/(VURTepi*PBvrg))) - KBOURT*SAURT*((ABURTepi/(VURTepi*PBvrg)) -
(ABURTex/(VURTex*PBvrg))) + Vmaxua*(ANURTepi/(VURTepi*PBvrg))/(Kmlu +
(ANURTepi/(VURTepi*PBvrg))) - Vmax2ua*(ABURTepi/(VURTepi*PBvrg))/(Km2lu +
(ABURTepi/VURTepi)) -
VmaxGua*(ABURTepi/(VURTepi*PBvrg))*CGSHua/(KmG1*(ABURTepi/(VURTepi*PBvrg)) +
KmG2*CGSHua + CGSHua*(ABURTepi/(VURTepi*PBvrg))) -
KNOH*(ABURTepi/(VURTepi*PBvrg))*1E3
d/dt(ABMET2ua) = (Vmax2ua*ABURTepi/(VURTepi*PBvrg)/(Km2lu + (ABURTepi/VURTepi)))/2
d/dt(ABMETGua) =
(VmaxGua*(ABURTepi/(VURTepi*PBvrg))*CGSHua/(KmG1*(ABURTepi/(VURTepi*PBvrg)) +
KmG2*CGSHua + CGSHua*(ABURTepi/(VURTepi*PBvrg))))/2
d/dt(ABNOHua) =  KNOH*(ABURTepi/(VURTepi*PBvrg))*1E3
init ABNOHua = 0
d/dt(ABURTex) = KBOURT*SAURT*((ABURTepi/VURTepi) - (ABURTex/(VURTex*PBvrg))) +
Qua*(CBart - (ABURTex/(VURTex*PBvrg)))
{differential equations for NAP in CA compartment, CA}
d/dt(ANCAa) = Q*(Cairin - (ANCAa/VCAa)) - KOCA*SACA*((ANCAa/VCAa)-
(ANCAmuc/(PMA*VCAmuc)))
d/dt(ANCAmuc) = KOCA*SACA*((ANCAa/VCAa) - (ANCAmuc/(PMA*VCAmuc))) -
KTRCA*SACA*((ANCAmuc/(VCAmuc*PMA)) - (ANCAepi/(VCAepi*Pvrg)))
d/dt(ANCAepi) = KTRCA*SACA*((ANCAmuc/(VCAmuc*PMA)) - (ANCAepi/(VCAepi*Pvrg))) -
KBOCA*SACA*((ANCAepi/(VCAepi*Pvrg)) - (ANCAex/(VCAex*Pvrg))) -
Vmaxca*(ANCAepi/(VCAepi*Pvrg))/(Kmlu + (ANCAepi/(VCAepi*Pvrg)))
d/dt(ANCAex) = KBOCA*SACA*((ANCAepi/(VCAepi*Pvrg)) - (ANCAex/(VCAex*Pvrg))) + Qca*(Cart -
(ANCAex/(VCAex*Pvrg)))
d/dt(AMETca) = Vmaxca*(ANCAepi/(VCAepi*Pvrg))/(Kmlu + (ANCAepi/(VCAepi*Pvrg)))
d/dt(GSHca) = Kgshca*(CGSHcab - (GSHca/VCAepi)) - Kge*GSHca -
VmaxGca*(ABCAepi/(VCAepi*PBvrg))*CGSHca/(KmG1*(ABCAepi/(VCAepi*PBvrg)) +
KmG2*CGSHca + CGSHca*(ABCAepi/(VCAepi*PBvrg)))
{differential equations for NO in CA compartment, CA}
d/dt(ABCAa) = Q*(CBairin - (ABCAa/VCAa)) - KOCA*SACA*((ABCAa/VCAa)-
(ABCAmuc/(PMA*VCAmuc)))
d/dt(ABCAmuc) = KOCA*SACA*((ABCAa/VCAa) - (ABCAmuc/(PMA*VCAmuc))) -
KTRCA*SACA*((ABCAmuc/(VCAmuc*PMA)) - (ABCAepi/(VCAepi*PBvrg)))
d/dt(ABCAepi) = KTRCA*SACA*((ABCAmuc/(VCAmuc*PMA)) - (ABCAepi/(VCAepi*PBvrg)))
+ Vmaxca*(ANCAepi/(VCAepi*Pvrg))/(Kmlu + (ANCAepi/(VCAepi*Pvrg))) -
KBOCA*SACA*((ABCAepi/(VCAepi*PBvrg)) - (ABCAex/(VCAex*PBvrg))) -
Vmax2ca*ABCAepi/(VCAepi*PBvrg)/(Km2lu + (ABCAepi/VCAepi)) -
VmaxGca*(ABCAepi/(VCAepi*PBvrg))*CGSHca/(KmG1*(ABCAepi/(VCAepi*PBvrg)) +
KmG2*CGSHca + CGSHca*(ABCAepi/(VCAepi*PBvrg))) - KNOH*(ABCAepi/(VCAepi*PBvrg))*1E3
d/dt(ABCAex) = KBOCA*SACA*((ABCAepi/(VCAepi*PBvrg)) - (ABCAex/(VCAex*PBvrg))) +
Qca*(CBart - (ABCAex/(VCAex*PBvrg)))
d/dt(ABMET2ca) = (Vmax2ca*ABCAepi/(VCAepi*PBvrg)/(Km2lu + (ABCAepi/VCAepi)))/2
d/dt(ABMETGca) =
(VmaxGca*(ABCAepi/(VCAepi*PBvrg))*CGSHca/(KmG1*(ABCAepi/(VCAepi*PBvrg)) +
KmG2*CGSHca + CGSHca*(ABCAepi/(VCAepi*PBvrg))))/2
d/dt(ABNOHca) =  KNOH*(ABCAepi/(VCAepi*PBvrg))*1E3
init ABNOHca = 0
{differential equations for NAP in TB compartment umoles, TB}
```

```
d/dt(ANTBa) = Q*(Cairin - (ANTBa/VTBa)) - KOTB*SATB*((ANTBa/VTBa)-
(ANTBmuc/(PMA*VTBmuc)))
d/dt(ANTBmuc) = KOTB*SATB*((ANTBa/VTBa) - (ANTBmuc/(PMA*VTBmuc))) -
KTRTB*SATB*((ANTBmuc/(VTBmuc*PMA)) - (ANTBmuc/(VTBmuc*PMA)))
d/dt(ANTBepi) = KTRTB*SATB*((ANTBmuc/(VTBmuc*PMA)) - (ANTBepi/(VTBepi*Pvrg))) -
KBOTB*SATB*((ANTBepi/(VTBepi*Pvrg)) - (ANTBex/(VTBex*Pvrg))) -
Vmaxtb*(ANTBepi/(VTBepi*Pvrg))/(Kmlu + (ANTBepi/(VTBepi*Pvrg)))
d/dt(ANTBex) = KBOTB*SATB*((ANTBepi/(VTBepi*Pvrg)) - (ANTBex/(VTBex*Pvrg))) + Qta*(Cart -
(ANTBex/(VTBex*Pvrg)))
d/dt(AMETtb) = Vmaxtb*(ANTBepi/(VTBepi*Pvrg))/(Kmlu + (ANTBepi/(VTBepi*Pvrg)))
d/dt(GSHtb) = Kgshtb*(CGSHtbb - (GSHtb/VTBepi)) - Kge*GSHtb -
VmaxGtb*(ABTBepi/(VTBepi*PBvrg))*CGSHtb/(KmG1*(ABTBepi/(VTBepi*PBvrg)) + KmG2*CGSHtb
+ CGSHtb*(ABTBepi/(VTBepi*PBvrg)))
{differential equations for NO in TB compartment umoles, TB}
d/dt(ABTBa) = Q*(CBairin - (ABTBa/VTBa)) - KOTB*SATB*((ABTBa/VTBa)-
(ABTBmuc/(PMA*VTBmuc)))
d/dt(ABTBmuc) = KOTB*SATB*((ABTBa/VTBa) - (ABTBmuc/(PMA*VTBmuc))) -
KTRTB*SATB*((ABTBmuc/(VTBmuc*PMA)) - (ABTBepi/(VTBepi*PBvrg)))
d/dt(ABTBepi) = KTRTB*SATB*((ABTBmuc/(VTBmuc*PMA)) - (ABTBepi/(VTBepi*PBvrg))) -
KBOTB*SATB*((ABTBepi/(VTBepi*PBvrg)) - (ABTBex/(VTBex*PBvrg))) +
Vmaxtb*(ANTBepi/(VTBepi*Pvrg))/(Kmlu + (ANTBepi/(VTBepi*Pvrg))) -
Vmax2tb*ABTBepi/(VTBepi*PBvrg)/(Km2lu + (ABTBepi/VTBepi)) -
VmaxGtb*(ABTBepi/(VTBepi*PBvrg))*CGSHtb/(KmG1*(ABTBepi/(VTBepi*PBvrg)) + KmG2*CGSHtb
+ CGSHtb*(ABTBepi/(VTBepi*PBvrg))) - KNOH* (ABTBepi/(VTBepi*PBvrg))*1E3
d/dt(ABMET2tb) = (Vmax2tb*(ABTBepi/(VTBepi*PBvrg))/(Km2lu + (ABTBepi/VTBepi)))/2
d/dt(ABMETGtb) =
(VmaxGtb*(ABTBepi/(VTBepi*PBvrg))*CGSHtb/(KmG1*(ABTBepi/(VTBepi*PBvrg)) +
KmG2*CGSHtb + CGSHtb*(ABTBepi/(VTBepi*PBvrg))))/2
d/dt(ABNOHtb) =  KNOH* (ABTBepi/(VTBepi*PBvrg))*1E3
init ABNOHtb = 0
d/dt(ABTBex) = KBOTB*SATB*(ABTBepi/(VTBepi*PBvrg) - (ABTBex/(VTBex*PBvrg))) + Qta*(CBart
- (ABTBex/(VTBex*PBvrg)))
{differential equations for NAP in PU compartment umoles, PU}
d/dt(ANPUa) = Q*(Cairin - (ANPUa/VPUa)) - KOPU*SAPU*((ANPUa/VPUa)-
(ANPUmuc/(PMA*VPUmuc)))
d/dt(ANPUmuc) = KOPU*SAPU*((ANPUa/VPUa) - (ANPUmuc/(PMA*VPUmuc))) -
KTRPU*SAPU*((ANPUmuc/VPUmuc) - (ANPUepi/(VPUepi*Pvrg)))
d/dt(ANPUepi) = KTRPU*SAPU*((ANPUmuc/(VPUmuc*PMA)) - (ANPUepi/(VPUepi*Pvrg))) -
KBOPU*SAPU*((ANPUepi/(VPUepi*Pvrg)) - (ANPUex/(VPUex*Pvrg))) -
Vmaxpu*(ANPUepi/(VPUepi*Pvrg))/(Kmlu + (ANPUepi/(VPUepi*Pvrg)))
d/dt(ANPUex) = KBOPU*SAPU*((ANPUepi/(VPUepi*Pvrg)) - (ANPUex/(VPUex*Pvrg))) + Qtot*(Cart -
(ANPUex/(VPUex*Pvrg)))
d/dt(GSHpu) = Kgshpu*(CGSHpub - (GSHpu/VPUepi)) - Kge*GSHpu -
VmaxGpu*(ABPUepi/(VPUepi*PBvrg))*CGSHpu/(KmG1*(ABPUepi/(VPUepi*PBvrg)) +
KmG2*CGSHpu + CGSHpu*(ABPUepi/(VPUepi*PBvrg)))
d/dt(AMETpu) = Vmaxpu*(ANPUepi/(VPUepi*Pvrg))/(Kmlu + (ANPUepi/(VPUepi*Pvrg)))
{differential equations for NO in PU compartment umoles, PU}
d/dt(ABPUa) = Q*(Cairin - (ANPUa/VPUa)) - KOPU*SAPU*((ANPUa/VPUa)-
(ANPUmuc/(PMA*VPUmuc)))
```

```
d/dt(ABPUmuc) = KOPU*SAPU*((ABPUa/VPUa) - (ABPUmuc/(PMA*VPUmuc))) -
KTRPU*SAPU*((ABPUmuc/(VPUmuc*PMA)) - (ABPUepi/(VPUepi*PBvrg)))
 d/dt(ABPUepi) = KTRPU*SAPU*((ABPUmuc/(VPUmuc*PMA)) - (ABPUepi/(VPUepi*PBvrg))) -
KBOPU*SAPU*((ABPUepi/(VPUepi*PBvrg)) - (ABPUex/(VPUex*PBvrg))) +
Vmaxpu*(ANPUepi/(VPUepi*Pvrg))/(Kmlu + (ANPUepi/(VPUepi*Pvrg))) -
Vmax2pu*ABPUepi/(VPUepi*PBvrg)/(Km2lu + (ABPUepi/VPUepi)) -
VmaxGpu*(ABPUepi/(VPUepi*PBvrg))*CGSHpu/(KmG1*(ABPUepi/(VPUepi*PBvrg)) +
KmG2*CGSHpu + CGSHpu*(ABPUepi/(VPUepi*PBvrg))) - KNOH*(ABPUepi/(VPUepi*PBvrg))*1E3
 d/dt(ABPUex) = KBOPU*SAPU*((ABPUepi/(VPUepi*PBvrg)) - (ABPUex/(VPUex*PBvrg))) +
Qtot*(CBart - (ABPUex/(VPUex*PBvrg)))
d/dt(ABMET2pu) = (Vmax2pu*(ABPUepi/(VPUepi*PBvrg))/(Km2lu + (ABPUepi/VPUepi)))/2
d/dt(ABMETGpu) =
(VmaxGpu*(ABPUepi/(VPUepi*PBvrg))*CGSHpu/(KmG1*(ABPUepi/(VPUepi*PBvrg)) +
KmG2*CGSHpu + CGSHpu*(ABPUepi/(VPUepi*PBvrg))))/2
d/dt(ABNOHpu) = KNOH*(ABPUepi/(VPUepi*PBvrg))*1E3
init ABNOHpu  =  0
{Sum of  Lung NAP}
d/dt(ANex) = Qtot*((Cart-Cvurtex) + (Cart-Cvcaex) + (Cart-Cvtbex) + (Cart-Cvpuex))
{Sum of Lung NO}
d/dt(ABex) = Qtot*((CBart-CBvurtex) +(CBart-CBvcaex) + (CBart-CBvtbex) + (CBart-CBvpuex))
{NAP ex respiratory tract, umoles}
init Af = 0
init Al = 0
Limit Al >= 0
init Am = 0
Limit Am >= 0
init Avrg = 0
Limit Avrg >= 0
init Ablood = 0
init GSHl = 6.0*Vl
{NO oxide ex respiratory tract, umoles}
init ABf = 0
init ABler = 0
Limit ABler >= 0
init ABlcy = 0
Limit ABlcy >= 0
init ABm = 0
Limit ABm >= 0
init ABvrg = 0
Limit ABvrg >= 0
init ABblood = 0
{umoles NAP metabolized}
init AMETl = 0
{umoles NO ex rt metabolized  EH, GST and P450 pathways}
init ABMETl2 = 0
init ABMETGl = 0
```

```
{AUCs NAP}
init AUCvtot = 0
init AUCvl = 0
{AUCs NO}
init AUCBvtot = 0
init AUCBvl = 0
{tissue flows mL/min}
Qtot = (0.012*Age^3 - 1.2144*Age^2 + 40.32*Age + 44.144)*1000/60
Qalv = 0.82*Qtot
Qf = 0.0528*Qtot
Ql = 0.0795*Qtot
Qm = 0.0304*Qtot
Qvrg = 0.837*Qtot
{tissue volumes mL}
BW = -1.9*Age^4 + 72.8*Age^3 - 813.1*Age^2 + 5535.6*Age + 4453.7
Vf = 0.0165*Age^5 - 1.9784*Age^4 + 51.963*Age^3 - 459.38*Age^2 + 1566.8*Age + 1004.2
Vl = 0.0072*Age^5 - 0.3975*Age^4 + 7.9052*Age^3 - 65.625*Age^2 + 262.02*Age + 157.2
Vm = - 0.0623*Age^5 + 2.3433*Age^4 - 26.559*Age^3 + 144.75*Age^2 + 339.84*Age + 1648.2
Vvrg = BW - (Vf + Vl + Vm + Vlu + Vblood)
Vblood = 0.075*BW
{blood/air and tissue/blood partition coefficients, NAP}
Pb = 571
Pl = 7.0
Pf = 160.0
Pm = 4.0
Pvrg = 4.0
{blood/air and tissue/blood partition coefficients, NO}
PBb = 571
PBl = 7.0
PBf = 22.9
PBm = 4.0
PBvrg = 4.0
{calculated concentrations of NAP umol/mL}
Cblood = Ablood/Vblood
Cart = Cvex
Cvf = Af/(Vf*Pf)
Cvl = Al/(Vl*Pl)
Cvm = Am/(Vm*Pm)
Cvvrg = Avrg/(Vvrg*Pvrg)
Cvtot = (Ql*Cvl + Qf*Cvf + Qm*Cvm + Qvrg*Cvvrg)/Qtot
Cairin = exposure/(24.45*1E3)
CGSHl = GSHl/Vl
CGSHlb = 6.0
{calculated concentrations of NO umol/mL}
```

```
CBblood = ABblood/Vblood
CBart = CBvex
CBvf = ABf/(Vf*PBf)
CBvler = ABler/(Vl*PBl)
CBvlcy = ABlcy/(Vl*PBl)
CBvm = ABm/(Vm*PBm)
CBvvrg = ABvrg/(Vvrg*PBvrg)
CBvtot = (Ql*CBvlcy + Qf*CBvf + Qm*CBvm + Qvrg*CBvvrg)/Qtot
CBairin = exposureB/(24.45*1E3)
{differential equations for NAP uptake and metabolism, umoles}
d/dt(Al) = Ql*(Cart - Cvl) - Vmaxl*Cvl/(Km + Cvl)
d/dt(Af) = Qf*(Cart - Cvf)
d/dt(Am) = Qm*(Cart - Cvm)
d/dt(Avrg) = Qvrg*(Cart - Cvvrg)
d/dt(Ablood) = Ql*Cvl + Qf*Cvf + Qm*Cvm + Qvrg*Cvvrg + Qtot*Cvex
{differential equations for NO uptake and metabolism, umoles}
d/dt(ABf) = Qf*(CBart - CBvf)
d/dt(ABm) = Qm*(CBart - CBvm)
d/dt(ABvrg) = Qvrg*(CBart - CBvvrg)
d/dt(ABblood) = Ql*CBvlcy + Qf*CBvf + Qm*CBvm + Qvrg*CBvvrg + Qtot*CBvex
d/dt(ABler) = Vmaxl*Cvl/(Km + Cvl) - Kec*(CBvler - CBvlcy) - Vmaxl2*CBvler/(Km2ih + CBvler)
d/dt(ABlcy) =  Kec*(CBvler - CBvlcy) + Ql*(CBart - CBvlcy) - VmaxGl*CBvlcy*CGSHl/(KmG2*CBvlcy
+ KmG1*CGSHl + CBvlcy*CGSHl)
d/dt(GSHl) = Kgshl*(CGSHlb - (GSHl/Vl)) - Kge*GSHl -  VmaxGl*CBvlcy*CGSHl/(KmG1*CBvlcy +
KmG2*CGSHl + CBvlcy*CGSHl) - KNOH*CBvlcy*1E3
{amount of BD metabolized in liver to NO,  umoles}
d/dt(AMETl)  =  Vmaxl*Cvl/(Km+ Cvl)
{amount of NO metabolized in liver and lung to diol,  umoles}
d/dt(ABMETl2)  = (Vmaxl2*CBvler/(Km2ih + CBvler))/2
 {amount of NO metabolized in liver and lung to GSH conjugate,  umoles}
d/dt(ABMETGl)  = (VmaxGl*CBVlcy*CGSHl/(KmG1*CBvlcy + KmG2*CGSHl + CGSHl*CBvlcy))/2
{amount of NO rearranged to NOH, umoles}
d/dt(ABNOHl) = KNOH*CBvlcy*1E3
init ABNOHl = 0
{AUCs for NAP, umolmin/mL}
d/dt(AUCvtot) = Cvtot
d/dt(AUCvl) = Cvl
{AUCs for NO, umolmin/mL}
d/dt(AUCBvtot) = CBvtot
d/dt(AUCBvl) = CBvlcy
```

E.5.6 Model Code for BD/BMO RT 0-5 yr Child

```
METHOD Stiff

STARTTIME = 0
STOPTIME= 2880
DT = 0.0001
{Butadiene (BD) in upper respiratory tract compartment (URT) umoles}
init ANURTa = 0
init ANURTmuc = 0
init ANURTepi = 0
Limit ANURTepi >= 0
init ANURTex = 0
init AMETua = 0
{Butadienemonoxide (BMO) in upper respiratory compartment (URT) umoles}
init ABURTa = 0
init ABURTmuc = 0
init ABURTepi = 0
init GSHua = 1.0*VURTepi
init ABURTex = 0
init ABMET2ua = 0
init ABMETGua = 0
init ABMET3ua = 0
Limit ABMET3ua >= 0
{BD in conducting airways compartment (CA), umoles}
init ANCAa = 0
init ANCAmuc = 0
init ANCAepi = 0
init ANCAex = 0
init AMETca = 0
{BMO in conducting airways compartment (CA), umoles}
init ABCAa = 0
init ABCAmuc = 0
init ABCAepi = 0
init ABCAex = 0
init GSHca = 1.0*VCA
init ABMET2ca = 0
init ABMETGca = 0
init ABMET3ca = 0
{BD in transitional bronchioles compartment (TB), umoles}
init ANTBa = 0
init ANTBmuc = 0
init ANTBepi = 0
init ANTBex = 0
```

```
init AMETtb = 0
{BMO in transitional bronchioles compartment (TB), umoles}
init ABTBa = 0
init ABTBmuc = 0
init ABTBepi = 0
init GSHtb = 1.0*VTBepi
init ABTBex = 0
init ABMET2tb = 0
init ABMETGtb = 0
init ABMET3tb = 0
{BD in pulmonary compartment (PU), umoles}
init ANPUa = 0
init ANPUmuc = 0
init ANPUepi = 0
init ANPUex = 0
init ANex = 0
init AMETpu = 0
{BMO in pulmonary compartment (PU), umoles}
init ABPUa = 0
init ABPUmuc = 0
init ABPUepi = 0
init ABPUex = 0
init ABex = 0
init GSHpu = 1.0*VPU
init ABMET2pu = 0
init ABMETGpu = 0
init ABMET3pu = 0
{model equations}
Q = RPM*TVOL
Cairin = exposure/(24.36*1E3)
VURTepi = SAURT*WUA
VURTmuc = SAURT*WSMua
VURTex = SAURT*WUAs
VCAmuc = SACA*WSMca
VCAepi = SACA*WCA
VCAex = SACA*WCAs
VTBmuc = SATB*WSMtb
VTBepi = SATB*WTA
VTBex = SATB*WTAs
VPUmuc = SAPU*WSMpu
VPUepi = SAPU*WPA
VPUex = SAPU*WTAs
Vlu = 59.213 + 123.99*Age - 20.31*Age^2 + 1.5069*Age^3 - 0.0346*Age^4
VURT = 0.0026*Vlu
```

```
VCA = 0.018*Vlu
VTB = 0.043*Vlu
VPU = 0.937*Vlu
Curtepil = (ANURTepi/VURTepi)
Ccaepil = (ANCAepi/VCAepi)
Ctbepil = (ANTBepi/VTBepi)
Cpuepil = (ANPUepi/VPUepi)
Cvurtex = (ANURTex/(VURTex*Pvrg))
Cvcaex = (ANCAex/(VCAex*Pvrg))
Cvtbex = (ANTBex/(VTBex*Pvrg))
Cvpuex = (ANPUex/(VPUex*Pvrg))
Cvex = (ANURTex+ANCAex+ANTBex+ANPUex)/((VURTex+VCAex+VTBex+VPUex)*Pvrg)
CBurtepil = (ABURTepi/VURTepi)
CBcaepil = (ABCAepi/VCAepi)
CBtbepil = (ABTBepi/VTBepi)
CBpuepil = (ABPUepi/VPUepi)
CBvurtex = (ABURTex/(VURTex*PBvrg))
CBvcaex = (ABCAex/(VCAex*PBvrg))
CBvtbex = (ABTBex/(VTBex*PBvrg))
CBvpuex = (ABPUex/(VPUex*PBvrg))
CBvex = (ABURTex+ABCAex+ABTBex+ABPUex)/((VURTex+VCAex+VTBex+VPUex)*PBvrg)
CGSHuab = 2.5
CGSHcab = 2.0
CGSHtbb =  1.0
CGSHpub = 1.0
Exposure = IF TIME <= 1440 THEN 1 ELSE 0
ExposureB = IF TIME <= 1440 THEN 0 ELSE 0
Age = 3.0
{upper respiratory tract constants}
PMA = 30 {mucus:air partition coeff}
KOURT = 1980 {mass transfer coeffs., cm/min}
KTRURT = 19.2
KBOURT = 19.2
KOCA = 181
KTRCA = 19.2
KBOCA = 19.2
KOTB = 158
KTRTB = 19.2
KBOTB = 19.2
KOPU = 158
KTRPU = 19.2
KBOPU = 19.2
KMUC = 0.001 {diffusion constants, cm2/min}
KSQM = 0.0002
```

KG = 6.0

SAURT = VURT/WUA {surface areas, cm2}

SACA = VCA/WCA

SATB = VTB/WTA

SAPU = VPU/WPA

VURTa = 0.00035*TLC {luminal volumes, cm3}

VCAa = 0.0105*TLC

VTBa = 0.042*TLC

VPUa = 0.944*TLC

TLC = 236.5 + 282*Age - 4.775*Age^2 + 0.285*Age^3

RPM = 53.5*(BW/1000)^-0.26 {breaths/min}

TVOL = 35.45 + 33.56*Age - 1.47*Age^2 + 0.0793*Age^3 {tidal volume mL/breath}

{thicknesses (W) of upper airways epithelium (UA), submucosa (UAs);mucus (SM); conducting airways epi (CA), submucosa (CAs); transitional airways epi (TA), submucosa (TAs); and pulmonary airways epi (PA), cm}

WUA = 0.005

WSMua = 0.001

WSMca = 0.0005

WSMtb = 0.0002

WSMpu = 0.0001

WCA = 0.0025

WTA = 0.001

WPA = 0.0005

WUAs = 0.01

WCAs = 0.005

WTAs = 0.002

Qua = 0.0025*Qtot {blood flow to the URT region}

Qca = 0.0075*Qtot {blood flow to the CA}

Qta = 0.0067*Qtot {blood flow to the TA}

{metabolic constants umol/min, umol/mL, based on Csanady et al. 2003 scaled to smaller BWs, 1 = EH, 2 = conj, 3 = oxid}

Vmaxua = 9.09E-3*3.0*VURTepi*(7E4/BW)^0.25/60

Vmaxca = 9.09E-3*3.0*VCAepi*(7E4/BW)^0.25/60

Vmaxtb = 9.09E-3*3.0*VTBepi*(7E4/BW)^0.25/60

Vmaxpu = 9.09E-3*3.0*VPUepi*(7E4/BW)^0.25/60

Vmaxl = 7.08E-2*14.5*Vl*(7E4/BW)^0.25/60

Vmaxl2 = 1.1*14.5*Vl*(7E4/BW)^0.25/60 {EH}

K1ua = 0.1914*3.0*VURTepi*(7E4/BW)^-0.25/60

K1ca = 0.1914*3.0*VCAepi*(7E4/BW)^-0.25/60

K1tb = 0.1914*3.0*VTBepi*(7E4/BW)^-0.25/60

K1pu = 0.1914*3.0*VPUepi*(7E4/BW)^-0.25/60

Kgsh = 0.012 {GSH /min}

Kge = 0.15/60

VmaxGl = 2.71*58*Vl*(7E4/BW)^0.25/60 {umol/min/liver, GST}

K2ua = 0.1536*54*VURTepi*(7E4/BW)^-0.25/60 {umol/min/URT}

```
K2ca = 0.1536*54*VCAepi*(7E4/BW)^-0.25/60 {umol/min/CA}
K2tb = 0.1536*54*VTBepi*(7E4/BW)^-0.25/60 {umol/min/TB}
K2pu = 0.1536*54*VPUepi*(7E4/BW)^-0.25/60 {umol/min/PU}
MPl = 14.5 {mg microsomal protein /mL tissue}
MPlu = 3.0 {mg microsomal protein/mL tissue}
CPl = 58 {mg cytosolic protein/mL tissue}
CPlu = 54 {mg cytosolic protein/mL tissue}
Km = 0.00514 {umol/mL}
Kmlu = 0.002
Km2 = 0.58
Km2ih = 0.116
KmG1 = 0.1 {GSH}
KmG2 = 10.4 {BMO}
Kec = 400
Vmaxua3 = 0.0066*0.2/(7E4/BW)^0.25/60
Vmaxca3 = 0.1986*0.2/(7E4/BW)^0.25/60
Vmaxtb3 = 0.7947*0.2/(7E4/BW)^0.25/60
Vmaxpu3 = 1.7/(7E4/BW)^0.25/60
Vmaxl3 = 14.8/(7E4/BW)^0.25/60
Km3 = 0.0156
 {differential equations for BD in URT compartment, URT}
d/dt(ANURTa) = Q*(Cairin - (ANURTa/VURTA)) - KOURT*SAURT*((ANURTa/VURTA)-
(ANURTmuc/(PMA*VURTmuc)))
d/dt(ANURTmuc) = KOURT*SAURT*((ANURTa/VURTa) - (ANURTmuc/(PMA*VURTmuc))) -
KTRURT*SAURT*((ANURTmuc/(VURTmuc*PMA)) - (ANURTepi/(VURTepi*Pvrg)))
d/dt(ANURTepi) = KTRURT*SAURT*((ANURTmuc/(VURTmuc*PMA)) - (ANURTepi/(VURTepi*Pvrg)))
- KBOURT*SAURT*((ANURTepi/(VURTepi*Pvrg)) - (ANURTex/(VURTex*Pvrg))) -
Vmaxua*(ANURTepi/(VURTepi*Pvrg))/(Kmlu + (ANURTepi/(VURTepi*Pvrg)))
d/dt(ANURTex) = KBOURT*SAURT*((ANURTepi/VURTepi) - (ANURTex/VURTex)) + Qua*(Cart -
(ANURTex/(VURTex*Pvrg)))
d/dt(AMETua) = Vmaxua*(ANURTepi/(VURTepi*Pvrg))/(Kmlu + (ANURTepi/(VURTepi*Pvrg)))
d/dt(GSHua) = Kgsh*(CGSHuab - (GSHua/VURTepi)) - Kge*GSHua - K2ua*ABURTepi
{differential equations for BMO in URT compartment, URT}
d/dt(ABURTa) = Q*(CBairin - (ABURTa/VURTa)) - KOURT*SAURT*((ABURTa/VURTa)-
(ABURTmuc/(PMA*VURTmuc)))
d/dt(ABURTmuc) = KOURT*SAURT*((ABURTa/VURTa) - (ABURTmuc/(PMA*VURTmuc))) -
KTRURT*SAURT*((ABURTmuc/(VURTmuc*PMA)) - (ABURTepi/(VURTepi*PBvrg)))
 d/dt(ABURTepi) = KTRURT*SAURT*((ABURTmuc/(VURTmuc*PMA)) -
(ABURTepi/(VURTepi*PBvrg))) - KBOURT*SAURT*((ABURTepi/(VURTepi*PBvrg)) -
(ABURTex/(VURTex*PBvrg))) + Vmaxua*(ANURTepi/(VURTepi*PBvrg))/(Kmlu +
(ANURTepi/(VURTepi*PBvrg))) - K1ua*ANURTepi - K2ua*ANURTepi -
Vmaxua3*(ABURTepi/(VURTepi*PBvrg))/(Km3 + (ABURTepi/(VURTepi*PBvrg)))
d/dt(ABMET2ua) = K1ua*ANURTepi
d/dt(ABMETGua) = K2ua*ANURTepi
d/dt(ABMET3ua) = Vmaxua3*(ABURTepi/(VURTepi*PBvrg))/(Km3 + (ABURTepi/(VURTepi*PBvrg)))
d/dt(ABURTex) = KBOURT*SAURT*((ABURTepi/VURTepi) - (ABURTex/(VURTex*PBvrg))) +
Qua*(CBart - (ABURTex/(VURTex*PBvrg)))
```

{differential equations for BD in CA compartment, CA}

d/dt(ANCAa) = Q*(Cairin - (ANCAa/VCAa)) - KOCA*SACA*((ANCAa/VCAa)-(ANCAmuc/(PMA*VCAmuc)))

d/dt(ANCAmuc) = KOCA*SACA*((ANCAa/VCAa) - (ANCAmuc/(PMA*VCAmuc))) - KTRCA*SACA*((ANCAmuc/(VCAmuc*PMA)) - (ANCAepi/(VCAepi*Pvrg)))

d/dt(ANCAepi) = KTRCA*SACA*((ANCAmuc/(VCAmuc*PMA)) - (ANCAepi/(VCAepi*Pvrg))) - KBOCA*SACA*((ANCAepi/(VCAepi*Pvrg)) - (ANCAex/(VCAex*Pvrg))) - Vmaxca*(ANCAepi/(VCAepi*Pvrg))/(Kmlu + (ANCAepi/(VCAepi*Pvrg)))

d/dt(ANCAex) = KBOCA*SACA*((ANCAepi/(VCAepi*Pvrg)) - (ANCAex/(VCAex*Pvrg))) + Qca*(Cart - (ANCAex/(VCAex*Pvrg)))

d/dt(AMETca) = Vmaxca*(ANCAepi/(VCAepi*Pvrg))/(Kmlu + (ANCAepi/(VCAepi*Pvrg)))

d/dt(GSHca) = Kgsh*(CGSHcab - (GSHca/VCAepi)) - Kge*GSHca - K2ca*ABCAepi

{differential equations for BMO in CA compartment, CA}

d/dt(ABCAa) = Q*(CBairin - (ABCAa/VCAa)) - KOCA*SACA*((ABCAa/VCAa)-(ABCAmuc/(PMA*VCAmuc)))

d/dt(ABCAmuc) = KOCA*SACA*((ABCAa/VCAa) - (ABCAmuc/(PMA*VCAmuc))) - KTRCA*SACA*((ABCAmuc/(VCAmuc*PMA)) - (ABCAepi/(VCAepi*PBvrg)))

d/dt(ABCAepi) = KTRCA*SACA*((ABCAmuc/(VCAmuc*PMA)) - (ABCAepi/(VCAepi*PBvrg)))

+ Vmaxca*(ANCAepi/(VCAepi*Pvrg))/(Kmlu + (ANCAepi/(VCAepi*Pvrg))) - KBOCA*SACA*((ABCAepi/(VCAepi*PBvrg)) - (ABCAex/(VCAex*PBvrg))) - K1ca*ABCAepi - K2ca*ABCAepi - Vmaxca3*(ABCAepi/(VCAepi*PBvrg))/(Km3 + (ABCAepi/(VCAepi*PBvrg)))

d/dt(ABCAex) = KBOCA*SACA*((ABCAepi/(VCAepi*PBvrg)) - (ABCAex/(VCAex*PBvrg))) + Qca*(CBart - (ABCAex/(VCAex*PBvrg)))

d/dt(ABMET2ca) = K1ca*ABCAepi

d/dt(ABMETGca) = K2ca*ABCAepi

d/dt(ABMET3ca) = Vmaxca3*(ABCAepi/(VCAepi*PBvrg))/(Km3 + (ABCAepi/(VCAepi*PBvrg)))

{differential equations for BD in TB compartment umoles, TB}

d/dt(ANTBa) = Q*(Cairin - (ANTBa/VTBa)) - KOTB*SATB*((ANTBa/VTBa)-(ANTBmuc/(PMA*VTBmuc)))

d/dt(ANTBmuc) = KOTB*SATB*((ANTBa/VTBa) - (ANTBmuc/(PMA*VTBmuc))) - KTRTB*SATB*((ANTBmuc/(VTBmuc*PMA)) - (ANTBmuc/(VTBmuc*PMA)))

d/dt(ANTBepi) = KTRTB*SATB*((ANTBmuc/(VTBmuc*PMA)) - (ANTBepi/(VTBepi*Pvrg))) - KBOTB*SATB*((ANTBepi/(VTBepi*Pvrg)) - (ANTBex/(VTBex*Pvrg))) - Vmaxtb*(ANTBepi/(VTBepi*Pvrg))/(Kmlu + (ANTBepi/(VTBepi*Pvrg)))

d/dt(ANTBex) = KBOTB*SATB*((ANTBepi/(VTBepi*Pvrg)) - (ANTBex/(VTBex*Pvrg))) + Qta*(Cart - (ANTBex/(VTBex*Pvrg)))

d/dt(AMETtb) = Vmaxtb*(ANTBepi/(VTBepi*Pvrg))/(Kmlu + (ANTBepi/(VTBepi*Pvrg)))

d/dt(GSHtb) = Kgsh*(CGSHtbb - (GSHtb/VTBepi)) - Kge*GSHtb - K2tb*ABTBepi

{differential equations for BMO in TB compartment umoles, TB}

d/dt(ABTBa) = Q*(CBairin - (ABTBa/VTBa)) - KOTB*SATB*((ABTBa/VTBa)-(ABTBmuc/(PMA*VTBmuc)))

d/dt(ABTBmuc) = KOTB*SATB*((ABTBa/VTBa) - (ABTBmuc/(PMA*VTBmuc))) - KTRTB*SATB*((ABTBmuc/(VTBmuc*PMA)) - (ABTBepi/(VTBepi*PBvrg)))

d/dt(ABTBepi) = KTRTB*SATB*((ABTBmuc/(VTBmuc*PMA)) - (ABTBepi/(VTBepi*PBvrg))) - KBOTB*SATB*((ABTBepi/(VTBepi*PBvrg)) - (ABTBex/(VTBex*PBvrg))) + Vmaxtb*(ANTBepi/(VTBepi*Pvrg))/(Kmlu + (ANTBepi/(VTBepi*Pvrg))) - K1tb*ABTBepi - K2tb*ABTBepi - Vmaxtb3*(ANTBepi/(VTBepi*Pvrg))/(Km3 + (ANTBepi/(VTBepi*Pvrg)))

d/dt(ABMET2tb) = K1tb*ABTBepi

d/dt(ABMETGtb) = K2tb*ABTBepi

```
d/dt(ABMET3tb) =  Vmaxtb3*(ANTBepi/(VTBepi*Pvrg))/(Km3 + (ANTBepi/(VTBepi*Pvrg)))
d/dt(ABTBex) = KBOTB*SATB*(ABTBepi/(VTBepi*PBvrg) - (ABTBex/(VTBex*PBvrg))) + Qta*(CBart
- (ABTBex/(VTBex*PBvrg)))
{differential equations for BD in PU compartment umoles, PU}
d/dt(ANPUa) = Q*(Cairin - (ANPUa/VPUa)) - KOPU*SAPU*((ANPUa/VPUa)-
(ANPUmuc/(PMA*VPUmuc)))
d/dt(ANPUmuc) = KOPU*SAPU*((ANPUa/VPUa) - (ANPUmuc/(PMA*VPUmuc))) -
KTRPU*SAPU*((ANPUmuc/VPUmuc) - (ANPUepi/(VPUepi*Pvrg)))
d/dt(ANPUepi) = KTRPU*SAPU*((ANPUmuc/(VPUmuc*PMA)) - (ANPUepi/(VPUepi*Pvrg))) -
KBOPU*SAPU*((ANPUepi/(VPUepi*Pvrg)) - (ANPUex/(VPUex*Pvrg))) -
Vmaxpu*(ANPUepi/(VPUepi*Pvrg))/(Kmlu + (ANPUepi/(VPUepi*Pvrg)))
d/dt(ANPUex) = KBOPU*SAPU*((ANPUepi/(VPUepi*Pvrg)) - (ANPUex/(VPUex*Pvrg))) + Qtot*(Cart -
(ANPUex/(VPUex*Pvrg)))
d/dt(GSHpu) = Kgsh*(CGSHpub - (GSHpu/VPUepi)) - Kge*GSHpu - K2pu*ABPUepi
d/dt(AMETpu) = Vmaxpu*(ANPUepi/(VPUepi*Pvrg))/(Kmlu + (ANPUepi/(VPUepi*Pvrg)))
{differential equations for BMO in PU compartment umoles, PU}
d/dt(ABPUa) = Q*(Cairin - (ANPUa/VPUa)) - KOPU*SAPU*((ANPUa/VPUa)-
(ANPUmuc/(PMA*VPUmuc)))
d/dt(ABPUmuc) = KOPU*SAPU*((ABPUa/VPUa) - (ABPUmuc/(PMA*VPUmuc))) -
KTRPU*SAPU*((ABPUmuc/(VPUmuc*PMA)) - (ABPUepi/(VPUepi*PBvrg)))
 d/dt(ABPUepi) = KTRPU*SAPU*((ABPUmuc/(VPUmuc*PMA)) - (ABPUepi/(VPUepi*PBvrg))) -
KBOPU*SAPU*((ABPUepi/(VPUepi*PBvrg)) - (ABPUex/(VPUex*PBvrg))) +
Vmaxpu*(ANPUepi/(VPUepi*Pvrg))/(Kmlu + (ANPUepi/(VPUepi*Pvrg))) - K1pu*ABPUepi -
K2pu*ABPUepi - Vmaxpu3*(ABPUepi/(VPUepi*Pvrg))/(Km3 + (ABPUepi/(VPUepi*Pvrg)))
 d/dt(ABPUex) = KBOPU*SAPU*((ABPUepi/(VPUepi*PBvrg)) - (ABPUex/(VPUex*PBvrg))) +
Qtot*(CBart - (ABPUex/(VPUex*PBvrg)))
d/dt(ABMET2pu) = K1pu*ABPUepi
d/dt(ABMETGpu) =  K2pu*ABPUepi
d/dt(ABMET3pu) =  Vmaxpu3*(ABPUepi/(VPUepi*Pvrg))/(Km3 + (ABPUepi/(VPUepi*Pvrg)))
{Sum of  Lung BD}
d/dt(ANex) = Qtot*((Cart-Cvurtex) + (Cart-Cvcaex) + (Cart-Cvtbex) + (Cart-Cvpuex))
{Sum of Lung BMO}
d/dt(ABex) = Qtot*((CBart-CBvurtex) +(CBart-CBvcaex) + (CBart-CBvtbex) + (CBart-CBvpuex))
{BD ex respiratory tract, umoles}
init Af = 0
init Al = 0
init Am = 0
init Avrg = 0
init Ablood = 0
init GSHl = 6.0*Vl
{BMO oxide ex respiratory tract, umoles}
init ABf = 0
init ABler = 0
init ABlcy = 0
init ABm = 0
init ABvrg = 0
init ABblood = 0
```

{umoles BD metabolized}
init AMETl = 0
{umoles BMO ex rt metabolized EH, GST and P450 pathways}
init ABMETl2 = 0
init ABMETGl = 0
init ABMETl3 = 0
{AUCs BD}
init AUCvtot = 0
init AUCvl = 0
{AUCs BMO}
init AUCBvtot = 0
init AUCBvl = 0
{tissue flows mL/min}
Qtot = (0.012*Age^3 - 1.2144*Age^2 + 40.32*Age + 44.144)*(1000/60)
Qalv = (17.874*Age + 39.785)*(1000/60)
Qf = 0.0528*Qtot
Ql = 0.0795*Qtot
Qm = 0.0304*Qtot
Qvrg = 0.837*Qtot
{tissue volumes mL}
BW = - 1.9*Age^4 + 72.8*Age^3 - 813.1*Age^2 + 5535.6*Age + 4453.7
Vf = 0.0165*Age^5 - 1.9784*Age^4 + 51.963*Age^3 - 459.38*Age^2 + 1566.8*Age + 1004.2
Vl = 0.0072*Age^5 - 0.3975*Age^4 + 7.9052*Age^3 - 65.625*Age^2 + 262.02*Age + 157.52
Vm = -0.0623*Age^5 + 2.3433*Age^4 - 26.559*Age^3 + 144.75*Age^2 +339.84*Age + 1648.2
Vvrg = BW - (Vf + Vl + Vm + Vlu + Vblood)
Vblood = 0.075*BW
{blood/air and tissue/blood partition coefficients, BD}
Pb = 1.5
Pl = 5.49
Pf = 118.2
Pm = 5.26
Pvrg = 5.34
{blood/air and tissue/blood partition coefficients, BMO}
PBb = 60
PBl = 0.6545
PBf = 1.808
PBm = 0.6533
PBvrg = 0.6348
{calculated concentrations of BD umol/mL}
Cblood = Ablood/Vblood
Cart = Cvex
Cvf = Af/(Vf*Pf)
Cvl = Al/(Vl*Pl)
Cvm = Am/(Vm*Pm)

Cvvrg = Avrg/(Vvrg*Pvrg)
Cvtot = (Ql*Cvl + Qf*Cvf + Qm*Cvm + Qvrg*Cvvrg)/Qtot
Cairin = exposure/(24.45*1E3)
CGSHl = GSHl/Vl
CGSHlb = 6.0
{calculated concentrations of BMO umol/mL}
CBblood = ABblood/Vblood
CBart = CBvex
CBvf = ABf/(Vf*PBf)
CBvler = ABler/(Vl*PBl)
CBvlcy = ABlcy/(Vl*PBl)
CBvm = ABm/(Vm*PBm)
CBvvrg = ABvrg/(Vvrg*PBvrg)
CBvtot = (Ql*CBvlcy + Qf*CBvf + Qm*CBvm + Qvrg*CBvvrg)/Qtot
CBairin = exposureB/(24.45*1E3)
{differential equations for BD uptake and metabolism, umoles}
d/dt(Al) = Ql*(Cart - Cvl) - Vmaxl*Cvl/(Km + Cvl)
d/dt(Af) = Qf*(Cart - Cvf)
d/dt(Am) = Qm*(Cart - Cvm)
d/dt(Avrg) = Qvrg*(Cart - Cvvrg)
d/dt(Ablood) = Ql*Cvl + Qf*Cvf + Qm*Cvm + Qvrg*Cvvrg + Qtot*Cvex
{differential equations for BMO uptake and metabolism, umoles}
d/dt(ABf) = Qf*(CBart - CBvf)
d/dt(ABm) = Qm*(CBart - CBvm)
d/dt(ABvrg) = Qvrg*(CBart - CBvvrg)
d/dt(ABblood) = Ql*CBvlcy + Qf*CBvf + Qm*CBvm + Qvrg*CBvvrg + Qtot*CBvex
d/dt(ABler) = Vmaxl*Cvl/(Km + Cvl) - Kec*(CBvler - CBvlcy) - Vmaxl2*CBvler/(Km2ih + CBvler)
d/dt(ABlcy) = Kec*(CBvler - CBvlcy) + Ql*(CBart - CBvlcy) - VmaxGl*CBvlcy*CGSHl/(KmG2*CBvlcy + KmG1*CGSHl + CBvlcy*CGSHl)
d/dt(GSHl) = Kgsh*(CGSHlb - (GSHl/Vl)) - Kge*GSHl - VmaxGl*CBvlcy*CGSHl/(KmG2*CBvlcy + KmG1*CGSHl + CBvlcy*CGSHl) - Vmaxl3*CBvlcy/(Km3 + CBvlcy)
{amount of BD metabolized in liver to BMO, umoles}
d/dt(AMETl) = Vmaxl*Cvl/(Km+ Cvl)
{amount of BMO metabolized in liver and lung to diol, umoles}
d/dt(ABMETl2) = Vmaxl2*CBvler/(Km2ih + CBvler)
{amount of bmo metabolized in liver and lung to GSH conjugate, umoles}
d/dt(ABMETGl) = VmaxGl*CBVlcy*CGSHl/(KmG2*CBvlcy + KmG1*CGSHl + CGSHl*CBvlcy)
{amount of BMO oxidized to DEB, umoles}
d/dt(ABMETl3) = Vmaxl3*CBvlcy/(Km3 + CBvlcy)
{AUCs for BD, umolmin/mL}
d/dt(AUCvtot) = Cvtot
d/dt(AUCvl) = Cvl
{AUCs for BMO, umolmin/mL}
d/dt(AUCBvtot) = CBvtot
d/dt(AUCBvl) = CBvlcy

E.5.7 Model Code for BD/BMO/DEB 0-5 Yr Child

```
METHOD Stiff

STARTTIME = 0
STOPTIME= 48
DT = 0.001
{butadiene BD, moles}
init Af = 0
init Al = 0
init Am = 0
init Avrg = 0
init Abr = 0
init Apu = 0
{butadienemonoxide BMO, moles}
init ABf = 0
init ABler = 0
init ABlcy = 0
init ABm = 0
init ABvrg = 0
init ABbr = 0
init ABpu = 0
{diepoxybutane DEB, moles}
init ACf = 0
init ACl = 0
init ACm = 0
init ACvrg = 0
init ACbr = 0
init ACpu = 0
{moles of GSH in liver and lung}
init GSHl = 5.9E-3*Vl
GSHl0 = 5.9E-3*Vl
init GSHlu = 1.12E-3*Vlu
GSHlu0 = 1.12E-3*Vlu
Kgsh = 0.72
Kge = 0.15
CGSHl = GSHl/Vl
CGSHlu = GSHlu/Vlu
{moles butadiene metabolized}
init Ametl = 0
init Ametpu = 0
init Ametbr = 0
{moles of butadienemonoxide metabolized}
init ABmetl1 = 0
init ABmetl2 = 0
```

```
init ABmetpu1 = 0
init ABmetpu2 = 0
init ABmetbr1 = 0
init ABmetbr2 = 0
init ABmetl3 = 0
init ABmetpu3 = 0
init ABmetbr3 = 0
{area under the venous blood concn x time curve, butadiene}
init AUCvtot = 0
init AUCvl = 0
init AUCvpu = 0
init AUCvbr = 0
init AUCvlung = 0
{area under the venous blood concn x time curve, butadienemonoxide}
init AUCBvtot = 0
init AUCBvl =  0
init AUCBvpu = 0
init AUCBvbr = 0
init AUCBvlung = 0
{area under the venous blood concn x time curve, diepoxybutene}
init AUCCvtot = 0
init AUCCvl = 0
init AUCCvpu = 0
init AUCCvbr = 0
init AUCCvlung = 0
{tissue flows L/hr}
Qtot = 0.012*Age^3 - 1.2144*Age^2 + 40.32*Age + 44.144
Qalv = 17.874*Age + 39.785
Qf = 0.0528*Qtot
Ql = 0.0795*Qtot
Qm = 0.0304*Qtot
Qvrg = 0.837*Qtot
Qpu = 0.93*Qtot
Qbr = 0.07*Qtot
Age = 0.0
{tissue volumes L}
BW = (-1.9*Age^4 + 72.8*Age^3 - 813.1*Age^2 + 5535.6*Age +4453.7)/1000
Vf = (0.0165*Age^5 - 1.9784*Age^4 + 51.963*Age^3 - 459.38*Age^2 + 1566.8*Age + 1004.2)/1000
Vl = (0.0072*Age^5 - 0.3975*Age^4 + 7.9052*Age^3 - 65.625*Age^2 + 262.02*Age + 157.52)/1000
Vm = (-0.0623*Age^5 + 2.3433*Age^4 - 26.559*Age^3 + 144.75*Age^2 + 339.84*Age + 1648.2)/1000
Vvrg = BW - (Vf + Vl + Vm +Vlu)
Vlu = (-0.0346*Age^4 + 1.5069*AGe^3 - 20.31*Age^2 + 123.99*AGe + 59.213)/1000
Vpu = 0.9*Vlu
Vbr = 0.1*Vlu
```

{blood/air and tissue/blood partition coefficients, butadiene}
Pb = 1.5
Pl = 5.49
Pf = 118.2
Pm = 5.26
Pvrg = 5.34
Ppu = 4.02
Pbr = 4.02
{blood/air and tissue/blood partition coefficients, butadienemonoxide}
PBb = 60
PBl = 0.6545
PBf = 1.8083
PBm = 0.6533
PBvrg = 0.6348
PBpu = 0.4725
PBbr = 0.4725
{blood/air and tissue/blood partition coefficients, diepoxybutene}
PCb = 300
PCl = 0.70
PCf = 0.715
PCm = 0.697
PCvrg = 0.6
PCpu = 0.6
PCbr = 0.6
{butadiene oxidation metabolic parameters, mol/hr, mol/L}
Vmaxlu = 9.0E-9*Vlu*3E3*(70/BW)^0.25
Vmaxbr = 0.1*Vmaxlu
Vmaxpu = 0.9*Vmaxlu
Vmaxl = 7.08E-8*Vl*1.45E4*(70/BW)^0.25
Km = 5.14E-6
Kmlu = 2.0E-6
{butadienemonoxide metabolic parameters, mol/hr, mol/L, /hr; 1 = hydrolysis, 2 = conjugation, 3 = oxidation}
Vmaxl1 = 1.1E-6*Vl*1.45E4*(70/BW)^0.25
Km1 = 5.8E-4
Km1ih = 0.2*Km1
Kec = 400
Vmaxl2 = 2.71E-6*Vl*5.8E4*(70/BW)^0.25
Km2 = 1.04E-2
Km2bmo = 1E-4
k1 = 0.1914*3E3*Vlu*(70/BW)^-0.25
k2 = 0.1536*5.8E4*Vlu*(70/BW)^-0.25
Vmaxl3 = 1.48e-5*(70/BW)^0.25
Vmaxpu3 = 1.7E-6*(70/BW)^0.25

```
Vmaxbr3 = 2.0E-7*(70/BW)^0.25
Km3 = 1.56E-5
Km3ih = 0.2*Km3
{diepoxybutene elimination constant, /hr}
Ke = 0.6*(70/BW)-0.25
{exposure in ppm converted to moles}
Cair = IF TIME <= 24 THEN 1*(1E-6/25.45) ELSE 0
{calculated concentrations of butadiene}
Cart = (Qpu*Cvpu + Qbr*Cvbr)/Qtot
Cvf = Af/(Vf*Pf)
Cvl = Al/(Vl*Pl)
Cvm = Am/(Vm*Pm)
Cvvrg = Avrg/(Vvrg*Pvrg)
Cvpu = Apu/(Vpu*Ppu)
Cvbr = Abr/(Vbr*Pbr)
Cvtot = (Ql*Cvl + Qf*Cvf + Qm*Cvm + Qvrg*Cvvrg)/Qpu
Cvipu = (Qalv*Cair + Qpu*Cvtot)/((Qalv/Pb) + Qpu)
Cexh = Cvipu/Pb
{calculated concentrations of butadienemonoxide}
CBart = (Qpu*CBvpu + Qbr*CBvbr)/Qtot
CBvf = ABf/(Vf*PBf)
CBvler = ABler/(Vl*PBl)
CBvlcy = ABlcy/(Vl*PBl)
CBvm = ABm/(Vm*PBm)
CBvvrg = ABvrg/(Vvrg*PBvrg)
CBvpu = ABpu/(Vpu*PBpu)
CBvbr = ABbr/(Vbr*PBbr)
CBvtot = (Ql*CBvlcy + Qf*CBvf + Qm*CBvm + Qvrg*CBvvrg)/Qtot
CBair = CBvtot/PBb
CBvipu = (Qalv*CBair + Qpu*CBvtot)/((Qalv/PBb) + Qpu)
CBexh = CBvipu/PBb
{calculated concentrations of diepoxybutene}
CCart = (Qpu*CCvpu + Qbr*CCvbr)/Qtot
CCvf = ACf/(Vf*PCf)
CCvl = ACl/(Vl*PCl)
CCvm = ACm/(Vm*PCm)
CCvvrg = ACvrg/(Vvrg*PCvrg)
CCvpu = ACpu/(Vpu*PCpu)
CCvbr = ACbr/(Vbr*PCbr)
CCvtot = (Ql*CCvl +Qf*CCvf + Qm*CCvm + Qvrg*CCvvrg)/Qtot
CCair = CCvtot/PCb
CCvipu = (Qalv*CCair + Qpu*CCvtot)/((Qalv/PCb) + Qpu)
CCexh = CCvipu/PCb
{differential equations for butadiene uptake and metabolism}
```

```
d/dt(Apu) = Qpu*(Cvipu - Cvpu) - Vmaxpu*Cvpu/(Kmlu + Cvpu)
d/dt(Abr) = Qbr*(Cart - Cvbr) - Vmaxbr * Cvbr/(Kmlu + Cvbr)
d/dt(Al) = Ql*(Cart - Cvl) - Vmaxl*Cvl/(Km + Cvl)
d/dt(Af) = Qf*(Cart - Cvf)
d/dt(Am) = Qm*(Cart - Cvm)
d/dt(Avrg) = Qvrg*(Cart - Cvvrg)
{amount of butadiene metabolized in liver and lung}
d/dt(Ametl)  = Vmaxl*Cvl/(Km + Cvl)
d/dt(Ametpu) = Vmaxpu*Cvpu/(Kmlu + Cvpu)
d/dt(Ametbr) = Vmaxbr*Cvbr/(Kmlu + Cvbr)
{AUCs for butadiene}
d/dt(AUCvtot) = Cvtot
d/dt(AUCvl) = Cvl
d/dt(AUCvpu) = Cvpu
d/dt(AUCvbr) = Cvbr
d/dt(AUCvlung) =Cvpu + Cvbr
{differential equations for butadienemonoxide metabolism}
d/dt(ABpu) = Qpu*(CBart - CBvpu) + Vmaxpu*Cvpu/(Kmlu + Cvpu) -k1*ABpu -k2*ABpu -
Vmaxpu3*CBvpu/(Km3 + CBvpu)
d/dt(ABbr) = Qbr*(CBart - CBvbr) + Vmaxbr * Cvbr/(Kmlu + Cvbr) -k1*ABbr -k2*ABbr -
Vmaxbr3*CBvbr/(Km3 + CBvbr)
d/dt(ABler) =  Vmaxl*Cvl/(Km + Cvl) - Kec*(CBvler - CBvlcy) - Vmaxl1*CBvler/(Km1ih + CBvler)  -
Vmaxl3*CBvler/(Km3ih + CBvler)
d/dt(ABlcy) = Ql*(CBart - CBvlcy)+ Kec*(CBvler - CBvlcy) - Vmaxl2*CBvlcy*CGSHl/(Km2*CGSHl +
Km2bmo*CBvlcy + CGSHl*CBvlcy)
d/dt(ABf) = Qf*(CBart - CBvf)
d/dt(ABm) = Qm*(CBart - CBvm)
d/dt(ABvrg) = Qvrg*(CBart - CBvvrg)
{AUCs for butadienemonoxide}
d/dt(AUCBvtot) = CBvtot
d/dt(AUCBvl) = CBvler + CBvlcy
d/dt(AUCBvpu) = CBvpu
d/dt(AUCBvbr) = CBvbr
d/dt(AUCBvlung) = CBvpu + CBvbr
{amounts of butadienemonoxide metabolized in liver and lung}
d/dt(ABmetl1) = Vmaxl1*CBvler/(Km1ih + CBvler)
d/dt(ABmetl2) = Vmaxl2*CBvlcy*CGSHl/(Km2*CGSHl + Km2bmo*CBvlcy + CGSHl*CBvlcy)
d/dt(ABmetpu1) = k1*ABpu
d/dt(ABmetpu2) = k2*ABpu
d/dt(ABmetbr1) = k1*ABbr
d/dt(ABmetbr2) = k2*ABbr
d/dt(ABmetl3) = Vmaxl3*CBvler/(Km3ih + CBvler)
d/dt(ABmetpu3) = Vmaxpu3*CBvpu/(Km3 + CBvpu)
d/dt(ABmetbr3) = Vmaxbr3*Cbvbr/(Km3 + CBvbr)
{differential equations for diepoxybutene}
```

```
d/dt(ACpu) = Qpu*(CCart - CCvpu) + Vmaxpu3*(ABpu/Vpu)/(Km3 + (ABpu/Vpu)) - Ke*ACpu
d/dt(ACbr) = Qbr*(CCart - CCvbr) + Vmaxbr3*(ABbr/Vbr)/(Km3 + (ABbr/Vbr)) - Ke*ACbr
d/dt(ACl) = Ql*(CCart - CCvl) + Vmaxl3*CBvler/(Km3ih + CBvler) - Ke*ACl
d/dt(ACf) = Qf*(CCart - CCvf)
d/dt(ACm) = Qm*(CCart - CCvm)
d/dt(ACvrg) = Qvrg*(CCart - CCvvrg)
{AUCs for diepoxybutene}
d/dt(AUCCvtot) = CCvtot
d/dt(AUCCvl) = CCvl
d/dt(AUCCvpu) = CCvpu
d/dt(AUCCvbr) = CCvbr
d/dt(AUCCVlung) = CCvpu + CCvbr
{differential equation for GSH}
d/dt(GSHl) = Kgsh*Vl*(GSHl0 - CGSHl) - Kge*GSHl - Vmaxl2*CBvlcy*Cgshl/(Km2*CGSHl +
Km2bmo*CBvlcy + CGSHl*CBvlcy)
d/dt(GSHlu) = Kgsh*Vlu*(GSHlu0 - CGSHlu) - Kge*GSHlu
```

E.5.8 Model Code for Styrene/SO RT (Sarangapani et al*. 2002) 0-5 yr Child*

METHOD Stiff

```
STARTTIME = 0
STOPTIME= 2880
DT = 0.0001
{Styrene in upper respiratory tract compartment (URT) umoles}
init ANURTa = 0
init ANURTmuc = 0
init ANURTepi = 0
init ANURTex = 0
init AMETurt = 0
{Styrene oxide in upper respiratory compartment (URT) umoles}
init ABURTa = 0
init ABURTmuc = 0
init ABURTepi = 0
init ABURTer = 0
init ABURTcy = 0
init GSHua = 1.0*VURTepi
init ABURTex = 0
init AMET2urt = 0
init AMET3urt = 0
{Styrene in conducting airways compartment (CA), umoles}
init ANCAa = 0
```

```
init ANCAmuc = 0
init ANCAepi = 0
init ANCAex = 0
{Styrene oxide in conducting airways compartment (CA), umoles}
init ABCAa = 0
init ABCAmuc = 0
init ABCAepi = 0
init ABCAex = 0
{Styrene in terminal bronchioles compartment (TB), umoles}
init ANTBa = 0
init ANTBmuc = 0
init ANTBepi = 0
init ANTBex = 0
init AMETtb = 0
{Styrene oxide in terminal bronchioles compartment (TB), umoles}
init ABTBa = 0
init ABTBmuc = 0
init ABTBepi = 0
init ABTBer = 0
init ABTBcy = 0
init GSHtb = 1.0*VTBepi
init ABTBex = 0
init AMET2tb = 0
init AMET3tb = 0
{Styrene in pulmonary compartment (PU), umoles}
init ANPUa = 0
init ANPUmuc = 0
init ANPUepi = 0
init ANPUex = 0
init ANex = 0
{Styrene oxide in pulmonary compartment (PU), umoles}
init ABPUa = 0
init ABPUmuc = 0
init ABPUepi = 0
init ABPUex = 0
init ABex = 0
{model equations}
Q = RPM*TVOL
Cairin = exposure/(24.36*1E3)
VURTepi = SAURT*WUA
VURTmuc = SAURT*WSMua
VURTex = SAURT*WUAs
VCAmuc = SACA*WSMca
VCAepi = SACA*WCA
```

```
VCAex = SACA*WCAs
VTBmuc = SATB*WSMtb
VTBepi = SATB*WTA
VTBex = SATB*WTAs
VPUmuc = SAPU*WSMpu
VPUepi = SAPU*WPA
VPUex = SAPU*WTAs
Vlu = 59.213 + 123.99*Age - 20.31*Age^2 + 1.5069*Age^3 - 0.0346*Age^4
VURT = 0.0026*Vlu
VCA = 0.018*Vlu
VTB = 0.043*Vlu
VPU = 0.937*Vlu
Curtepil = (ANURTepi/VURTepi)
Ccaepil = (ANCAepi/VCAepi)
Ctbepil = (ANTBepi/VTBepi)
Cpuepil = (ANPUepi/VPUepi)
Cvurtex = (ANURTex/(VURTex*Pvrg))
Cvcaex = (ANCAex/(VCAex*Pvrg))
Cvtbex = (ANTBex/(VTBex*Pvrg))
Cvpuex = (ANPUex/(VPUex*Pvrg))
Cvex = (ANURTex+ANCAex+ANTBex+ANPUex)/((VURTex+VCAex+VTBex+VPUex)*Pvrg)
CBurtepil = (ABURTepi/VURTepi)
CBcaepil = (ABCAepi/VCAepi)
CBtbepil = (ABTBepi/VTBepi)
CBpuepil = (ABPUepi/VPUepi)
CBvurtex = (ABURTex/(VURTex*PBvrg))
CBvcaex = (ABCAex/(VCAex*PBvrg))
CBvtbex = (ABTBex/(VTBex*PBvrg))
CBvpuex = (ABPUex/(VPUex*PBvrg))
CBvex = (ABURTex+ABCAex+ABTBex+ABPUex)/((VURTex+VCAex+VTBex+VPUex)*PBvrg)
GSHuab = 2.5
GSHtbb = 1.0
Exposure = IF TIME <= 1440 THEN 1 ELSE 0
ExposureB = IF TIME <= 1440 THEN 0 ELSE 0
Age = 3.0
{upper respiratory tract constants}
PMA = 30 {mucus:air partition coeff}
KOURT = 1980 {mass transfer coeffs., cm/min}
KTRURT = 19.2
KBOURT = 19.2
KOCA = 181
KTRCA = 19.2
KBOCA = 19.2
KOTB = 158
```

KTRTB = 19.2
KBOTB = 19.2
KOPU = 158
KTRPU = 19.2
KBOPU = 19.2
KMUC = 0.001 {diffusion constants, cm2/min}
KSQM = 0.0002
KG = 6.0
SAURT = VURT/WUA {surface areas, cm2}
SACA = VCA/WCA
SATB = VTB/WTA
SAPU = VPU/WPA
VURTa = 0.00035*TLC {luminal volumes, cm3}
VCAa = 0.0105*TLC
VTBa = 0.042*TLC
VPUa = 0.944*TLC
TLC = 236*5 + 282*Age - 4.775*Age^2 + 0.285*Age^3
RPM = 53.5*(BW/1000)^-0.26 {breaths/min}
TVOL = 35.45 + 33.56*Age - 1.47*Age^2 + 0.0793*Age^3 {tidal volume mL/breath}

{thicknesses (W) of upper airways epithelium (UA), submucosa (UAs);mucus (SM); conducting airways epi (CA), submucosa (CAs); transitional airways epi (TA), submucosa (TAs); and pulmonary airways epi (PA), cm}

WUA = 0.005
WSMua = 0.001
WSMca = 0.0005
WSMtb = 0.0002
WSMpu = 0.0001
WCA = 0.0025
WTA = 0.001
WPA = 0.0005
WUAs = 0.01
WCAs = 0.005
WTAs = 0.002
Qua = 0.0025*Qtot {blood flow to the URT region}
Qca = 0.0075*Qtot {blood flow to the CA}
Qta = 0.0067*Qtot {blood flow to the TA}

{metabolic constants umol/min, umol/mL, based on Csanady et al. 2003 scaled to smaller BWs}

Vmaxua = 4.17E-5*VURTepi*(70/BW)^0.25
Vmaxtb = 4.17E-5*VTBepi*(70/BW)^0.25
Vmaxl = 0.033*Vl*(70/BW)^0.25
Vmaxl2 = 0.075*Vl*(70/BW)^0.25 {EH}
Vmaxua2 = 0.0112*VURTepi*(70/BW)^0.25
Vmaxtb2 = 0.0112*VTBepi*(70/BW)^0.25
Kgsh = 0.012 {/min}

```
VmaxGl = 0.467*Vl*(70/BW)^0.25 {umol/min/liver, GST}
VmaxGua = 1.36*VURTepi*(70/BW)^0.25 {umol/min/URT}
VmaxGtb = 1.36*VTBepi*(70/BW)^0.25 {umol/min/TB}
MPl = 23 {mg microsomal protein /mL tissue}
MPlu = 3.8 {mg microsomal protein/mL tissue}
CPl = 45 {mg cytosolic protein/mL tissue}
CPlu = 43 {mg cytosolic protein/mL tissue}
Km1 = 0.01 {umol/mL}
Km2 = 0.01
Kmlu1 = 0.0175
Kmlu2 = 0.0156
KmG1 = 0.1 {GST}
KmG2 = 2.5 {SO}
Kec = 400
 {differential equations for ST in URT compartment, URT}
d/dt(ANURTa) = Q*(Cairin - (ANURTa/VURTA)) - KOURT*SAURT*((ANURTa/VURTA)-
(ANURTmuc/(PMA*VURTmuc)))
d/dt(ANURTmuc) = KOURT*SAURT*((ANURTa/VURTa) - (ANURTmuc/(PMA*VURTmuc))) -
KTRURT*SAURT*((ANURTmuc/(VURTmuc*PMA)) - (ANURTepi/(VURTepi*Pvrg)))
d/dt(ANURTepi) = KTRURT*SAURT*((ANURTmuc/(VURTmuc*PMA)) - (ANURTepi/(VURTepi*Pvrg)))
- KBOURT*SAURT*((ANURTepi/(VURTepi*Pvrg)) - (ANURTex/(VURTex*Pvrg))) -
Vmaxua*(ANURTepi/(VURTepi*Pvrg))/(Kmlu1 + (ANURTepi/(VURTepi*Pvrg)))
d/dt(ANURTex) = KBOURT*SAURT*((ANURTepi/VURTepi) - (ANURTex/VURTex)) + Qua*(Cart -
(ANURTex/(VURTex*Pvrg)))
d/dt(AMETurt) = Vmaxua*(ANURTepi/(VURTepi*Pvrg))/(Kmlu1 + (ANURTepi/(VURTepi*Pvrg)))
d/dt(GSHua) = Kgsh*(GSHuab - (GSHua/VURTepi)) -
VmaxGua*(ABURTcy/VURTepi)*(GSHua/VURTepi)/(KmG1*(ABURTcy/VURTepi) +
KmG2*(GSHua/VURTepi) + (ABURTcy/VURTepi)*(GSHua/VURTepi))
{differential equations for ST oxide in URT compartment, URT}
d/dt(ABURTa) = Q*(CBairin - (ABURTa/VURTa)) - KOURT*SAURT*((ABURTa/VURTa)-
(ABURTmuc/(PMA*VURTmuc)))
d/dt(ABURTmuc) = KOURT*SAURT*((ABURTa/VURTa) - (ABURTmuc/(PMA*VURTmuc))) -
KTRURT*SAURT*((ABURTmuc/(VURTmuc*PMA)) - (ABURTepi/(VURTepi*PBvrg)))
d/dt(ABURTepi) = KTRURT*SAURT*((ABURTmuc/(VURTmuc*PMA)) -
(ABURTepi/(VURTepi*PBvrg))) - KBOURT*SAURT*((ABURTepi/(VURTepi*PBvrg)) -
(ABURTex/(VURTex*PBvrg))) + Vmaxua*(ANURTepi/(VURTepi*PBvrg))/(Kmlu1 +
(ANURTepi/(VURTepi*PBvrg)))
d/dt(ABURTer) = Vmaxua*(ANURTepi/(VURTepi*Pvrg))/(Kmlu1 + (ANURTepi/(VURTepi*Pvrg))) -
Kec*((ABURTer/(VURTepi*PBvrg)) - (ABURTcy/(VURTepi*PBb))) -
Vmaxua2*(ABURTer/(VURTepi*PBvrg))/(Kmlu2 + (ABURTer/(VURTepi*PBvrg)))
d/dt(AMET2urt) = Vmaxua2*(ABURTer/(VURTepi*PBvrg))/(Kmlu2 + (ABURTer/(VURTepi*PBvrg)))
d/dt(ABURTcy) = Kec*((ABURTer/(VURTepi*PBvrg)) - (ABURTcy/(VURTepi*PBb))) + Qua*(CBart -
ABURTcy/(VURTepi*PBvrg)) -
VmaxGua*(ABURTcy/VURTepi)*(GSHua/VURTepi)/(KmG1*(ABURTcy/VURTepi) +
KmG2*(ABURTcy/VURTepi) + KmG2*(GSHua/VURTepi) + (ABURTcy/VURTepi)*(GSHua/VURTepi))
d/dt(AMET3urt) = VmaxGua*(ABURTcy/VURTepi)*(GSHua/VURTepi)/(KmG1*(ABURTcy/VURTepi) +
KmG2*(ABURTcy/VURTepi) + KmG2*(GSHua/VURTepi) + (ABURTcy/VURTepi)*(GSHua/VURTepi))
```

d/dt(ABURTex) = KBOURT*SAURT*((ABURTepi/VURTepi) - (ABURTex/(VURTex*PBvrg))) + Qua*(CBart - (ABURTex/(VURTex*PBvrg)))

{differential equations for ST in CA compartment, CA}

d/dt(ANCAa) = Q*(Cairin - (ANCAa/VCAa)) - KOCA*SACA*((ANCAa/VCAa)-(ANCAmuc/(PMA*VCAmuc)))

d/dt(ANCAmuc) = KOCA*SACA*((ANCAa/VCAa) - (ANCAmuc/(PMA*VCAmuc))) - KTRCA*SACA*((ANCAmuc/(VCAmuc*PMA)) - (ANCAepi/(VCAepi*Pvrg)))

d/dt(ANCAepi) = KTRCA*SACA*((ANCAmuc/(VCAmuc*PMA)) - (ANCAepi/(VCAepi*Pvrg))) - KBOCA*SACA*((ANCAepi/(VCAepi*Pvrg)) - (ANCAex/(VCAex*Pvrg)))

d/dt(ANCAex) = KBOCA*SACA*((ANCAepi/(VCAepi*Pvrg)) - (ANCAex/(VCAex*Pvrg))) + Qca*(Cart - (ANCAex/(VCAex*Pvrg)))

{differential equations for ST oxide in CA compartment, CA}

d/dt(ABCAa) = Q*(CBairin - (ABCAa/VCAa)) - KOCA*SACA*((ABCAa/VCAa)-(ABCAmuc/(PMA*VCAmuc)))

d/dt(ABCAmuc) = KOCA*SACA*((ABCAa/VCAa) - (ABCAmuc/(PMA*VCAmuc))) - KTRCA*SACA*((ABCAmuc/(VCAmuc*PMA)) - (ABCAepi/(VCAepi*PBvrg)))

d/dt(ABCAepi) = KTRCA*SACA*((ABCAmuc/(VCAmuc*PMA)) - (ABCAepi/(VCAepi*PBvrg))) - KBOCA*SACA*((ABCAepi/(VCAepi*PBvrg)) - (ABCAex/(VCAex*PBvrg)))

d/dt(ABCAex) = KBOCA*SACA*((ABCAepi/(VCAepi*PBvrg)) - (ABCAex/(VCAex*PBvrg))) + Qca*(CBart - (ABCAex/(VCAex*PBvrg)))

{differential equations for ST in TB compartment umoles, TB}

d/dt(ANTBa) = Q*(Cairin - (ANTBa/VTBa)) - KOTB*SATB*((ANTBa/VTBa)-(ANTBmuc/(PMA*VTBmuc)))

d/dt(ANTBmuc) = KOTB*SATB*((ANTBa/VTBa) - (ANTBmuc/(PMA*VTBmuc))) - KTRTB*SATB*((ANTBmuc/(VTBmuc*PMA)) - (ANTBmuc/(VTBmuc*PMA)))

d/dt(ANTBepi) = KTRTB*SATB*((ANTBmuc/(VTBmuc*PMA)) - (ANTBepi/(VTBepi*Pvrg))) - KBOTB*SATB*((ANTBepi/(VTBepi*Pvrg)) - (ANTBex/(VTBex*Pvrg))) - Vmaxtb*(ANTBepi/(VTBepi*Pvrg))/(Kmlu1 + (ANTBepi/(VTBepi*Pvrg)))

d/dt(ANTBex) = KBOTB*SATB*((ANTBepi/(VTBepi*Pvrg)) - (ANTBex/(VTBex*Pvrg))) + Qta*(Cart - (ANTBex/(VTBex*Pvrg)))

d/dt(AMETtb) = Vmaxtb*(ANTBepi/(VTBepi*Pvrg))/(Kmlu1 + (ANTBepi/(VTBepi*Pvrg)))

d/dt(GSHtb) = Kgsh*(GSHtbb - (GSHtb/VTBepi)) - VmaxGtb*(ABTBcy/VTBepi)*(GSHtb/VTBepi)/(KmG1*(ABTBcy/VTBepi) + KmG2*(GSHtb/VTBepi) + (ABTBcy/VTBepi)*(GSHtb/VTBepi))

{differential equations for ST oxide in TB compartment umoles, TB}

d/dt(ABTBa) = Q*(CBairin - (ABTBa/VTBa)) - KOTB*SATB*((ABTBa/VTBa)-(ABTBmuc/(PMA*VTBmuc)))

d/dt(ABTBmuc) = KOTB*SATB*((ABTBa/VTBa) - (ABTBmuc/(PMA*VTBmuc))) - KTRTB*SATB*((ABTBmuc/(VTBmuc*PMA)) - (ABTBepi/(VTBepi*PBvrg)))

d/dt(ABTBepi) = KTRTB*SATB*((ABTBmuc/(VTBmuc*PMA)) - (ABTBepi/(VTBepi*PBvrg))) - KBOTB*SATB*((ABTBepi/(VTBepi*PBvrg)) - (ABTBex/(VTBex*PBvrg))) + Vmaxtb*(ANTBepi/(VTBepi*Pvrg))/(Kmlu1 + (ANTBepi/(VTBepi*Pvrg)))

d/dt(ABTBer) = Vmaxtb*(ANTBepi/(VTBepi*Pvrg))/(Kmlu1 + (ANTBepi/(VTBepi*Pvrg))) - Kec*((ABTBer/(VTBepi*PBvrg)) - (ABTBcy/(VTBepi*PBvrg))) - Vmaxtb2*(ABTBer/(VTBepi*PBvrg))/(Kmlu2 + (ABTBer/(VTBepi*PBvrg)))

d/dt(AMET2tb) = Vmaxtb2*(ABTBer/(VTBepi*PBvrg))/(Kmlu2 + (ABTBer/(VTBepi*PBvrg)))

d/dt(ABTBcy) = Kec*((ABTBer/(VTBepi*PBvrg)) - (ABTBcy/(VTBepi*PBvrg))) + Qta*(CBart - (ABTBcy/(VTBepi*PBvrg))) - VmaxGtb*(ABTBcy/VTBepi)*(GSHtb/VTBepi)/(KmG1*(ABTBcy/VTBepi) + KmG2*(ABTBcy/VTBepi) + KmG2*(GSHtb/VTBepi) + (ABTBcy/VTBepi)*(GSHtb/VTBepi))

d/dt(AMET3tb) = VmaxGtb*(ABTBcy/VTBepi)*(GSHtb/VTBepi)/(KmG1*(ABTBcy/VTBepi) + KmG2*(ABTBcy/VTBepi) + KmG2*(GSHtb/VTBepi) + (ABTBcy/VTBepi)*(GSHtb/VTBepi))

d/dt(ABTBex) = KBOTB*SATB*((ABTBepi/(VTBepi*PBvrg)) - (ABTBex/(VTBex*PBvrg))) + Qta*(CBart - (ABTBex/(VTBex*PBvrg)))

{differential equations for ST in PU compartment umoles, PU}

d/dt(ANPUa) = Q*(Cairin - (ANPUa/VPUa)) - KOPU*SAPU*((ANPUa/VPUa)-(ANPUmuc/(PMA*VPUmuc)))

d/dt(ANPUmuc) = KOPU*SAPU*((ANPUa/VPUa) - (ANPUmuc/(PMA*VPUmuc))) - KTRPU*SAPU*((ANPUmuc/VPUmuc) - (ANPUepi/(VPUepi*Pvrg)))

d/dt(ANPUepi) = KTRPU*SAPU*((ANPUmuc/(VPUmuc*PMA)) - (ANPUepi/(VPUepi*Pvrg))) - KBOPU*SAPU*((ANPUepi/(VPUepi*Pvrg)) - (ANPUex/(VPUex*Pvrg)))

d/dt(ANPUex) = KBOPU*SAPU*((ANPUepi/(VPUepi*Pvrg)) - (ANPUex/(VPUex*Pvrg))) + Qtot*(Cart - (ANPUex/(VPUex*Pvrg)))

{differential equations for ST oxide PU compartment umoles, PU}

d/dt(ABPUa) = Q*(Cairin - (ANPUa/VPUa)) - KOPU*SAPU*((ANPUa/VPUa)-(ANPUmuc/(PMA*VPUmuc)))

d/dt(ABPUmuc) = KOPU*SAPU*((ABPUa/VPUa) - (ABPUmuc/(PMA*VPUmuc))) - KTRPU*SAPU*((ABPUmuc/(VPUmuc*PMA)) - (ABPUepi/(VPUepi*PBvrg)))

d/dt(ABPUepi) = KTRPU*SAPU*((ABPUmuc/(VPUmuc*PMA)) - (ABPUepi/(VPUepi*PBvrg))) - KBOPU*SAPU*((ABPUepi/(VPUepi*PBvrg)) - (ABPUex/(VPUex*PBvrg)))

d/dt(ABPUex) = KBOPU*SAPU*((ABPUepi/(VPUepi*PBvrg)) - (ABPUex/(VPUex*PBvrg))) + Qtot*(CBart - (ABPUex/(VPUex*PBvrg)))

{Sum of Lung Styrene}

d/dt(ANex) = Qtot*((Cart-Cvurtex) + (Cart-Cvcaex) + (Cart-Cvtbex) + (Cart-Cvpuex))

{Sum of Lung Styrene Oxide}

d/dt(ABex) = Qtot*((CBart-CBvurtex) +(CBart-CBvcaex) + (CBart-CBvtbex) + (CBart-CBvpuex))

{ST ex respiratory tract, umoles}

init Af = 0

init Al = 0

init Am = 0

init Avrg = 0

init Ablood = 0

init GSHl = 6.0*Vl

{ST oxide ex respiratory tract, umoles}

init ABf = 0

init ABl = 0

init ABler = 0

init ABlcy = 0

init ABm = 0

init ABvrg = 0

init ABblood = 0

{umoles ST metabolized}

init Ametl = 0

{umoles ST oxide ex rt metabolized by EH and GST pathways}

init ABmetl = 0

init ABmetGl = 0

```
{AUCs}
init AUCvtot = 0
init AUCvl = 0
{AUCs BaP oxide}
init AUCBvtot = 0
init AUCBvl = 0
{tissue flows mL/min}
Qtot = (0.012*Age^3 - 1.2144*Age^2 + 40.32*Age + 44.144)*(1000/60)
Qalv = (17.874*Age + 39.785)*(1000/60)
Qf = 0.0528*Qtot
Ql = 0.0795*Qtot
Qm = 0.0304*Qtot
Qvrg = 0.837*Qtot
{tissue volumes mL}
BW = - 1.9*Age^4 + 72.8*Age^3 - 813.1*Age^2 + 5535.6*Age + 4453.7
Vf = 0.0165*Age^5 - 1.9784*Age^4 + 51.963*Age^3 - 459.38*Age^2 + 1566.8*Age + 1004.2
Vl = 0.0072*Age^5 - 0.3975*Age^4 + 7.9052*Age^3 - 65.625*Age^2 + 262.02*Age + 157.52
Vm = -0.0623*Age^5 + 2.3433*Age^4 - 26.559*Age^3 + 144.75*Age^2 +339.84*Age + 1648.2
Vvrg = BW - (Vf + Vl + Vm + Vlu + Vblood)
Vblood = 0.075*BW
{blood/air and tissue/blood partition coefficients, ST}
Pb = 48
Pl = 2.0
Pf = 50
Pm = 1.3
Pvrg = 1.3
{blood/air and tissue/blood partition coefficients, ST oxide}
PBb = 2000
PBl = 1.0
PBf = 14.0
PBm = 0.6
PBvrg = 0.6
{calculated concentrations of ST umol/mL}
Cblood = Ablood/Vblood
Cart = Cvex
Cvf = Af/(Vf*Pf)
Cvl = Al/(Vl*Pl)
Cvm = Am/(Vm*Pm)
Cvvrg = Avrg/(Vvrg*Pvrg)
Cvtot = (Ql*Cvl + Qf*Cvf + Qm*Cvm + Qvrg*Cvvrg)/Qtot
Cairin = exposure/(24.45*1E3)
CGSHl = GSHl/Vl
GSHlb = 6.0
{calculated concentrations of ST oxide umol/mL}
```

CBblood = ABblood/Vblood

CBart = CBvex

CBvf = ABf/(Vf*PBf)

CBvl = ABl/(Vl*PBl)

CBler = ABler/Vl

CBlcy = ABlcy/Vl

CBvm = ABm/(Vm*PBm)

CBvvrg = ABvrg/(Vvrg*PBvrg)

CBvtot = (Ql*CBvl + Qf*CBvf + Qm*CBvm + Qvrg*CBvvrg)/Qtot

CBairin = exposureB/(24.45*1E3)

{differential equations for ST uptake and metabolism, umoles}

d/dt(Al) = Ql*(Cart - Cvl) - Vmaxl*Cvl/(Km1 + Cvl)

d/dt(Af) = Qf*(Cart - Cvf)

d/dt(Am) = Qm*(Cart - Cvm)

d/dt(Avrg) = Qvrg*(Cart - Cvvrg)

d/dt(Ablood) = Ql*Cvl + Qf*Cvf + Qm*Cvm + Qvrg*Cvvrg + Qtot*Cvex

{differential equations for ST oxide uptake and metabolism, umoles}

d/dt(ABl) = Ql*(CBart - CBvl) + Vmaxl*Cvl/(Km1 + Cvl) - Vmaxl2*CBvl/(Km2 + CBvl)

d/dt(ABf) = Qf*(CBart - CBvf)

d/dt(ABm) = Qm*(CBart - CBvm)

d/dt(ABvrg) = Qvrg*(CBart - CBvvrg)

d/dt(ABblood) = Ql*CBvl + Qf*CBvf + Qm*CBvm + Qvrg*CBvvrg + Qtot*CBvex

d/dt(ABler) = Vmaxl*Cvl/(Km1 + Cvl) - Kec*((ABler/(Vl*PBl)) - (ABlcy/(Vl*PBl))) - Vmaxl2*(ABler/(Vl*PBl))/(Km2 + (ABler/(Vl*PBl)))

d/dt(ABlcy) = Kec*((ABler/(Vl*PBl)) - (ABlcy/(Vl*PBl))) + Ql*(CBart - ABlcy/(Vl*PBl)) - (VmaxGl*(ABlcy/Vl)*(GSHl/Vl)/(KmG1*(ABlcy/Vl) + KmG2*(ABlcy/Vl) + KmG2*(GSHl/Vl) + (ABlcy/Vl)*(GSHl/Vl)))

d/dt(GSHl) = Kgsh*(GSHlb - (GSHl/Vl)) - VmaxGl*(ABlcy/Vl)*(GSHl/Vl)/(KmG1*(ABlcy/Vl) + KmG2*(GSHl/Vl) + (ABlcy/Vl)*(GSHl/Vl))

{amount of ST metabolized in liver to Styrene oxide, umoles}

d/dt(Ametl) = Vmaxl*Cvl/(Km1 + Cvl)

{amount of ST oxide metabolized in liver and lung to diol, umoles}

d/dt(ABmetl) = Vmaxl2*CBvl/(Km2 + CBvl)

{amount of ST oxide metabolized in liver and lung to GSH conjugate, umoles}

d/dt(ABmetGl) = VmaxGl*CBlcy*CGSHl/(KmG1*CBlcy + KmG2*CBlcy + KmG2*CGSHl + CGSHl*CBlcy)

{AUCs for ST, umolmin/mL}

d/dt(AUCvtot) = Cvtot

d/dt(AUCvl) = Cvl

{AUCs for ST oxide, umolmin/mL}

d/dt(AUCBvtot) = CBvtot

d/dt(AUCBvl) = CBvl

E.5.9 Model Code for Vinyl Chloride 0-5 yr Child

METHOD Stiff

```
STARTTIME = 0
STOPTIME = 48
DT = 0.005
{vinyl chloride moles or equivalents}
init Af = 0
init Al = 0
init Am = 0
init Avrg = 0
init Abr = 0
init Apu = 0
init Areactive = 0
init ACO2 = 0
init Aconj = 0
init ADNAad = 0
init AGI = 0
init AGSH = 0.058*Vl
init AMET = 0
init AUCrm = 0
init RISKM =0
init RISKG = 0
{tissue flows L/hr}
Qtot = 0.012*Age^3 - 1.2144*Age^2 + 40.324*Age + 44.414
Qalv = 17.875*Age + 39.785
Qf = 0.0528*Qtot
Ql = 0.0795*Qtot
Qm = 0.0304*Qtot
Qvrg = 0.837*Qtot
Qpu = 0.93*Qtot
Qbr = 0.07*Qtot
{tissue volumes L}
Vf = (0.0165*Age^5 - 1.9784*Age^4 + 51.963*Age^3 - 459.38*Age^2 + 1566.8*Age + 1004.2)/1000
Vl = (0.0072*Age^5 - 0.3975*Age^4 + 7.9052*Age^3 - 65.624*Age^2 + 262.02*Age + 157.52)/1000
Vvrg = BW - (Vf+Vl+Vm+Vlu)
Vlu = (-0.0346*Age^4 + 1.5069*Age^3 - 20.13*Age^2 + 123.99*Age + 59.213)/1000
Vm = (-0.0623*Age^5 + 2.3433*Age^4 - 26.559*Age^3 + 144.75*Age^2 + 339.84*Age + 1648.2)/1000
Vpu = 0.90*Vlu
Vbr = 0.10*Vlu
{blood/air and tissue/blood partition coefficients, vinyl chloride}
Pb = 1.16
Pl = 1.45
```

```
Pf = 20.7
Pm = 0.83
Pvrg = 1.45
Ppu = 1.45
Pbr = 1.45
{calculated concentrations of vinyl chloride}
Cart = (Qpu*Cvpu + Qbr*Cvbr)/Qtot
Cvf = Af/(Vf*Pf)
Cvl = Al/(Vl*Pl)
Cvm = Am/(Vm*Pm)
Cvvrg = Avrg/(Vvrg*Pvrg)
Cvpu = Apu/(Vpu*Ppu)
Cvbr = Abr/(Vbr*Pbr)
Cvtot = (Ql*Cvl + Qf*Cvf + Qm*Cvm + Qvrg*Cvvrg)/Qpu
Cvipu = (Qalv*Cair + Qpu*Cvtot)/((Qalv/Pb) + Qpu)
Cexh = Cvipu/Pb
{exposure in ppm converted to moles}
Cair = IF TIME <= 24 THEN 1*(1E-6/25.45) ELSE 0
{constants and conversions}
BW = (-1.9*Age^4 + 72.8*Age^3 - 813.1*Age^2 + 5535.6*Age + 4453.7)/1000
Age = 0.0
MW = 62.5
Vmax1c = 4.0
Vmax2c = 0.1
Km1 = 1.0
Km2 = 10.0
KGSMc = 0.13
KFEEc = 35.0
KCO2c = 1.6
KOC = 28.5
KBC = 0.12
KS = 2000
KA = 3.0
GSO = 0.058
H2O = 55.0
KGSM = KGSMc/BW^0.25
KFEE = KFEEc/BW^0.25
KO = KOC*BW^0.75
KB = KBC/BW^0.25
KCO2 = KCO2c/BW^0.25
Vmax1 = Vmax1c*BW^0.75
Vmax2 = Vmax2c*BW^0.75
Vmax1M = Vmax1c*(BW^0.75)/(1000*MW)
Vmax2M = Vmax2c*(BW^0.75)/(1000*MW)
```

```
KmM = Km1/(1000*MW)
Km2M = Km2/(1000*MW)
{differential equations for vinyl chloride uptake and metabolism}
d/dt(Apu) = Qpu*(Cvipu - Cvpu)
d/dt(Abr) = Qbr*(Cart - Cvbr)
d/dt(Al) = Ql*(Cart - Cvl) - Vmax1M*(Al/Vl)/(KmM + (Al/Vl)) - Vmax2M*(Al/Vl)/(Km2M + (Al/Vl)) +
KA*AGI
d/dt(Areactive) = Vmax1M*(Al/Vl)/(KmM + (Al/Vl)) + Vmax2M*(Al/Vl)/(Km2M + (Al/Vl)) -
KGSM*(AGSH/Vl)*(Areactive/Vl) - KFEE*(Areactive/Vl) - KCO2*(Areactive/Vl)*H2O*Vl
d/dt(AGSH) = KO*(KS +GSO)/(KS + (AGSH/Vl))
d/dt(ACO2) = KCO2*(Areactive/Vl)*H2O*Vl
d/dt(ADNAad) = KFEE*(Areactive/Vl)
d/dt(Aconj) = KGSM*(AGSH/Vl)*(Areactive/Vl)
d/dt(Af) = Qf*(Cart - Cvf)
d/dt(Am) = Qm*(Cart - Cvm)
d/dt(Avrg) = Qvrg*(Cart - Cvvrg)
d/dt(AMET) = Vmax1M*Cvl/(KmM + Cvl) + Vmax2M*Cvl/(Km2M + Cvl)
d/dt(AUCrm) = (Areactive/Vl)*TIME
d/dt(AGI) = - KA*AGI
d/dt(RISKM) = ADNAad/Vl
d/dt(RISKG) = Aconj/Vl
```

E.5.10 Model Code for TCE 0-5 yr child

METHOD Auto

```
STARTTIME = 0
STOPTIME= 120
DT = 0.001
{TCE moles}
init Af = 0
init Al = 0
init Am = 0
init Avrg = 0
init Abr = 0
init Apu = 0
init Alu = 0
init Akid = 0
init Astom = 0
init Agi = 0
init AUCvtot = 0
init Aexh = 0
{CH moles}
```

```
init ABl = 0
init ABbody = 0
init ABlu = 0
init ABkid = 0
init AUCBvtot = 0
init AUCBlu = 0
init ABurine = 0
{TCA moles}
init ACl = 0
init ACbody = 0
init AClu = 0
init ACkid = 0
init AUCCl = 0
init AUCCvtot = 0
init AUCCl = 0
init ACurine = 0
{TCOH moles}
init ADl = 0
init ADbody = 0
init ADlu = 0
init ADkid = 0
init AUCDvtot = 0
init ADurine = 0
{TCOG moles}
init AEl = 0
init AEbody = 0
init AElu = 0
init AEkid = 0
init AUCEvtot = 0
init AUCEkid = 0
init AEurine = 0
init AEfec = 0
{moles of TCE metabolized}
init Ametl1 = 0
{tissue flows L/hr}
Qtot = 0.012*Age^3 - 1.2144*Age^2 + 40.324*Age + 44.414
Qalv = 17.875*Age + 39.785
Qf = 0.0528*Qtot
Ql = 0.0795*Qtot
Qm = 0.0304*Qtot
Qvrg = 0.687*Qtot
Qpu = 0.93*Qtot
Qbr = 0.07*Qtot
Qkid = 0.15*Qtot
```

Qbody = 0.24*Qtot
{tissue volumes, L}
Vf = (0.0165*Age^5 - 1.9784*Age^4 + 51.963*Age^3 - 459.38*Age^2 + 1566.8*Age + 1004.2)/1000
Vl = (0.0072*Age^5 - 0.3975*Age^4 + 7.9052*Age^3 - 65.624*Age^2 + 262.02*Age + 157.52)/1000
Vm = (-0.0623*Age^5 + 2.3433*Age^4 - 26.559*Age^3 + 144.75*Age^2 + 339.84*Age + 1648.2)/1000
Vvrg = BW - (Vf + Vl + Vm + Vlu + Vkid)
Vpu = 0.9*Vlu
Vbr = 0.1*Vlu
Vlu = (-0.0346*Age^4 + 1.5069*Age^3 - 20.31*Age^2 + 123.99*Age + 59.213)/1000
Vkid = (0.000973*Age^5 - 0.0561*Age^4 + 1.1729*Age^3 - 10.34*Age^2 + 44.604*Age + 28.291)/1000
Vbody = BW
{blood/air and tissue/blood partition coefficients, TCE}
Pb = 15.91
Pl = 1.73
Pf = 36.38
Pm = 2.36
Pvrg = 1.73
Ppu = 2.61
Pbr = 2.61
Pkid = 2.07
{blood/air and tissue/blood partition coefficients, CH}
PBl = 1.42
PBlu = 1.65
PBbody = 1.35
PBkid = 0.98
{tissue/blood partition coefficients, TCA}
PCl = 1.18
PClu = 0.54
PCbody = 0.88
PCkid = 0.74
{blood/air and tissue/blood partition coefficients, TCOH}
PDl = 1.30
PDlu = 0.78
PDbody = 1.11
PDkid = 1.02
{blood/air and tissue/blood partition coefficients, TCOG}
PEl = 0.56
PElu = 1.06
PEbody = 1.11
PEkid = 1.44
{TCE oxidation metabolic parameters, mol/hr, mol/L, /hr, fraction}
Vmaxl1C = 3.04E-5
Vmaxl1 = Vmaxl1C*BW^0.75
Km1 = 1.37E-5

```
{CH conversion to TCA and TCOH}
PTCA = 115*BW
PTCOH = 309*BW
KUB = 0.06*BW
{TCOH conversion to TCA and TCOG}
KTCA = 10
Vmaxl2C = 1.11E-4
Vmaxl2 = Vmaxl2C*BW^0.75
Km2 = 1.06E-4
KUD = 1.14*BW
KUC = 1.55*BW
KFE = 4.61*BW
KUE = 32.8*BW
Age = 5.0
BW = (-1.9*Age^4 + 72.8*Age^3 - 813.1*Age^2 + 5535.6*Age + 4453.7)/1000
{exposure in ppm converted to moles}
Cair = IF TIME <= 24 THEN 1*(1E-6/24.45) ELSE 0.0
Cart = (Qpu*Cvpu + Qbr*Cvbr)/Qtot
Cvf = Af/(Vf*Pf)
Cvl = Al/(Vl*Pl)
Cl = Al/Vl
Cvm = Am/(Vm*Pm)
Cvvrg = Avrg/(Vvrg*Pvrg)
Cvpu = Apu/(Vpu*Ppu)
Cvbr = Abr/(Vbr*Pbr)
Cvkid = Akid/(Vkid*Pkid)
Cvtot = (Ql*Cvl + Qf*Cvf + Qm*Cvm + Qvrg*Cvvrg + Qkid*Cvkid)/Qpu
Cvipu = (Qalv*Cair + Qpu*Cvtot)/((Qalv/Pb) + Qpu)
Cexh = Cvipu/Pb
A = Sum(Alu,Al,Am,Akid,Agi,Aexh)
Mass = Sum(A,B,C,D,E)
{calculated concentrations of CH}
CBart = CBvlu
CBvl = ABl/(Vl*PBl)
CBvlu = ABlu/(Vlu*PBlu)
CBlu = ABlu/Vlu
CBvbody = ABbody/(Vbody*PBbody)
CBvkid = ABkid/(Vkid*PBkid)
CBvtot = (Ql*CBvl + Qbody*CBvbody + Qkid*CBvkid)/Qtot
B = Sum(ABlu,ABl,ABkid,ABbody,ABurine)
{calculated concentrations of TCA}
CCart = CCvlu
CCvl = ACl/(Vl*PCl)
CCl = ACl/Vl
```

```
CCvlu = AClu/(Vlu*PClu)
CCvbody = ACbody/(Vbody*PCbody)
CCvkid = ACkid/(Vkid*PCkid)
CCvtot = (Ql*CCvl + Qbody*CCvbody + Qkid*CCvkid)/Qtot
C = Sum(AClu,ACl,ACkid,ACbody,ACurine)
{calculated concentrations of TCOH}
CDart = CDvlu
CDvl = ADl/(Vl*PDl)
CDl = ADl/Vl
CDvlu = ADlu/(Vlu*PDlu)
CDvbody = ADbody/(Vbody*PDbody)
CDvkid = ADkid/(Vkid*PDkid)
CDvtot = (Ql*CDvl + Qbody*CDvbody + Qkid*CDvkid)/Qtot
D = Sum(ADlu,ADl,ADkid,ADbody,ADurine)
{calculated concentrations of TCOG}
CEart = CEvlu
CEvl = AEl/(Vl*PEl)
CEvlu = AElu/(Vlu*PElu)
CEvbody = AEbody/(Vbody*PEbody)
CEvkid = AEkid/(Vkid*PEkid)
CEkid = AEkid/Vkid
CEvtot = (Ql*CEvl + Qbody*CEvbody  + Qkid*CDvkid)/Qtot
E = Sum(AElu,AEl,AEkid,AEbody,AEfec,AEurine)
{differential equations for TCE uptake, metabolism, and excretion}
d/dt(Astom) = -Astom*3.09 -Astom*2.18
d/dt(Agi) = Astom*2.18 - Agi*0.044
d/dt(Apu) = Qpu*(Cvipu - Cvpu)
d/dt(Abr) = Qbr*(Cart - Cvbr)
d/dt(Alu) = Apu + Abr
d/dt(Al) = Ql*(Cart - Cvl) - Vmaxl1*Cvl/(Km1 + Cvl) + Agi*0.044 + Astom*3.09
d/dt(Af) = Qf*(Cart - Cvf)
d/dt(Am) = Qm*(Cart - Cvm)
d/dt(Avrg) = Qvrg*(Cart - Cvvrg)
d/dt(Akid) = Qkid*(Cart - Cvkid)
{amount of TCE metabolized in liver}
d/dt(Ametl1)  = Vmaxl1*Cvl/(Km1 + Cvl)
d/dt(AUCvtot) = Cvtot
d/dt(Aexh) = Cexh*Qalv
{differential equations for CH metabolism}
d/dt(ABlu) = Qtot*(CBvtot - CBvlu)
d/dt(ABl) = Ql*(CBart - CBvl) + Vmaxl1*Cvl/(Km1 + Cvl) - ABl*PTCA - ABl*PTCOH
d/dt(ABbody) = Qbody*(CBart - CBvbody)
d/dt(ABkid) = Qkid*(CBart - CBvkid) - ABkid*KUB
d/dt(ABurine) = ABkid*KUB
```

```
{AUCs for CH}
d/dt(AUCBlu) = CBlu
d/dt(AUCBvtot) = CBvtot
{differential equations for TCA}
d/dt(AClu) = Qtot*(CCvtot - CCvlu)
d/dt(ACl) = Ql*(CCart - CCvl) + ABl*PTCA + ADl*KTCA
d/dt(ACbody) = Qbody*(CCart - CCvbody)
d/dt(ACkid) = Qkid*(CCart - CCvkid) - ACkid*KUC
d/dt(ACurine) = ACkid*KUC
{AUCs for TCA}
d/dt(AUCCl) = CCl
d/dt(AUCCvtot) = CCvtot
{differential equations for TCOH}
d/dt(ADlu) = Qtot*(CDvtot - CDvlu)
d/dt(ADl) = Ql*(CDart - CDvl) + ABl*PTCOH - ADl*KTCA - 2.73E-3*CDvl/(Km2 + CDvl)
d/dt(ADbody) = Qbody*(CDart - CDvbody)
d/dt(ADkid) = Qkid*(CDart - CDvkid) - ADkid*KUD
d/dt(AUCDvtot) = CDvtot
d/dt(ADurine) = ADkid*KUD
{differential equations for TCOG}
d/dt(AElu) = Qtot*(CEvtot - CEvlu)
d/dt(AEl) = Ql*(CEart - CEvl) + 2.73E-3*CDvl/(Km2 + CDvl) - AEl*KFE
d/dt(AEbody) = Qbody*(CEart - CEvbody)
d/dt(AEkid) = Qkid*(CEart - CEvkid)  -  AEkid*KUE
d/dt(AEurine) = AEkid*KUE
d/dt(AEfec) = AEl*KFE
d/dt(AUCEkid) = CEkid
d/dt(AUCEvtot) = CEvtot
```

E.5.11 Model Code for Styrene/SO (Csanady et al*. 2003) 0-6 yr Child*

METHOD Stiff

```
STARTTIME = 0
STOPTIME= 48
DT = 0.001
{Styrene mmol}
init Aluc = 0 {conducting airways}
init Alua = 0  {alveoli}
init Alubld = 0 {lung blood}
init Aven = 0  {venous blood}
init Aart = 0  {arterial blood}
```

```
init Afat = 0
init Avrg = 0
init Amusc = 0
init Al = 0
init Amet1luc = 0
init Amet1lua = 0
init Amet1l = 0
{Styrene oxide, mmol}
init ABluc = 0
init ABlua = 0
init ABlubld = 0
init ABven = 0
init ABart = 0
init ABfat = 0
init ABvrg = 0
init ABmusc = 0
init ABler = 0
init ABlcy = 0
init ABmet2luc = 0
init ABmet2lua = 0
init ABmet2l = 0
init ABmet3luc = 0
init ABmet3lua = 0
init ABmet3l = 0
init AUCBluc = 0
init AUCBlua = 0
init AUCBl = 0
{Model parameters}
BW = (-1.9*Age^4 + 72.8*Age^3 - 813.1*Age^2 + 5535.6*Age + 4453.7)/1000 {kg, L}
Qalv = 0.82*Qtot {L/hr}
Qtot = 0.012*Age^3 - 1.2144*Age^2 + 40.324*Age + 44.414
tcap = 7.45E-6 {dm}
Scap = 115 {dm2/kg}
Dst = 4.4E-4 {dm2/hr}
Dso = 4.3E-4
{Flows, L/hr}
Qfat = 0.053*Qtot
Ql = 0.0795*Qtot
Qvrg = Qtot - (Qfat + Ql + Qmusc)
Qmusc = 0.03*Qtot
{Volumes, L}
Vart  = 0.0178*BW
Vven = 0.0533*BW
Vlubld = 0.0079*BW
```

Vfat = 0.19*BW
Vlu = (-0.0346*Age^4 + 1.5069*Age^3 - 20.31*Age^2 + 123.99*Age + 59.213)/1000
Vluc = fs*Vlu
Vlua = (1-fs)*Vlu
Vl = (0.0072*Age^5 - 0.3975*Age^4 + 7.9052*Age^3 - 65.624*Age^2 + 262.02*Age + 157.52)/1000
Vvrg = BW - (Vfat + Vl + Vart + Vven + Vlubld + Vmusc + Vlu)
Vmusc = (-0.0623*Age^5 + 2.3433*Age^4 - 26.559*Age^3 + 144.75*Age^2 + 339.84*Age + 1648.2)/1000
{Partition coeffs styrene, dimensionless}
Pb = 70.0
Pfat = 93.8
Pl = 2.71
Plu = 1.46
Pvrg = 2.60
Pmusc = 1.96
{Partition coeffs styrene oxide, dimensionless}
PbB = 2370
PBfat = 6.1
PBl = 2.6
PBlu = 1.9
PBvrg = 2.6
PBmusc = 1.5
{Concentrations ST mmol/L}
Exposure = IF TIME < 24 THEN 1*(1E-3/24.45) ELSE 0
Age = 0.542
fs = 0.1
Cair = exposure
Cart = Aart/Vart
Cven = Aven/Vven
Cfat = Afat/Vfat
Cl = Al/Vl
Cvrg = Avrg/Vvrg
Cmusc = Amusc/Vmusc
Clubld = Alubld/Vlubld
Cluc = Aluc/(fs*Vlu)
Clua = Alua/((1-fs)*Vlu)
{Concentrations SO, mmol/L}
CBart = ABart/Vart
CBven = ABven/Vven
CBfat = ABfat/Vfat
CBvrg = ABvrg/Vvrg
CBmusc = ABmusc/Vmusc
CBlcy = ABlcy/Vl
CBvlcy = ABlcy/(Vl*Pl)
CBluc = ABluc/(fs*Vlu)

```
CBlua = ABlua/((1-fs)*Vlu)
CBlubld = ABlubld/Vlubld
Qendo = Vmaxl2*1E3*Vl/(Kml2app - Kml2eh)
a = CBlcy - Kml2eh + (Vmaxl1*1E3*Vl*Cl/(Qendo*(Pl*Kml1 + Cl))) - (Vmaxl2*1E3*Vl/Qendo)
CBendo = 0.5*(a + (a^2 + 4*Kml2eh*(CBlcy + Vmaxl1*1E3*Vl*Cl/Qendo*(Pl*Kml1 + Cl)))^0.5)
{GSH}
init GSHluc = fs*GSHlu0
init GSHlua = (1-fs)*GSHlu0
init GSHl = GSHl0
CGSHluc = GSHluc*fs/Vluc
CGSHlua = GSHlua*(1-fs)/Vlua
CGSHl = GSHl/Vl
fGSH = 0.75
GSHlu0 = 1.95*Vlu
GSHl0 = 5.9*Vl
{Biochemical parameters, mmol/hr/mL, mmol/L; 1 = P450, 2 = EH, 3 = GST}
Vmaxl1 = 0.002*(70/BW)^0.25
Kml1 = 0.01
Vmaxl2 = 0.0045*(70/BW)^0.25
Kml2eh = 0.001
Kml2app = 0.01
Vmaxl3 = 0.028*(70/BW)^0.25
Kml3G = 0.1
Kml3so = 2.5
Kdl = 0.2
Vmaxlu1 = 2.5E-6*(70/BW)^0.25
Kmlu1 = 0.0175
Vmaxlu2 = 6.73E-4*(70/BW)^0.25
Kmlu2 = 0.0156
Vmaxlu3 = 0.082*(70/BW)^0.25
Kmlu3G = 0.1
Kmlu3so = 2.5
Kdlu = 2.0
{Differential equations for styrene}
d/dt(Aluc) = Qalv*(Cair*fs + fs*(1 - fs)*(Clua/Pb) - (fs + fs*(1-fs))*Cluc/Pb) -
Vmaxlu1*1E3*Vlu*fs*Cluc/(Kmlu1 + Cluc)
d/dt(Alua) = Qalv*(Cair*(1-fs) - (1-fs)*Clua/Pb) - Vmaxlu1*1E3*Vlu*(1-fs)*Clua/(Kmlu1 + Clua) -
(Scap*Dst/tcap)*(Clua/Plu - Clubld)
d/dt(Alubld) = (Scap*Dst/tcap)*(Clua/Plu - Clubld) + Qtot*(Cven - Clubld)
d/dt(Aart) = Qtot*(Clubld - Cart)
d/dt(Afat) = Qfat*(Cart - Cfat/Pfat)
d/dt(Avrg) = Qvrg*(Cart - Cvrg/Pvrg)
d/dt(Amusc) = Qmusc*(Cart - Cmusc/Pmusc)
d/dt(Al) = Ql*(Cart - Cl/Pl) - Vmaxl1*1E3*Vl*Cl/(Pl*Kml1 + Cl)
d/dt(Aven) = (Qfat*Cfat/Pfat  + Qvrg*Cvrg/Pvrg + Ql*Cl/Pl + Qmusc*Cmusc/Pmusc) - Qtot*Cven
```

d/dt(Amet1luc) = Vmaxlu1*1E3*Vlu*fs*Cluc/(Kmlu1 + Cluc)

d/dt(Amet1lua) = Vmaxlu1*1E3*Vlu*(1-fs)*Clua/(Kmlu1 + Clua)

d/dt(Amet1l) = Vmaxl1*1E3*Vl*Cl/(Pl*Kml1 + Cl)

{Differential equations for styrene oxide, B}

d/dt(ABluc) = Vmaxlu1*1E3*Vlu*(1-fs)*Cluc/(Kmlu1 + Cluc) - Vmaxlu2*1E3*Vlu*fs*CBluc/(Kmlu2 + CBluc) - Vmaxlu3*1E3*Vlu*fs*CBluc*CGSHluc/(Kmlu3so*CGSHluc + Kmlu3G*CBluc + CBluc*CGSHluc)

d/dt(ABlua) = Vmaxlu1*1E3*Vlu*(1-fs)*Clua/(Kmlu1 + Clua) - Vmaxlu2*1E3*Vlu*(1-fs)*CBlua/(Kmlu2 + CBlua) - Vmaxlu3*1E3*Vlu*(1-fs)*CBlua*CGSHlua/(Kmlu3so*CGSHlua + Kmlu3G*CBlua + CBlua*CGSHlua) - (Scap*Dso/tcap)*(CBlua/PBlu - CBlubld)

d/dt(ABlubld) = (Scap*Dso/tcap)*(CBlua/PBlu - CBlubld) + Qtot*(CBven - CBlubld)

d/dt(ABart) = Qtot*(CBlubld - CBart)

d/dt(ABfat) = Qfat*(CBart - CBfat/PBfat)

d/dt(ABvrg) = Qvrg*(CBart - CBvrg/PBvrg)

d/dt(ABmusc) = Qmusc*(CBart - CBmusc/PBmusc)

d/dt(ABlcy) = Ql*(CBart - CBvlcy) + Qendo*(CBendo - CBlcy) - Vmaxl3*1E3*Vl*CBlcy*CGSHl/(Kml3so*CGSHl + Kml3G*CBlcy + CBlcy*CGSHl)

d/dt (ABler) = Vmaxl1*1E3*Vl*Cl/(Pl*Kml1 + Cl) - Qendo*(CBendo - CBlcy) - Vmaxl2*CBendo*1E3*Vl/(Kml2eh + CBendo)

d/dt(ABven) = (Qfat*CBfat/PBfat + Qvrg*CBvrg/PBvrg + Ql*CBvlcy/PBl + Qmusc*CBmusc/PBmusc) - Qtot*CBven

d/dt(ABmet2luc) = Vmaxlu2*1E3*Vlu*fs*CBluc/(Kmlu2 + CBluc)

d/dt(ABmet2lua) = Vmaxlu2*1E3*Vlu*(1-fs)*CBlua/(Kmlu2 + CBlua)

d/dt(ABmet2l) = Vmaxl2*CBendo*1E3*Vl/(Kml2eh + CBendo)

d/dt(ABmet3luc) = Vmaxlu3*1E3*Vlu*fs*CBluc*CGSHluc/(Kmlu3so*CGSHluc + Kmlu3G*CBluc + CBluc*CGSHluc)

d/dt(ABmet3lua) = Vmaxlu3*1E3*Vlu*(1-fs)*CBlua*CGSHlua/(Kmlu3so*CGSHlua + Kmlu3G*CBlua + CBlua*CGSHlua)

d/dt(ABmet3l) = Vmaxl3*1E3*Vl*CBlcy*CGSHl/(Kml3so*CGSHl + Kml3G*CBlcy + CBlcy*CGSHl)

d/dt(AUCBluc) = CBluc

d/dt(AUCBlua) = CBlua

d/dt(AUCBl) = CBlcy

{differential equations GSH, no circadian term included}

d/dt(GSHluc) = fs*Kdlu*Vluc*(fGSH*1.95 - CGSHluc) - Vmaxlu3*1E3*Vlu*fs*CBluc*CGSHluc/(Kmlu3so*CGSHluc + Kmlu3G*CBluc + CBluc*CGSHluc)

d/dt(GSHlua) = (1-fs)*Kdlu*Vlua*(fGSH*1.95 - CGSHlua) - Vmaxlu3*1E3*Vlu*(1-fs)*CBlua*CGSHlua/(Kmlu3so*CGSHlua + Kmlu3G*CBlua + CBlua*CGSHlua)

d/dt(GSHl) = Kdl*Vl*(fGSH*5.9 - CGSHl) - Vmaxl3*1E3*Vl*CBlcy*CGSHl/(Kml3so*CGSHl + Kml3G*CBlcy + CBlcy*CGSHl)

E.5.12 Model Code for DCM 0-5 yr Child

METHOD Stiff

```
STARTTIME = 0
STOPTIME=48
DT = 0.001
{dichloromethane moles}
init Af = 0
init Al = 0
init Am = 0
init Avrg = 0
init Abr = 0
init Apu = 0
init Agi = 0
{moles dichloromethane metabolized by MFO pathway}
init Ametl1 = 0
init Ametpu1 = 0
init Ametbr1 = 0
{moles of dichloromethane metabolized by GST pathway}
init Ametl2 = 0
init Ametpu2 = 0
init Ametbr2 = 0
{tissue flows L/hr}
Qtot = 0.012*Age^3 - 1.2144*Age^2 + 40.324*Age + 44.414
Qalv = (17.875*Age) + 39.785
Qf = 0.0528*Qtot
Ql = 0.0795*Qtot
Qm = 0.0304*Qtot
Qvrg = 0.837*Qtot
Qpu = 0.93*Qtot
Qbr = 0.07*Qtot
{tissue volumes L}
Vf = (0.0165*Age^5 - 1.9784*Age^4 + 51.963*Age^3 - 459.38*Age^2 + 1566.8*Age + 1004.2)/1000
Vl = (0.0072*Age^5 - 0.3975*Age^4 + 7.9052*Age^3 - 65.624*Age^2 + 262.02*Age + 157.52)/1000
Vm = (-0.0623*Age^5 + 2.3433*Age^4 - 26.559*Age^3  + 144.75*Age^2 + 339.84*Age + 1648.2)/1000
Vvrg = BW - (Vf + Vl + Vm + Vlu)
Vlu = (-0.0346*Age^4 + 1.5069*Age^3 - 20.31*Age^2 + 123.99*Age + 59.213)/1000
Vpu = 0.9*Vlu
Vbr = 0.1*Vlu
BW = (-1.9*age^4 + 72.8*Age^3 - 813.1*Age^2 + 5535.6*Age + 4453.70)/1000
Age = 0
{blood/air and tissue/blood partition coefficients, dichloromethane}
Pb = 9.09
```

```
Pl = 0.824
Pf = 7.239
Pm = 1.09
Pvrg = 0.788
Ppu = 0.552
Pbr = 0.552
{dichloromethane oxidation metabolic parameters, mol/hr, mol/L}
Vmaxbr = 0.1*1.46E-3*Vmaxl
Vmaxpu = 0.9*1.46E-3*Vmaxl
Vmaxl = 8.58E-5*BW^0.7
Km = 8.7E-6
{dichloromethane GST conjugation /hr}
Kfl = 1.26*BW^-0.3
Kfpu = 0.9*0.242*Kfl
Kfbr = 0.1*0.242*Kfl
{uptake of DCM gfrom GI tract to liver, /hr}
KAI = 0.5
{exposure in ppm converted to moles/L}
Cair = IF TIME <= 6 THEN 10*(1E-6/25.45) ELSE 0
{calculated concentrations of dichloromethane}
Cart = (Qpu*Cvpu + Qbr*Cvbr)/Qtot
Cvf = Af/(Vf*Pf)
Cvl = Al/(Vl*Pl)
Cvm = Am/(Vm*Pm)
Cvvrg = Avrg/(Vvrg*Pvrg)
Cvpu = Apu/(Vpu*Ppu)
Cvbr = Abr/(Vbr*Pbr)
Cvtot = (Ql*Cvl + Qf*Cvf + Qm*Cvm + Qvrg*Cvvrg)/Qpu
Cvipu = (Qalv*Cair + Qpu*Cvtot)/((Qalv/Pb) + Qpu)
Cexh = Cvipu/Pb
{differential equations for dichloromethane uptake and metabolism}
d/dt(Agi) = - KAI*Agi
d/dt(Apu) = Qpu*(Cvipu - Cvpu) - Vmaxpu*Cvpu/(Km + Cvpu) - Kfpu*Apu
d/dt(Abr) = Qbr*(Cart - Cvbr) - Vmaxbr * Cvbr/(Km + Cvbr) - Kfbr*Abr
d/dt(Al) = Ql*(Cart - Cvl) - Vmaxl*Cvl/(Km + Cvl) - Kfl*Al + KAi*Agi
d/dt(Af) = Qf*(Cart - Cvf)
d/dt(Am) = Qm*(Cart - Cvm)
d/dt(Avrg) = Qvrg*(Cart - Cvvrg)
{amount of dichloromethane metabolized by MFO pathway in liver and lung}
d/dt(Ametl1)  = Vmaxl*(Al/Vl)/(Km + (Al/Vl))
d/dt(Ametpu1) = Vmaxpu*(Apu/Vpu)/(Km + (Apu/Vpu))
d/dt(Ametbr1) = Vmaxbr*(Abr/Vbr)/(Km + (Abr/Vbr))
{amount of dichloromethane metabolized by GST pathway in liver and lung}
d/dt(Ametl2)  = Kfl*Al
```

```
d/dt(Ametpu2) = Kfpu*Apu
d/dt(Ametbr2) = Kfbr*Abr
Ametpu2k = Ametpu2/BW
Ametbr2k = Ametbr2/BW
```

E.5.13 Model Code for Ethylene/Ethylene oxide 0-6 yr Child

METHOD Stiff

```
STARTTIME = 0
STOPTIME=48
DT = 0.001
{ethylene moles}
init Af = 0
Limit Af >= 0
init Al = 0
Limit Al >= 0
init Am = 0
Limit Am >= 0
init Avrg = 0
Limit Avrg >= 0
init Alubld = 0
Limit Alubld >= 0
init Aart = 0
Limit Aart >= 0
init Aven = 0
Limit Aven >= 0
{ethylene oxide moles}
init ABf = 0
Limit ABf >= 0
init ABl = 0
Limit ABl >= 0
init ABm = 0
Limit ABm >= 0
init ABvrg = 0
Limit ABvrg >= 0
init ABlubld = 0
Limit Ablubld >= 0
init ABart = 0
Limit Abart >= 0
init ABven = 0
Limit ABven >= 0
```

```
{adducts formed}
init Hbadd = 0
init DNAadd = 0
Khb = 4.5E-5
Kdna = 9.4E-5
Keldna = 0.0077
ter = 3024
{moles ethylene metabolized}
init Amet = 0
{moles of ethylene oxide metabolized}
init ABmet = 0
{area under the venous blood concn x time curve, ethylene}
init AUCvtot = 0
init AUCvl = 0
init AUCvlubld = 0
{area under the venous blood concn x time curve, ethylene oxide}
init AUCBvtot = 0
init AUCBvl = 0
init AUCBvlubld = 0
{tissue flows L/hr}
Qtot = 0.012*Age^3 - 1.2144*Age^2 + 40.324*Age + 44.414
Qalv = 0.82*Qtot
Qf = 0.053*Qtot
Ql = 0.0795*Qtot
Qm = 0.03*Qtot
Qvrg = Qtot - (Qf + Ql + Qm)
{tissue volumes, L}
BW = (-1.9*Age^4 + 72.8*Age^3 - 813.1*Age^2 + 5535.6*Age + 4453.7)/1000
Vf = (0.0165*Age^5 - 1.9784*Age^4 + 51.963*Age^3 - 459.38*Age^2 + 1566.8*Age + 1004.2)/1000
Vl = (0.0072*Age^5 - 0.3975*Age^4 + 7.9052*Age^3 - 65.624*Age^2 + 262.02*Age + 157.52)/1000
Vm = (-0.0623*Age^5 + 2.3433*Age^4 - 26.559*Age^3 + 144.75*Age^2 + 339.84*Age + 1648.2)/1000
Vvrg = BW - (Vf + Vl + Vm + Vart + Vven + Vlubld)
Vart = 0.0178*BW
Vven = 0.0533*BW
Vlubld = 0.0079*BW
t = TIME
{blood/air and tissue/blood partition coefficients, ethylene}
Pb = 0.22
Pl = 2.05
Pf = 8.73
Pm = 2.95
Pvrg = 2.18
{blood/air and tissue/blood partition coefficients, ethylene oxide}
PBb = 61
```

```
PBl = 0.89
PBf = 0.70
PBm = 1.08
PBvrg = 1.03
{ethylene oxidation metabolic parameters, clearance L/hr}
Clr = 74.9*(70/BW)^0.25
{ethylene oxide metabolic parameters, clearance L/hr}
CBlr = 1.53*(70/BW)^0.25
Feo = 0.8
{exposure in ppm converted to, mmoles/L}
Cair = IF TIME <= 24 THEN 1*(1E-3/25.45) ELSE 0
CBair = IF TIME <= 24 THEN 0.01*(1E-3/25.45) ELSE 0
Age = 0
{calculated concentrations of ethylene}
Cart = Aart/Vart
Cven = Aven/Vven
Clubld = Alubld/Vlubld
Cvlubld = Alubld/(Vlubld*Pb)
Cvf = Af/(Vf*Pf)
Cvl = Al/(Vl*Pl)
Cl = Al/Vl
Cvl = Al/(Vl*Pl)
Cvm = Am/(Vm*Pm)
Cvvrg = Avrg/(Vvrg*Pvrg)
Cvtot = (Ql*Cvl + Qf*Cvf + Qm*Cvm + Qvrg*Cvvrg)/Qtot
Cexh = Cvlubld/Pb
{calculated concentrations of ethylene oxide}
CBart = ABart/Vart
CBven = ABven/Vven
CBvf = ABf/(Vf*PBf)
CBl = ABl/Vl
CBvl = ABl/(Vl*PBl)
CBvm = ABm/(Vm*PBm)
CBlubld = ABlubld/Vlubld
CBvlubld = ABlubld/(Vlubld*Pb)
CBvvrg = ABvrg/(Vvrg*PBvrg)
CBvtot = (Ql*CBvl + Qf*CBvf + Qm*CBvm + Qvrg*CBvvrg)/Qtot
CBair = CBvtot/PB
Chb = Hbadd*(1- t/(2*ter)) {circulating Hb adducts}
 {differential equations for ethylene uptake and metabolism}
d/dt(Alubld) = Qalv*(Cair - Cvlubld) + Qtot*(Cven - Cvlubld)
d/dt(Aart) = Qtot*(Clubld - Cart) + 4.71E-7*BW
d/dt(Aven) = (Ql*Cvl + Qf*Cvf + Qm*Cvm + Qvrg*Cvvrg) - Qtot*Cven
d/dt(Al) = Ql*(Cart - Cvl) - Clr*Cvl
```

```
d/dt(Af) = Qf*(Cart - Cvf)
d/dt(Am) = Qm*(Cart - Cvm)
d/dt(Avrg) = Qvrg*(Cart - Cvvrg)
{amount of ethylene metabolized in liver}
d/dt(Amet)  = Clr*Cvl
{AUCs for ethylene}
d/dt(AUCvtot) = Cvtot
d/dt(AUCvl) = Cvl
d/dt(AUCvlubld) = Cvlubld
{differential equations for ethylene oxide metabolism}
d/dt(ABlubld) = Qalv*(CBair*Feo - CBvlubld) + Qtot*(CBven - CBvlubld)
d/dt(ABart) = Qtot*(CBlubld - CBart)
d/dt(ABven) = (Ql*CBvl + Qf*CBvf + Qm*CBvm + Qvrg*CBvvrg) - Qtot*CBven
d/dt(ABl) = Ql*(CBart - CBvl) + Clr*Cvl - CBlr*CBvl
d/dt(ABf) = Qf*(CBart - CBvf)
d/dt(ABm) = Qm*(CBart - CBvm)
d/dt(ABvrg) = Qvrg*(CBart - CBvvrg)
d/dt(Hbadd) = (Vart*CBart + Vven*CBven + Vlubld*CBlubld)*Khb
d/dt(DNAadd) = (Vart*CBart + Vven*CBven + Vlubld*CBlubld)*Kdna - Keldna*DNAadd
{AUCs for ethylene oxide}
d/dt(AUCBvtot) = CBvtot
d/dt(AUCBvl) = CBvl
d/dt(AUCBvlubld) = CBvlubld
{amounts of ethylene oxide metabolized in liver}
d/dt(ABmet) = CBlr*CBvl
```

E.5.14 Model Code for Styrene/SO RT Model of Csanady et al*. (2003) Adult*

METHOD Stiff

```
STARTTIME = 0
STOPTIME= 48
DT = 0.001
{Styrene mmol}
init Aluc = 0 {conducting airways}
init Alua = 0  {alveoli}
init Alubld = 0 {lung blood}
init Aven = 0  {venous blood}
init Aart = 0  {arterial blood}
init Afat = 0
init Avrg = 0
init Amusc = 0
```

```
init Al = 0
init Amet1luc = 0
init Amet1lua = 0
init Amet1lu = 0
init Amet1l = 0
{Styrene oxide, mmol}
init ABluc = 0
init ABlua = 0
init ABlubld = 0
init ABven = 0
init ABart = 0
init ABfat = 0
init ABvrg = 0
init ABmusc = 0
init ABler = 0
init ABlcy = 0
init ABmet2luc = 0
init ABmet2lua = 0
init ABmet2l = 0
init ABmet3luc = 0
init ABmet3lua = 0
init ABmet3l = 0
init AUCBluc = 0
init AUCBlua = 0
init AUCBl = 0
{Hb adduct, DNA Adduct}
Init Hbadd = 0
d/dt(Hbadd) = (Vart*CBart +  Vven*CBven + Vlubld*CBlubld)*Kher
init DNAadd =0
d/dt(DNAadd) = (Vart*CBart +  Vven*CBven + Vlubld*CBlubld)*Kfdna - Keldna*DNAadd
Kher = 4.5E-5
Kfdna = 3.7E-5
Keldna = 0.0077
{Model parameters}
BW = 70 {kg, L}
Qalv = 300 {L/hr}
Qtot = 372
tcap = 7.45E-6 {dm}
Scap = 115 {dm2/kg}
Dst = 4.4E-4 {dm2/hr}
Dso = 4.3E-4
{Flows, L/hr}
Qfat = 0.05*Qtot
Ql = 0.26*Qtot
```

```
Qvrg = 0.44*Qtot
Qmusc = 0.25*Qtot
{Volumes, L}
Vch = 3E3
Vart  = 0.0178*BW
Vven = 0.0533*BW
Vlubld = 0.0079*BW
Vfat = 0.19*BW
Vlu = 0.0076*BW
Vluc = fs*Vlu
Vlua = (1-fs)*Vlu
Vl = 0.026*BW
Vvrg = 0.042*BW
Vmusc = 0.541*BW
{Partition coeffs styrene, dimensionless}
Pb = 70.0
Pfat = 93.8
Pl = 2.71
Plu = 1.46
Pvrg = 2.60
Pmusc = 1.96
{Partition coeffs styrene oxide, dimensionless}
PbB = 2370
PBfat = 6.1
PBl = 2.6
PBlu = 1.9
PBvrg = 2.6
PBmusc = 1.5
{Concentrations ST mmol/L}
Exposure  = IF TIME < 24 THEN 1*(1E-3/24.45) ELSE 0
fs = 0.1
Cair = exposure
Cart = Aart/Vart
Cven = Aven/Vven
Cfat = Afat/Vfat
Cl = Al/Vl
Cvrg = Avrg/Vvrg
Cmusc = Amusc/Vmusc
Clubld = Alubld/Vlubld
Cluc = Aluc/(fs*Vlu)
Clua = Alua/((1-fs)*Vlu)
Cexalv = (fs*(2.0-fs)*(Cluc/Pb) + (1.0-fs)*(1.0-fs)*(Clua/Pb))/factor
Cexpul = (fs*(2.0-fs)*(Cluc/Pb) + (1.0-fs)*(1.0-fs)*(Clua/Pb))/factor + 1/3*Cairp
factor = 1
```

```
Cairp = Cair*(24.45/1E-3)
{Concentrations SO, mmol/L}
CBart = ABart/Vart
CBven = ABven/Vven
CBfat = ABfat/Vfat
CBvrg = ABvrg/Vvrg
CBmusc = ABmusc/Vmusc
CBlcy = ABlcy/Vl
CBvlcy = ABlcy/(Vl * Pl)
CBluc = ABluc/(fs*Vlu)
CBlua = ABlua/((1-fs)*Vlu)
CBlubld = ABlubld/Vlubld
Qendo = Vmaxl2*1E3*Vl/(Kml2app - Kml2eh)
a = CBlcy - Kml2eh + Vmaxl1*1E3*Vl*Cl/Qendo*(Pl*Kml1 + Cl) - Vmaxl2*1E3*Vl/Qendo
CBendo = 0.5*(a + (a^2 + 4*Kml2eh*(CBlcy + Vmaxl1*1E3*Vl*Cl/Qendo*(Pl*Kml1 + Cl)))^0.5)
{GSH}
init GSHluc = fs*GSHlu0
init GSHlua = (1-fs)*GSHlu0
init GSHl = GSHl0
CGSHluc = GSHluc*fs/Vluc
CGSHlua = GSHlua*(1-fs)/Vlua
CGSHl = GSHl/Vl
fGSH = 0.75
GSHlu0 = 1.95*Vlu
GSHl0 = 5.9*Vl
{Biochemical parameters, mmol/hr/mL, mmol/L; 1 = P450, 2 = EH, 3 = GST}
Vmaxl1 = 0.002
Kml1 = 0.01
Vmaxl2 = 0.0045
Kml2eh = 0.001
Kml2app = 0.01
Vmaxl3 = 0.028
Kml3G = 0.1
Kml3so = 2.5
Kdl = 0.2
Vmaxlu1 = 2.5E-6
Kmlu1 = 0.0175
Vmaxlu2 = 6.73E-4
Kmlu2 = 0.0156
Vmaxlu3 = 0.082
Kmlu3G = 0.1
Kmlu3so = 2.5
Kdlu = 2.0
{Differential equations for styrene}
```

d/dt(Aluc) = Qalv*(Cair*fs + fs*(1 - fs)*(Clua/Pb) - (fs + fs*(1-fs))*Cluc/Pb) - Vmaxlu1*1E3*Vlu*fs*Cluc/(Kmlu1 + Cluc)

d/dt(Alua) = Qalv*(Cair*(1-fs) - (1-fs)*Clua/Pb) - Vmaxlu1*1E3*Vlu*(1-fs)*Clua/(Kmlu1 + Clua) - (Scap*Dst/tcap)*(Clua/Plu - Clubld)

d/dt(Alubld) = (Scap*Dst/tcap)*(Clua/Plu - Clubld) + Qtot*(Cven - Clubld)

d/dt(Aart) = Qtot*(Clubld - Cart)

d/dt(Afat) = Qfat*(Cart - Cfat/Pfat)

d/dt(Avrg) = Qvrg*(Cart - Cvrg/Pvrg)

d/dt(Amusc) = Qmusc*(Cart - Cmusc/Pmusc)

d/dt(Al) = Ql*(Cart - Cl/Pl) - Vmaxl1*1E3*Vl*Cl/(Pl*Kml1 + Cl)

d/dt(Aven) = (Qfat*Cfat/Pfat + Qvrg*Cvrg/Pvrg + Ql*Cl/Pl + Qmusc*Cmusc/Pmusc) - Qtot*Cven

d/dt(Amet1luc) = Vmaxlu1*1E3*Vlu*fs*Cluc/(Kmlu1 + Cluc)

d/dt(Amet1lua) = Vmaxlu1*1E3*Vlu*(1-fs)*Clua/(Kmlu1 + Clua)

d/dt(Amet1lu) = Vmaxlu1*1E3*Vlu*fs*Cluc/(Kmlu1 + Cluc) + Vmaxlu1*1E3*Vlu*(1-fs)*Clua/(Kmlu1 + Clua)

d/dt(Amet1l) = Vmaxl1*1E3*Vl*Cl/(Pl*Kml1 + Cl)

{Differential equations for styrene oxide, B}

d/dt(ABluc) = Vmaxlu1*1E3*Vlu*(1-fs)*Cluc/(Kmlu1 + Cluc) - Vmaxlu2*1E3*Vlu*fs*CBluc/(Kmlu2 + CBluc) - Vmaxlu3*1E3*Vlu*fs*CBluc*CGSHluc/(Kmlu3so*CGSHluc + Kmlu3G*CBluc + CBluc*CGSHluc)

d/dt(ABlua) = Vmaxlu1*1E3*Vlu*(1-fs)*Clua/(Kmlu1 + Clua) - Vmaxlu2*1E3*Vlu*(1-fs)*CBlua/(Kmlu2 + CBlua) - Vmaxlu3*1E3*Vlu*(1-fs)*CBlua*CGSHlua/(Kmlu3so*CGSHlua + Kmlu3G*CBlua + CBlua*CGSHlua) - (Scap*Dso/tcap)*(CBlua/PBlu - CBlubld)

d/dt(ABlubld) = (Scap*Dso/tcap)*(CBlua/PBlu - CBlubld) + Qtot*(CBven - CBlubld)

d/dt(ABart) = Qtot*(CBlubld - CBart)

d/dt(ABfat) = Qfat*(CBart - CBfat/PBfat)

d/dt(ABvrg) = Qvrg*(CBart - CBvrg/PBvrg)

d/dt(ABmusc) = Qmusc*(CBart - CBmusc/PBmusc)

d/dt(ABlcy) = Ql*(CBart - CBvlcy) + Qendo*(CBendo - CBlcy) - Vmaxl3*1E3*Vl*CBlcy*CGSHl/(Kml3so*CGSHl + Kml3G*CBlcy + CBlcy*CGSHl)

d/dt(ABler) = Vmaxl1*1E3*Vl*Cl/(Pl*Kml1 + Cl) - Qendo*(CBendo - CBlcy) - Vmaxl2*1E3*Vl*CBendo/(Kml2eh + CBendo)

d/dt(ABven) = (Qfat*CBfat/PBfat + Qvrg*CBvrg/PBvrg + Ql*CBvlcy/PBl + Qmusc*CBmusc/PBmusc) - Qtot*CBven

d/dt(ABmet2luc) = Vmaxlu2*1E3*Vlu*fs*CBluc/(Kmlu2 + CBluc)

d/dt(ABmet2lua) = Vmaxlu2*1E3*Vlu*(1-fs)*CBlua/(Kmlu2 + CBlua)

d/dt(ABmet2l) = Vmaxl2*1E3*Vl*CBendo/(Kml2eh + CBendo)

d/dt(ABmet3luc) = Vmaxlu3*1E3*Vlu*fs*CBluc*CGSHluc/(Kmlu3so*CGSHluc + Kmlu3G*CBluc + CBluc*CGSHluc)

d/dt(ABmet3lua) = Vmaxlu3*1E3*Vlu*(1-fs)*CBlua*CGSHlua/(Kmlu3so*CGSHlua + Kmlu3G*CBlua + CBlua*CGSHlua)

d/dt(ABmet3l) = Vmaxl3*1E3*Vl*CBlcy*CGSHl/(Kml3so*CGSHl + Kml3G*CBlcy + CBlcy*CGSHl)

d/dt(AUCBluc) = CBluc

d/dt(AUCBlua) = CBlua

d/dt(AUCBl) = CBlcy

{differential equations GSH, no circadian term included}

d/dt(GSHluc) = fs*Kdlu*Vluc*(fGSH*1.95 - CGSHluc) - Vmaxlu3*1E3*Vlu*fs*CBluc*CGSHluc/(Kmlu3so*CGSHluc + Kmlu3G*CBluc + CBluc*CGSHluc)

d/dt(GSHlua) = (1-fs)*Kdlu*Vlua*(fGSH*1.95 - CGSHlua) - Vmaxlu3*1E3*Vlu*(1-fs)*CBlua*CGSHlua/(Kmlu3so*CGSHlua + Kmlu3G*CBlua + CBlua*CGSHlua)

d/dt(GSHl) = Kdl*Vl*(fGSH*5.9 - CGSHl) - Vmaxl3*1E3*Vl*CBlcy*CGSHl/(Kml3so*CGSHl + Kml3G*CBlcy + CBlcy*CGSHl)

E.5.15 Model Code for Carbon tetrachloride 0-6 yr Child

METHOD Stiff

STARTTIME = 0
STOPTIME=48
DT = 0.001
{CCl4 moles}
init Af = 0
init Al = 0
init Am = 0
init Avrg = 0
init Abr = 0
init Apu = 0
{moles CCl4 metabolized}
init Ametl = 0
init AUCvtot = 0
init AUCvl = 0
{tissue flows L/hr}
Qtot = 0.012*Age^3 - 1.2144*Age^2 + 40.324*Age + 44.414
Qalv = 0.82*Qtot
Qf = 0.053*Qtot
Ql = 0.0795*Qtot
Qm = 0.03*Qtot
Qvrg = Qtot -(Qf + Ql + Qm)
Qpu = 0.93*Qtot
Qbr = 0.07*Qtot
{tissue volumes L}
Vf = (0.0165*Age^5 - 1.9784*Age^4 + 51.963*Age^3 - 459.38*Age^2 + 1566.8*Age + 1004.2)/1000
Vl = (0.0072*Age^5 - 0.3975*Age^4 + 7.9052*Age^3 - 65.624*Age^2 + 262.02*Age + 157.52)/1000
Vm = (-0.0623*Age^5 + 2.3433*Age^4 - 26.559*Age^3 + 144.75*Age^2 + 339.84*Age + 1648.2)/1000
Vvrg = BW - (Vf + Vl + Vm + Vlu)
Vlu = (-0.0346*Age^4 + 1.5069*Age^3 - 20.31*Age^2 + 123.99*AGe + 59.213)/1000
Vpu = 0.9*Vlu
Vbr = 0.1*Vlu
BW = (-1.9*Age^4 + 72.8*Age^3 - 813.1*Age^2 + 5535.6*Age + 4453.7)/1000

```
{blood/air and tissue/blood partition coefficients, CCl4}
Pb = 4.52
Pl = 3.14
Pf = 79.4
Pm = 1.00
Pvrg = 1.00
Ppu = 1.00
Pbr = 1.00
{CCl4 oxidation metabolic parameters, mol/hr/mg protein, mol/L, mol/hr}
Vmax = 1.35E-7*(70/BW)^0.25
Vmaxl = Vmax*Vl*23.0*1E3
Km = 5.68E-5
{exposure in ppm converted to moles/L}
Cair = IF TIME <= 24 THEN 1*(1E-6/25.45) ELSE 0
Age = 5
{calculated concentrations of CCl4}
Cart = (Qpu*Cvpu + Qbr*Cvbr)/Qtot
Cvf = Af/(Vf*Pf)
Cvl = Al/(Vl*Pl)
Cvm = Am/(Vm*Pm)
Cvvrg = Avrg/(Vvrg*Pvrg)
Cvpu = Apu/(Vpu*Ppu)
Cvbr = Abr/(Vbr*Pbr)
Cvtot = (Ql*Cvl + Qf*Cvf + Qm*Cvm + Qvrg*Cvvrg)/Qpu
Cvipu = (Qalv*Cair + Qpu*Cvtot)/((Qalv/Pb) + Qpu)
Cexh = Cvipu/Pb
{differential equations for CCl4 uptake and metabolism}
d/dt(Apu) = Qpu*(Cvipu - Cvpu)
d/dt(Abr) = Qbr*(Cart - Cvbr)
d/dt(Al) = Ql*(Cart - Cvl) - Vmaxl*Cvl/(Km + Cvl)
d/dt(Af) = Qf*(Cart - Cvf)
d/dt(Am) = Qm*(Cart - Cvm)
d/dt(Avrg) = Qvrg*(Cart - Cvvrg)
{amount of CCl4 metabolized in liver}
d/dt(Ametl)  = Vmaxl*Cvl/(Km + Cvl)
d/dt(AUCvtot) = Cvtot
d/dt(AUCvl) = Cvl
```

E.5.16 Model Code for Toluene 0-6 yr Child

```
METHOD Stiff

STARTTIME = 0
STOPTIME=48
DT = 0.001
{Toluene moles}
init Af = 0
init Al = 0
init Am = 0
init Avrg = 0
init Abr = 0
init Apu = 0
{moles toluene metabolized}
init Ametl = 0
init AUCvtot = 0
init AUCvl = 0
{tissue flows,  L/hr}
Qtot = 0.012*Age^3 - 1.2144*Age^2 + 40.324*Age + 44.414
Qalv = 0.82*Qtot
Qf = 0.053*Qtot
Ql = 0.0795*Qtot
Qm = 0.03*Qtot
Qvrg = Qtot -(Qf + Ql + Qm)
Qpu = 0.93*Qtot
Qbr = 0.07*Qtot
{tissue volumes,  L}
Vf = (0.0165*Age^5 - 1.9784*Age^4 + 51.963*Age^3 - 459.38*Age^2 + 1566.8*Age + 1004.2)/1000
Vl = (0.0072*Age^5 - 0.3975*Age^4 + 7.9052*Age^3 - 65.624*Age^2 + 262.02*Age + 157.52)/1000
Vm = (-0.0623*Age^5 + 2.3433*Age^4 - 26.559*Age^3 + 144.75*Age^2 + 339.84*Age + 1648.2)/1000
Vvrg = BW - (Vf + Vl + Vm + Vlu)
Vlu = (-0.0346*Age^4 + 1.5069*Age^3 - 20.31*Age^2 + 123.99*AGe + 59.213)/1000
Vpu = 0.9*Vlu
Vbr = 0.1*Vlu
BW = (-1.9*Age^4 + 72.8*Age^3 - 813.1*Age^2 + 5535.6*Age + 4453.7)/1000
{blood/air and tissue/blood partition coefficients, toluene}
Pb = 15.6
Pl = 2.98
Pf = 65.8
Pm = 1.37
Pvrg = 2.66
Ppu = 2.66
Pbr = 2.66
```

```
{toluene oxidation metabolic parameters, mol/hr, mol/L}
Vmaxl = 5.2E-5*BW*(70/BW)^0.25
Km = 5.97E-6
{exposure in ppm converted to moles/L}
Cair = IF TIME <= 24 THEN 1*(1E-6/25.45) ELSE 0
Age = 5
{calculated concentrations of toluene}
Cart = (Qpu*Cvpu + Qbr*Cvbr)/Qtot
Cvf = Af/(Vf*Pf)
Cvl = Al/(Vl*Pl)
Cvm = Am/(Vm*Pm)
Cvvrg = Avrg/(Vvrg*Pvrg)
Cvpu = Apu/(Vpu*Ppu)
Cvbr = Abr/(Vbr*Pbr)
Cvtot = (Ql*Cvl + Qf*Cvf + Qm*Cvm + Qvrg*Cvvrg)/Qpu
Cvipu = (Qalv*Cair + Qpu*Cvtot)/((Qalv/Pb) + Qpu)
Cexh = Cvipu/Pb
{differential equations for toluene uptake and metabolism}
d/dt(Apu) = Qpu*(Cvipu - Cvpu)
d/dt(Abr) = Qbr*(Cart - Cvbr)
d/dt(Al) = Ql*(Cart - Cvl) - Vmaxl*Cvl/(Km + Cvl)
d/dt(Af) = Qf*(Cart - Cvf)
d/dt(Am) = Qm*(Cart - Cvm)
d/dt(Avrg) = Qvrg*(Cart - Cvvrg)
{amount of toluene metabolized in liver and AUCs in blood and liver}
d/dt(Ametl)  = Vmaxl*Cvl/(Km + Cvl)
d/dt(AUCvtot) = Cvtot
d/dt(AUCvl) = Cvl
```

E.5.17 Model Code for Xylene 0-6 Yr Child

```
METHOD Stiff

STARTTIME = 0
STOPTIME=48
DT = 0.001
{Xylene moles}
init Af = 0
init Al = 0
init Am = 0
init Avrg = 0
init Abr = 0
```

```
init Apu = 0
{moles xylene metabolized}
init Ametl = 0
init AUCvtot = 0
init AUCvl = 0
{tissue flows, L/hr}
Qtot = 0.012*Age^3 - 1.2144*Age^2 + 40.324*Age + 44.414
Qalv = 0.82*Qtot
Qf = 0.053*Qtot
Ql = 0.0795*Qtot
Qm = 0.03*Qtot
Qvrg = Qtot -(Qf + Ql + Qm)
Qpu = 0.93*Qtot
Qbr = 0.07*Qtot
{tissue volumes, L}
Vf = (0.0165*Age^5 - 1.9784*Age^4 + 51.963*Age^3 - 459.38*Age^2 + 1566.8*Age + 1004.2)/1000
Vl = (0.0072*Age^5 - 0.3975*Age^4 + 7.9052*Age^3 - 65.624*Age^2 + 262.02*Age + 157.52)/1000
Vm = (-0.0623*Age^5 + 2.3433*Age^4 - 26.559*Age^3 + 144.75*Age^2 + 339.84*Age + 1648.2)/1000
Vvrg = BW - (Vf + Vl + Vm + Vlu)
Vlu = (-0.0346*Age^4 + 1.5069*Age^3 - 20.31*Age^2 + 123.99*AGe + 59.213)/1000
Vpu = 0.9*Vlu
Vbr = 0.1*Vlu
BW = (-1.9*Age^4 + 72.8*Age^3 - 813.1*Age^2 + 5535.6*Age + 4453.7)/1000
{blood/air and tissue/blood partition coefficients, xylene}
Pb = 26.4
Pl = 3.02
Pf = 77.8
Pm = 3.00
Pvrg = 4.42
Ppu = 4.42
Pbr = 4.42
{Xylene oxidation metabolic parameters, mol/hr, mol/L}
Vmaxl = 7.9E-5*BW*(70/BW)^0.25
Km = 1.88E-6
{exposure in ppm converted to moles/L}
Cair = IF TIME <= 24 THEN 1*(1E-6/25.45) ELSE 0
Age = 5
{calculated concentrations of xylene}
Cart = (Qpu*Cvpu + Qbr*Cvbr)/Qtot
Cvf = Af/(Vf*Pf)
Cvl = Al/(Vl*Pl)
Cvm = Am/(Vm*Pm)
Cvvrg = Avrg/(Vvrg*Pvrg)
Cvpu = Apu/(Vpu*Ppu)
```

```
Cvbr = Abr/(Vbr*Pbr)
Cvtot = (Ql*Cvl + Qf*Cvf + Qm*Cvm + Qvrg*Cvvrg)/Qpu
Cvipu = (Qalv*Cair + Qpu*Cvtot)/((Qalv/Pb) + Qpu)
Cexh = Cvipu/Pb
{differential equations for xylene uptake and metabolism}
d/dt(Apu) = Qpu*(Cvipu - Cvpu)
d/dt(Abr) = Qbr*(Cart - Cvbr)
d/dt(Al) = Ql*(Cart - Cvl) - Vmaxl*Cvl/(Km + Cvl)
d/dt(Af) = Qf*(Cart - Cvf)
d/dt(Am) = Qm*(Cart - Cvm)
d/dt(Avrg) = Qvrg*(Cart - Cvvrg)
{amount of xylene metabolized in liver and AUCs in blood and liver}
d/dt(Ametl)  = Vmaxl*Cvl/(Km + Cvl)
d/dt(AUCvtot) = Cvtot
d/dt(AUCvl) = Cvl
```

E.5.18 Model Code for Toluene-Xylene Mixed Exposure 0-6 Yr Child

```
METHOD Stiff

STARTTIME = 0
STOPTIME=48
DT = 0.001
{Toluene moles}
init Af = 0
Limit Af >= 0
init Al = 0
Limit Al >= 0
init Am = 0
Limit Am >= 0
init Avrg = 0
Limit Avrg >= 0
init Abr = 0
Limit Abr >= 0
init Apu = 0
Limit Apu >= 0
{Xylene moles}
init ABf = 0
Limit ABf >= 0
init ABl = 0
Limit ABl >= 0
init ABm = 0
```

```
Limit ABm >= 0
init ABvrg = 0
Limit ABvrg >= 0
init ABbr = 0
Limit ABbr >= 0
init ABpu = 0
Limit ABpu >= 0
{moles toluene metabolized}
init Ametl = 0
init AUCvtot = 0
init AUCvl = 0
{moles xylene metabolized}
init ABmetl = 0
init AUCBvtot = 0
init AUCBvl = 0
{tissue flows L/hr}
Qtot = 0.012*Age^3 - 1.2144*Age^2 + 40.324*Age + 44.414
Qalv = Qtot
Qf = 0.053*Qtot
Ql = 0.0795*Qtot
Qm = 0.03*Qtot
Qvrg = Qtot - (Qf + Ql + Qm)
Qpu = 0.93*Qtot
Qbr = 0.07*Qtot
{tissue volumes L}
Vf = (0.0165*Age^5 - 1.9784*Age^4 + 51.963*Age^3 - 459.38*Age^2 + 1566.8*Age + 1004.2)/1000
Vl = (0.0072*Age^5 - 0.3975*Age^4 + 7.9052*Age^3 - 65.624*Age^2 + 262.02*Age + 157.52)/1000
Vm = (-0.0623*Age^5 + 2.3433*Age^4 - 26.559*Age^3 + 144.75*Age^2 + 339.84*Age + 1648.2)/1000
Vvrg = BW - (Vf + Vl + Vm + Vlu)
Vlu = (-0.0346*Age^4 + 1.5069*Age^3 - 20.31*Age^2 + 123.99*AGe + 59.213)/1000
Vpu = 0.9*Vlu
Vbr = 0.1*Vlu
BW = (-1.9*Age^4 + 72.8*Age^3 - 813.1*Age^2 + 5535.6*Age + 4453.7)/1000
{blood/air and tissue/blood partition coefficients, toluene}
Pb = 15.6
Pl = 2.98
Pf = 65.8
Pm = 1.37
Pvrg = 2.66
Ppu = 2.66
Pbr = 2.66
{blood/air and tissue/blood partition coefficients, xylene}
PBb = 26.4
PBl = 3.02
```

```
PBf = 77.8
PBm = 3.00
PBvrg = 4.42
PBpu = 4.42
PBbr = 4.42
{toluene oxidation metabolic parameters, mol/hr, mol/L}
Vmaxl = 5.2E-5*BW*(70/BW)^0.25
Km = 5.97E-6
Ki = 3.8E-6
{xylene oxidation metabolic parameters, mol/hr, mol/L}
Vmaxl2 = 7.9E-5*BW*(70/BW)^0.25
Km2 = 1.88E-6
K2i = 5.6E-6
{toluene exposure in ppm converted to moles/L}
Cair = IF TIME <= 8 THEN 10*(1E-6/25.45) ELSE 0
{xylene exposure in ppm converted to moles/L}
CBair = IF TIME <= 8 THEN 1*(1E-6/25.45) ELSE 0
Age = 5
{calculated concentrations of toluene}
Cart = (Qpu*Cvpu + Qbr*Cvbr)/Qtot
Cvf = Af/(Vf*Pf)
Cvl = Al/(Vl*Pl)
Cvm = Am/(Vm*Pm)
Cvvrg = Avrg/(Vvrg*Pvrg)
Cvpu = Apu/(Vpu*Ppu)
Cvbr = Abr/(Vbr*Pbr)
Cvtot = (Ql*Cvl + Qf*Cvf + Qm*Cvm + Qvrg*Cvvrg)/Qpu
Cvipu = (Qalv*Cair + Qpu*Cvtot)/((Qalv/Pb) + Qpu)
Cexh = Cvipu/Pb
{calculated concentrations of xylene}
CBart = (Qpu*CBvpu + Qbr*CBvbr)/Qtot
CBvf = ABf/(Vf*PBf)
CBvl = ABl/(Vl*PBl)
CBvm = ABm/(Vm*PBm)
CBvvrg = ABvrg/(Vvrg*PBvrg)
CBvpu = ABpu/(Vpu*PBpu)
CBvbr = ABbr/(Vbr*PBbr)
CBvtot = (Ql*CBvl + Qf*CBvf + Qm*CBvm + Qvrg*CBvvrg)/Qpu
CBvipu = (Qalv*CBair + Qpu*CBvtot)/((Qalv/PBb) + Qpu)
CBexh = CBvipu/PBb
{differential equations for toluene uptake and metabolism}
d/dt(Apu) = Qpu*(Cvipu - Cvpu)
d/dt(Abr) = Qbr*(Cart - Cvbr)
d/dt(Al) = Ql*(Cart - Cvl) - Vmaxl*Cvl/(Km*(1 +  CBvl/K2i) + Cvl)
```

```
d/dt(Af) = Qf*(Cart - Cvf)
d/dt(Am) = Qm*(Cart - Cvm)
d/dt(Avrg) = Qvrg*(Cart - Cvvrg)
{differential equations for xylene uptake and metabolism}
d/dt(ABpu) = Qpu*(CBvipu - CBvpu)
d/dt(ABbr) = Qbr*(CBart - CBvbr)
d/dt(ABl) = Ql*(CBart - CBvl) - Vmaxl2*CBvl/(Km2*(1 +  Cvl/Ki) + CBvl)
d/dt(ABf) = Qf*(CBart - CBvf)
d/dt(ABm) = Qm*(CBart - CBvm)
d/dt(ABvrg) = Qvrg*(CBart - CBvvrg)
{amount of toluene metabolized in liver, AUCs in blood and liver}
d/dt(Ametl)  = Vmaxl*Cvl/(Km*(1 +  CBvl/K2i) + Cvl)
d/dt(AUCVtot) = Cvtot
d/dt(AUCvl) = Cvl
{amount of xylene metabolized in liver, AUCs in blood and liver}
d/dt(ABmetl)  = Vmaxl2*CBvl/(Km2*(1 +  Cvl/Ki) + CBvl)
d/dt(AUCBVtot) = CBvtot
d/dt(AUCBvl) = CBvl
```

References

Abbas R and Fisher JW (1997). A physiologically based pharmacokinetic model for trichloroethylene and its metabolites, chloral hydrate, trichloroacetate, dichloroacetate, trichloroethanol, and trichloroethanol glucuronide in B6C3F1 mice. Toxicol Appl Pharmacol 147(1): 15-30.

Andersen ME and Jarabek AM (2001). Nasal tissue dosimetry-issues and approaches for "Category 1" gases: a report on a meeting held in Research Triangle Park, NC, February 11-12, 1998. Inhal Toxicol 13(5): 415-35.

Appelman LM, ten Berge WF and Reuzel PG (1982). Acute inhalation toxicity study of ammonia in rats with variable exposure periods. Am Ind Hyg Assoc J 43(9): 662-665.

Brown JP (2001). Physiological and Anatomical Parameters for Infants and Children's PBPK Modeling. Children's Environmental Health--Developing a Risk Assessment Framework. Office of Environmental Health Hazard Assessment, California Environmental Protection Agency. Monterey (CA), April.

Bylin G, Lagerstrand L, Hedenstierna G and Wagner PD (1995). Variability in airway conductance and lung volume in subjects with asthma. Clin Physiol 15(3): 207-218.

Champeix J and Catilina P (1967). Les Intoxicants par l'Acroléine. Paris, France: Masson and Co.

Chen CW and Blancato JN (1989). Incorporation of biological information in cancer risk assessment: Example--vinyl chloride. Cell Biol Toxicol 5(4): 417-444.

Clewell HJ, Gentry PR, Covington TR, Sarangapani R and Teeguarden JG (2004). Evaluation of the potential impact of age- and gender-specific pharmacokinetic differences on tissue dosimetry. Toxicol Sci 79(2): 381-393.

Clewell HJ, Gentry PR, Gearhart JM, Allen BC and Andersen ME (1995). Considering pharmacokinetic and mechanistic information in cancer risk assessments for environmental contaminants: Examples with vinyl chloride and trichloroethylene. Chemosphere 31(1): 2561-2578.

Clewell RA, Merrill EA, Yu KO, Mahle DA, Sterner TR, Fisher JW and Gearhart JM (2003a). Predicting neonatal perchlorate dose and inhibition of iodide uptake in the rat during lactation using physiologically-based pharmacokinetic modeling. Toxicol Sci 74(2): 416-436.

Corley RA, Mendrala AL, Smith FA, Staats DA, Gargas ML, Conolly RB, Andersen ME and Reitz RH (1990). Development of a physiologically based pharmacokinetic model for chloroform. Toxicol Appl Pharmacol 103(3): 512-527.

Csanady GA, Denk B, Putz C, Kreuzer PE, Kessler W, Baur C, Gargas ML and Filser JG (2000). A physiological toxicokinetic model for exogenous and endogenous ethylene and ethylene oxide in rat, mouse, and human: Formation of 2-hydroxyethyl adducts with hemoglobin and DNA. Toxicol Appl Pharmacol 165(1): 1-26.

Csanady GA, Kessler W, Hoffmann HD and Filser JG (2003). A toxicokinetic model for styrene and its metabolite styrene-7,8-oxide in mouse, rat and human with special emphasis on the lung. Toxicol Lett 138(1-2): 75-102.

Dorne JL and Renwick AG (2005a). The refinement of uncertainty/safety factors in risk assessment by the incorporation of data on toxicokinetic variability in humans. Toxicol Sci 86(1): 20-26.

Dorne JL, Walton K and Renwick AG (2001). Uncertainty factors for chemical risk assessment: Human variability in the pharmacokinetics of CYP1A2 probe substrates. Food Chem Toxicol 39(7): 681-696.

Dourson ML and Stara JF (1983). Regulatory history and experimental support of uncertainty (safety) factors. Regul Toxicol Pharmacol 3(3): 224-238.

Evelo CT, Oostendorp JG, ten Berge WF and Borm PJ (1993). Physiologically based toxicokinetic modeling of 1,3-butadiene lung metabolism in mice becomes more important at low doses. Environ Health Perspect 101(6): 496-502.

Fisher JW, Mahle D and Abbas R (1998). A human physiologically based pharmacokinetic model for trichloroethylene and its metabolites, trichloroacetic acid and free trichloroethanol. Toxicol Appl Pharmacol 152(2): 339-59.

Frederick CB, Bush ML, Lomax LG, Black KA, Finch L, Kimbell JS, Morgan KT, Subramaniam RP, Morris JB and Ultman JS (1998). Application of a hybrid computational fluid dynamics and physiologically based inhalation model for interspecies dosimetry extrapolation of acidic vapors in the upper airways. Toxicol Appl Pharmacol 152(1): 211-231.

Gearhart JM, Mahle DA, Greene RJ, Seckel CS, Flemming CD, Fisher JW and Clewell 3rd. HJ (1993). Variability of physiologically based pharmacokinetic (PBPK) model parameters and their effects on PBPK model predictions in a risk assessment for perchloroethylene (PCE). Toxicol Lett 68(1-2): 131-144.

Gentry PR, Covington TR and Clewell 3rd. HJ (2003). Evaluation of the potential impact of pharmacokinetic differences on tissue dosimetry in offspring during pregnancy and lactation. Regul Toxicol Pharmacol 38(1): 1-16.

Georgieva AV, Kimbell JS and Schlosser PM (2003). A distributed-parameter model for formaldehyde uptake and disposition in the rat nasal lining. Inhal Toxicol 15(14): 1435-1463.

Gerde P, Medinsky MA and Bond JA (1991). The retention of polycyclic aromatic hydrocarbons in the bronchial airways and in the alveolar region--A theoretical comparison. Toxicol Appl Pharmacol 107(2): 239-252.

Gerde P, Muggenburg BA, Lundborg M and Dahl AR (2001). The rapid alveolar absorption of diesel soot-adsorbed benzo[a]pyrene: Bioavailability, metabolism and dosimetry of an inhaled particle-borne carcinogen. Carcinogenesis 22(5): 741-749.

Gillis CA, Keenan RE, Carlson-Lynch HL and Price PS (1997). Characterization of the inter-individual (UFH) factors: Alternative models and approaches [Abstract #1053]. The Toxicologist 36: 207.

Ginsberg G, Hattis D, Russ A and Sonawane B (2004b). Physiologically based pharmacokinetic (PBPK) modeling of caffeine and theophylline in neonates and adults: Implications for assessing children's risks from environmental agents. J Toxicol Environ Health A 67(4): 297-329.

Ginsberg G, Hattis D, Sonawane B, Russ A, Banati P, Kozlak M, Smolenski S and Goble R (2002). Evaluation of child/adult pharmacokinetic differences from a database derived from the therapeutic drug literature. Toxicol Sci 66(2): 185-200.

Haddad S, Beliveau M, Tardif R and Krishnan K (2001). A PBPK modeling-based approach to account for interactions in the health risk assessment of chemical mixtures. Toxicol Sci 63(1): 125-131.

Haddad S, Poulin P and Krishnan K (2000). Relative lipid content as the sole mechanistic determinant of the adipose tissue:blood partition coefficients of highly lipophilic organic chemicals. Chemosphere 40(8): 839-843.

Hanna LM, Lou SR, Su S and Jarabek AM (2001). Mass transport analysis: Inhalation RfC methods framework for interspecies dosimetric adjustment. Inhal Toxicol 13(5): 437-463.

Hattis D (1996a). Variability in susceptibility--how big, how often, for what responses to what agents? Environ Toxicol Pharmacol 2: 135-145.

Hattis D (1996b). Human interindividual variability in susceptibility to toxic effects: From annoying detail to a central determinant of risk. Toxicology 111(1-3): 5-14.

Hine CH, Meyers F, Ivanhoe F, Walker S and Takahashi GH (1961). Simple Tests of Respiratory Function and Study of Sensory Response in Human Subjects Exposed to Respiratory Tract Irritants. Proceedings of the 5th Air Pollution Medical Research Conference: Symposium on Human Exposures to Air Pollutants. California State Deparment of Health. 20-38. Berkeley (CA).

Horstman D, Roger LJ, Kehrl H and Hazucha M (1986). Airway sensitivity of asthmatics to sulfur dioxide. Toxicol Ind Health 2(3): 289-298.

ICRP (1994). Human Respiratory Tract Model for Radiological Protection. International Commission on Radiological Protection. Publication 66 Annals of the ICRP. Tarrytown (NY): Elsevier Science Inc. 24(1-3).

Johanson G and Filser JG (1993). A physiologically based pharmacokinetic model for butadiene and its metabolite butadiene monoxide in rat and mouse and its significance for risk extrapolation. Arch Toxicol 67(3): 151-163.

Jonsson F and Johanson G (2001). A Bayesian analysis of the influence of GSTT1 polymorphism on the cancer risk estimate for dichloromethane. Toxicol Appl Pharmacol 174(2): 99-112.

Kapeghian JC, Mincer HH, Jones AB, Verlangieri AJ and Waters IW (1982). Acute inhalation toxicity of ammonia in mice. Bull Environ Contam Toxicol 29(3): 371-378.

Kelson TL, Secor McVoy JR and Rizzo WB (1997). Human liver fatty aldehyde dehydrogenase: Microsomal localization, purification, and biochemical characterization. Biochim Biophys Acta 1335(1-2): 99-110.

Kimbell JS, Overton JH, Subramaniam RP, Schlosser PM, Morgan KT, Conolly RB and Miller FJ (2001b). Dosimetry modeling of inhaled formaldehyde: Binning nasal flux predictions for quantitative risk assessment. Toxicol Sci 64(1): 111-121.

Kimbell JS, Subramaniam RP, Gross EA, Schlosser PM and Morgan KT (2001a). Dosimetry modeling of inhaled formaldehyde: Comparisons of local flux predictions in the rat, monkey, and human nasal passages. Toxicol Sci 64(1): 100-110.

Kohn MC and Melnick RL (1993). Species differences in the production and clearance of 1,3-butadiene metabolites: A mechanistic model indicates predominantly physiological, not biochemical, control. Carcinogenesis 14(4): 619-628.

Krasovskii GN (1976). Extrapolation of experimental data from animals to man. Environ Health Perspect 13: 51-58.

Lester D, Greenberg LA and Adams WR (1963). Effects of single and repeated exposures of humans and rats to vinyl chloride. Am Ind Hyg Assoc J 24: 265-275.

Licata AC, Dekant W, Smith CE and Borghoff SJ (2001). A physiologically based pharmacokinetic model for methyl tert-butyl ether in humans: Implementing sensitivity and variability analyses. Toxicol Sci 62(2): 191-204.

Loizou GD (2001). The application of physiologically based pharmacokinetic modeling in the analysis of occupational exposure to perchloroethylene. Toxicol Lett 124(1-3): 59-69.

Lyman WJ (1982). Octanol/Water Partition Coefficient. In: Handbook of Chemical Property Estimation Methods. Lyman W. J., Reehl W. F. and Rosenblatt D. H. McGraw-Hill Book Co. New York (NY): Chapter 1.

MacEwen J, Theodore J and Vernot EH. (1970). *Human Exposure to EEL Concentration of Monomethylhydrazine*. AMRL-TR-1970; 70-102, 23. SysteMed Corp.

Mitchell DY and Petersen DR (1989). Oxidation of aldehydic products of lipid peroxidation by rat liver microsomal aldehyde dehydrogenase. Arch Biochem Biophys 269(1): 11-17.

Moir D, Viau A, Chu I, Withey J and McMullen E (1998). Pharmacokinetics of benzo[a]pyrene in the rat. J Toxicol Environ Health A 53(7): 507-530.

O'Connor G, Sparrow D, Taylor D, Segal M and Weiss S (1987). Analysis of dose-response curves to methacholine. An approach suitable for population studies. Am Rev Respir Dis 136(6): 1412-1417.

OSHA. (1997). *Occupational Exposure to Methylene Chloride. Final Rule. 29 CFR Parts 1919, 1915, and 1926*. RIN 1218-AA98 Vol 62(7): 1493-1619. Washington (DC): Federal Register, Occupational Safety and Health Administration, United States Department of Labor. www.osha.slc.gov/sltc/methylenechloride/index.html.

Overton JH, Kimbell JS and Miller FJ (2001). Dosimetry modeling of inhaled formaldehyde: The human respiratory tract. Toxicol Sci 64(1): 122-134.

Paterson S and Mackay D (1989). Correlation of tissue, blood, and air partition coefficients of volatile organic chemicals. Br J Ind Med 46(5): 321-328.

Pelekis M, Gephart LA and Lerman SE (2001). Physiological-model-based derivation of the adult and child pharmacokinetic intraspecies uncertainty factors for volatile organic compounds. Regul Toxicol Pharmacol 33(1): 12-20.

Pelekis M, Nicolich MJ and Gauthier JS (2003). Probabilistic framework for the estimation of the adult and child toxicokinetic intraspecies uncertainty factors. Risk Anal 23(6): 1239-1255.

Philippin C, Gilgen A and Grandjean E (1970). [Toxicological and physiological investigation on acroleine inhalation in the mouse]. Int Arch Arbeitsmed 26(4): 281-305.

Price K, Haddad S and Krishnan K (2003). Physiological modeling of age-specific changes in the pharmacokinetics of organic chemicals in children. J Toxicol Environ Health A 66(5): 417-433.

Prodan L, Suciu I, Pislaru V, Ilea E and Pascu L (1975). Experimental acute toxicity of vinyl chloride (monochloroethene). Ann N Y Acad Sci 246: 154-158.

Ramesh A, Greenwood M, Inyang F and Hood DB (2001). Toxicokinetics of inhaled benzo[a]pyrene: Plasma and lung bioavailability. Inhal Toxicol 13(6): 533-555.

Renwick AG, Dorne JL and Walton K (2000). An analysis of the need for an additional uncertainty factor for infants and children. Regul Toxicol Pharmacol 31(3): 286-296.

Renwick AG and Lazarus NR (1998). Human variability and noncancer risk assessment--An analysis of the default uncertainty factor. Regul Toxicol Pharmacol 27(1): 3-20.

Sams C, Loizou GD, Cocker J and Lennard MS (2004). Metabolism of ethylbenzene by human liver microsomes and recombinant human cytochrome P450s (CYP). Toxicol Lett 147(3): 253-260.

Sarangapani R, Gentry PR, Covington TR, Teeguarden JG and Clewell 3rd. HJ (2003). Evaluation of the potential impact of age- and gender-specific lung morphology and ventilation rate on the dosimetry of vapors. Inhal Toxicol 15(10): 987-1016.

Sarangapani R, Teeguarden JG, Cruzan G, Clewell 3rd. HJ and Andersen ME (2002). Physiologically based pharmacokinetic modeling of styrene and styrene oxide respiratory-tract dosimetry in rodents and humans. Inhal Toxicol 14(8): 789-834.

Sarangapani R, Teeguarden JG, Gentry PR, Clewell 3rd. HJ, Barton HA and Bogdanffy MS (2004). Interspecies dose extrapolation for inhaled dimethyl sulfate: A PBPK model-based analysis using nasal cavity N7-methylguanine adducts. Inhal Toxicol 16(9): 593-605.

Silver SD and McGrath FP (1948). A comparison of acute toxicities of ethylene imine and ammonia in mice. J Ind Hyg Toxicol 30(1): 7-9.

Sun JD, Wolff RK and Kanapilly GM (1982). Deposition, retention, and biological fate of inhaled benzo[a]pyrene adsorbed onto ultrafine particles and as a pure aerosol. Toxicol Appl Pharmacol 65(2): 231-244.

Sun JD, Wolff RK, Kanapilly GM and McClellan RO (1984). Lung retention and metabolic fate of inhaled benzo[a]pyrene associated with diesel exhaust particles. Toxicol Appl Pharmacol 73(1): 48-59.

Sweeney LM, Shuler ML, Quick DJ and Babish JG (1996). A preliminary physiologically based pharmacokinetic model for naphthalene and naphthalene oxide in mice and rats. Ann Biomed Eng 24(2): 305-320.

Tardif R, Charest-Tardif G, Brodeur J and Krishnan K (1997). Physiologically based pharmacokinetic modeling of a ternary mixture of alkyl benzenes in rats and humans. Toxicol Appl Pharmacol 144(1): 120-134.

Tardif R, Lapare S, Charest-Tardif G, Brodeur J and Krishnan K (1995). Physiologically-based pharmacokinetic modeling of a mixture of toluene and xylene in humans. Risk Anal 15(3): 335-342.

Thrall KD, Vucelick ME, Gies RA, Zangar RC, Weitz KK, Poet TS, Springer DL, Grant DM and Benson JM (2000). Comparative metabolism of carbon tetrachloride in rats, mice, and hamsters using gas uptake and PBPK modeling. J Toxicol Environ Health A 60(8): 531-548.

U.S.EPA. (1992a). *Acute Inhalation Toxicity of Acrolein in Hamsters*. EPA/OTS: 88-920002323. Washington (DC), United States Environmental Protection Agency.

U.S.EPA. (1992b). *Acute Inhalation Toxicity of Acrolein Vapor by One and Four-Hour Exposures* EPA/OTS: 88-920001468S. Washington (DC), United States Environmental Protection Agency.

U.S.EPA. (1994a). *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* EPA/600/8-90/066F. Washington (DC) , United States Environmental Protection Agency. http://www.epa.gov/iris/subst/0364.htm

U.S.EPA. (2004). *An Examination of EPA Risk Assessment Principles and Practices*. EPA/100/B-04/001. Washington (DC), United States Environmental Protection Agency. http://www.epa.gov/OSA/pdfs/ratf-final.pdf.

Uebori M and Imamura K (2004). Analysis of aliphatic and aromatic carbonyl compounds in ambient air by LC/MS/MS. Anal Sci 20(10): 1459-1462.

Verberk MM (1977). Effects of ammonia in volunteers. Int Arch Occup Environ Health 39(2): 73-81.

Weil CS (1972). Statistics vs safety factors and scientific judgment in the evaluation of safety for man. Toxicol Appl Pharmacol 21(4): 454-463.

Wiersma DA and Roth RA (1983). The prediction of benzo[a]pyrene clearance by rat liver and lung from enzyme kinetic data. Mol Pharmacol 24(2): 300-308.

Willems BA, Melnick RL, Kohn MC and Portier CJ (2001). A physiologically based pharmacokinetic model for inhalation and intravenous administration of naphthalene in rats and mice. Toxicol Appl Pharmacol 176(2): 81-91.

Appendix F: Estimating Human Equivalent Concentrations Using the U.S. EPA Default Approach

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Appendix F. Estimating Human Equivalent Concentrations Using the U.S. EPA Default Approach

F.1 Estimating Human Equivalent Concentrations Using the U.S. EPA Default Approach

The United States Environmental Protection Agency (U.S. EPA) Human Equivalent Concentration (HEC) approach (U.S.EPA, 1994a) is designed to adjust the dose in an animal inhalation experiment to the dose that a human would receive at the same air concentration. The adjustment is based on some of the physiological differences between humans and animals. The Office of Environmental Health Hazard Assessment has recommended the necessary physiological parameters for children from the literature needed to adjust the dose in an animal inhalation experiment to the dose that children would receive at the same air concentration.

The U.S. EPA HEC approach was initially adopted by OEHHA for derivation of chronic inhalation Reference Exposure Levels (RELs). The U.S. EPA has proposed a number of different HEC schemes depending on the physicochemical characteristics of the substance (reactive gases, water soluble gases, water-insoluble gases, and particles) and on the site of toxic action (respiratory effects and systemic effects). For both the U.S. EPA Reference Exposure Concentrations (RfCs) and earlier OEHHA chronic RELs, the U.S. EPA default HEC approach was used when more data-intensive methods and specific parameters were unavailable.

The U.S. EPA HEC methods are presented in detail in U.S. EPA (1994a) and will be briefly reviewed here (Section F.1). Modifications to the U.S. EPA method developed by OEHHA to incorporate child-specific parameters are also described (Section F.2).

The U.S. EPA HEC method assumed that interspecies toxicokinetic differences were adequately accounted for by the method and thus the value of the interspecies uncertainty factor (UF_A) was reduced from 10 to $\sqrt{10}$. However, the U.S. EPA HEC procedure deals only with deposition of the original material. It does not consider interspecies differences in distribution of the parent compound after absorption into the respiratory system, in metabolism, or in the distribution of metabolites. The present guidance therefore regards this procedure as providing only a partial estimate of toxicokinetic differences, and an additional uncertainty factor of at least 2 is recommended (*i.e.* the full value of UF_A would be 6.28 if, as is most often the case, there is no reduction of the toxicodynamic component of interspecies uncertainty). A larger uncertainty factor to account for remaining toxicokinetic differences may be warranted in special cases where evidence indicates a larger interspecies toxickinetic difference (with humans being the more sensitive species).

F.1.1 Gases with Respiratory Effects

The regional gas dose ratio (RGDR) is calculated as the relative minute volume (MV) to relative surface area (SA) for the lung region of concern:

$$RGDR = (MV_a/MV_h) / (SA_a/SA_h)$$

Default lung surface area estimates presented by U.S. EPA (1994a) are used (Table F.1.1).

TABLE F.1.1. DEFAULT LUNG SURFACE AREA ESTIMATES

Species	Extrathoracic Surface Area (cm^2)	Tracheobronchial Surface Area (cm^2)	Pulmonary Surface Area (cm^2)
Guinea pig	30	200	9,000
Hamster	14	20	3,000
Human	200	3,200	540,000
Mouse	3	3.5	500
Rabbit	30	300	59,000
Rat	15	22.5	3,400

U.S. EPA, 1994a

Minute volume (volume inhaled per minute) is the product of inhaled volume and respiratory rate. Minute volumes (MV) in L/min for five animal species were estimated from body weights (BW) in kg with allometric relationships presented by U.S. EPA (1994):

$$\log_e(MV) = b_0 + b_1 \log_e(BW)$$

where b_0 and b_1 are empirically derived factors from a database of MV and BW values for various species and strains.

Body weights were estimated from the published experimental study under review or, when necessary, from strain and gender specific default values presented by U.S. EPA (1994a). Intercept (b_0) and slope (b_1) values are presented in Table F.1.2.

TABLE F.1.2. INTERCEPT AND SLOPE PARAMETERS FOR ESTIMATING MINUTE VOLUME FROM BODY WEIGHT

Species	b_0	b_1
Guinea pig	-1.191	0.516
Hamster	-1.054	0.902
Mouse	0.326	1.05
Rabbit	-0.783	0.831
Rat	-0.578	0.821

F.1.2 Gases with Systemic Effects

Gases leading to systemic health effects were calculated using the default assumptions used by the U.S. EPA for all systemic RfCs developed to date. The default methodology adjusts the average exposure concentration by the regional gas dose ratio (RGDR), which for systemically-acting gases is assumed to be the ratio of the animal blood:air partition coefficient $(H_{b/g})_A$ to the human blood:air partition coefficient $(H_{b/g})_H$. The following formulae describe the calculation of the RGDR and HEC:

$$RGDR = (H_{b/g})_A / (H_{b/g})_H$$

$$HEC = \text{Average exposure concentration} \times (H_{b/g})_A / (H_{b/g})_H$$

Where the relevant blood:air coefficients are unknown, U.S. EPA recommends assuming that $(H_{b/g})_A$ is equal to $(H_{b/g})_H$ and thus the RGDR for systemic effects is assumed to equal one. This assumption was used for all RfCs that have been developed for systemically-acting gases. Chemical-specific data, where available, were used to estimate the HEC for additional REL values determined by OEHHA. Where species-specific, but not chemical-specific, data were available, the default assumption of RGDR = 1 was used. Where both species-specific and chemical-specific data were lacking, no HEC calculation was used, and a 10-fold interspecies UF was applied.

F.1.3 Particulates with Respiratory Effects

The U.S. EPA HEC method for particulates (U.S.EPA, 1994a) estimates fractional deposition in different lung regions for both animal species and humans, and calculates the regional deposited dose ratio (RDDR) as the ratio of animal fractional deposition to human fractional deposition. Fractional deposition is assumed to be dependent on minute volume, mass median aerodynamic diameter (MMAD), sigma g, and prior deposition in regions through which the particles have already passed. Deposition efficiency (DE), which is unaffected by prior deposition, is calculated from minute volume, MMAD, and sigma g using a fitted logistic function. The function uses impaction diameter (x) estimated from MMAD and minute volume and is fitted for a given species with two parameters (α and β, Table F.1.3):

$$\text{Flow rate (Q)} \approx MV / 30$$

$$x = MMAD^2 \times Q$$

$$DE = 1 / (1 + e^{\alpha + \beta \log_{10} x})$$

Then, fractional deposition is determined by sequentially determining deposition in extrathoracic (ET), tracheobronchial (TB), and pulmonary (PU) regions.

U.S. EPA RDDR software (U.S. EPA, 1994a) has been used to calculate RDDR and HEC for OEHHA RELs for particulates with respiratory effects. Parameters used include experimentally-determined values for the particle distribution, characterized by the mass median aerodynamic diameter (MMAD) and geometric standard deviation (sigma g), the experimental species, and

experimentally-determined or estimated body weights. Minute volumes are estimated from body weights and default estimates of lung surface areas were used. Deposition and RDDRs are estimated for different lung regions.

TABLE F.1.3. PARAMETERS FOR DEPOSITION EFFICIENCY EQUATION

Species	α (ET)	β (ET)	α (TB)	β (TB)	α (PU)	β (PU)
Human	7.13	-1.96	3.30	-4.59	0.52	-1.39
Rat	6.60	-5.52	1.87	-2.09	2.24	-9.46
Mouse	0.66	-2.17	1.63	-2.93	1.12	-3.20
Hamster	1.97	-3.50	1.87	-2.86	1.15	-7.22
Guinea pig	2.25	-1.28	2.52	-0.87	0.75	-0.56
Rabbit	4.31	-1.63	2.82	-2.28	2.58	-1.99

F.2 Human Equivalent Concentration Calculation for Children

OEHHA examined differences related to postnatal development of the lung, including such factors as differences in respiratory frequency, minute volume, lung surface area, lung deposition, and lung compliance. We also noted other factors such as mouth vs. nasal breathing habits and differences in physical activity. Different scenarios can lead to somewhat different results, but, in general, most differences between children and adults are no greater than several-fold in magnitude. The patterns of postnatal development indicate that susceptibility may change throughout childhood, and exposure during the first year of life may be of special concern.

OEHHA compares the human adult physiological and anatomical parameters used by U.S. EPA with the same parameters for children. We then examine the difference that the use of these child specific parameters would make in the HEC calculations. We thus determine if the HEC adjustment to a NOAEL derived from an animal study is protective of children. We also propose adjustment of some of the U.S. EPA HEC factors based on our reevaluation of studies and newer literature.

F.2.1 Respiratory Differences between Children and Adults

Various factors can affect particle deposition. The respiratory tract is often considered to consist of three anatomically and functionally distinct units: (a) the extra-thoracic (ET - from the mouth and nose to the larynx); (b) the tracheo-bronchial (TB – from the larynx through the conducting airways; and (c) the alveolar (AL – the gas exchange zone). In general, more serious pollution-related health outcomes are related to effects in the TB and AL regions. The patterns of particle deposition in the respiratory tract do not, however, correspond well to the categories used to classify particles (PM10, fine (PM2.5) and coarse (PM10 – PM2.5) fractions). Generally, larger particles demonstrate a greater fractional deposition in the ET and upper TB areas, while smaller

particles show greater deposition in the deep lung (lower TB and AL). These regional patterns reflect principally the mechanisms of deposition that differentially influence particles by size.

Mechanisms of nonfibrous particle deposition include: (i) gravitational settling, for particles more dense than air; (ii) impaction on the wall of a bronchus or bronchiole, due to inertia maintained when the airstream changes direction at an anatomical bend or bifurcation; (iii) diffusion related to Brownian motion; and (iv) electrostatic attraction, which is generally considered of lesser importance than the other three. Settling and diffusion are more important for particles less than about 3 μm, while inertial impaction generally affects larger particles, particularly in the ET and upper TB area (Foster, 1999). For ultrafine particles (with diameters <0.1 μm in diameter), diffusion represents the dominant mode of deposition.

The ET region and especially the nose effectively filter out a large fraction of inhaled particles, mainly those above 1 μm in diameter, and also ultrafine particles. In general, inertial impaction predominates in the ET region, so increasing particle size and increasing flow rates will tend to increase particle deposition. However, fractional deposition of ultrafine particles (inhaled at flow rates between 5.9 and 22 liters/min) in the nose has also been reported to be very high (in excess of 93%) (Swift and Strong, 1996).

In the TB and AL areas, increased depth of breathing tends to enhance the deposition of fine particles, while an increased respiratory rate has the opposite effect (Foster, 1999). Exercise and increased respiratory rates also tend to result in greater deposition in larger, central airways, and less in the AL region (Foster, 1999). Using inert particles 1, 3, and 5 μm in diameter, Kim et al. (1996) showed that, even in healthy adults, there is striking heterogeneity of deposition patterns, with airway surface doses 2 to 16.6 times greater in large airways and up to 4.5 times greater in small airways than in the alveolar region for larger (3 and 5 μm) particles. A similar, but less pronounced, pattern was also observed for particles of 1 μm diameter.

Among healthy adults, airway caliber (measured by specific airway resistance) appears to be an important determinant of particle deposition, with a generally inverse relationship between airway diameter and deposition efficiency (Bennett et al., 1996). This may result from the decreased cross-sectional distance that particles have to traverse (by inertial velocity, gravitational settling, or diffusion) before depositing. Women tended to display a greater deposition fraction than men of 3-5 um particles (perhaps because of a smaller respiratory tract anatomy overall)., particularly in the ET and TB regions (Kim and Hu, 1998).

Individuals with asthma and chronic obstructive lung disease experience greater fractional deposition of fine particles (1 μm in diameter) than individuals with healthy, normal lungs, with the degree of particle retention roughly proportionate to the severity of airway obstruction (Kim and Kang, 1997). Anderson et al. (1990) showed a similar increase in deposition efficiency of fine and ultrafine particles, defined here as those with 0.02 – 0.24 μm in diameter, in several individuals with asthma and COPD relative to healthy subjects.

In such individuals, one can observe focal hyperdeposition of particles, often in sites of airflow limitation in central airways, even when nominal ambient particle concentrations are relatively low (Foster, 1999). Airway hyperresponsiveness, which is one of the hallmarks of asthma, is likewise associated with enhanced regionalization of deposition to the central airways (Foster,

1999). The work of Kim and Kang (1997) indicates that such dose amplification can occur because individuals with obstructive lung disease: (1) ventilate only a portion of their lungs, (2) experience increased deposition compared with healthy individuals, and (3) if symptomatic, tend to have increased minute ventilation. Assessing these factors together, Kim and Kang (1997) estimate that such individuals may have more than three-fold greater total lung deposition than healthy subjects, with this enhanced deposition concentrated in small areas of the lung.

One group of investigators modeled short-term particle deposition in various regions of the respiratory tract using a dosimetry model developed by the International Committee on Radiological Protection (Snipes et al., 1997). They identified large differences in deposition between the ET, TB and AL regions. Daily deposition of all particle sizes was estimated to be greater (by one to three orders of magnitude) in the TB compared with the AL region.

Results of the deposition modeling forming the basis for the report by Snipes et al. (1997) are presented in slightly different form in the 1996 U.S. EPA Criteria Document for particulate matter (U.S. EPA, 1996; vol II, chapter 10). For normal adult males in the general population exposed to a Phoenix-like aerosol (tending to coarse mode), the model predicted daily deposition of 2 and 6 μg/day of fine and coarse mode particles, respectively, in the bronchi, 3 (fine) and 4 (coarse) μg/day in the bronchioles, and 17 (fine) and 12 (coarse) in the alveolar region. Particle doses were estimated to increase substantially in all zones of the lower respiratory tract among "mouth breathers (U.S. EPA, 1996). Higher doses were also predicted to occur as a result of light or heavy work (involving increased breathing rates). Somewhat lower doses were estimated to result from exposure to a Philadelphia-like aerosol, which is characterized by a particle distribution favoring smaller particles. The model employed in these deposition exercises is based on average doses and does not take into account the potential impacts of age, gender, disease states or inter-individual variations in anatomy, ventilation patterns, short-term peak exposures, and so forth.

The human respiratory system undergoes developmental changes thoughout childhood. Full lung maturity may not occur until the age of 20 or 25 (Yu and Xu, 1987).

The structural development of the respiratory system varies markedly among species (Mauderly, 2000). Humans as well as rabbits and dogs have developed alveoli at birth, but these structures have not yet developed their mature form, and undergo septal wall thinning and capillary fusion postnatally. Humans form 80% of alveoli postnatally (Plopper and Fanucchi, 2004). Human alveolar multiplication can continue until about 8 years of age (Boyden, 1971). Development of intra-acinar vessels also occurs postnatally (Boyden, 1971). Guinea pigs and sheep have morphologically mature alveoli at birth that only increase in number and size after birth. At birth rats, mice, and hamsters have immature lungs that lack developed alveoli. Thus different species are at markedly different stages of development and may differ in susceptibility to toxicants during the early postnatal period.

There are significant anatomic and physiological differences between the developing lungs of children and those of mature adults (Snodgrass, 1992). These include differences in the size and shape of the conducting airways, the number and orientation of physiologically active gas

exchange regions, and ventilation rates. Though the basic structure of the airways is established *in utero*, most of the alveoli (≈ 85%) develop in infancy and early childhood. Alveolar multiplication coincides with incorporation of elastin and collagen in the lung, which are responsible for the mature lung's mechanical properties (Lipsett, 1995). With growth and development other patterns of anatomical differences emerge. For instance, TB airways increase in diameter and length until adulthood. Lung volume expands disproportionately in relation to the increasing number of alveoli during somatic growth, indicating enlargement of individual alveoli (Murray, 1986).

Because of differences in anatomy, activity, and ventilation patterns, children are likely to inhale and retain larger quantities of pollutants per unit body surface area than adults (Adams, 1993). Phalen et al. (1985) developed a model incorporating airway dimensions measured in lung casts of people (aged 11 days to 21 years) and predicted that particle deposition efficiency would be inversely related to body size, which would tend to accentuate differences in exposure related to activity and ventilation patterns. Phalen et al. (1985) estimated that 5 micron diameter particles will deposit in a 6-fold higher dose per kilogram body weight in the tracheobronchial region in a resting newborn compared to a resting adult. Corroborative evidence for this was provided by Oldham et al. (1997), who found that in models of the proximal TB airways (i.e., the trachea and the first two bronchial bifurcations) of 4- and 7-year-old children and an adult, deposition efficiencies for radiolabelled particles 1.2, 4.5, 9.7 and 15.4 μm in median aerodynamic diameter were greater in the child models in almost all cases. As expected, particle deposition efficiency increased markedly with increasing particle size in this model system. For instance, in the model of the four-year-old child, the deposition efficiency increased from 0.3% to 10.7% when the smallest and largest particle sizes were used, respectively.

Inhalation experiments comparing particle deposition patterns in children and adults have produced somewhat inconsistent results. Schiller-Scotland et al. (1994) reported greater fractional deposition in healthy children, aged 3 – 14 years, compared with adults, when breathing 1, 2 or 3 μm particles spontaneously through a mouthpiece. The differences were greater with the larger particles. However, as noted by the authors, these children were breathing more deeply than expected, which is a common tendency when breathing through a mouthpiece. This propensity may result in greater time-dependent deposition of fine particles (by sedimentation and diffusion). Schiller-Scotland et al. (1994) also noted that, among the older children (mean age = 10.9 years) who were capable of controlled breathing in time with a metronome, particle deposition was inversely related to body height, so that the shorter children demonstrated greater fractional deposition (for 1 and 2 μm particles, the only categories analyzed in this manner). In contrast, Bennett and Zeman (1998) found no significant differences between children (7 – 14 yr), adolescents (14 to 18 yr), and young adults (19 – 35 yr) in deposition (measured as deposition fraction or rate) of 2 μm particles during spontaneous breathing at rest. Unlike the study by Schiller-Scotland et al. (1994), this investigation tailored the participants' mouthpiece breathing patterns to those measured during unencumbered breathing, in order to control for the tendency to breathe more deeply through a mouthpiece. Another difference between the study by Bennett and Zeman (1998) and that by Schiller-Scotland et al. (1994) is that the former did not include very young children, who would have had difficulty in mimicking their normal breathing patterns while using a mouthpiece. However, Schiller Scotland et al.

(1994) found that older children (mean age = 10.9 years) as well as the younger ones (mean age = 5.3 years) also showed increased fractional particle deposition relative to adults.

Children demonstrate lower absolute minute ventilation at rest than adults, despite having higher breathing rates. Relative to lung volume, however, children demonstrate a higher minute ventilation than adults. Thus, Bennett and Zeman (1998) noted that children tended to have a somewhat greater normalized deposition rate (by about 35%) than the combined group of adolescents and adults, suggesting that children at rest would receive higher doses of particles per unit of lung surface area than adults. This tendency might be additionally enhanced by activity patterns, as children spend more time than adults in activities requiring elevated ventilation rates. However, it is unknown whether flow-dependent deposition mechanisms operative at higher ventilation rates in children would offset the decreases that would occur in time-dependent mechanisms (sedimentation and diffusion). If this offset does occur, then particle deposition would likely be shifted more towards the larger, more central airways, which would tend to increase the dose per surface area in children versus adults (Bennett and Zeman, 1998).

The above studies suggest that children may experience proportionately greater particle deposition than adults. It is also possible that, especially in very young children, immature respiratory defenses may result in lower clearance rates in relation to those observed in adults. For instance, Sherman et al. (1977) reported that alveolar macrophages of neonatal rabbits (1 day old) ingested significantly fewer bacteria than older animals (7 days). To the extent that this phenomenon may also apply across species and to nonbiological particles, the immaturity of the neonatal human lung may result in slower and less complete particle clearance.

In summary, there is substantial evidence to conclude that childhood exposures may differ significantly from those experienced by adults. In some cases doses received by children may be substantially greater than those received by adults. However, the differences may be complex and change somewhat over the period of lung development.

F.2.2 Calculation of Adult and Child HECs

The regional gas dose ratio (RGDR) for gases with respiratory effects is calculated as the relative minute volume (MV) to relative surface area (SA) for the lung region of concern.

Minute volume (volume inhaled per minute) is calculated as the product of tidal volume and respiratory frequency. Using empirical formulas for humans,

$$\text{Tidal volume (cm}^3\text{)} = 21.7 + 35.15t - 0.64\,t^2$$

and

$$\text{Respiratory frequency (per minute)} = 15.17 / (0.25t + 0.5) + 11.75,$$

where t is age in years (Hofmann, 1982). Minute volumes (MV) in L/min for five animal species were estimated from body weights (see Section 1.1 and Table 2).

F.2.2.1 Gases with Extrathoracic Effects

Many pollutants fall into the category of gases with extrathoracic effects. These include ammonia, chlorine, formaldehyde, hydrogen chloride, and hydrogen sulfide. Data to estimate child nasopharyngeal surface area are very limited. A simple assumption is that growth of the extrathoracic surface area is proportional to body weight, body surface area, or overall lung surface area.

The approach applied here uses estimates of head volume derived from head growth charts to estimate relative extrathoracic surface area. It assumes that overall extrathoracic surface area is proportional to the surface area of a horizontal plane through the nasopharyngeal region. Based on these assumptions, children are predicted to have lower extrathoracic exposures than adults (Table F.2.1).

TABLE F.2.1. RELATIVE MINUTE VOLUME (MV) TO SURFACE AREA (SA) RATIOS FOR PULMONARY, TRACHEOBRONCHIAL, AND EXTRATHORACIC SPACES IN CHILDREN

A. Chronic Exposure

Age Range (years)	Pulmonary Relative MV/SA	Tracheobronchial Relative MV/SA	Extrathoracic Relative MV/SA
0 to 1	3.0	0.5	0.5
1 to 2	2.0	0.5	0.5
2 to 4	1.5	0.6	0.6
4 to 8	1.5	0.8	0.7
8 to 15	1.3	0.9	0.9
15-25	1.1	1.0	1.0

B. Acute Exposure

Age (years)	Pulmonary Relative MV/SA[1]	Tracheobronchial Relative MV/SA[2]	Extrathoracic Relative MV/SA[3]
0	3.8	0.5	0.5
1	2.2	0.5	0.5
2	1.8	0.5	0.5
4	1.6	0.7	0.6
8	1.4	0.8	0.8
15	1.2	1.0	0.9

[1]Pulmonary calculations based on the lung growth model of Yu and Xu (1987).

[2]Tracheobronchial calculations based on the data of Phalen *et al*. (1985). Calculations are based on flux per surface area in accordance with the U.S. EPA HEC methodology, and do not take into account increased absorption and greater particle deposition due to much greater relative tracheobronchial surface area in children. For example, Phalen *et al*. (1985) predicted a 6-fold increased tracheobronchial deposition of 5-micron particles in newborns compared with adults.

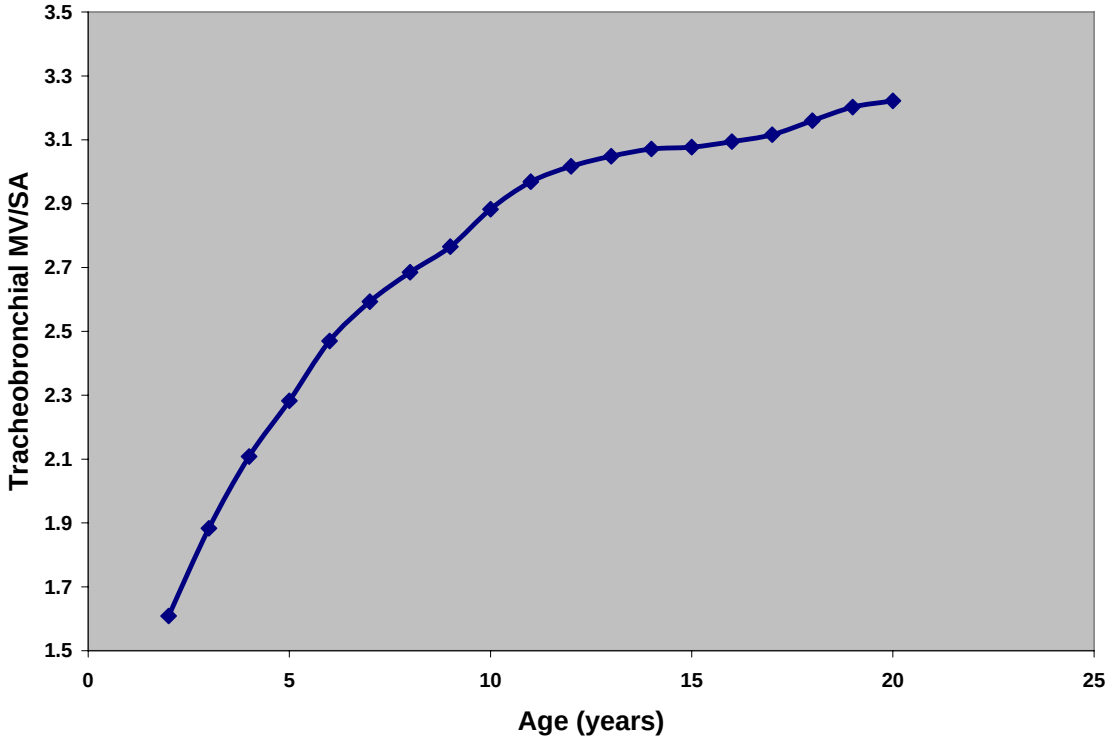
[3]Extrathoracic calculations based on head growth data of Tanner in Dattani and Preece (1978). The increase in extrathoracic surface area is presumed to be proportional to the increase in head volume.

Using the HEC model, the observed concentration divided by the appropriate relative MV/SA factor may be used as an estimate of equivalent childhood exposure. Thus in terms of relative MV/SA, pulmonary effects are predicted to be greater in children, whereas tracheobronchial and extrathoracic effects are predicted to be less in children. The approach does not take into account other differences between adults and children, such as differences in deposition, mouth breathing, and susceptibility.

F.2.2.2 Gases with Tracheobronchial Effects

Other pollutant gases, such as chlorine dioxide and toluene diisocyanate, have primarily tracheobronchial effects. Good data are available to estimate child tracheobronchial surface areas. Figure F.2-1 below depicts changes in the relative ratio of minute volume to tracheobronchial surface area as children age. This approach results in lower tracheobronchial regional gas doses for children than adults (Table F.2.1).

FIGURE F.2-1. CHANGES IN MINUTE VOLUME/TRACHEOBRONCHIAL SURFACE AREA WITH AGE.



F.2.2.3 Gases with Pulmonary Effects

For gases with pulmonary effects, an opposite result is obtained. There are good data to estimate child pulmonary surface areas. As shown in Figure F.2-2, the number of alveoli increases dramatically from birth to age 8. Figure F.2-3 depicts changes in the relative ratio of minute volume to tracheobronchial surface area as children age. This approach results in higher regional gas doses for children than adults (Table F.2.1). This is most pronounced in newborns and infants.

FIGURE F.2-2. INCREASE IN NUMBER OF ALVEOLI FROM BIRTH TO AGE 8.

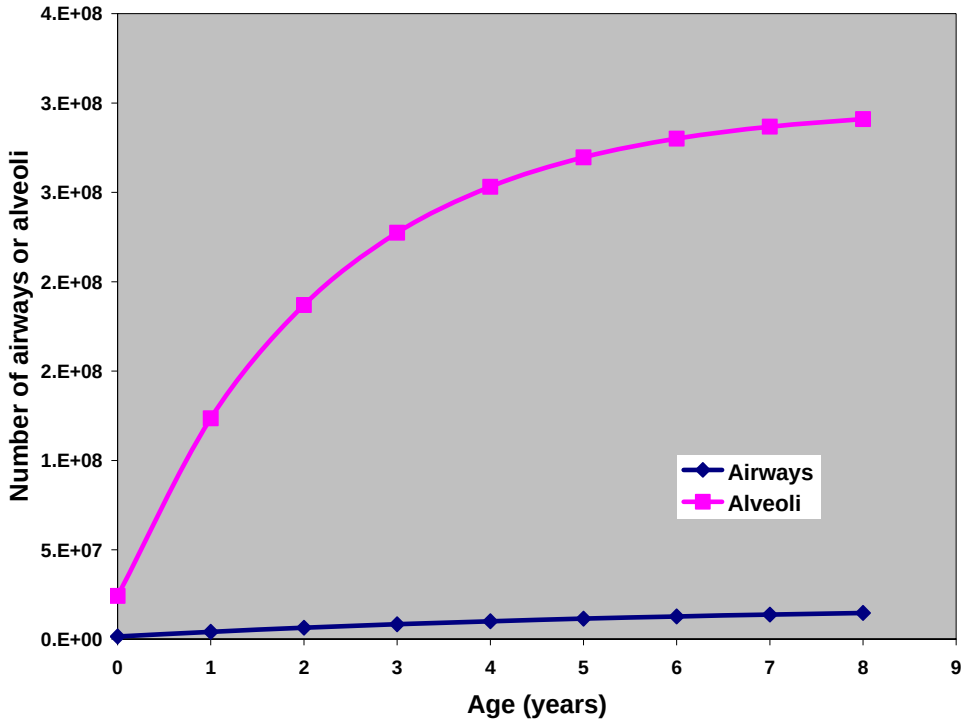
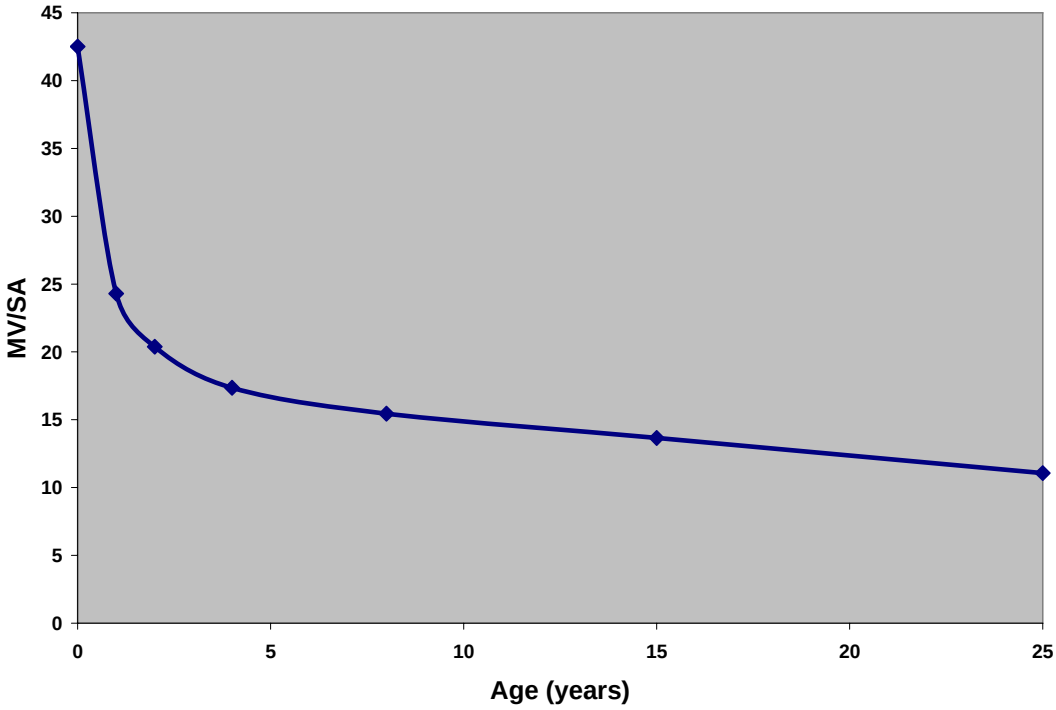


FIGURE F.2-3. DECLINE IN MINUTE VOLUME/TRACHEOBRONCHIAL SURFACE AREA WITH AGE.



F.2.2.4 Vapors with Systemic Effects

The RGDR calculation for systemic effects assumes:

$$RGDR = \lambda_{animal} / \lambda_{human}$$

where λ is the blood to air partition coefficient.

Experimental data for the blood to air partition coefficient were used. A default blood to air partition coefficient value of 1 was used where chemical-specific data were unavailable. Appropriate methods to account for differences between adults and children have not been developed.

F.2.2.5 Particulates/Aerosols/Mists

Deposition efficiency differs as a function of age, as do minute volume, surface area, and body weight. Total deposition fractions tend to be higher in children than adults (Oldham et al., 1997). Deposition fractions of 2 µm particles were 73% in a 7 month old and 38% in an adult (Musante and Martonen, 2000). Children under 8 years of age have the highest deposition fractions. Both tracheobronchial and pulmonary deposition fractions are higher in children. Children may receive a 3-fold higher deposited dose than adults.

Tracheobronchial deposition is inversely proportional to age. Alveolar deposition is maximal at age 4 to 6 as a result of later alveolar development. Aerosol deposition in the nose is also predicted to be greater in children than in adults (Phalen et al., 1989).

As noted earlier, the minute volume to respiratory surface area may be higher or lower for children relative to adults, depending on the region of interest. Thus the net relative RDDR may increase or offset the effect of increased deposition in children, depending on the region of interest.

F.3 Conclusions

Differences between children and adults for relative minute volume to surface area ratios are 4-fold or less. Such differences may be already accounted for in many cases by the 10-fold intraspecies uncertainty factor to protect sensitive subpopulations. There may be cases, however, where other factors lead to greater exposures or susceptibility among children. In these cases, children may be affected at concentrations more than 10-fold lower than concentrations affecting adults. Increased deposition among children can be addressed by child-specific deposition modeling. Known differences in susceptibility should be addressed separately.

References

Adams WC. (1993). *Measurement of Breathing Rate and Volume in Routinely Performed Daily Activities. Final Report*. Contract No. A033-205. California Air Resources Board, California Environmental Protection Agency.

Anderson PJ, Wilson JD and Hiller FC (1990). Respiratory tract deposition of ultrafine particles in subjects with obstructive or restrictive lung disease. Chest 97(5): 1115-1120.

Bennett WD and Zeman KL (1998). Deposition of fine particles in children spontaneously breathing at rest. Inhal Toxicol 10(9): 831-842.

Bennett WD, Zeman KL and Kim C (1996). Variability of fine particle deposition in healthy adults: Effect of age and gender. Am J Respir Crit Care Med 153(5): 1641-1647.

Boyden EA (1971). The structure of the pulmonary acinus in a child of six years and eight months. Am J Anat 132(3): 275-299.

Dattani M and Preece M (1978). Physical Growth and Development. In: Textbook of Pediatrics. Forfar J. and Arnell C. Churchill Livingstone. Edinburgh: 363.

Foster WM (1999). Deposition and Clearance of Inhaled Particles. In: Air Pollution and Health. Holgate S. T., Samet J. M., Koren H. S. and Maynard R. L. Academic Press. San Diego (CA): 295-324.

Hofmann W (1982). Mathematical model for the postnatal growth of the human lung. Respir Physiol 49(1): 115-129.

Kim CS and Hu SC (1998). Regional deposition of inhaled particles in human lungs: comparison between men and women. J Appl Physiol 84(6): 1834-1844.

Kim CS, Hu SC, DeWitt P and Gerrity TR (1996). Assessment of regional deposition of inhaled particles in human lungs by serial bolus delivery method. J Appl Physiol 81(5): 2203-2213.

Kim CS and Kang TC (1997). Comparative measurement of lung deposition of inhaled fine particles in normal subjects and patients with obstructive airway disease. Am J Respir Crit Care Med 155(3): 899-905.

Lipsett MJ (1995). Air Pollution: Effects in Children. In: Environmental Medicine. Brooks S. M., Gochfeld M. and Jackson R. Mosby-Year Book, Inc. Chicago (IL).

Mauderly JL (2000). Animal models for the effect of age on susceptibility to inhaled particulate matter. Inhal Toxicol 12(9): 863-900.

Murray JF (1986). The Normal Lung: The Basis for Diagnosis and Treatment of Pulmonary Disease, 2nd Edition. Philadelphia (PA): WB Saunders Co.

Musante CJ and Martonen TB (2000). Computer simulations of particle deposition in the developing human lung. J Air Waste Manag Assoc 50(8): 1426-1432.

Oldham MJ, Mannix RC and Phalen RF (1997). Deposition of monodisperse particles in hollow models representing adult and child-size tracheobronchial airways. Health Phys 72(6): 827-834.

Phalen RF, Oldham MJ, Beaucage CB, Crocker TT and Mortensen JD (1985). Postnatal enlargement of human tracheobronchial airways and implications for particle deposition. Anat Rec 212(4): 368-380.

Phalen RF, Oldham MJ and Mautz WJ (1989). Aerosol deposition in the nose as a function of body size. Health Phys 57 (Suppl 1): 299-305.

Plopper CG and Fanucchi MV (2004). Development of Airway Epithelium. In: The Lung: Development, Aging and The Environment Harding R., Pinkerton K. and Plopper C. G. Elsevier Academic Press. San Diego (CA).

Schiller-Scotland CF, Hlawa R and Gebhart J (1994). Experimental data for total deposition in the respiratory tract of children. Toxicol Lett 72(1-3): 137-144.

Sherman M, Goldstein E, Lippert W and Wennberg R (1977). Neonatal lung defense mechanisms: A study of the alveolar macrophage system in neonatal rabbits. Am Rev Respir Dis 116(3): 433-440.

Snodgrass WR (1992). Physiological and Biochemical Differences Between Children and Adults as Determinants of Toxic Response to Environmental Pollutants. In: Similarities and Differences Between Children and Adults: Implications For Risk Assessment. Guzelian P. S., Henry C. J. and Olin S. S. ILSI Press. Washington (DC): 35-42.

Swift DL and Strong JC (1996). Nasal deposition of ultrafine 218Po aerosols in human subjects. J Aerosol Sci 27(7): 1125-1132.

U.S.EPA. (1994a). *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* EPA/600/8-90/066F. Washington (DC) , United States Environmental Protection Agency. http://www.epa.gov/iris/subst/0364.htm

Yu CP and Xu GB (1987). Predictive models for deposition of inhaled diesel exhaust particles in humans and laboratory species. Res Rep Health Eff Inst(10): 3-22.

Appendix G. Value of the Haber's Law Exponent (n) for various gases and vapors for acute RELs developed using OEHHA (1999) procedures

TABLE G1. VALUE OF THE HABER'S LAW EXPONENT (*N*) FOR VARIOUS GASES AND VAPORS FOR ACUTE RELS DEVELOPED USING OEHHA (1999)[1] PROCEDURES

Chemical	*n*	Species/Effect (site of action)	References, Comments
Acrolein	1.2	rat/lethality (local irritant)	U.S. EPA (1992a; U.S.EPA, 1992b)[2]
Acrylonitrile	1.1	rat/lethality (systemic)	(Dudley and Neal, 1942; Appel et al., 1981)[3]
Allyl chloride	0.5	rat/lethality (local irritant)	Adams *et al.* (1940)[2]
Ammonia	4.6	Human/irritation	Rosenbaum *et al.*(1993)
	2.02	rat/lethality (local irritant)	Appelman *et al.*(1982)
Arsine	2.2	rat/lethality (systemic)	IRDC (1985)[2] for 0.5 to 1 hr (n dependent on exposure duration)
	1.0	rat/lethality (systemic)	IRDC (1985)[2] for 4 hr to 1 hr (n dependent on exposure duration)
	2	mice/lethality (systemic)	Levvy (1947)
Benzene	2	not given	AICE (1989)
Bromine	2.2	mice/lethality (local irritant)	Bitron & Aharoson (1978)[3]
Carbon monoxide	1	not given	AICE (1989)
Carbon tetrachloride	2.8	rat/lethality (systemic)	Adams *et al.*(1952)[3]
Chlorine	2.8	rat/lethality (local irritant)	Zwart & Woutersen (1988)[2] for 0.5 hr to 1 hr (n dependent on exposure duration)
	1.0	rat/lethality (local irritant)	Zwart & Woutersen (1988)[2] for 4 hr to 1 hr (n dependent on exposure duration)
	1.3	mouse/lethality (local irritant)	Zwart & Woutersen (1988)[2]
	3.5	mouse/lethality (local irritant)	Bitron & Aharoson (1978)[3]
Chlorine pentafluoride	2	rat, mouse, dog, monkey/lethality (local irritant)	Darmer *et al*. (1972) [3]
Crotonaldehyde	1.2	rat/lethality (local irritant)	Rinehart (1967) [3]
Dibutyl hexamethylene-diamine	1	rat/lethality (local irritant)	Kennedy & Chen (1984)[3]
1,2-dichloroethylene	2	(not applicable)/lethality (systemic)	U.S.EPA (1996), based on the mid-point range of n values from lethality data of [3]
Dimethyldichloro-silane	2	(not applicable)/lethality (local irritant)	U.S.EPA (1996), based on the mid-point range of n values from lethality data of [3]
Ethylene dibromide	1.2	rat/lethality (systemic)	(Rowe *et al.*, 1952b)[3]
Ethylene imine	1.1	rat, guinea pig/lethality (local irritant)	(Carpenter *et al.*, 1948)[3]
Fluorine	1.9	rat/lethality (local irritant)	U.S.EPA (1996), derived from LC_{50} data of Keplinger & Suissa (1968)

Chemical	*n*	Species/Effect (site of action)	References, Comments
	1.8	mouse/lethality (local irritant)	U.S. EPA (1996), derived from LC_{50} data of Keplinger & Suissa (1968)
	1.6	guinea pig/lethality (local irritant)	U.S.EPA (1996), derived from LC_{50} data of Keplinger & Suissa 1968)
Formaldehyde	2	not given	AICE (1989)
Hydrazine	2	(not applicable)/lethality (systemic)	U.S.EPA (1996), based on the mid-point range of n values from lethality data of [3]
Hydrogen chloride	1	rat, mouse/lethality (local irritant)	Darmer (1972)[3]
	1.5	rat/lethality (local irritant)	Hartzell & Johnson (1985)[2]
Hydrogen cyanide	2.7	numerous species/lethality (systemic)	Barcroft (1931)[3]
Hydrogen fluoride	2	rabbits, guinea pigs/ lethality (local irritant)	Machle (1934)[3]
Hydrogen fluoride (low humidity)	1	rat/lethality (local irritant)	Haskell Lab. (1988)[2]
Hydrogen sulfide	2.2	cat, rabbit/lethality (systemic/local irritant)	Lehmann (1892)[3]
	8.2	lethality (systemic/local irritant)	Arts (1989)
Methyl bromide	4.0	severe morbidity (systemic/local irritant)	Pharmaco: LSR, (1994) as cited in DPR (2004)[2], DPR (1996)
	1	not given	AICE (1989)
Methylene chloro-bromide	1.6	rat/lethality (systemic)	Torkelson (1960)[3]
Methyl hydrazine	1.0	squirrel monkey/lethality (systemic and local irritant)	Haun (1970)[2]
	1.0	dog/lethality (systemic and local irritant)	Haun (1970)[2]
Methyl isocyanate	1.1	human/eye irritation	Mellon Institute (1963)[2]
	0.5	rat/lethality (local irritant)	Kimmerle & Eben (1964)[2]
	0.7	rat/lethality (local irritant)	DOW Chemical (1990)[2]
Methyl mercaptan	2	(Not applicable)/lethality (systemic and local irritant)	U.S.EPA (1996), based on the mid-point range of n values from lethality data of [3]
Methyl t-butyl ether	2.0	lethality (systemic)	Snam Progretti (1980) as cited in ten Berg et al., (1986)[3]
Nitrogen dioxide	3.5	guinea pig, mouse, dog, rat, rabbit/lethality (local irritant)	Hine *et al.*, (1970)[3]
Nitric acid	3.5	not applicable (local irritant)	U.S.EPA (1996), based on NO_2 from Hine *et al.* (1970)
Perfluoroisobutylene	1.2	rat/lethality (local irritant)	Smith *et al.* (1982)[3]
Phosgene	1	lethality (local irritant)	Rinehart & Hatch (1964)
Propylene oxide	2.2	rat/lethality (local irritant)	Rowe *et al.* (1956)[2]

Chemical	*n*	Species/Effect (site of action)	References, Comments
	1.5	guinea pig/lethality (local irritant)	Rowe *et al.* (1956)[2]
Sulfur dioxide	1	not given	AICE (1989)
Tetrachloroethylene	2.0	rat/lethality (systemic)	Rowe *et al* (1952a)[3]
Toluene	2.5	not given	AICE (1989)
Trichloroethylene	0.8	rat/lethality (systemic)	Adams *et al.* (1951)[3]

[1] OEHHA (1999a); [2]derived by OEHHA; [3]derived by ten Berge (1986)

References

Adams EM, Spencer HC and Irish DD (1940). The acute vapor toxicity of allyl chloride. J Ind Hyg Toxicol 22(2): 79-86.

Adams EM, Spencer HC, Rowe VK, Mc CD and Irish DD (1952). Vapor toxicity of carbon tetrachloride determined by experiments on laboratory animals. A M A Arch Ind Hyg Occup Med 6(1): 50-66.

Adams EM, Spencer HC, Rowe VK, Mc CDD and Irish DD (1951). Vapor toxicity of trichloroethylene determined by experiments on laboratory animals. A M A Arch Ind Hyg Occup Med 4(5): 469-481.

AICE. (1989). *Guidelines for Chemical Process Quantitative Risk Analysis*. pp. 148-159. New York (NY): Center for Chemical Process Safety, American Institute of Chemical Engineers.

Appel KE, Peter H, Bolt M and Bolt HM (1981). Interaction of acrylonitrile with hepatic microsomes of rats and men. Toxicol Lett 7(4-5): 335-339.

Appelman LM, ten Berge WF and Reuzel PG (1982). Acute inhalation toxicity study of ammonia in rats with variable exposure periods. Am Ind Hyg Assoc J 43(9): 662-665.

Arts JH, Zwart A, Schoen ED and Klokman-Houweling JM (1989). Determination of concentration-time-mortality relationships versus LC_{50}'s according to OECD guideline 403. Exp Pathol 37(1-4): 62-66.

Barcroft J (1931). The toxicity of atmospheres containing hydrocyanic acid gas. J Hyg 31(1): 1-34.

Bitron MD and Aharonson EF (1978). Delayed mortality of mice following inhalation of acute doses of CH_2O, SO_2Cl_2, and Br_2. Am Ind Hyg Assoc J 39(2): 129-138.

Carpenter CP, Smyth Jr. HF and Shaffer CB (1948). The acute toxicity of ethylene imine to small animals. J Ind Hyg Toxicol 30: 2-6.

Darmer Jr. KI, Haun CC and MacEwen JD (1972). The acute inhalation toxicology of chlorine pentafluoride. Am Ind Hyg Assoc J 33(10): 661-668.

Dow Chemical (1990). Unpublished experiments on methyl isocyanate. Dow Chemical Company.

DPR. (1996). *Summary of Pesticide Use Report Data 1996 Indexed by Commodity*. Sacramento (CA): Department of Pesticide Regulation, California Environmental Protection Agency.

Dudley HC and Neal PA (1942). Toxicology of acrylonitrile (vinyl cyanide). I. A study of the acute toxicity. J Ind Hyg Toxicol 24(2): 27-36.

Hartzell CR and Johnson GV (1985). In vivo MAC values and in vitro experimentation. Anesth Analg 64(4): 386-387.

Haun CC, MacEwen JD, Vernot EH and Eagan GF (1970). Acute inhalation toxicity of monomethylhydrazine vapor. Am Ind Hyg Assoc J 31(6): 667-677.

Hine CH, Meyers FH and Wright RW (1970). Pulmonary changes in animals exposed to nitrogen dioxide, effects of acute exposures. Toxicol Appl Pharmacol 16(1): 201-213.

IRDC. (1985). *Three Acute Inhalation Toxicity Studies of Arsine on Rats (Final Report)*

Report No. 533-001, 533-002, and 533-003. Mattawan (MI): International Research & Development Corporation.

Kennedy Jr. GL and Chen HC (1984). Inhalation toxicity of dibutylhexamethylenediamine in rats. Food Chem Toxicol 22(6): 425-429.

Keplinger ML and Suissa LW (1968). Toxicity of fluorine short-term inhalation. Am Ind Hyg Assoc J 29(1): 10-18.

Kimmerle G and Eben A (1964). [On the toxicity of methylisocyanate and its quantitative determination in the air]. Arch Toxikol 20: 235-241.

Lehmann KB (1892). Experimentele Studien uber den Einfluss technisch und hygienisch wichtiger Gase und Dampfe auf den Organismus [German]. Arch Hyg 14: 135-189.

Levvy GA (1947). A study of arsine poisoning. Quart J Exp Physiol 34: 47-67.

Machle W, Thamann F, Kitzmiller K and Cholak J (1934). The effects of the inhalation of hydrogen fluoride. I. The response following exposure to high concentrations. J Ind Hyg 16(2): 129-145.

Mellon Institute (1963). Special report 26-23: Methyl Isocyanate. Pittsburgh (PA): Mellon Institute.

OEHHA. (1999a). *The Air Toxics Hot Spots Program Risk Assessment Guidelines. Part I: Technical Support Document for Determination of Acute Reference Exposure Levels for Airborne Toxicants*. Air Toxicology and Epidemiology Section, Office of Environmental Health Hazard Assessment, California Environmental Protection Agency. http://www.oehha.ca.gov/air/acute_rels/acuterel.html#download.

Pharmaco: LSR I (1994). An Up-and-Down Acute Inhalation Toxicity Study of Methyl Bromide in the Dog (Four Exposure Phase). E. Millstone(NJ), Pharmaco: LSR, Inc.

Rinehart WE (1967). The effect on rats of single exposures to crotonaldehyde vapor. Am Ind Hyg Assoc J 28(6): 561-566.

Rinehart WE and Hatch T (1964). Concentration-time product (Ct) as an expression of dose in sublethal exposures to phosgene. Am Ind Hyg Assoc J 25: 545-553.

Rosenbaum JR, Alexeeff GV and Lewis DC (1993). Use of benchmark dose methodology to combine data sets in the development of an acute REL for ammonia [Abstract]. The Toxicologist 13: 282.

Rowe VK, Dd M, Spencer HC, Adams EM and Irish DD (1952a). Vapor toxicity of tetrachloroethylene for laboratory animals and human subjects. A M A Arch Ind Hyg Occup Med 5(6): 566-579.

Rowe VK, Hollingsworth RL, Oyen F, McCollister DD and Spencer HC (1956). Toxicity of propylene oxide determined on experimental animals. AMA Arch Ind Health 13(3): 228-236.

Rowe VK, Spencer HC, Mc CD, Hollingsworth RL and Adams EM (1952b). Toxicity of ethylene dibromide determined on experimental animals. A M A Arch Ind Hyg Occup Med 6(2): 158-173.

Smith LW, Gardner RJ and Kennedy Jr. GL (1982). Short-term inhalation toxicity of perfluoroisobutylene. Drug Chem Toxicol 5(3): 295-303.

Snam Progretti (1980). Research reports on MTBE: Toxicological data.

ten Berge WF, Zwart A and Appelman LM (1986). Concentration-time mortality response relationship of irritant and systemically acting vapours and gases. J Hazard Mater 13: 301-309.

Torkelson TR, Oyen F and Rowe VK (1960). The toxicity of bromochloromethane (methylene chlorobromide) as determined on laboratory animals. Am Ind Hyg Assoc J 21: 275-286.

U.S.EPA. (1992a). *Acute Inhalation Toxicity of Acrolein in Hamsters*. EPA/OTS: 88-920002323. Washington (DC), United States Environmental Protection Agency.

U.S.EPA. (1992b). *Acute Inhalation Toxicity of Acrolein Vapor by One and Four-Hour Exposures* EPA/OTS: 88-920001468S. Washington (DC), United States Environmental Protection Agency.

U.S.EPA (1996). Integrated Risk Information System (IRIS) Database

Washington (DC), United States Environmental Protection Agency. http://www.epa.gov/IRIS. August 7, 2006.

Zwart A and Woutersen RA (1988). Acute inhalation toxicity of chlorine in rats and mice time-concentration-mortality relationships and effects on respiration. Journal of Hazardous Materials 19(2): 195-208.