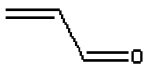
Appendix C-1
Chemical Toxicity Summaries
For Tier 1 Toxic Air Contaminants

Acrolein



107-02-8

I. Physical and Chemical Properties

Description	Colorless or yellow liquid with piercing, disagreeable odor
Molecular formula	C_3H_4O
Molecular weight	56.1
Air concentration conversion	1 ppm = 2.3 mg/m^3 @ 25°C

II. Overview

Data both in animals *in vivo* and using human tissue *in vitro* strongly suggest that acrolein may exacerbate asthma (Roux et al., 1999; Borchers et al., 1998; Borchers et al., 1999a and 1999b; Leikauf et al., 1989a and 1989b). These studies are presented in the following sections. As described in Section II of the Introduction, OEHHA considers asthma to impact children more than adults, and thus substances that either exacerbate or induce asthma should be considered for listing under SB 25. 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). As noted in Section II.C of this document, hospitalization is not a discretionary activity and is an indication of the severity of the asthma. 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. Although it is very difficult to measure acrolein in ambient air, it appears that acrolein is an important component of air pollution, and that acrolein exposures are significant. In addition, model predictions indicate that typical urban air concentrations of acrolein exceed the chronic Reference Exposure Level (REL), which was developed to protect the public from respiratory toxicity. Thus, acrolein ranked high in the initial prioritization of TACs (see Table 1 of the Introduction). For these reasons acrolein was considered a priority chemical for evaluation of potential differential effects on infants and children.

III. Principal Sources of Exposure

Acrolein is used as a chemical intermediate in the production of acrylic acid and its esters. It 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, 1985; 1995). According to the California Department of Pesticide

Regulation's (DPR's) annual pesticide use reports for 1996-1999, over 300,000 pounds of acrolein are applied in California each year, the majority of which is used on rights of way. In addition, acrolein is produced from the combustion of fossil fuels, tobacco smoke, and pyrolyzed animal and vegetable fats (IARC, 1985). As a byproduct of fires, it is one of several acute toxicants to which firefighters are exposed. Acrolein is also formed from atmospheric reactions of 1,3-butadiene. In addition to mobile source tailpipe emissions, acrolein is emitted by stationary sources. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Progam in California based on the most recent inventory were estimated to be 54,565 pounds of acrolein (CARB, 2000).

Because acrolein is extremely reactive, it is very difficult to measure in ambient air. It has been suggested that many of the measurements that have been reported in the literature underestimate actual acrolein concentrations. The California Air Resources Board (CARB) has little data on ambient concentrations of acrolein. A CARB study conducted in the early 1990s in Woodland, California, reported results of 13 outdoor measurements of acrolein (many of which were below the quantifiable limit) that ranged from 2 $\mu g/m^3$ to 8.6 $\mu g/m^3$. Indoor levels averaged 7.1 $\mu g/m^3$, and ranged up to 29 $\mu g/m^3$ (CARB, 2001). The U.S. EPA compiled data from 1961 to 1980 for two urban locations that reported a mean concentration of 14.3 $\mu g/m^3$ (6.2 ppb) with a range of concentrations from 8.2 to 24.6 $\mu g/m^3$ (3.6 to 10.7 ppb) (U.S. EPA, 1993). Acrolein has been measured in smoky indoor air. In bars and restaurants, levels between 2.3 and 275 $\mu g/m^3$ have been reported (IARC, 1995: citing Triebig and Zober, 1984; Löfroth et al., 1989). In residences where wood stoves were used, concentrations from 0.7-6.0 $\mu g/m^3$ have been reported (IARC, 1995: citing Highsmith et al., 1988). IARC (1995) noted that the acrolein concentrations in the smoke from various cigarettes ranged from 3-220 μg/cigarette. Levels as high as 463-684 μg/cigarette were reported in Japan (Kuwata et al., 1979). Jones et al. (1999) reported concentrations of acrolein in mainstream smoke (defined as smoke that is directly exhaled from the smoker) ranging from 10 – 140 μg per cigarette, and estimated concentrations in sidestream smoke (i.e., smoke emitted from the smoldering tobacco between puffs) in the range of 100 – 1700 μg per cigarette. Acrolein has also been detected in exhaust gases from both gasoline engines (0.05-27.7 mg/m^3) and diesel engines (0.12-0.21 mg/m^3) (IARC, 1995). Grosjean et al. (2001) calculated emission factors (by regression analysis of experimental data) of 0.11 mg/km from light duty vehicles and 0.72 mg/km from heavy-duty vehicles.

The U.S. EPA's Cumulative Exposure Project (CEP) provides modeling data for 148 hazardous air pollutants, including acrolein (U.S. EPA, 2000; 1990 data). The CEP data for California, primarily from urban counties, indicate that the estimated statewide annual average ambient concentration of acrolein is 0.15 $\mu g/m^3$ (95^{th} percentile = 0.3 $\mu g/m^3$). Pratt et al. (2000) examined 1990 CEP data, in addition to monitoring data when available, to assess air toxics in Minnesota. Using a hazard quotient approach, the concentrations calculated from the monitoring and modeling data were compared to cancer and noncancer health benchmark values. Pratt et al. (2000) reported that for acrolein (for which there were only modeled data), 70% of the census tracts studied exceeded the health benchmark (in this case, 0.02 $\mu g/m^3$). In addition, Pratt et al. (2000) estimated a screening level total noncancer hazard index by summing all of the noncancer hazard quotients (over all endpoints). They found that acrolein was by far the most important contributor to the noncancer hazard index. The apportionment of the hazard index for an average Minnesotan showed that acrolein accounted for 89% of the hazard index,

followed by formaldehyde at 6%, with each of the other pollutants accounting for less than 1% of the hazard index. As part of this study, Pratt et al. also did a comparison of modeling and monitoring data, where possible. While there were no monitoring data available for comparison for acrolein, Pratt et al. did find that overall there was a tendency for the modeling results to underpredict measured values.

In spite of the uncertainties regarding acrolein concentrations, it does appear that acrolein is an important irritating component of air pollution. The limited data available on ambient levels of acrolein indicate that ambient concentrations of acrolein are above the chronic REL (based on respiratory toxicity) of 0.06 $\mu g/m^3$ (0.03 ppb). Indoor air levels also have the potential to be above the chronic REL.

IV. Potential for Differential Effects

A. Summary of Key Human Studies

The argument for greater impacts of acrolein exposure in children than in adults rests on the ability of this compound to exacerbate asthma. While there are no *in vivo* human studies linking acrolein to asthma, two *in vitro* studies provide mechanistic data suggestive of a linkage.

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 minutes 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 preexposed 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.

Mucus hypersecretion is one of the hallmarks of inflammatory airway disorders, including asthma. Borchers et al. (1999b) examined the effect of acrolein on mucus glycoprotein (mucin) gene expression in airway epithelial cells. Cultured cells were treated for 4 hours with 0.01-100 nM acrolein. Borchers et al. reported that *in vitro*, acrolein can act directly on epithelial cells to increase mucin mRNA levels, or indirectly through inflammatory mediators released after acrolein exposure.

B. Summary of Key Animal Studies

In vivo animal studies provide support for the contention that acrolein exposure may exacerbate asthma. First, acrolein induces bronchial hyperresponsiveness, a key characteristic of asthma, in guinea pigs. Leikauf et al. (1989a) investigated the onset and time course of increases in pulmonary resistance and bronchial responsiveness to intravenous acetylcholine in guinea pigs exposed to acrolein. First, animals were exposed to <0.01 (sham), 0.31, 0.67, 0.94, or 1.26 ppm acrolein for 2 hours. Pulmonary resistance was immediately increased (first measured 5 minutes after cessation of acrolein exposure) and

returned to base-line within 25 minutes (0.31 ppm) and 60 minutes (1.26 ppm) post exposure. Second, bronchohyperresponsiveness was assessed by measuring the change in specific total pulmonary resistance induced by acetycholine either before (control) or 1, 2, 6, or 24 hours after a 2-hour exposure to 0.94 ppm acrolein. In contrast to the transient increase in base-line pulmonary resistance observed in the first experiment, acrolein exposures of greater than 0.94 ppm produced a persistant change in bronchoresponsiveness to intravenous acetylcholine. Increased bronchoresponsiveness was evident at 1 hour and became maximal 2-4 hours after exposure. The concentration of acetylcholine necessary to double pulmonary resistance (ED200) decreased from 104.2 ± 7.3 (µg/kg/min) prior to acrolein exposure to 79.6 ± 15.9 at 1 hour after cessation of acrolein exposure. At two hours, ED200 was 32.5 ± 7.9. This effect persisted with significant increases in bronchoresponsiveness at 6 and 24 hours following acrolein exposure.

Leikauf et al. (1989b) confirmed the results of the previous study, and also observed an association between acrolein-induced bronchial hyperresponsiveness and increased sulfidopeptide leukotriene (LT) C_4 concentration in lung lavage fluid of guinea pigs. Sulfidopeptide leukotrienes are bronchoconstrictive lipid mediators thought to have an important role in the pathophysiology of asthma. In this study, guinea pigs were exposed to 1.3 ppm acrolein for 2 hours. Following acrolein exposure, bronchial responsiveness to intravenous acetylcholine was determined after administration of a leukotriene receptor antagonist (L-649,923) or leukotriene formation inhibitors (L-651,392 and U-60,257). Both the leukotriene receptor antagonist and the leukotriene formation inhibitors attenuated acrolein-induced hyperresponsiveness and bronchoconstriction. In addition, the leukotriene inhibitor L-651,392 reduced the increase of immunoreactive LTC_4 concentrations in lavage fluid following acrolein exposure.

As previously noted, mucous hypersecretion is one of the hallmarks of respiratory diseases, including asthma. Borchers et al. (1998) studied mucin gene expression and mucus hypersecration in respiratory tissues of rats exposed to acrolein. Animals were exposed to 3 ppm acrolein, 6 hours per day, 5 days per week, for 2 weeks. Results of this study demonstrate that acrolein exposure induces mucous cell hyperplasia and metaplasia in airway surface epithelium and airway lumen, accompanied by increased mucin mRNA and mucin glycoproteins. The percentage of mucous cells increased to approximately the same level in small (≤0.8-mm-diameter) and large (>0.8-mm-diameter) airways, yet the magnitude of the effect was greater in the small airways where mucous cells are normally rare or absent. In small airways, cells increased 270-fold in exposed animals (from 0.02% of cells in control animals) while in large airways, mucus cells increased 26-fold (from 0.23% of cells in controls). In the trachea (large airway), mucin mRNA increased within 2 days of exposure and was accompanied by an increase in mucin glycoproteins on the surface of the airways and submucosal gland epithelium compared to controls. In the lung (small airway), increases in mucin mRNA and mucin glycoproteins were observed on days 5 and 9 of exposure. Increased mucin glycoproteins were detected within the lumen and airway epithelium of the lung on day 12.

Borchers et al. (1999a) examined the effects of acrolein exposure on mucin gene expression in mice. In this study, animals were exposed to 3.0 ppm acrolein, 6 hours/day, 5 days/week for 3 weeks. Acrolein increased mucin mRNA levels in the lung in a time-dependent manner, becoming significantly greater than controls at day 12 (5-fold increase), and increasing further after 3 weeks of exposure (10-fold

increase). Mucin glycoproteins were found in cytoplasmic granules of mucous cells, on apical surface epithelium and in the airway lumen of exposed mice, but were not found in unexposed mice or mice exposed up to one week. The acrolein-induced increase in mucin mRNA and mucin glycoproteins was associated with a significant increase in macrophages (indicative of an inflammatory response) recovered in bronchoalveolar lavage (BAL) fluid. The magnitude of macrophage cell increase was correlated with the increase in mucin mRNA levels. While the macrophage effect developed over 2-3 weeks exposure, acrolein caused an immediate increase in neutrophils in BAL fluid, observed on day 1 of exposure; however by day 5 and for the remainder of the experiment neutrophil numbers were similar in control and exposed animals.

V. Additional Information

A. Other Respiratory Toxicity

Although not all respiratory irritants are associated with the exacerbation of asthma, it is interesting to note that acrolein is a potent irritant of the respiratory tract and eyes in both animals and humans. In animal studies, chronic exposure of rats to acrolein (0.4 to 5 ppm) resulted in bronchopneumonia, obstructive lesions in small and large airways, histological changes to the nasal turbinates (increased submucosal lymphoid aggregates) as well as the pulmonary epithelium and mucosa, rhinitis, lung lesions, epithelial necrosis of the peribronchiolar and bronchiolar regions, alveolitis, hemmorhage, hyperplasia and metaplasia of the airway epithelium, and inflammatory alterations (Kutzman, 1981; Kutzman et al., 1985; Feron et al., 1978; Lyon et al., 1970; Leach et al., 1987). In mice, acrolein exposure (1.7 ppm; 6 hours/day; 5 days) produced severe exfoliation and squamous metaplasia of the respiratory epithelium, and ulceration of the olfactory epithelium (Buckley et al., 1984). Similar respiratory effects have also been reported in monkeys, dogs, hamsters and rabbits (Feron et al., 1978; Lyon et al., 1970). The RD_{50} (concentration required for depression of the respiratory rate of mice by 50%) was estimated as 1.7 ppm (Kane et al., 1979).

B. Regulatory Background

Acrolein is a federal hazardous air pollutant and was identified as a toxic air contaminant in California in April 1993 under AB 2728. OEHHA has adopted an acute non-cancer reference exposure level (REL) of 0.19 $\mu g/m^3$ (0.09 ppb) and a chronic REL of 0.06 $\mu g/m^3$ (0.03 ppb) for acrolein (OEHHA, 1999; OEHHA, 2001). Acrolein is not listed under Proposition 65.

In 1985, The International Agency for Research on Cancer (IARC) reviewed the available data on acrolein and found inadequate evidence in both humans and experimental animals to evaluate the potential carcinogenicity of acrolein to humans (IARC, 1985; IARC, 1995). 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 by U.S. EPA as a Group C substance, with possible human carcinogenic potential (U.S. EPA, 1994).

VI. Conclusions

In vivo data in animals and *in vitro* data using human tissue strongly suggest that acrolein may exacerbate asthma (Roux et al., 1999; Borchers et al., 1998; Borchers et al., 1999a and 1999b; Leikauf et al., 1989a and 1989b). Asthma is a disease that disproportionately impacts children (see Introduction Section III.). Therefore, OEHHA has placed acrolein into Tier 1.

VII. References

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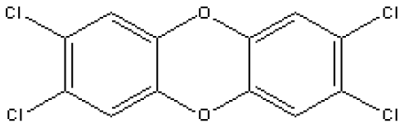
Polychlorinated Dibenzo-*p*-dioxins (PCDDs), Dibenzofurans (PCDFs) and Biphenyls (PCBs)

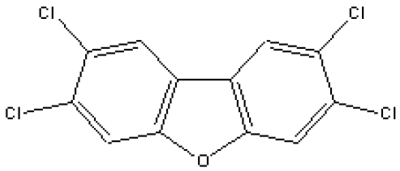
Including: all 2,3,7,8-chlorinated PCDDs and PCDFs, and PCBs

I. Selected structures:

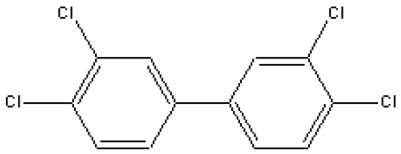
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD)



2,3,7,8-Tetrachlorodibenzofuran (TCDF)



3,3´,4,4´-tetrachlorobiphenyl (PCB #77)



Selected Physical and Chemical Properties:

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD)

Description	White crystalline powder at 25°C
Molecular formula	$C_{12}H_4Cl_4O_2$
Molecular weight	321.97 g
Water solubility	1.93 ng/100 ml at 22°C
Log P (octanol-water)	6.80
Air concentration conversion	Not available

2,3,7,8-Tetrachlorodibenzofuran (TCDF)

Molecular formula	$C_{12}H_4Cl_4O$
Molecular weight	305.975 g
Water solubility	69.2 ng/100 ml at 26°C
Log P (octanol-water)	6.53
Air concentration conversion	Not available

3,3',4,4'-tetrachlorobiphenyl (PCB #77)

Molecular formula	$C_{12}H_6Cl_4$
Molecular weight	291.99 g
Water solubility	56.9 ng/100 ml at 25°C
Log P (octanol-water)	6.63
Air concentration conversion	Not available

II. Overview

There are a number of studies which indicate that developing fetuses and newborns, particularly breast-fed infants, represent a segment of the population particularly vulnerable to exposure to polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs). Concern about dioxins and dioxin-like compounds is justified not only because of their toxicity but also because of their very long biological and environmental persistence. Emissions into the air, the majority of environmental emissions, of these toxic air pollutants results in subsequent deposition onto crops, grass and feed. Deposited dioxins are either eaten by humans directly or eaten by livestock and become a source of contamination for humans in beef, poultry and dairy products. In addition, these persistent compounds accumulate in breast milk, and are thus transferred to the feeding infant. Since human exposure *in utero* and in infancy to PCDDs, PCDFs and PCBs represents a serious concern for children's health, this class of chemicals is considered a priority for evaluation of potential differential effects on infants and children.

- Immunotoxicology is identified as one of the key toxicological endpoints of concern for infants and children (see Introduction Section III). Immune system toxicity appears to be among the most sensitive responses (Birnbaum, 1994). Effects on immune development from perinatal exposure to dioxin and dioxin-like chemicals may be more dramatic or persistent than that following exposure during adult life (Holladay, 1999). Functional developmental immunotoxicity was observed in children exposed both pre- and post-natally during the rice oil poisoning episode with PCDFs and PCBs in Yu-Cheng, Taiwan (Guo *et al.*, 1995; Schecter *et al.*, 1996). Similar effects were also observed in children as a result of background exposure to these chemicals (Gladen *et al.*, 2000; Nagayama *et al.*, 1998a; Nagayama *et al.*, 1998b; Papke, 1998; Patandin *et al.*, 1998; Patandin *et al.*, 1999b; Vartiainen *et al.*, 1998; Weisglas-Kuperus *et al.*, 2000).

- Developmental toxicity represents another key toxicological endpoint of concern for infants and children (see Introduction Section III). Dioxins and dioxin-like chemicals are potent teratogens in animals. Detectable concentrations of PCBs and dioxins have been found in amniotic fluid, placenta and fetal tissue samples, and infants who are breast-fed can have blood levels of PCBs and dioxins greater than the corresponding maternal levels (Feeley and Brouwer, 2000). Evidence of transplacental transfer has been obtained from analysis of PCDDs and PCDFs in fetal tissue (Kreuzer *et al.*, 1997; Schecter *et al.*, 1996).

- PCDDs, PCDFs, and PCBs are transferred to infants from the mother during breast feeding. This route appears to be the most important route of exposure for humans, resulting in about 50 times the daily dose of dioxin toxic equivalents (TEQ) in breast-fed infants compared to adults (Patandin *et al.*, 1999a). Lanting *et al.* (1998a) clearly identified lactation as a major source of the PCB body burden of 42 month-old children. Forty-two month-old children who had been fully breast-fed for at least six weeks as babies, had plasma median PCB levels 4.5-times as high as that in formula-fed children (0.81 µg/L vs. 0.18 µg/L). Children receive greater exposures to environmental pollutants present in air, food, and water because they inhale or ingest more air, food, or water on a per kg

body weight basis than do adults (Mott, 1995; OEHHA, 2000). This holds true especially for lipophilic compounds like the PCDDs, PCDFs and PCBs because, in addition to the increased dose through inhalation and ingestion of contaminated food, these compounds are transferred through breast milk, which is often the sole source of nutrition in the infant.

- Exposure of infants and children to carcinogenic chemicals is a general concern since, as discussed in the introduction to this report, exposure to carcinogens early in life may result in higher tumor incidence and shorter latency than exposure as an adult. 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is a multisite, multispecies animal carcinogen, and is probably a human carcinogen. Populations occupationally or accidentally exposed to chemicals contaminated with dioxin have demonstrated increased incidences of soft-tissue sarcoma and non-Hodgkin's lymphoma (Mukerjee, 1998; Birnbaum, 1994). In addition, evidence from rodent studies indicates that *in utero* exposure to a single dose of TCDD was sufficient to promote mammary carcinogenesis when animals were dosed at day 50 with a mammary carcinogen (Brown *et al.*, 1998).

- Developmental neurotoxicity has been shown in animals, and there is evidence of this effect in humans in epidemiological studies of children exposed *in utero* to the non-coplanar PCBs. The PCBs can be grouped into two categories by mechanism of toxicity. The non-coplanar PCBs have predominantly neurotoxic effects. The coplanar PCBs have dioxin-like effects and act through the aryl hydrocarbon receptor. Like polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs), the coplanar polychlorinated biphenyls (PCBs) appear to have a significant number of toxic effects mediated through their interaction with the aryl hydrocarbon (*Ah*) receptor. These PCB congeners are substituted in the para and at least 2 of the meta positions but not at any of the ortho positions, and are structurally similar to TCDD. PCBs with more than one chlorine in the ortho positions lack some effects exerted by non- and mono-ortho PCBs and show a partially different spectrum of toxic effects (Safe, 1994), indicating that a second mechanism of toxicity exists for non-coplanar PCBs, which probably acts outside of the *Ah* receptor pathway.

III. Principal Sources of Exposure

PCDDs and PCDFs are generated as by-products from various combustion and chemical processes. PCDDs, PCDFs and PCBs are produced during incomplete combustion of chlorine containing wastes like municipal solid waste, sewage sludge, and hospital and hazardous wastes. Various metallurgical processes involving heat, and burning of coal, wood, petroleum products and used tires for energy generation also generate PCDDs. Industrial and municipal processes in which naturally occurring phenolic compounds are chlorinated can produce PCDDs; the best example is chlorine bleaching of wood pulp in the manufacture of paper products. These PCDDs and PCDFs end up as water contaminants and generally not air contaminants. It should be noted that most pulp mills in the U.S. have switched to a bleaching process that produces little or no PCDDs and PCDFs.

U.S. EPA (2000a) conducted an extensive review of contemporary formation (as opposed to reservoir) sources of dioxin release to the environment. The U.S. EPA report (U.S. EPA, 2000a) divides sources of PCDD/PCDFs into two subclasses: 1) contemporary formation sources (sources which have

essentially simultaneous formation and release) and 2) reservoir sources (materials or places that contain previously formed PCDD/PCDFs or dioxin-like PCBs that are re-released to the environment). The inventory of estimated dioxin releases in the United States prepared by U.S. EPA (2000a), classified by media and source category, for 1987 and 1995 is shown in Table 1. Values in the table are reported as dioxin equivalents, or TEQ. The equivalency factors (WHO98) used in the calculation are those in the 1998 World Health Organization update to the previously established equivalency factors (I-TEF) for dioxins, furans, and dioxin-like PCBs (Van den Berg *et al*., 1998). This inventory lists estimated emissions of chlorinated dioxins and furans only. Environmental releases of PCDD/PCDFs in the United States occur from a wide variety of sources, but these are dominated by releases to the air from combustion sources including waste incineration, industrial power generation, and vehicle fuel combustion. Vehicle fuel combustion is considered to make a significant contribution to general ambient dioxin levels in urban areas, and to contribute particularly to the higher dioxin levels experienced near freeways and similar high-traffic areas (Hunt *et al*., 1997). Among different classes of vehicles, diesel fueled vehicles contribute nearly five times as much dioxins (Table 1), in spite of their smaller numbers than gasoline fueled vehicles. The 1995 inventory indicates that quantifiable emissions of PCDD/PCDFs from combustion sources are more than an order of magnitude greater than quantifiable emissions from all other categories combined (U.S. EPA, 2000a). Total environmental releases of PCDD/PCDFs in the United States in 1995, expressed as TEQ-WHO, were estimated at 2,835 g TEQ. Air emissions contributed 2,705 g TEQ in that year, while emissions to water contributed 20 g TEQ and releases to land contributed 110 g TEQ. While there is substantial uncertainty in the emissions estimates for any specific source, it appears that air emissions represent more than 95 % of all emissions across all sources in the 1995 inventory of the PCDD/PCDFs in the United States.

U.S. EPA (2000a) compared emission of dioxins into the environment from quantifiable sources in 1987 and 1995, and found an approximately 80% reduction over this time interval. Their best estimates of releases of chlorinated dioxins and dibenzofurans to all environmental media (except products) were approximately 2,800 g TEQ (WHO) in 1995 and 13,500 g TEQ –(WHO) in 1987. This decrease was primarily due to decreased emissions of dioxins and related compounds into the atmosphere from municipal and medical waste incinerators. Emission reductions resulted partly from improved combustion and emission controls applied to these sources, in response to regulatory initiatives. For instance, the California Air Resources Board developed an airborne toxic control measure for dioxins from medical waste incinerators in 1990, which reduced emissions from these sources by 99%. In addition to improved controls on operating incinerators, a number of facilities were closed. More recently promulgated regulations and those currently under development by US EPA should result in some additional reduction in emissions from major combustion sources of dioxin-like compounds.

Ambient air concentrations for urban and rural areas in the United States were reported in 1995 to be 0.050 and 0.022 pg I-TEQ/m^3 for all measured PCDDs and PCDFs. TCDD levels for the same monitored area were 0.007 and 0.003 pg/m^3 (U.S. EPA, 2000b).

Table 1: Quantitative Inventory of Environmental Releases of Dioxins in the United States

Emission source category		**Reference year 1995**			**Reference year 1987**		
Confidence Rating[a]		A	B	C	A	B	C
RELEASES (g TEQ/yr) TO AIR							
Waste Incineration[f]							
Municipal waste incineration			1,250		8,877		
Hazardous waste incineration			5.8			5.0	
Boilers/industrial furnaces				0.39			0.78
Medical waste/pathological incineration				488			2,590
Crematoria				9.11[e]			5.5[e]
Sewage sludge incineration			14.8			6.1	
Tire combustion				0.11			0.11
Power/Energy Generation							
Vehicle fuel combustion	- leaded[b]		2.0		37.5		
	- unleaded			5.9			3.6
	- diesel			35.5			27.8
Wood combustion	- residential			62.8[e]			89.6[e]
	- industrial		27.6			26.4	
Coal combustion – utility			60.1			50.8	
Oil combustion – industrial/utility				10.7			17.8
Other High Temperature Sources							
Cement kilns (hazardous waste burning)			156.1		117.8		
Lightweight aggregate kilns burning hazardous waste				3.3[e]			2.4[e]
Cement kilns (non hazardous waste burning)				17.8			13.7
Petroleum refining catalyst regeneration				2.21			2.24
Cigarette combustion				0.8			1.0
Carbon reactivation furnaces				0.08[e]			0.06[e]
Kraft recover boilers			2.3			2.0	
Minimally Controlled or Uncontrolled Combustion							
Forest, brush, and straw fires[d]			208[e]		170[e]		
Metallurgical Processes							
Metal Smelting/refining							
Ferrous:	- Sintering plants		28.0				32.7
Nonferrous:	- Primary copper		< 0.5[e]			< 0.5[e]	
	- Secondary aluminum			29.1			16.3
	- Secondary copper			271			983
	- Secondary lead		1.72			1.29	
Drum and barrel reclamation				0.08			0.08
Chemical Manufacturing /Processing Sources							

Ethylene dichloride/vinyl chloride		11.2 [e]				
Total Quantified Releases To Air[c]	**2,705**			**13,081**		

Table 1: Quantitative Inventory of Environmental Releases of Dioxins in the United States (continued)

Emission source category	**Reference year 1995**			**Reference year 1987**		
Confidence Rating[a]	A	B	C	A	B	C
RELEASES (g TEQ/yr) TO WATER						
Chemical Manufacturing/Processing Sources						
Bleached chemical wood pulp and paper mills	19.5			356		
Ethylene dichloride/vinyl chloride		0.43 [e]				
Total Quantified Releases To Water[f]	**19.93**			**356**		
RELEASES (g TEQ/yr) TO LAND						
Chemical Manufacturing/Processing Sources						
Bleached chemical wood pulp and paper mill sludge	1.4			14.1		
Ethylene dichloride/vinyl chloride		0.73 [e]				
Municipal wastewater treatment sludge	76.6			76.6		
Commercially marketed sewage sludge	2.6			2.6		
2,4-Dichlorophenoxy acetic acid	28.9			33.4		
Total Quantified Releases To Land[c]	**110.23**			**126.7**		
OVERALL QUANTIFIED RELEASES TO THE OPEN and CIRCULATING ENVIRONMENT	**2,835**			**13,564**		

a. Characterization of the source category judged to be adequate for quantitative estimation with:
   A = High confidence in the emission factor and high confidence in activity level.
   B = Medium confidence in the emission factor and at least medium confidence in activity level.
   C = Low confidence in either the emission factor and/or the activity level.
b. Leaded fuel production and the manufacture of motor vehicle engines requiring leaded fuel for highway use have been prohibited in the United States.
c. TOTAL reflects only the total of the estimates made in U.S. EPA (2000a).
d. It is not known what fraction, if any, of the estimated emissions from forest fires represents a "reservoir" source. The estimated emissions may be solely the result of combustion.
e. Congener-specific emissions data were not available; the I-TEQ_{DF} emission estimate was used as a surrogate for the TEQ_{DF}-WHO98 emission estimate.
f. Pulp and paper mill sludge incinerators were included within estimate for Wood Combustion - Industrial.

(Source : U.S. EPA, 2000a)

Emissions of dioxin-like compounds in California by county in 1999 are shown in Table 2. For the year 1999, Sacramento County had the highest emission for PCDDs and PCDFs with 5.4 lbs./year for chlorinated PCDFs alone. However, Contra Costa County had the highest emission of total PCBs, with 9.7 lbs./year. PCDD/PCDF emission data from California Air Resource Board (CARB) and U.S. Environmental Protection Agency (U.S. EPA) are not directly comparable, since CARB reports air emission in pounds per year of total and some isomers of PCDDs and PCDFs while U.S. EPA reports air emission of PCDDs and PCDFs as total g TEQ per year.

Table 2: CALIFORNIA EMISSION INVENTORY: DIOXINS, DIBENZOFURANS, PCBS for data base year 1999

County	Pollutant	lbs/year	g/year	g TEQ/year (WHO-97)
LOS ANGELES	2,3,7,8-Tetrachlorodibenzo-p-dioxin	0.000149	6.79E-02	6.79E-02
	Total TEQ of the 15 PCDDs/Fs*	-	-	7.56E-02
	PCDFs (chlorinated)	0.00056	2.55E-01	-
	PCDDs total $^{w/o}$	0.010803	4.92E+00	-
	PCDDs total ^{w}	0.001311	5.97E-01	-
	Polychlorinated biphenyls (PCBs)	4.17509	1.90E+03	-
SACRAMENTO	2,3,7,8-Tetrachlorodibenzo-p-dioxin	3.83E-06	1.75E-03	1.75E-03
	Total TEQ of the 15 PCDDs/Fs*	-	-	5.89E+01
	PCDFs (chlorinated)	5.42022	2.47E+03	-
	PCDDs total $^{w/o}$	2.4E-08	1.09E-05	-
	PCDDs total ^{w}	0.039012	1.78E+01	-
	Polychlorinated biphenyls (PCBs)	0.444033	2.02E+02	-
TUOLUMNE	PCDFs (chlorinated)	1.251765	5.70E+02	-
	PCDDs total ^{w}	0.020105	9.16E+00	-
KERN	2,3,7,8-Tetrachlorodibenzo-p-dioxin	3.46E-06	1.58E-03	1.58E-03
	Total TEQ of the 15 PCDDs/Fs*	-	-	7.18E-02
	PCDFs (chlorinated)	0.008751	3.99E+00	-
	PCDDs total ^{w}	0.000741	3.37E-01	-
	Polychlorinated biphenyls (PCBs)	1.73	7.88E+02	-
SAN BERNARDINO	2,3,7,8-Tetrachlorodibenzo-p-dioxin	1.76E-06	8.02E-04	8.02E-04
	Total TEQ of the 15 PCDDs/Fs*	-	-	1.89E-03
	PCDFs (chlorinated)	9.57E-05	4.36E-02	-
	PCDDs total $^{w/o}$	1.36E-05	6.20E-03	-
	PCDDs total ^{w}	0.001421	6.47E-01	-
MADERA	PCDFs (chlorinated)	0.01165	5.31E+00	-
	PCDDs total ^{w}	0.000035	1.59E-02	-
SHASTA	PCDFs (chlorinated)	0.010083	4.59E+00	-
	PCDDs total $^{w/o}$	6.01E-05	2.74E-02	-
	PCDDs total ^{w}	0.000847	3.86E-01	-
	Polychlorinated biphenyls (PCBs)	2.15501	9.82E+02	-

CONTRA COSTA	Polychlorinated biphenyls (PCBs)	9.681	4.41E+03	-

(See next page for footnotes.)

[w/o] Dioxins, total, excluding individual isomers reported (PCDDs)

[w] Dioxins, total, with individual isomers also reported (PCDDs)

* Total TEQ of the 15 PCDDs/Fs for which a Toxicity Equivalent Factor is applicable:

1,2,3,4,6,7,8-Heptachlorodibenzo-p-dioxin
1,2,3,4,6,7,8-Heptachlorodibenzofuran
1,2,3,4,7,8,9-Heptachlorodibenzofuran
1,2,3,4,7,8-Hexachlorodibenzo-p-dioxin
1,2,3,4,7,8-Hexachlorodibenzofuran
1,2,3,6,7,8-Hexachlorodibenzo-p-dioxin
1,2,3,6,7,8-Hexachlorodibenzofuran
1,2,3,7,8,9-Hexachlorodibenzo-p-dioxin
1,2,3,7,8,9-Hexachlorodibenzofuran
1,2,3,7,8-Pentachlorodibenzo-p-dioxin
1,2,3,7,8-Pentachlorodibenzofuran
2,3,4,6,7,8-Hexachlorodibenzofuran
2,3,4,7,8-Pentachlorodibenzofuran
2,3,7,8-Tetrachlorodibenzo-p-dioxin
2,3,7,8-Tetrachlorodibenzofuran

(Source : CARB, 1999)

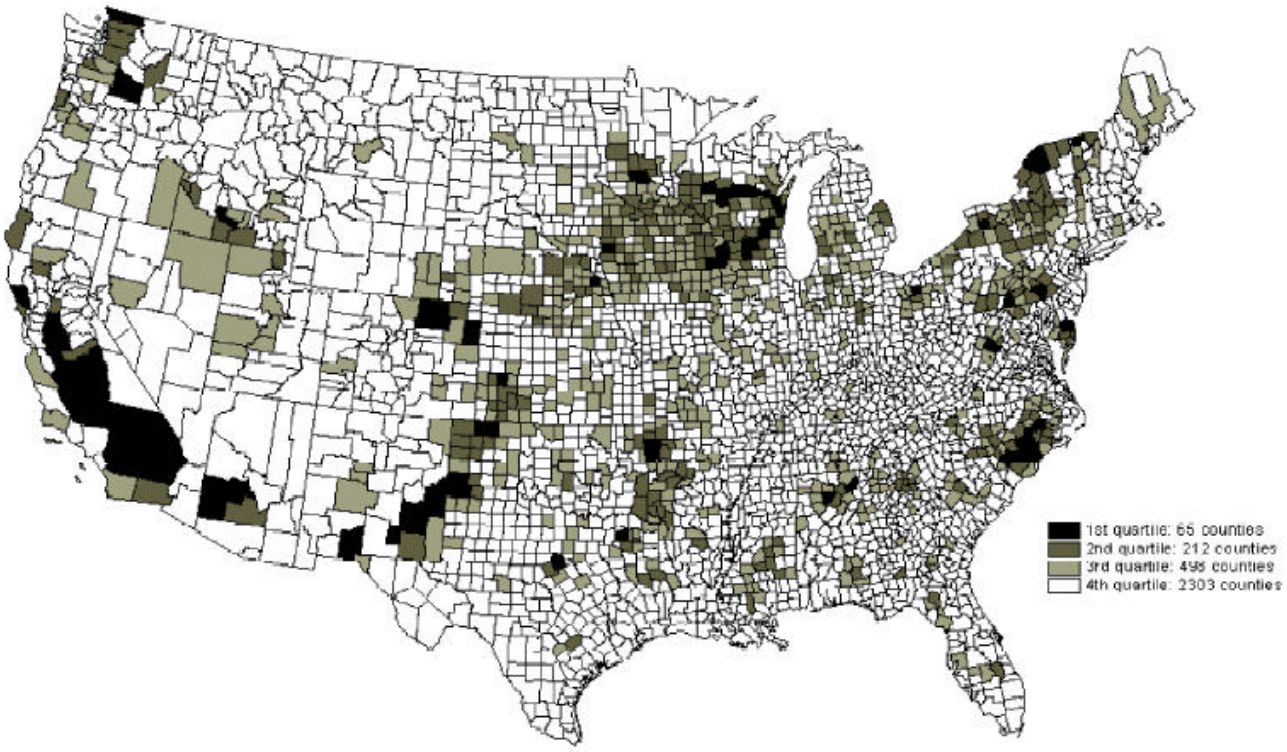
Concerns about dioxins and dioxin-like compounds are justified not only because of toxicity but also because of their very long biological and environmental persistence. These toxic air pollutants settle in grass and feed, which are then eaten and become a source of contamination for humans in livestock, poultry and dairy products. In addition, these persistent compounds accumulate in breast milk and then are transferred to the feeding infant.

Exposure to dioxins and dioxin-like compounds can occur through several pathways. The most important route for human exposure to PCDDs, PCDFs and PCBs is food consumption, contributing over 90% of total exposure, with products of animal origin and fish making the greatest contribution to this exposure (Liem *et al.*, 2000). Therefore, consumption habits may play a major role in the intake of dioxins and dioxin-like compounds. It must be stressed that the PCDDs and PCDFs as well as PCBs were largely originally airborne. The U.S. EPA report cited above (2000a) concluded that "*The environmental releases of CDD/CDFs ... are dominated by releases to the air from combustion sources. The current (i.e., 1995) inventory indicates that quantifiable emissions from combustion sources are more than an order of magnitude greater than quantifiable emissions from all other categories combined* ".

U.S. EPA (2000b) examined the geographical distribution of emissions contributing to the total dioxin TEQ in the food supply. The major contributors to dioxins entering the human food supply in the U.S. (48 contiguous states) were identified by combining CDD/CDF/PCB concentration values from the EPA meat/milk surveys with food production data for beef, pork, chicken, eggs and dairy products by

county (from USDA and State agricultural records), expressed as production of animal fat. The 3,048 counties in the database were sorted in descending order and divided into four groups, with each group encompassing 25 percent of the total. The top 65 counties account for 25 percent of the total TEQ. The second, third, and fourth quartiles included 212, 498, and 2,303 counties, respectively. These findings are shown in the map presented in U.S. EPA (2000b) and shown below (Figure 1). (For discussion of the TEQ definition used in this description and the accompanying figure, refer to the explanation on page 4 given in relation to the US emissions inventory)

Figure 1: Total TEQ production in meats, eggs and dairy products, categorized by quartile.



(Source : U.S. EPA, 2000b)

Most commercial food growing occurs in rural areas where there is no large dioxin reservoir source in the soil. (Some known contaminated sites contribute to dioxin levels in homegrown produce and livestock used by certain small communities, but such food materials do not enter the general commercial market. Also some reservoir sources in agricultural areas may result from earlier use of pesticides or herbicides, but inventory data [U.S. EPA, 2000a] suggest that this is not a major contributor to the overall input of dioxins into the food supply.) It therefore follows that the dominant pathway resulting in dioxin exposure for domestic meat and dairy animals is air deposition onto feed crops. Thus, the dominant sources of general population exposure to dioxin are the ambient air concentrations in the areas flagged by this analysis, and control of airborne contamination must occur to decrease PCDD, PCDF and PCB exposures via food intake. U.S. EPA, CARB and local air districts are currently engaged in measurement and analyses to further characterize these dioxin sources.

In general on a body weight basis, intake of dioxins and dioxin-like compounds is highest during childhood, drops during adolescence, and stabilizes in adults of about 20 years of age (Liem *et al.*, 2000; Patandin *et al.*, 1999a; Schecter *et al.*, 2001). When normalized by body weight, exposure is found to decrease with childhood age due primarily to increasing body weight (Liem *et al.*, 2000). In the U.S., estimated daily intake in WHO-TEQ (World Health Organization – toxic equivalents) declines with age. Schecter *et al.* (2001) estimated a mean daily intake of 42 pg TEQ/kg body weight for breast-fed infants during the first year of life For children aged 1-11 years, males and females aged 12-19 years, and adult men and women aged 20-79 years, the estimated daily TEQ intakes were 6.2, 3.5 and 2.7, and 2.4 and 2.2 pg/kg body weight, respectively (Schecter *et al.*, 2001). Note that the oral Reference Exposure Level for dioxins is 10 pg/kg-day. Infant exposures exceed this oral REL by four-fold.

For newborns and fetuses however, maternal exposure and body burden need to be considered since *in utero* and lactational exposure represent important exposure pathways (Feeley and Brouwer, 2000). To better understand exposure of infants via lactation, at least two parameters need to be considered: the level of chemicals in breast-milk in the United States, and elimination kinetics from the mother during breast-feeding. Over 80% of the human milk samples examined by Angulo *et al.* (1999) contained PCB congeners #28, 138, 170 and 180, and over 70% of the human milk samples contained PCB congeners #52, 153, 187 and 188. PCB #28 demonstrated the highest milk concentration with 1.626 ppb, and PCB #183 the lowest with 0.109 ppb. In this study, PCB congener levels were significantly associated with birthplace, the location of industrial facilities, smoking, the consumption of a varied diet, meat, fish or industrially processed foods, number of children and lactation periods. Dioxins in breast milk were monitored in Tennessee in 1990, and averaged 18.8 ppt WHO-TEQ (per gram fat). In the Los Angeles region, data collected in 1987 showed dioxin levels in breast milk of 20.2 ppt WHO-TEQ (LaKind *et al.*, 2001). Schecter *et al.* (2001) reported breast milk levels for PCDDs, PCDFs and coplanar PCBs of 0.257, 0.089 and 0.075 pg TEQ/g whole weight with a mean lipid content of 3.70 % in 1996 in Binghamton, NY.

Newborns of active smoking mothers had higher plasma PCB levels than newborns of passive smoking mothers during pregnancy. Prenatal uptake of PCB was significantly less in newborns of non-smoking families compared to infants borne to active smoking mothers and passive smoking mothers (Lackmann *et al.*, 2000).

Although intake from inhalation of dioxins and dioxin-like compounds is low, there are some cases where inhalation can be a potential contributor to the uptake of these chemicals. It has been reported that indoor air is a more important source of dioxin-like compounds (Currado and Harrad, 1998). In a limited survey in Birmingham and Midlands, United Kingdom, on average, 9.0 ng total PCB/m^3 were measured in indoor air versus 0.31 ng total PCB/m^3 in outdoor air. This trend was not modified even when samples were collected near and away from a harbor during dredging of contaminated sediments (Vorhees *et al.*, 1997). Similarly, there was more PCB contamination in house dust than in the yard soil in a neighborhood close to contaminated sediment fill from a harbor (Vorhees *et al.*, 1999). Although the yard soil and the house dust showed similar profile for PCB contamination, house dust contained almost 10 times as much PCB as the yard soil (260-23,000 ng/g in house dust versus 15-1800 ng/g in

yard soil). Thus it appears that when considering inhalation as an uptake route for PCBs, indoor air can be an important contributor to human contamination.

IV. Potential for Differential Effects

A. Summary of the Key Human Studies

a) PCDD, PCDF and coplanar PCB-induced effects

(1) Immunotoxicological effects

Dioxins and dioxin-like PCBs have been associated with immunotoxicity. It was reported that, in Dutch preschool children, the immunotoxic effects of perinatal background exposure to PCBs and dioxins persist into childhood and might be associated with a greater susceptibility to infectious diseases (Weisglas-Kuperus *et al.*, 2000). In this prospective study, on 207 healthy mother-infant pairs, prenatal PCB exposure was associated with an increased number of lymphocytes, T-cells, and $CD_3CD_8(+)$ (cytotoxic), $CD_4(+)CD_{45}RO(+)$ (memory), T-cell receptor (TcR) αB(+), and $CD_3(+)$HLA-DR(+) (activated) T cells (Table 3) and lower antibody levels to mumps and measles at preschool age. Alteration in the developing stage of immune cells (change in cell population ratio) may indicate detrimental effects on the immune system. Any intrinsic (hormonal) or extrinsic (chemical) insult on thymocyte maturation during critical periods of thymocyte selection for self-recognition may have significant and detrimental consequences on immune function in postnatal life (Blaylock *et al*., 1992). For instance, exposure to PCBs and dioxins may change the kinetics of thymocyte maturation and skew the thymocyte differentiation toward CD8+ phenotypically more mature TcR αB(+) T cells.

Prenatal PCB exposure was also associated with fewer cases of shortness of breath with wheeze. In addition, current PCB body burden was related to a higher prevalence of recurrent middle-ear infections and of chicken pox and to a lower prevalence of allergic reactions. A higher breast milk dioxin TEQ was associated with a higher prevalence of coughing, chest congestion, and phlegm. These results are consistent with suppression of the immune system. In this study, the median concentration in breast milk for dioxins was approximately 35 pg TEQ/g milk fat. Planar PCB and mono-ortho PCB median concentrations were both approximately 14 pg TEQ/g milk fat. The median plasma concentrations of PCBs in maternal, umbilical cord and 42 month old children were 2, 0.4, and 0.39 µg/L, respectively.

Chao *et al.* (1997) reported a higher incidence of middle-ear diseases, in comparison to control, in a follow-up study of the episode of poisoning from ingestion of rice oil contaminated with PCB and PCDF in Yu-Cheng, central Taiwan during 1978 and 1979. These children were born between 1978 and 1985 of mothers who had consumed contaminated oils before their children were born. The 8–9 year old children had a risk ratio for middle-ear diseases of 5.8 ($p = 0.051$) and the group of 10 – 11 year olds had a risk ratio of 4.1 ($p = 0.032$) (Chao *et al.*, 1997). These children had serum blood levels of 2,3,4,7,8-pentachlorodibenzofuran (PnCDF) ranging from 1200-1400 ng/kg lipid and of 1,2,3,4,7,8-hexachloro-dibenzofuran (HxCDF) ranging from 2800 - 3200 ng/kg lipid. The reference group, Yu-Cheng children with normal middle ear, had PnCDF and HxCDF serum blood levels ranging from 200-400 and 400–800 ng/kg lipid respectively. Although blood PCDF was associated with an increase

middle-ear infection rate, blood PCB levels were not found to be associated with middle-ear disease in this study.

Table 3: Results of the white blood cell counts and the immunologic marker analysis (n = 85) in relation to prenatal PCB exposure.

	Absolute counts Percentiles (10^9/L)			Prenatal PCB exposure			
				S PCB maternal		S PCB cord	
	5th	50th	95th	Pearson correlation[a]	p Value	Pearson correlation[a]	p Value
White blood cells							
Monocytes	0.3	0.5	0.9	0.04	0.73	0.09	0.48
Granulocytes	2.2	4.1	7.5	0.14	0.22	0.15	0.20
Lymphocytes	2.2	4.1	6.6	0.25	0.02*	0.22	0.05*
T-cells markers							
CD3+	1.4	2.7	4.6	0.25	0.02*	0.21	0.07
CD3+CD4+	0.8	1.7	2.7	0.19	0.08	0.16	0.17
CD3+CD8+	0.4	0.9	1.7	0.27	0.01*	0.24	0.04*
CD4+CD45RA+	0.3	1.0	1.9	0.12	0.26	0.04	0.77
CD4+45RO+	0.2	0.4	0.6	0.25	0.02*	0.26	0.02*
TcR aB+	1.1	2.5	4.2	0.25	0.02*	0.20	0.08
TcR yS+	0.1	0.2	0.4	0.17	0.12	0.15	0.20
CD3 +HLA-DR+	0.1	0.3	0.5	0.26	0.02*	0.32	0.005*
B-cell markers							
CD 19/20+	0.4	0.9	1.7	0.12	0.28	0.15	0.20
NK-cell markers							
CD16+ n/or CD56+/CD3-	0.1	0.3	1.1	0.13	0.23	0.11	0.31

a : After logarithmic transformation of both variables involved. * Significant at the $p \leq 0.05$ level.
(Sources: Weisglas-Kuperus *et al.*, 2000)

In an investigation of 36 Japanese mother-children pairs, Nagayama *et al.* (1998b) reported a positive correlation between the PCDD, PCDF and coplanar PCB concentrations (TEQ) in breast milk and the CD4+/CD8+ lymphocyte ratio for these breast-fed babies. One year after birth, peripheral blood samples were obtained from 36 healthy babies to measure lymphocyte subsets by immunofluorescence using monoclonal antibody against CD3 (mature T cells), CD4 (helper T cells), CD8 (suppressor/cytotoxic T cells), CD20 (B cells), and HLA-DR (activated T cells). Breast milk samples taken about 3 months after birth were analyzed for PCDDs, PCDFs, and PCBs. Breast milk TEQ concentrations averaged 27 ppt on a fat weight basis. Postnatal exposure was then estimated as a product of breast milk intake and breast milk concentration. Analysis of variance was applied to evaluate the relationship between postnatal breastmilk exposure to PCDD, PCDF and coplanar PCB and lymphocyte subsets in peripheral blood. Mean TEQ intake was 34 ng/kg-day (range 6-84). The authors report that TEQ intake correlated positively with the percentage of CD4+ T cells and negatively

with CD8+ T cells. The ratio of CD4+ to CD8+ T cells showed a significant increase with increase TEQ intake ($p = 0.025$). This study indicates an impact of dioxin TEQ on the functioning of the immune system in infants. In contrast to the result reported by Nagayama *et al*. (1998b) in infants exposed to dioxins, a reduction in $CD4_+$ T helper cells was observed (U.S. EPA, 2000c) in several human studies of cohorts exposed to polyhalogenated aromatic hydrocarbons (PHAHs). Although the fluctuations in the immune cell population were generally within the "normal" range in cohorts exposed to PHAHs, and may not translate into clinical effects, it is important to note that such cells have an important role in regulating immune responses. For instance, reduction/increase in immune cell population in clinical diseases is associated with immunosuppression. The CD4+/CD8+ T cell ratio is an indication of the stage of development of T-helper cells and cytotoxic T lymphocytes. Nagayama *et al*. (1998b) also note that CD4+/CD8+ T cell ratio is one of the most sensitive biomarkers for the exposure to highly toxic PCDDs, PCDFs and coplanar PCBs.

(2) Developmental effects

A number of developmental effects including teratogenicity have been associated with exposure to dioxins in animals. Several investigations of humans have tried to evaluate developmental effects in people exposed environmentally. Patandin *et al*. (1998) evaluated growth of 207 children by measuring birth weight, and weight, height and head circumference at 10 days, and 3, 7, 10, and 42 months of age and evaluating any association of these parameters with background exposure to PCBs and dioxins. About half the children were breast-fed and half were formula-fed. Prenatal exposure (based on umbilical cord and maternal plasma levels) to "background" PCB level was significantly associated with reduced growth for the first 3 months as measured by weight, length, and head circumference. However, the same association was not noted for the breast-fed children (estimated from the analysis of PCB and dioxin concentrations in milk), which the authors note could be interpreted as a protective effect of breast-feeding nutrition on a number of health outcomes in infants (Patandin *et al.*, 1998). In this study, cord and maternal plasma PCB levels (based on PCB congeners #118, 138, 153 and 180) were both significantly associated with lower growth rate. Furthermore, infants with high cord plasma PCB levels (0.80 µg/L, the 90^{th} percentile) weighed significantly less (165 g less; $p \leq 0.05$) compared with infants with low cord plasma PCB levels (0.20 µg/L, the 10^{th} percentile).

Similarly, in a study of 167 pregnant women, breast milk contamination by PCDDs/PCDFs (a measure of the mother's body burden and indirectly of prenatal exposure) was tentatively associated with low birth weight (Vartiainen *et al.*, 1998). Breast milk was analyzed for the seventeen 2,3,7,8-substituted PCDDs and PCDFs, three coplanar PCBs, and 23 mono-ortho, di-ortho and non-coplanar PCBs. Concentrations of PCDDs and PCDFs in breast milk averaged 26 pg/g fat and the sum of PCB concentrations averaged approximately 500 ng/g fat. Using Pearsson's correlation 2-tailed test, the birth weight for all children grouped together ($p<0.02$), and in boys separately ($p<0.04$) but not girls, was slightly decreased with increasing concentrations of 2,3,4,7,8-pentachlorodibenzofuran, 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin, and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. However, when the analysis was restricted to primiparae, there was no statistically significant correlation between birth weight and the concentrations of PCDDs/PCDFs in the mother's milk (Vartiainen *et al.*, 1998). Also, in the same study, no correlation was found between the weight of the child and PCBs, PCB-TEQs, or individual

PCB congeners in the whole group or among primiparae, or among boys or girls. The authors note that the correlation of birth weight in boys and dioxin contamination of the mother (as assessed by breast milk concentrations) may or may not be real due to the lack of correlation in girls or in primiparae.

Altered sex ratio of offspring has also been reported as a health effect of environmental exposure to PCDDs and PCDFs. Mocarelli *et al.* (2000), in a follow-up study of the Seveso, Italy, accident, found an association between lower sex ratio (male/female) in children and increasing TCDD concentrations in serum samples from their fathers ($p = 0.008$). This effect started at concentrations less than 20 ng/kg body weight, and fathers exposed when they were younger than 19 years of age sired significantly more girls than boys (sex ratio 0.38 [95% CI 0.30-0.47]). The median concentration of dioxin in fathers in this study was similar to doses that induce epididymal impairments in rats, and is about 20 times the estimated average concentration of TCDD currently found in human beings in industrialized countries.

(3) Thyroid hormone effects

In the same cohort described above by Nagayama *et al.* (1998b), Nagayama *et al.* (1998a) reported a negative correlation between polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzofurans and coplanar polychlorinated biphenyls concentrations in breast milk (in TEQ) and the levels of triiodothyronine (T_3) and thyroxin (T_4) in the blood of 36 breast-fed Japanese babies. Blood samples, taken one year after birth, were analyzed for serum T_3, T_4, and TSH by radioimmunoassay. Analysis of variance indicated a significant negative correlation between total TEQ intake and serum T_3 ($p< 0.037$) and serum T_4 ($p< 0.018$). In this study, breast milk TEQ concentration averaged 27 ppt on a fat weight basis.

In an epidemiological study on exposure of 320 children 7-10 years of age to a toxic waste incineration plant, Osius *et al.* (1999) found a statistically significant positive association between the mono-ortho congener PCB 118 in blood and thyroid stimulating hormone (TSH) as well as statistically significant negative relationships between PCBs 138, 153, 180, 183, and 187 and free T_3. Free T_4 level was not associated with the PCB congeners considered. The geometric mean of blood concentrations for PCBs 138, 153 and 180 was 0.39 µg/L.

b) Non-coplanar PCB-induced effects

(1) Neurobehavioral effects

The non-dioxin-like PCBs have been associated with developmental neurotoxicity in animals, an effect of particular concern for infants and children. There are a number of epidemiological studies suggesting that PCB exposure is associated in humans with developmental neurotoxicity.

Winneke *et al.*, (1998) administered the Bayley Scale Index Development, version II (BSID II) mental development index test to 7-month old infants in a cohort covering 171 healthy mother-infant pairs. The BSID II is an established psychodevelopmental tool which has been applied in several PCB studies. The BSID consists of three scales: the Mental Development Index (MDI), the Psychomotor Development Index (PDI) and the Behavior Rating Scale. Winneke *et al.* (1998) administered only the

first two tests. The MDI assesses the child's level of cognitive development (memory, learning and problem solving), language development (expressive/receptive language, vocalization), and personal/social development. The motor scale assesses fine and gross motor development.

Winneke *et al*. (1998) reported that cord plasma and breast milk mean sum PCB-concentrations for the three non-coplanar di-ortho substituted PCBs monitored (PCB congeners #138, 153 and 180) were 0.55 ng/ml and 427 ng/g fat respectively. These concentrations correspond to those reported in recent studies of central European cohorts (Lanting *et al.*, 1998c; Schade and Heinzow, 1998). Winneke *et al*. (1998) found a significant negative association between the PCB contamination of maternal milk and the mental development index of 7-month old infants ($p < 0.05$).

These results agree with a similar study in North Carolina (Gladen *et al.*, 1988). In the Winneke *et al*. (1998) and the Gladen *et al*. (1988) studies, only maternal milk PCB was related to cognitive/motor outcome; cord plasma PCB was not. On the other hand Jacobson *et al.*, (1985) in Michigan found an association of impaired mental development with PCB levels in cord plasma but not in milk.

A cohort of 418 Dutch children, half breast-fed and half formula-fed, were evaluated for potential effects on neurological development of background exposure to PCBs (Huisman *et al*., 1995). Maternal and cord blood were analyzed for the non-coplanar PCB congeners # 118, 138, 153, and 180, as a measure of prenatal exposure. Of these PCB congeners, only PCB #118 is a mono-ortho substituted PCB; the remaining PCB congeners are di-ortho substituted with no or very little affinity for the Ah receptor. Breast milk samples were also obtained and the fat analyzed for PCBs and dioxins (seventeen 2,3,7,8-chlorinated dioxins and furans, three coplanar PCBs and 23 non-coplanar PCBs). Formula milk was also obtained for the formula-fed babies and analyzed for the same set of congeners. At 18 months of age, neurological examinations of the children were conducted focusing on motor function. Each toddler was classified as normal, mildly abnormal (e.g., presence of signs such as slight asymmetry, hyper- or hypotonia), or abnormal (e.g., presence of overt neurological problems). A list of 57 neurological items was scored for "optimality" and a total score calculated for each child. Special emphasis was placed on fluency of movement, which the authors note is a good indicator of the integrity of brain function. Chi-square, Student's T-test, and the Mann-Whitney U tests were used to compare groups. The effect of PCB and dioxin exposure was evaluated by multiple linear regression analyses. The independent variables were PCB and dioxin levels, social, perinatal, and obstetric variables. The dependent variables were the neurological optimality score and fluency score. A small but statistically significant effect of PCB exposure *in utero* on the neurological optimality score was noted. There was also an effect of paternal smoking on neurological optimality score. There was no association between PCB or dioxin exposure via breast milk with the neurological optimality score, despite the higher doses of dioxins and PCBs in these children. The authors speculate this could be a protective effect of breast-feeding on brain development.

Lanting *et al*. (1998b) evaluated the neurological condition of the same cohort used by Huisman *et al* (1995) and reported that although some small neurological effects were observed in prenatally exposed children at 18 months of age, no effect of pre- or postnatal exposure to PCBs or dioxins was associated with neurological adverse effect in children 42 months of age. In this cohort, the median summed concentrations of PCBs were 0.4 and 2.0 µg/L for umbilical cord and maternal plasma, and 0.4 µg/L in

plasma of 42-month old children. Concentrations of dioxins in breast milk were 28.8 ng TEQ dioxin/kg fat, and of coplanar PCBs were 14.5 ng TEQ /kg fat.

However, others have reported persistent neurological effects of non-coplanar PCB exposures. Patandin *et al.* (1999b), in a follow-up of the Dutch PCB/dioxin study, reported that exposure *in utero* to "background" PCB concentrations is associated with poorer cognitive functioning (cognitive abilities and verbal comprehension) in preschool 42-month-old children (n = 395). Prenatal PCB exposure was estimated from the sum of PCBs 118, 138, 153, and 180 ($\sum$PCB) in maternal plasma during pregnancy. The investigators used the Kaufman Assessment Battery for Children (KABC), and Reynell Language Developmental Scales (RLDS). The KABC tests both sequential problem solving and simultaneous processing ability; the RLDS is primarily an assessment of language ability. After controlling for confounders, prenatal PCB exposure, measured as the maternal plasma PCB levels, was significantly associated for all children with lower scores on the overall cognitive scale ($p < 0.005$) and the sequential and simultaneous processing scales (both $p < 0.02$) of the KABC. This was highly significant for the formula-fed babies, but the breast-fed babies showed much less of an effect and most associations were not significant. In the formula-fed group, there was also a significant association between prenatal exposure to PCB and low scores on the verbal comprehension scale of the RLDS ($p < 0.03$). Cord plasma PCB concentrations were also significantly associated with the simultaneous processing score of the KABC in the whole group, and significantly associated with the RDLS verbal comprehension scale in the formula-fed group. The highest exposed group ($\sum$PCB $\geq$ 3 µg/L) scored 4 points lower than the lowest exposed group ($\sum$PCB < 1.5 µg/L) on all 3 scales of the KABC ($p < 0.05$). In this study, lactation and current (42-month-old infant estimated body burden) exposure to PCBs and dioxins were not related to 42-month cognitive performance. Thus, the prenatal exposure appears to be more important for effects on cognitive development.

In a follow-up study of a group of 418 infants from birth up to 6 years of age, Boersma and Lanting (2000) concluded that prenatal exposure to PCBs has subtle negative effects on neurological and cognitive development of the child up to school-age. They also showed evidence that breast-feeding, despite a greater intake of PCBs and dioxins compared to formula-fed babies, counteracts these adverse developmental effects of *in utero* exposure to PCBs and dioxins. Median maternal and umbilical cord plasma sum PCB-concentrations were 2.2 and 0.43 µg/L respectively. For breast milk, sum PCB and sum dioxins median concentrations were 405 µg/kg fat and 29 ng TEQ/kg fat respectively.

Moreover, Jacobson and Jacobson (1996), in a follow-up of the Michigan study (Jacobson *et al.*, 1990) administered a battery of IQ and achievement tests to 212 eleven-year-old children. These children were born to mothers who were known to have consumed Lake Michigan fish contaminated with PCBs. Each species of fish was weighted according to degree of contamination with PCBs as reported in the database from the U.S. Environmental Protection Agency. Each child was tested individually at 11 years of age with the Wechsler Intelligence Scales for Children IQ Test, the spelling and arithmetic subtests of the Wide Range Achievement Test and the word- and passage-comprehension subtests of the Woodcock Reading Mastery Tests. The authors reported a significant association between prenatal exposure to PCBs and lower full-scale and verbal IQ scores. The

strongest effects were related to memory and attention. In this study, children most highly exposed (≥ 1.25 µg PCB/g of fat expressed in terms of maternal milk contamination) were three times as likely to have low average IQ scores and twice as likely to be at least two years behind in reading comprehension.

Stewart *et al.* (2000) demonstrated that neonates born of mothers (n=141) who consumed at least 40 lbs of Lake Ontario fish over their lifetime demonstrated a significant linear relationship between the most heavily chlorinated PCBs measured in umbilical cord plasma and performance impairments on the Habituation and Autonomic clusters of the Neonatal Behavioral Assessment Scale (NBAS) at 25-48 hours after birth (Table 4). The controls consisted of 152 women known not to have eaten fish from Lake Ontario. The most highly prenatally exposed neonates, as evaluated by the umbilical cord PCB level, exhibited poorer performance in a significantly greater proportion of the NBAS scales (Stewart *et al.*, 2000). Less chlorinated PCBs, DDE, Mirex, HCB, lead, and mercury were not related to NBAS performance. These results corroborated earlier findings; the most heavily chlorinated PCB congeners (hepta-, octa-, and nonachlorinated biphenyls) are most strongly correlated with breast milk levels. It appears that the chlorination and persistence of PCBs may be important factors both for exposure assessment and for neurobehavioral toxicity.

Table 4: Dose-response relationships between the concentration of highly chlorinated PCBs (ng/g fat) and performance on the habituation, autonomic, and reflex clusters of the Neonatal Behavioral Assessment Scale (NBAS) at 25 - 48 h after birth

NBAS performance	Highly chlorinated PCBs (ng/g fat)				Linear trend analysis
	0 (ND)	> 0	> 24	> 133	
Habituation (48 h postnatal)	7.34	7.60	7.06	6.80	F (1, 221) = 3.95 $p < 0.05$
Autonomic (48 h postnatal)	6.02	6.35	5.48	5.72	F (1, 261) = 4.40 $p < 0.05$
Abnormal reflexes	2.3	2.75	3.0	2.85	F (1, 262) = 2.81 $p = 0.095$

(Source : Stewart *et al.*, 2000)

B. Summary of the Key Animal Studies

a) PCDD, PCDF and coplanar PCB-induced effects

(1) Immunotoxicological effects

Delayed immunotoxicological effects were demonstrated in experiments on TCDD-exposed dams (Nohara *et al.*, 2000). Pregnant dams were administered a single oral dose of 12.5-800 ng /kg body weight TCDD on gestation day (GD) 15. The thymus and spleen of pups, from dams exposed to 800

ng/kg TCDD, contained 102.0 and 62.7 pg TCDD/g tissue on post-natal day (PND) 21, respectively, and the amounts decreased thereafter. In the thymus, dose-dependent CYP1A1 mRNA induction was clearly observed on PND 5 in pups of dams exposed to 50-800 ng/kg TCDD. The induction was gradually decreased on PND 21 and 49. CYP1A1 mRNA induction in the spleen was very weak. Splenocyte number, on PND 49 (puberty), decreased in a dose-dependent manner in pups of dams exposed to 12.5-800 ng/kg TCDD. The alteration in spleen cellularity by TCDD was not detected on PND 21 (weaning) or 120 (adulthood). The results showed an effect of perinatal exposure to low doses of TCDD on the immune system, which is apparent in the spleen around puberty and likely to be unrelated to Ah receptor-dependent gene expression (Nohara *et al.*, 2000).

Exposure *in utero* to TCDD can cause persistent immunotoxicological effects. In Gehrs *et al.* (1997), timed-bred pregnant F344 rats were dosed with 0 or 1.0 µg/kg TCDD by gavage on GD 14. One day after birth, litters were cross-fostered to produce control, placental-only, lactational-only, and placental/lactational exposure groups. The organ weights and thymic and splenic phenotypes of these pups were assayed 1, 2, or 3 weeks post-partum, while the delayed-type hypersensitivity (DTH) response was assessed in 5-month-old males. Increased liver/body weight ratios, decreased percentages of thymic $CD3^+/CD4^-CD8^-$ cells, and increased percentages of thymic $CD3^+/CD4^-CD8^+$ cells were seen through 3 weeks old in both genders after TCDD exposure. These data are presented in Table 5 through Table 9. The severity of the effects was related to the route of exposure (i.e. placental/lactational > lactational > placental). The delayed-type hypersensitivity (DTH) response to bovine serum albumin (BSA) was suppressed in the males receiving both placental and lactational exposure. In a second set of experiments, TCDD exposure (3.0 µg/kg) increased spleen/body weight ratio, decreased the thymus/body weight ratio (in males), and decreased the percentage of splenic $CD3^+/CD4^-CD8^-$ cells in both the TCDD-exposed male and female pups when tested at 14 - 17 weeks (Table 9). TCDD suppressed DTH response to BSA in both genders (Gehrs *et al.*, 1997).

Table 5: Effects of TCDD on 1-week-old male rat pups whose dams were dosed orally on gestational day 14[a] (From Gehrs *et al*., 1997)

	Route of TCDD exposure[b, c]			
	Control	**Placental**	**Lactational**	**Placental/lactational**
Body weight (g)	7.73 ± 0.65	7.77 ± 0.41	7.92 ± 0.42	7.28 ± 0.38
Relative organ weights (mg/g body wt.)				
Spleen	3.63 ± 0.23	3.76 ± 0.21	3.63 ± 0.14	3.13 ± 0.13
Thymus	1.60 ± 0.13	1.69 ± 0.08	1.55 ± 0.11	1.37 ± 0.09
Liver	27.2 ± 1.8	29.4 ± 1.4	33.8 ± 1.8*	31.1 ± 1.3
Splenic cellularity (× 10^6)	9.8 ± 1.6	10.0 ± 0.9	8.6 ± 1.2	7.9 ± 1.3
Thymic cellularity (× 10^6)	21.0 ± 3.9	19.7 ± 3.2	16.0 ± 2.9	14.0 ± 2.1
Thymocyte phenotype				
Percentage $CD3^+$	24.8 ± 0.7	24.5 ± 1.1	27.1 ± 0.5	28.0 ± 0.9
% $CD4^+CD8^-$	11.4 ± 0.6	11.5 ± 0.4	10.8 ± 0.4	11.5 ± 0.4
% $CD4^+CD8^+$	75.6 ± 0.4	73.0 ± 1.2	72.7 ± 2.2	70.3 ± 1.2*
% $CD4^-CD8^-$	2.4 ± 0.1	1.8 ± 0.3*	1.1 ± 0.1**	1.0 ± 0.1**
% $CD4^-CD8^+$	10.6 ± 0.4	13.8 ± 1.3	15.6 ± 2.1	17.3 ± 1.3*

[a] Results expressed as means ± S.E.
[b] Dams were given 1.0 µg TCDD/kg or vehicle control.
[c] There were five animals (1/litter) in each exposure group. For the placental and the lactational exposure groups, the litters were cross-fostered on postnatal day 1.
* $p < 0.05$ versus vehicle control; ** $p < 0.01$ versus vehicle control.

Table 6: Effects of TCDD on 1-week-old female rat pups whose dams were dosed orally on gestational day 14[a] (From Gehrs *et al.*, 1997)

	Route of TCDD exposure[b, c]			
	Control	**Placental**	**Lactational**	**Placental/lactational**
Body weight (g)	9.38 ± 0.20	8.92 ± 0.54	8.89 ± 0.24	8.07 ± 0.53
Relative organ weights (mg/g body wt.)				
Spleen	3.52 ± 0.26	3.58 ± 0.34	3.64 ± 0.06	3.17 ± 0.21
Thymus	2.09 ± 0.09	2.02 ± 0.14	1.70 ± 0.10	1.77 ± 0.14
Liver	25.9 ± 1.0	28.8 ± 1.4	31.8 ± 1.8*	32.8 ± 1.1*
Splenic cellularity ($\times 10^6$)	17.7 ± 1.9	12.5 ± 1.8	16.2 ± 2.0	11.8 ± 2.4
Thymic cellularity ($\times 10^6$)	33.7 ± 1.9	24.1 ± 3.4	21.9 ± 2.7*	17.4 ± 2.6**
Thymocyte phenotype				
Percentage $CD3^+$	31.6 ± 0.6	32.7 ± 0.7	30.0 ± 0.6	32.1 ± 1.0
% $CD4^+CD8^-$	5.8 ± 0.3	5.7 ± 0.4	5.5 ± 0.5	5.3 ± 0.4
% $CD4^+CD8^+$	84.6 ± 0.6	83.0 ± 0.8	82.9 ± 1.1	78.9 ± 2.0*
% $CD4^-CD8^-$	1.1 ± 0.1	1.1 ± 0.1	0.6 ± 0.0**	0.7 ± 0.1**
% $CD4^-CD8^+$	8.6 ± 0.4	10.3 ± 0.8	11.1 ± 1.1	15.1 ± 1.5**

[a] Results expressed as means ± S.E.

[b] Dams were given 1.0 µg TCDD/kg or vehicle control.

[c] There were five animals (1/litter) in each exposure group. For the placental and the lactational exposure groups, the litters were cross-fostered on postnatal day 1.

* $p < 0.05$ versus vehicle control; ** $p < 0.01$ versus vehicle control.

Table 7: Effects of TCDD on 2-week-old rat pups whose dams were dosed orally on gestational day 14[a] (From Gehrs *et al.*, 1997)

	Male pups[b]		Female pups[b]	
	Control	**Perinatal TCDD[c]**	**Control**	**Perinatal TCDD[c]**
Body weight (g)	19.7 ± 0.6	17.4 ± 0.6	20.0 ± 0.9	16.2 ± 1.1
Relative organ weights (mg/g body wt.)				
Spleen	3.31 ± 0.1	3.55 ± 0.11	3.27 ± 0.1	3.22 ± 0.15
Thymus	2.56 ± 0.1	1.98 ± 0.09	2.60 ± 0.1	2.27 ± 0.26
Liver	27.1 ± 0.5	30.0 ± 0.5*	29.3 ± 0.1	33.2 ± 0.9*
Splenic cellularity ($\times 10^6$)	32.6 ± 4.3	28.5 ± 1.7	25.7 ± 2.4	15.4 ± 2.8
Thymic cellularity ($\times 10^6$)	81.3 ± 3.5	51.9 ± 3.4**	82.2 ± 7.7	55.5 ± 9.0
Thymocyte phenotype				
Percentage $CD3^+$	25.0 ± 0.4	23.4 ± 0.3	23.8 ± 0.2	25.5 ± 1.2
% $CD4^+CD8^-$	17.0 ± 1.5	11.9 ± 0.4**	10.1 ± 0.6	7.4 ± 0.5*
% $CD4^+CD8^+$	70.1 ± 1.6	73.1 ± 0.8	75.0 ± 0.5	77.7 ± 0.9
% $CD4^-CD8^-$	1.4 ± 0.1	0.8 ± 0.0**	1.2 ± 0.1	0.7 ± 0.1**
% $CD4^-CD8^+$	11.6 ± 1.0	14.3 ± 0.5	13.7 ± 0.2	14.3 ± 0.3

[a] Results expressed as means ± S. E.

[b] There were five or six animals (1/litter) in each exposure group.

[c] Dams were given 1.0 µg TCDD/kg or vehicle control. The perinatal TCDD groups refer to placental/lactational exposure. In general, results in the placental and the lactational groups were intermediate between those in the control and the placental/lactational groups.

* $p < 0.05$ versus vehicle control; ** $p < 0.01$ versus vehicle control.

Table 8: Effects of TCDD on 3-week-old rat pups whose dams were dosed orally on gestational day 14[a] (From Gehrs *et al.*, 1997)

	Male pups[b]		Female pups[b]	
	Control	**Perinatal TCDD[c]**	**Control**	**Perinatal TCDD[c]**
Body weight (g)	**34.9 ± 1.8**	**33.1 ± 0.6**	**36.3 ± 0.4**	**31.1 ± 0.9****
Relative organ weights (mg/g body wt.)				
Spleen	3.18 ± 0.03	3.31 ± 0.07	3.85 ± 0.12	3.77 ± 0.15
Thymus	3.19 ± 0.06	2.91 ± 0.08	3.54 ± 0.24	3. l2 ± 0.15
Liver	40.2 ± 1.0	48.1 ± 1.5**	43.1 ± 0.8	50.2 ± 1.3**
Splenic cellularity (× 10^6)	58.4 ± 5.7	41.6 ± 2.0	80.4 ± 6.5	63.0 ± 4.3
Thymic cellularity (× 10^6)	185.3 ± 12.4	142.2 ± 20.5	240.8 ± 23.0	172.7 ± 14.2*
Thymocyte phenotype				
Percentage $CD3^+$	30.0 ± 0.9	34.9 ± 1.1**	33.7 ± 0.5	35.1 ± 1.3
% $CD4^+CD8^-$	14.5 ± 0.7	14.0 ± 0.3	24.8 ± 1.1	21.6 ± 0.8*
% $CD4^+CD8^+$	68.5 ± 0.7	66.0 ± 0.7	52.6 ± 1.0	55.1 ± 1.2
% $CD4^-CD8^-$	1.0 ± 0.1	0.6 ± 0.0**	1.3 ± 0.1	0.9 ± 0.1*
% $CD4^-$CD8+	16.1 ± 0.3	19.6 ± 0.6**	21.4 ± 0.2	22.4 ± 0.3*

[a] Results expressed as means ± S. E.

[b] There were five or six animals (1/litter) in each exposure group.

[c] Dams were given 1.0 µg TCDD/kg or vehicle control. The perinatal TCDD groups refer to placental/lactational exposure. In general, results in the placental and the lactational groups were intermediate between those in the control and the placental/lactational groups.

* $p < 0.05$ versus vehicle control; ** $p < 0.01$ versus vehicle control.

Table 9: Effects of TCDD on 14-week-old rats whose dams were dosed orally on gestational day 14[a] (From Gehrs *et al*., 1997)

	Male rats[b]		Female rats[b]	
	Control	**Perinatal TCDD**	**Control**	**Perinatal TCDD**
Body weight (g)	278.6 ± 5.0	264.5 ± 5.9	166.1 ± 2.6	167.7 ± 2.4
Relative organ weights (mg/g body wt.)				
Spleen	1.99 ± 0.03	2.44 ± 0.06**	2.40 ± 0.05	3.01 ± 0.09**
Thymus	0.96 ± 0.04	0.70 ± 0.02**	1.09 ± 0.05	1.04 ± 0.07
Liver	43.0 ± 1.2	37.3 ± 1.5*	36.2 ± 0.5	36.8 ± 0.3
Splenic cellularity ($\times 10^6$)	289.8 ± 7.5	269.3 ± 10.8	233.5 ± 15.2	276.8 ± 16.4
Splenocyte phenotype				
Percentage IgM^+	44.2 ± 0.6	41.8 ± 1.3	40.5 ± 0.9	38.4 ± 1.7
Percentage $CD3^+$	42.1 ± 1.1	40.9 ± 1.0	47.3 ± 1.1	48.2 ± 1.0
% $CD4^+CD8^-$	75.4 ± 0.8	77.1 ± 0.4	74.1 ± 1.0	76.1 ± 0.3
% $CD4^+CD8^+$	3.0 ± 0.1	2.9 ± 0.1	2.7 ± 0.3	3.0 ± 0.2
% $CD4^-CD8^-$	2.1 ± 0.0	1.8 ± 0.1**	2.0 ± 0.1	1.5 ± 0.1**
% $CD4^-CD8^+$	19.6 ± 0.8	18.2 ± 0.3	21.3 ± 0.8	19.4 ± 0.5

[a] Results expressed as means ± S.E.
[b] There were seven animals in each exposure group. The perinatal TCDD groups refer to placental/lactational exposure. The dams of these rats received 3.0 µg/kg TCDD on GD14. The dams of the control rats were untreated.
* $p < 0.05$ versus vehicle control; ** $p < 0.01$ versus vehicle control.

Gehrs and Smialowicz (1999) in a similar experiment demonstrated that the suppression of the DTH response in rats associated with perinatal TCDD exposure is persistent through late adulthood, occurs at a low dose (i.e. 0.1 µg TCDD/kg to the dam), and is more pronounced in males than females. In the first experiment, DTH response to BSA was tested in animals previously shown to have a suppressed DTH response at 4 months of age following 3 µg TCDD/kg dose to the dams at GD 14. The animals were retested at 8, 12, and 19 months of age. Male offspring had significantly suppressed DTH response at 4, 8, and 19 months of age ($p<0.05$); the trend in females was towards a suppressed DTH response but was only significant at 4 mo of age. In a second experiment, dams were given 0, 0.1, 0.3, or 1.0 µg TCDD/kg on GD 14, and lactational exposure was allowed through 4 weeks in the pups. Suppression of the DTH response to BSA was evident in males at 0.1 and 0.3 µg TCDD/kg (to the dam) group. Thus, this study shows that the perinatally exposed animals continued to have a suppressed immune response into late adulthood. DTH reactions are important in protection against invading microorganisms and certain tumors, as well as having a role in allergy. Thus, supression of DTH response results in an increased risk for infectious disease, and also neoplasms, while decreasing the risk of autoimmune disease.

In a chicken embryo study where eggs were injected with PCB #126 at various incubation stages, Fox and Grasman (1999) reported that lymphoid cell numbers were more sensitive to PCB #126 than immune organ masses. They also observed that the bursa of Fabricius (a dorsal outpocketing of the cloaca that controls antibody-mediated immunity in young birds) tended to be more sensitive than the thymus. Doses necessary to reduce the number of viable lymphoid cells in the thymus and bursa were

at least one order of magnitude lower with full-term incubation as compared to exposure only during later stages of incubation. The LD_{20} and LD_{50} for lymphocyte viability was estimated to be 0.21 and 1.01 ng PCB #126/g, respectively. Thymus mass dropped sharply between 0.13 and 0.32 ng/g, and lymphoid cell numbers in the thymus fell sharply between 0.051 and 0.13 ng/g. Bursa mass began to decrease at the lowest dose of 0.051 ng/g and reached a minimum at 0.32 ng/g. The number of viable cells decreased slightly at 0.051 ng/g and reached a minimum at the 0.13- and 0.32-ng/g doses.

(2) Developmental effects

In animal experiments, offspring of pregnant Long Evans rats treated on GD 15 with 1.0 µg/kg TCDD by gavage showed signs of reproductive developmental toxicity (Hamm *et al.*, 2000). These changes are indicative of disruption of the proper hormonal environment in the offspring. The effects seen may parallel those in adults, but whereas the responses may be reversible in adults, exposure of the fetus results in irreversible effects, including both anatomical and functional abnormalities. Starting at PND 32 male pups showed impaired growth of their seminal vesicles, which was associated with a dramatic decrease in the development of the epithelium. Gray *et al.* (1997) administered TCDD to Long Evans pregnant rats at gestational day 15 at dosage levels of 0.05, 0.2 or 0.8 µg/kg. Female rat offspring (80 days of age) had morphological reproductive tract alterations ($p < 0.05$) such as cleft phallus (significant at 0.8 µg TCDD/kg), temporary or permanent vaginal thread formation (significant at 0.2 and 0.8 µg TCDD/kg) and delayed vaginal opening (significant at 0.8 µg TCDD/kg).

In a cross-fostering study (Crofton *et al.* 2000) examined the progeny of rats treated with 6 mg/kg/day Aroclor 1254 (A1254) from GD 6 to PND 21. On the day of birth, half of the treated litters and half of the control litters were cross-fostered, resulting in the following groups: Ctrl/Ctrl (controls); A1254/A1254 (perinatal exposure); A1254/Ctrl (prenatal exposure only); and Ctrl/A1254 (postnatal exposure only). Rats exposed during their development, exhibited ototoxicity however, that effect was mostly observed in the group exposed during lactation (Table 10). They concluded that the critical period for developmental ototoxicity from Aroclor 1254 exposure is within the first few postnatal weeks in the rat.

Table 10: Perinatal Arochlor 1254 (6 mg/kg/day) treatment caused low frequency hearing loss that was due solely to postnatal exposure.

Frequency	Threshold, dB SPL (mean ± SE)			
	Ctrl/Ctrl	A1254/A1254	A1254/Ctrl	Ctrl/A1254
1 kHz	24	42*	28	46*
40 kHz	16	17	17	21

* indicates significant difference from the 1 kHz control group, $p < 0.05$; $n = 11 – 14$ litter/group (Source : Crofton *et al.*, 2000)

Offspring of pregnant Wistar rats administered a single oral dose of 10 µg/kg body weight PCB #126 or 100 µg/kg of PCB #77 on GD 15 showed signs of developmental toxicity (Faqi *et al.*, 1998). Male

offspring were killed on postnatal days 65 or 140. In the PCB #126 group, the age of vaginal opening was delayed in the female pups. Testis and brain weights, and daily sperm production were permanently increased and seminal vesicle weight was decreased in male offspring of the PCB #77-treated group. In male rats of the PCB #126 group, brain weights were permanently increased and ventral prostate weights permanently reduced. In both PCB groups, however, serum testosterone concentration was reduced only at adulthood. All these responses were significant at $p<0.05$. Faqi *et al.* (1998) concluded that PCB #126 elicited some TCDD-like developmental toxicity on the reproductive tract after exposure *in utero*. For the PCB #77, these authors hypothesized that the reproductive effects of *in utero* exposure to PCB #77 on male offspring may be attributed to neonatal hypothyroidism induced by the substance during early fetal development.

(3) Thyroid hormone effects

A number of studies indicate that dioxins and dioxin-like compounds decrease circulating thyroid hormone levels. A reduction of maternal serum thyroxin (T_4) levels can impair the brain development of the offspring (Glorieux *et al.*, 1988; Rovet *et al.*, 1987; Haddow *et al.*, 1999). Brain developmental damage appears to be inversely related to maternal serum T_4 levels in the first and second trimesters. Maternal serum free T_4 is able to pass through the placenta and is converted to tri-iodothyronine (T_3) in the fetal brain. The T_3 generated in situ is believed to be necessary for the development of brain, specifically the cerebral cortex, the extrapyramidal system, and the cochlea (Porterfield, 1994). The availability of a minimum level of maternal free T_4 is crucial for proper fetal brain development in the first and second trimesters, as the fetal thyroid is not fully mature and functional during that time period. A number of human studies have shown that pregnancy itself puts stress on the thyroid (Crooks *et al.*, 1967; Glinoer *et al.*, 1990; Brent, 1999). Consequently, insults on the maternal thyroid condition are particularly relevant to the issue of increased sensitivity of infants and children to dioxins and dioxin like compounds.

In a cross fostering study, Crofton *et al.* (2000) demonstrated that progeny of rats gavaged with 6 mg/kg/day Aroclor 1254 (A1254) from GD 6 to PND 21, exhibited hypothyroxinemia. On the day of birth, half of the treated litters and half of the control litters were cross-fostered, resulting in the following groups: Ctrl/Ctrl (controls); A1254/A1254 (perinatal exposure); A1254/Ctrl (prenatal exposure only); and Ctrl/A1254 (postnatal exposure only). Compared to the control, serum T_4 concentrations of offspring were sharply reduced at GD 21 in all A1254-exposed groups ($p < 0.05$). On PND 3, 7, 14, and 21, T_4 decrease was also significant in the A1254/A1254 and the Ctrl/A1254 groups ($p < 0.05$). Smaller but significant decreases in T_4 were observed in the A1254/Ctrl group on PND 3, 7, and 14. Thus, decrease in serum T_4 was mostly observed in the lactationally exposed group.

Viluksela *et al.* (1997) demonstrated that rats exposed orally to 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin (HpCDD) showed a dose-dependent statistically significant decrease (78% at the highest dose, 6,000 and 10,000 µg/kg HpCDD for females and males respectively) in the serum T_4 concentrations. The animals were divided into 7 treatment groups (n = 20 per sex per group). HpCDD dosages (Group 1 = 0; Group 2 = 18.5 females (F), 30.9 for males (M); Group 3 = 222 (F), 370 (M); group 4 = 1,333 (F), 2,222 (M); Group 5 = 4,000 (F), 6,667 (M); Group 6 = 6,000 (F), 10,000 (M)) in µg/kg were divided into four daily loading doses and six biweekly maintenance doses for 13 weeks. In group

7, the rats were administered TCDD in one total dose of 41.9 and 70 µg/kg for female and male rats respectively. Half the animals were sacrificed after the 13 week dosing schedule and the other half allowed a 13-week off-dose schedule. Dose-dependent enzyme induction was noted in the liver by measuring EROD activity ($p < 0.05$ for all treatment groups relative to controls). Serum T_4 levels were decreased in a dose-dependent manner at the three highest HpCDD doses and by TCDD ($p < 0.01$ to 0.001). There was a maximal decrease of 78 % in the males and 44 % in the females at the end of the 13-week dosing period. This decrease in serum T_4 continued through the off-dose period and was maximally 65 and 60 % in males and females in the HpCDD treatment groups. Serum T_4 came back towards normal in the TCDD group, which is consistent with its shorter half-life in this species. Serum T_3 concentrations were only slightly affected, and not significantly in either males or females. In a similar study, Viluksela *et al.* (1998) described a subchronic experiment in rats given a mixture of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), 1,2,3,7,8-pentaCDD (PCDD), 1,2,3,4,7,8-hexaCDD (HxCDD), and 1,2,3,4,6,7,8-heptaCDD (HpCDD), and in rats given PCDD or HxCDD (cumulative dosage 10 – 100 µg/kg). The dosing period was 13 weeks, and half of the animals were then put on a 13-week off-dose period. They reported a dose-dependent statistically significant decrease of serum T_4 concentrations (maximally by 69 %), with some reversibility in males during the off-dose period. Serum T_3 levels were not significantly affected.

Treatment with 25 µmol (single oral dose) tetrachlorobiphenyl (TCB, PCB #77) significantly reduced plasma T_4 levels up to 7 days after administration in non-pregnant rats and up to 4 days after administration in pregnant rats (Morse *et al.*, 1995). By 7 days after administration, plasma T_4 levels had returned to control levels in the TCB-treated pregnant rats. However, fetal plasma T_4 levels were significantly decreased from TCB-treated dams 7 days after TCB administration. This decrease in fetal T_4 was attributed to the 4-hydroxylated metabolite of TCB. Hepatic microsomal ethoxyresorufin-O-deethylase (EROD) activity was significantly induced in TCB-treated dams relative to controls at 4 and 7 days after administration, while no EROD activity was detected in hepatic microsomes from control or TCB treated fetal rats at day 20 of gestation.

An age-related effect was reported for the toxicity of Aroclor 1254 on thyroid hormone levels (Provost *et al.*, 1999). Dams were exposed to 1.25 or 12.5 ppm Aroclor 1254 from conception through postnatal day 15 or 30, and their offspring were tested at 15 or 30 days of age. T_4 concentrations were slightly elevated in 15-day-old pups of 1.25 ppm Aroclor 1254 exposed dams and significantly depressed ($p < 0.05$) in 15- and 30-day-old pups of 12.5 ppm Aroclor exposed dams. T_3 concentrations were not altered in 15-day-old rats but were significantly depressed ($p < 0.05$) in 30-day-old pups of dams treated with 1.25 and 12.5 ppm Aroclor 1254.

b) Non-coplanar PCB-induced effects

(1) Neurobehavioral effects

Exposure to the non-coplanar PCBs has been associated with neurodevelopmental toxicity in animals and humans. Roegge *et al.* (2000) exposed pregnant Long-Evans females to 0 or 6-mg/kg/day Arochlor 1254 (A1254) (p.o. in corn oil) from gestation day (GD) 6 to weaning at postnatal day (PND) 21. Results indicate that perinatal exposure to Aroclor 1254 (6 mg/kg) in Long Evans pregnant

rats may cause sex-specific deficits in spatial learning and memory in adult offspring (120 – 150 days of age). Compared to control males, the A1254-exposed males made significantly ($p < 0.01$) more working memory errors (2.15 +/- 0.13 and 3.20 +/- 0.18 errors (+/- SEM) for A1254 and control males, respectively) and reference memory errors (3.17 +/- 0.10 and 4.13+/-0.14 errors (+/- SEM) for control and A1254 males, respectively) on a 12-arm radial maze (RAM). A1254-exposed females were not impaired relative to control females on the RAM.

Pregnant rats were gavaged with 8 or 32 mg/kg/day 2,2',3,5',6-pentachlorobiphenyl (PCB #95) on gestation days 10-16 (Schantz *et al.*, 1997). Spatial learning and memory was assessed using an eight arm radial maze working memory task at 60 days of age and a T-maze delayed spatial alternation task at 140 days of age. Locomotor activity was evaluated at 35 and 100 days of age using an automated open-field. PCB #95-treated rats did not differ from controls on the T-maze delayed spatial alternation task. Offspring of rats dosed with PCB #95 showed normal levels of activity in the open-field test as juveniles, but were hypoactive as adults ($p < 0.05$). Interestingly, these rats also showed a faster acquisition of the working memory task on the radial arm maze, as measured by the number of errors made in subsequent sessions in the maze ($p < 0.05$). The authors attribute this effect to the affected rats using a different strategy to learn the maze, a "response patterning" which is seen in certain types of brain damage. It should be noted that in earlier experiments by these investigators (Schantz *et al.*, 1995), treatment of pregnant dams with other ortho-substituted PCBs (118, 153) produced offspring that were significantly hyperactive as adults and impaired in learning in the radial arm maze ($p < 0.05$)

When pregnant rats were gavaged with 6 mg/kg/day Aroclor 1254 from GD 6 to postnatal day 21 in a cross-fostering study, no difference between the Aroclor 1254-treated group and controls could be established when spatial learning was tested in the offspring at 3 months of age (Gilbert *et al.*, 2000). Provost *et al.* (1999) demonstrated dose- and age-dependent alterations in choline acetyltransferase (ChAT, an enzyme involved in the biosynthesis of acetylcholine) activity and in learning and memory in 15- and 30-day old offspring of dams exposed to 1.25 or 12.5 ppm Aroclor 1254 beginning at conception (Table 11)

Dietary exposure of dams to 1.25 ppm Aroclor 1254 during gestation and lactation significantly ($p < 0.05$) elevated ChAT activity in the hippocampus and basal forebrain of 15-day old offspring. Rats exposed to 12.5 ppm of Aroclor 1254 until 15 days of age demonstrated significant elevations of ChAT activity in the basal forebrain. At 30 days both 1.25 and 12.5 ppm Aroclor 1254 treatment groups displayed significantly depressed ChAT activity in both areas of the brain, indicating persistency of the PCB effect. Only the 12.5 ppm Aroclor 1254-treated group showed decrements in spatial learning, when rats were tested between 25 and 29 days of age.

Table 11. Effect of Arochlor 1254 on ChAT activity (nmol/mg protein/hr) in hippocampus and basal forebrain.

Arochlor 1254,	Hippocampus		Basal Forebrain	
ppm	15 Day	30 Day	15 Day	30 Day
0 (control)	57 ± 8 [a]	79 ± 5	146 ± 6	257 ± 10
1.25	130 ± 5 *	62 ± 2 *	307 ± 10 *	195 ± 12 *
12.5	63 ± 7	57 ± 3 *	162 ± 13	196 ± 11 *

- Significantly different from control ($p < 0.05$)

[a] Mean ± SEM of 64 rat pup brains

(Data extracted from figure 1; Provost *et al.*, 1999)

V. Additional Information

A. Carcinogenic effects

There are only a few cases where dioxin exposure of the general population has been documented; the incident in Seveso, Italy is one of them. In 1976, a chemical plant producing 2,4,5-trichlorophenol, experienced an explosion and fire releasing several chemicals including TCDD into the atmosphere in the vicinity of Seveso. The Seveso incident represents a unique event in the sense that exposure to dioxins was not limited to occupational exposure by workers but the whole population was affected by the TCDD release in the area surrounding the chemical plant. The population was exposed to different degrees depending on the distance and direction from the origin of the plume. Fifteen years after the industrial accident, Bertazzi *et al*. (1997) examined the cancer mortality among residents (20 to 74 years old) of Seveso by comparing populations living in dioxin contaminated areas (divided into three zones: highest, lower and lowest zone of exposure to dioxin, zone A, B, and R, respectively) with a population from neighboring noncontaminated areas (zone nonABR). No increase for all-cancer mortality, or major specific sites like respiratory cancer among males and breast cancer among females, was found. However, elevation in other specific cancer mortality was observed and could be associated with dioxin exposure. Table 12 summarizes cancer mortality for men and women living in zone B.

Table 12: Female and male deaths in zone B for selected causes, 1976-1991, ten years or more since first exposure (latency) and duration of exposure (length of stay in contaminated area)*

		Latency > 10 years		Length of stay > 10 years	
		Female	Male	Female	Male
All cancers	OBS	23	31	20	29
	RR	1.4	1.0	1.4	1.1
	(95% CI)	(0.9 –2.1)	(0.7 –1.4)	(0.8 –2.1)	(0.7 – 1.6)
Digestive cancer	OBS	10	12	9	12
	RR	1.5	1.0	1.6	1.2
	(95% CI)	(0.7 – 2.7)	(0.5 – 1.8)	(0.7 – 2.9)	(0.6 – 2.1)
Stomach cancer	OBS	5	X	4	
	RR	2.4	X	2.3	
	(95% CI)	(0.8 – 5.7)		(0.6 – 6.0)	
Lymphatic and hemopoietic	OBS	4	4	3	4
	RR	2.8	2.5	2.4	3.0
	(95% CI)	(0.7 – 7.1)	(0.7 –6.4)	(0.5 – 7.1)	(0.8 – 7.7)
Multiple myeloma	OBS	3		2	
	RR	15.9		11.0	
	(95% CI)	(3.2 – 46.5)		(1.2 – 39.6)	
Rectal cancer	OBS		4		4
	RR		6.2		7.2
	(95% CI)		(1.7 – 15.9)		(1.9 - 18.4)
Leukemia	OBS		2		2
	RR		3.4		3.9
	(95% CI)		(0.4 – 12.3)		(0.4 – 14.1)

* OBS = observed deaths.
(Source : Bertazzi *et al.*, 1997)

Increased mortality from stomach cancer (RR = 2.4; 95% CI = 0.8-5.7) was reported 10 years after the accident in women living in zone B although the RR did not reach statistical significance. In men, a statistically significant increase in mortality from rectal cancer was observed and was highest for latency greater than 10 years (RR = 6.2; 95% CI = 1.7-15.9), and length of stay greater than ten years (RR= 7.2; 95% CI = 1.9-18.4). Leukemia in men also appears elevated in Zone B (RR=3.1, 95%CI = 1.3-6.4) when evaluated as observed versus expected total cases over the years 1976-1991. The relative

risk for leukemia in men did not reach statistical significance when broken out by latency or length of stay in the contaminated area (RR = 3.4; 95% CI = 0.4 – 12.3), leading the authors to conclude that there was no clear time-related trend. Statistically significant elevated rates of multiple myeloma were observed in women in Zone B with the highest risks in those with > 10 years latency (RR = 15.9; 95% CI = 3.2 - 46.5). Hodgkin's disease in both genders (RR = 3.3; 95% CI = 0.4 - 11.9 in men; and RR = 6.5; 95% CI = 0.7 - 23.5 in women) appeared elevated although the elevation was not statistically significant.

In the young population (20,000 subjects aged 0 to 19 years old), some cases of cancer were also found (Pesatori *et al.*, 1993), including two ovarian cancers and Hodgkin's lymphoma; myeloid leukemia was elevated although not statistically significant (RR = 2.7; 95% CI = 0.7 - 11.4). Two cases of thyroid cancer were also reported (RR = 4.6; 95% CI = 0.6 - 32.7) in younger people.

None of the elevated cancer incidences in zone A, the area with the highest exposure, were statistically significant; however, this area also had the smallest population. Additionally, it should be noted that the Seveso population was exposed to 2–3 orders of magnitude the level of dioxin normally experienced by the general population of industrialized countries. In 1997, individuals living in the contaminated area at the time of the accident still experienced high level of plasma TCDD 20 years after the industrial accident in Seveso. Geometric means for plasma TCDD concentration for individuals who lived in zone A, B and nonABR (control zone) in 1976 were 53.2, 11.0 and 4.9 ppt, respectively. Women in these three groups represented the gender with the highest plasma TCDD contamination (Landi *et al.*, 1997). The authors concluded that the results indicate a positive association between dioxin exposure and certain cancers, but further study is needed to clarify this association. It should be noted that the length of follow-up of 15 years is still short. In addition, potential exposure miscalssification, and small sample size complicate the analysis.

Because dioxin is a potent potentiator but a weak initiator of cancer processes, exposure early in life theoretically should have less impact than when exposed later. However, Brown *et al.* (1998) suggested that prenatal exposure to dioxin and related compounds may increase sensitivity in adulthood to other chemical carcinogens. In an investigation of predisposition to mammary cancer, Brown *et al.* (1998) treated pregnant Sprague-Dawley rats on gestational day 15 with 1 µg/kg TCDD. Results indicate that prenatal TCDD exposure significantly increased terminal end buds and decreased lobules II in 50-day-old offspring. No alterations in mammary gland differentiation were observed in 21-day old offspring. Additionally, prenatal TCDD treatment was associated with an increased number of chemically induced (by DMBA) mammary adenocarcinomas in rats. These authors concluded that prenatal exposure to TCDD increased susceptibility to mammary cancer, which correlated with alteration of mammary gland differentiation based on the increased number of terminal end buds.

B. Mechanism of toxicity

Among the dioxin-like compounds that exert their toxic effects through the Aryl hydrocarbon (*Ah*) receptor are the coplanar polychlorinated biphenyls (PCB). These PCB congeners substituted in the para and at least 2 of the meta positions but not at any of the ortho positions are the most toxic PCBs. These congeners are structurally similar to TCDD. Introduction of one chlorine in the ortho position

results in a decrease in toxic potency, and PCBs with more than one chlorine in the ortho positions lack some effects exerted by non- and mono-ortho PCBs and show a partially different spectrum of toxic effects (Safe, 1994). PCB congeners that have little or no activity at the *Ah* receptor (non-dioxin-like PCBs) have been shown to accumulate in the brain following in vivo exposure and decrease dopamine content. These non-dioxin-like PCBs interfere with calcium homeostatic mechanisms and intracellular second messenger systems *in vitro* in neuronal cultures and brain subcellular fractions. Structure-activity relationship (SAR) studies based on measures of PCB-induced alterations in protein kinase C (PKC) translocation and Ca^{2+}-buffering, indicate that congeners with chlorine substitutions at the ortho-position are active *in vitro*, while non-ortho congeners are relatively inactive. Subsequent research has found that chlorine substitution patterns that favor non-co-planarity are associated with *in vitro* neurotoxicity (Tilson and Kodavanti, 1998). These results therefore seem to indicate a mechanism of toxicity for non-coplanar PCBs that is different than the interaction with the *Ah* receptor pathway.

C. Mechanistic evidence of age-related susceptibility in animals

Dioxin and dioxin-like compounds act primarily through the aryl hydrocarbon (*Ah*) receptor. The presence of the *Ah* receptor in the rat varies with the stage of development. *Ah* receptor protein levels in developing rat ventral and dorsolateral prostate decrease with age, declining approximately 70 % between postnatal days (PND) 1 and 21. ARNT (*Ah* receptor nuclear translocator) protein levels also decrease with age in dorsolateral, but not ventral prostate (Sommer *et al.*, 1999). This decrease is associated with a decrease in *Ah* receptor and ARNT mRNA. TCDD treatment in adult male rats (0.2, 1, 5, or 25 µg/kg by gavage, 24 h) decreased *Ah* receptor but not ARNT protein in ventral and dorsolateral prostate, vas deferens, and epididymis (Sommer *et al.*, 1999). This study also reported that *in utero* and lactational TCDD exposure of offspring (1.0 µg/kg to dam by gavage, on gestation day (GD) 15) did not alter ARNT levels but reduced prostatic *Ah* receptor protein levels on PND 7 and delayed the developmental decrease in *Ah* receptor protein in ventral and dorsolateral prostate. Also, pretreatment of rat pups for 24 hours with TCDD (5 µg/kg ip) down-regulated prostatic *Ah* receptor protein on PND 7, but not on PND 1. Thus, the authors concluded that prostatic *Ah* receptor and ARNT protein and mRNA levels are regulated with age, whereas only *Ah* receptor protein concentration is altered by TCDD exposure.

Age-related induction of cytochrome P4501A1, as indicated by the activity of hepatic microsomal ethoxyresorufin-O-deethylase (EROD), was reported by Morse *et al.* (1995). Hepatic EROD activity in PCB #77-treated dams was significantly induced relative to controls at 4 and 7 days after administration. No EROD activity was detected at GD 20 in hepatic microsomes in fetal rats from control or PCB #77-treated dams. Provost *et al.* (1999) demonstrated the influence of age on thyroid hormone levels in the offspring of dams treated with Aroclor 1254. T_3 concentrations were not altered in 15-day-old offspring, but were significantly depressed in 30-day-old offspring of 1.25 ppm and 12.5 ppm-treated dams.

Another indication of age-related difference comes from the *in vitro* testing of Ca^{2+}-uptake by subcellular brain preparations from Long-Evans rats. Aroclor 1254 inhibited Ca^{2+}-uptake by brain microsomes, and the inhibition increased with age (PND 7 < PND 21 < or = adults; IC50s = 21-34, 8-

20 and 10-14 µM, respectively) (Sharma *et al.*, 2000). In general, microsomal and mitochondrial Ca^{2+}-uptake in selected brain regions increased with age (PND 7 < PND 21 < or = adults).

D. Regulatory Background

a) Chronic RELs

For TCDD (OEHHA, 2000)

- The inhalation reference exposure level is 0.00004 µg/m^3 (40 pg/m^3).

- The oral reference exposure level is 10 pg/kg/day.

The critical effects for these RELs, which are based on studies by Kociba *et al.* (1978) are: mortality, decreased weight gain, depression of erythroid parameters, increased excretion of porphyrins and d-aminolevulinic acid, increase serum activities of alkaline phosphatase, gamma glutamyl transferase and glutamic-pyruvic transaminase, gross and histopathological changes in the liver, lymphoid tissue, lung and vascular tissues in rats (OEHHA, 2000).

b) Cancer Risk

(1) Cancer Risk for Polychlorinated Dibenzo-p-dioxins and Polychlorinated Dibenzofurans

See Table 13

(2) Cancer risk for PCBs

Unit risk factor: 5.7 E-4 (µg/m^3)$^{-1}$ (for use in cases where congeners with more than four chlorines do not comprise less than one-half percent of total PCBs.)

Unit risk factor: 2.0 E-5 (µg/m^3)$^{-1}$ (for use in cases where congeners with more than four chlorines comprise more than one-half percent of total PCBs.)

The Scientific Advisory Board (SAB) for the U.S. Environmental Protection Agency (US EPA) peer reviewed and approved the report *Dioxins reassessment: Exposure and Human Health Reassessment of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) and Related Compounds* in May 2001. This report identifies dioxin as a cause of cancer in laboratory animals and possibly in humans.

Table 13: Health Assessment Values for Dioxins and Dibenzofurans (OEHHA, 1999)

Congener	Unit Risk $(\mu g/m^3)^{-1}$	Oral Slope Factor $(mg/kg/day)^{-1}$
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin	3.8×10^{1}	1.3×10^{5}
1,2,3,7,8-Pentachlorodibenzo-*p*-dioxin	1.9×10^{1}	6.5×10^{4}
1,2,3,4,7,8-Hexachlorodibenzo-*p*-dioxin	3.8	1.3×10^{4}
1,2,3,6,7,8-Hexachlorodibenzo-*p*-dioxin	3.8	1.3×10^{4}
1,2,3,7,8,9-Hexachlorodibenzo-*p*-dioxin	3.8	1.3×10^{4}
1,2,3,4,6,7,8-Heptachlorodibenzo-*p*-dioxin	3.8×10^{-1}	1.3×10^{3}
1,2,3,4,5,6,7,8-Octachlorodibenzo-*p*-dioxin	3.8×10^{-2}	1.3×10^{2}
2,3,7,8-Tetrachlorodibenzofuran	3.8	1.3×10^{4}
1,2,3,7,8-Pentachlorodibenzofuran	1.9	6.5×10^{3}
2,3,4,7,8-Pentachlorodibenzofuran	1.9×10^{1}	6.5×10^{4}
1,2,3,4,7,8-Hexachlorodibenzofuran	3.8	1.3×10^{4}
1,2,3,6,7,8-Hexachlorodibenzofuran	3.8	1.3×10^{4}
1,2,3,7,8,9-Hexachlorodibenzofuran	3.8	1.3×10^{4}
2,3,4,6,7,8-Hexachlorodibenzofuran	3.8	1.3×10^{4}
1,2,3,4,6,7,8-Heptachlorodibenzofuran	3.8×10^{-1}	1.3×10^{3}
1,2,3,4,7,8,9-Heptachlorodibenzofuran	3.8×10^{-1}	1.3×10^{3}
1,2,3,4,5,6,7,8-Octachlorodibenzofuran	3.8×10^{-2}	1.3×10^{2}

[Linearized multistage procedure (GLOBAL79), fitted to male mouse hepatic adenoma and carcinoma data (NTP, 1982), body weight scaling, cross-route extrapolation (CDHS, 1986).]

VI. Conclusions

There are numerous reports indicating that *in utero* and postnatal exposure to PCDDs, PCDFs and PCBs can result in significant toxicity in young animals and infants and children. OEHHA has therefore placed chlorinated dioxins and dibenzofurans in Tier 1. Because airborne exposures to PCBs are extremely low, OEHHA has placed the PCBs in Tier 2. The deleterious outcomes of exposure to dioxins and PCBs even at low exposure levels can persist long after birth. Immunological and neurobehavioral adverse effects in children perinatally exposed to dioxins and dioxin-like compounds and non-coplanar PCBs, respectively, have been reported to persist up to school age. Hormonal changes demonstrated in animals exposed to PCDD and PCDF, particularly on thyroid hormones, may be related to birth weight decrease, alterations in brain development, and delayed sexual maturation. In addition, there is some evidence from animal studies that the presence of the *Ah* receptor (through which dioxin toxicity is mediated) and cytochrome P450 CYP1A1 (which is greatly induced by dioxins) may vary during development. Thus, differential susceptibility to the dioxins and dioxin-like chemicals throughout development seems plausible. Interaction with *Ah* and steroid receptors are possible mechanisms for the observed effects.

Current background levels of human exposure to dioxins in particular are within the range at which various toxic responses have been observed in animals. Exposure *in utero* is the direct consequence of the accumulated maternal body burdens, and food chain contamination, including contamination of breast milk, are sources of continuing exposure. Regulatory efforts should focus on the identification and control of environmental airborne sources, which are currently the major origin of food chain contamination.

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Lead and Compounds

CAS Registry Number: 7439-92-1

I. Physical and Chemical Properties

Description	Bluish-gray metal
Molecular formula	Pb
Molecular weight	207.2
Air concentration conversion	Not applicable

II. Overview

Epidemiological studies have shown that at low lead levels neurodevelopmental effects occur in children (Bellinger *et al*., 1984a, b; McMichael *et al*., 1994; Needleman *et al*., 1996). While the CDC and the U.S. EPA have identified a blood lead concentration of 10 µg/dL as the level of concern, no threshold levels for adverse effects have been identified for inorganic lead. To correlate blood lead with air lead levels, OEHHA developed slope factors of 1.8 µg/dL $(\mu g/m^3)^{-1}$ for adults and 4.2 µg/dL $(\mu g/m^3)^{-1}$ for children. These slope factors reflect the higher rate of lead uptake in children compared to adults.

Data from the National Health and Nutrition Examination Survey (NHANES III) indicate that approximately 5.9% of children one to two years of age already have blood lead levels exceeding 10 µg/dl. At an ambient level of 0.011 $\mu g/m^3$, an additional 4,560 of the 1.2 million one and two year old children will move above the 10 µg/dL threshold compared to zero air lead (extrapolated from Table 2). At an air lead concentration equivalent to the current ambient air standard of 1.5 $\mu g/m^3$, more than 45% of children aged one and two would have blood lead levels above the CDC guideline of 10 µg/dL according to the aggregate model employed by OEHHA. Thus while the state-wide ambient lead levels in 1998 were substantially below this level (0.011 $\mu g/m^3$; CARB, 2000a), we are concerned that children exposed to locally high emissions of lead from stationary sources are at substantial risk for adverse health effects.

III. Principal Sources of Exposure

At current ambient lead concentrations, airborne lead is on average a minor contributor to a child's overall lead exposure. The major airborne exposures to environmental lead appear to be localized sources including deterioration of lead-based painted surfaces, lead that has accumulated in dust and soil, and near point sources of air emissions. Deposition of particulate lead on food and water is another indirect route of exposure to airborne lead. The ambient air lead concentration in California in 1998 was 0.011 $\mu g/m^3$ as reported by the California Ambient Toxics Monitoring Network (CARB, 2000a). Annual lead emissions from stationary sources in 1998 reported as part of the Air Toxics Hotspots program were 233,797 lbs/yr (CARB, 2000b)

IV. Potential for Differential Effects

A large and growing body of evidence indicates that children are more sensitive to the neurotoxic effects of lead than are adults. Because low levels of lead exposure have been associated with developmental delays and decrements in intelligence, short term memory, perception integration, visual motor functioning, and behavior in children (Bellinger *et al*., 1984a, b; Needleman *et al*., 1996; Needleman and Gatsonis, 1990), lead is considered a priority chemical for evaluation of potential differential effects on infants and children.

A. Summary of Key Human Studies

Lead has been associated with adverse reproductive and developmental outcomes. Maternal blood lead levels of 10 to 15 µg/dL have been associated with pre-term delivery and low birthweight. Based on data from NHANES, Schwartz *et al*. (1986) reported small but significant reductions in early childhood growth with no apparent threshold across the range of 5-35 µg/dL. Blood lead levels of 10 µg/dL and below have been associated with decreased hearing acuity (Schwartz and Otto, 1987). Pre- and postnatal lead exposure have been negatively associated with measures of intelligence, such as IQ in infants and young children (OEHHA, 1997). These effects were noted at blood levels of 10-20 µg/dL and lower. Indeed, Lanphear *et al*. (2000) used NHANES III data on children 6-16 years of age and found an inverse association between cognitive functions measured on the Wide Range Achievement Test-Revised (WRAT-R) (reading, math, block design, digit span) and blood lead concentration in the range of <2.5 to <10 µg/dL. Significant inverse correlations were found between blood lead levels as low as < 5.0 µg/dL and decrements in math ($p = 0.03$) and reading ($p < 0.001$) (Table 3).

Lead's neurodevelopmental effects, observed at low to moderate exposure levels (30 µg/dL and below) include: decreased intelligence, short-term memory loss, reading and spelling underachievement, impaired visual motor functioning, poor perception integration, disruptive classroom behavior, and impaired reaction time (Bellinger *et al*., 1984a, b; Needleman *et al*., 1996).

Needleman *et al*. (1979), using lead levels in the teeth of first and second graders, found a significant association between increased dentine lead level and decrements in intelligence quotient (IQ). The association was still evident when the children were tested 5 and 11 years later (Bellinger *et al*., 1984b; Needleman *et al*., 1990). Needleman and Gatsonis (1990) undertook a meta-analysis of the published IQ-blood lead studies. The results suggested that each 1 µg/dL increase of blood lead results in a 0.24 point decrease in IQ.

Large, long-term, prospective studies were conducted in Boston, Cincinnati, and Port Pirie, Australia. One of the larger cohorts studied, from Boston, Massachusetts, included several hundred middle and upper-middle class children followed from birth to 10 years of age (Bellinger *et al*., 1984a, 1985, 1991, 1992; Stiles and Bellinger, 1993). These studies have consistently found an association between blood lead and IQ among different age cohorts. Among the more important findings are those of older children since their IQs may be better characterized in the standardized tests. For example, at age 10 years, the children were examined again using the Wechsler Intelligence Scale for Children-Revised

(WISC-R), a measure of cognitive function, as well as the Kaufman Test of Educational Achievement (KTEA) (Bellinger *et al*., 1992; Stiles and Bellinger, 1993). Higher levels of blood lead at 24 months of age were associated with significantly lower scores on FSIQ (full scale IQ) and verbal IQ. The authors observed a decrease of almost 6 points on FSIQ and 9 points on KTEA Battery Composite score for each 10 µg/dL increase in lead level at 24 months of age. These estimates include adjustments for maternal age, race, marital status, number of residence changes and home environment. Visual inspection of the results and analysis of an earlier data set (Schwartz, 1993) suggest a continuous response across the entire range of blood lead levels and the lack of any threshold. Children from lower socioeconomic status appeared to be more sensitive to effects at lower blood lead concentrations. A more recent study found that lead impacted high school classroom behavior (Needleman *et al*., 1996). Therefore, evidence from these studies suggests that both prenatal and postnatal exposure may be associated with adverse impacts on cognitive performance with effects from postnatal exposure persisting to at least 10 years of age. The effects of later postnatal exposure seem to be strongest.

Other large prospective studies of lead and neurodevelopment involve cohorts of inner-city children in Cincinnati, Ohio and children in Port Pirie, South Australia (McMichael *et al*., 1994). Although there are differences in socioeconomics and demographics, experimental techniques, statistical models, and patterns of exposure among the three large cohort studies, their findings are consistent. Among the more relevant findings, changes in IQ at ages 6 to 10 are associated with blood lead measured either cumulatively over several years or in a single year. In addition, the magnitudes of effect per µg/dL are similar among both the prospective and cross-sectional studies. Many of these studies report mean blood concentrations near 10 µg/dL.

Several meta-analyses have been conducted of the prospective studies relating low-level blood lead exposures to neurodevelopmental effects in young children. Researchers with the CDC (Thacker *et al*., 1992) reviewed 35 prenatal and early postnatal prospective cohort studies and concluded that the weight of evidence suggested an inverse relationship between lead and the intelligence of children. Pocock *et al*. (1994) reviewed several types of study to quantify the relationship between lead and IQ, including the WISC-R. The analysis concluded that for postnatal blood lead, both the cross-sectional and prospective studies indicate a significant inverse association between blood lead and IQ. In addition, Schwartz (1994) conducted meta-analyses of both longitudinal and cross-sectional neurodevelopmental studies and concluded that the two study designs were capturing similar effects.

To provide an estimate and range of risk, OEHHA performed a simplified meta-analysis (Hedges and Olkin, 1985) of cohort studies conducted in children older than 5 years. This age group was used because it is likely to provide the most accurate assessment of the impact of blood lead. Estimates of the mean effect were derived by weighting each of the regression coefficients by the inverse of its variance. This generated a mean decrease of 0.33 IQ points per µg/dL blood lead with a 95% confidence interval of 0.32 to 0.34 (Table 1). Thus, this central estimate suggests that a 1 µg/dL increase in postnatal blood lead is associated, on average, with a 0.33 point decrease in FSIQ. This level is close to the range of estimates derived from the earlier meta-analyses, cited above. OEHHA used this value in its identification of lead and lead compounds as a toxic air contaminant (OEHHA, 1997).

In addition to the general effect magnitude, the overall population-level impact of IQ is also important to consider. Grant and Davis (1989) have demonstrated that, if one shifts down a normal distribution of IQ scores (mean=100, standard deviation=16) by 4 points, the number of children scoring 80 or below increases by 50%. The impact of such a shift applies across the entire distribution of scores, reducing the number of children who score above the norm as well as increasing the number scoring well below the norm. Thus, while a 4-point IQ loss might not have much impact on an individual child, this decrease could have a significant public health impact in a community. Similarly, a shift of 3.3 points would increase the percent of children scoring 80 or below from 10.56% to 14.74%, a 39.5% increase.

Based on current information it is not possible to identify a clear threshold blood lead level associated with adverse health effects in humans. The level of concern where human neurodevelopmental effects are seen in children exposed either prenatally or postnatally has been identified at 10 µg/dL. However, as the evidence continues to grow, it is possible that future levels of concern may drop below 10 µg/dL. The consistency of findings lends strong support to the conclusion that neurodevelopmental effects are causally associated with blood lead, and that the CDC level of concern of 10 µg/dL is a reasonable action level.

B. Summary of Key Animal Studies

Many of the neurological and cardiovascular effects noted in humans have also been observed in experimental animals. Lead in the diet of pregnant mice resulted in marked reductions in fertility and retarded growth of pups. Embryotoxicity and fetotoxicity have been observed and the toxic effects depend on the day of gestation when lead was administered (Domingo, 1994; Ronis *et al*., 1998). Early postnatal lead exposure in rats causes long-lasting cholinergic deficits (Bielarczyk *et al*., 1994; 1996). Prolonged deficits in learning behavior have been observed in monkeys exposed to lead *in utero* (Newland *et al*., 1996). The enormous number of studies of the toxicity of lead in animals supports that observed in humans (ATSDR, 1999; OEHHA, 1997). The neurotoxic effects seen following postnatal exposure of experimental animals include altered neurochemistry, histopathology, delay in development of reflexes, poor performance in learning tasks, and other behavioral measures.

V. Additional Information

A. Other Toxicity

Anemia in adults has been reported at blood lead levels of 40-60 µg/dL (Baker *et al*., 1979), and in children at 30-40 µg/dL (Schwartz *et al*., 1990). Increased blood pressure in adults has been reported at blood lead concentrations as low as 10 µg/dL.

B. Regulatory Background

The current California Ambient Air Quality Standard is 1.5 $\mu g/m^3$ averaged over one month. A cancer unit risk factor of 1.2 E-5 $(\mu g/m^3)^{-1}$ was calculated for lead by OEHHA (1997) from rat kidney tumor incidence data (Azar *et al*., 1973).

The CDC and others have concluded that blood lead concentrations at or near 10 µg/dL present a public-health risk to infants, children and pregnant women (CDC, 1991; U.S. EPA, 1990; NRC, 1993). OEHHA concurred with this level of concern in our TAC document (OEHHA, 1997). This blood lead level is the CDC level of concern for communities as a whole, as well as for individuals.

VI. Conclusions

The neurotoxicity of lead has been well-characterized, and children are a sensitive subpopulation for this effect. Although average ambient concentrations are low, there are many children with blood lead levels above 10 µg/dl, the level of concern for effects in children. In addition, there are significant emissions of lead from stationary sources reporting under the Air Toxics Hot Spots program, and near-source exposures will be higher than ambient averages. Additional exposure, either ambient or near-source, pushes more children above the level of concern. Furthermore, new studies indicate impacts in children at blood lead levels below 10 µg/dl. For all these reasons, therefore, OEHHA has placed lead in Tier 1.

Table 1. Regression Coefficients Indicating Change in IQ per 1.0 µg/dL Increase in Blood Lead for Crude and Adjusted Models in Prospective Studies at Later Ages

Crude Model:		
Study	Intelligence Measure	Coefficient (s.e.)
Boston[a]	WISC-R (FSIQ)	-0.71 (0.25)
Cincinnati[b]	WISC-R (FSIQ)	-0.58 (0.13)
Adjusted Model:		
Study	Intelligence Measure	Coefficient (s.e.)
Boston[c]	WISC-R (FSIQ)	-0.58 (0.21)
Cincinnati[d]	WISC-R (FSIQ)	-0.33 (0.14)
Port Pirie[e, f]	WISC-R (FSIQ)	-0.24 (0.12)
Meta-Analyses:		
Study	Intelligence Measure	Coefficient (s.e.)
Needleman and Gatsonis[g]	Varied	-0.25 (0.04)
Schwartz[h]	Varied	-0.24 (0.04)
OEHHA[I]	WISC-R (FSIQ)	-0.33

a) Blood lead at age 2, WISC-R at age 10, unadjusted analysis.
b) Mean blood lead at age 6, WISC-R at age 6.5.
c) Adjusted for HOME score at 10 years, maternal age, race, marital status, and number of residence changes prior to 57 months.
d) Adjusted for HOME score, maternal IQ, birth weight, birth length, gender, and cigarette consumption during pregnancy.
e) Averaged blood lead at ages 0-4, linearized using PbB mean of 19.59, WISC-R at age 7.
f) Adjusted for sex, parent's level of education, maternal age at delivery, parental smoking status, SES, HOME score, birth weight, birth order, feeding method, duration of breast feeding and whether or not child's parents were still living together.
g) Meta-analysis of six cross-sectional studies of blood lead and intelligence.
h) Meta-analysis using same six cross-sectional studies and one additional prospective study by Bellinger *et al*. (1991).
i) Meta-analysis using the three above "Adjusted Models."

Sources: *Stiles and Bellinger (1993); Bellinger et al. (1992), Dietrich et al. (1993), Baghurst et al. (1992), Needleman and Gatsonis (1990), Schwartz (1993), OEHHA (1997)*

Table 2. Association Between Ambient Air Lead and the Expected Percent of One and Two Year Old Children Equal to or Above 10 mg/dL Blood Lead.

	GM = 3.14 *** GSD = 2.1	GM = 3.14 GSD = 1.8	GM = 2.5 GSD = 1.8
Average Air Lead Concentration ($\mu g/m^3$)	Percent ≥ 10 µg/dL	Percent ≥ 10 µg/dL	Percent ≥ 10 µg/dL
0.011*	4.3	0.95	0.79
0.055**	5.9	2.4	1.0
0.10	6.9	3.0	1.3
0.25	10.6	5.7	3.0
0.50	17.6	12.0	8.0
1.00	32.2	28.1	22.9
1.50	45.6	44.4	39.6

* California ambient air lead concentration for 1999 (CARB 2000a).

** National average air lead concentration during the period of data collection of NHANES III, Phase 2. Calculation assumes that baseline non-air sources of lead exposure including paint, household dust, soil, pottery, and tap water are constant.

*** Geometric Mean (GM) = 3.14 and Geometric Standard Deviation (GSD) = 2.1 are taken from NHANES III, Phase2, and represent the blood lead distribution for children ages one and two (Pirkle *et al*., 1998).

Table 3. Adjusted Coefficients of Blood Lead Concentration from Multiple Regression Analyses of Cognitive Function Scores at Various Cut-offs for Children and Adolescents, 6 to 16 Years of Age, NHANES III. Coefficient (std errors) Represents the Decrement Associated with Each 1 µg/dL Increase in Blood Lead Concentration.

Variable	Total Sample n = 4,853	< 10 µg/dL n = 4,681	< 7.5 µg/dL n = 4,526	< 5.0 µg/dL n = 4,043	< 2.5 µg/dL n = 2,467
Math	-0.70 (0.17) (p < 0.001)	-0.89 (0.32) (p = 0.008)	-1.06 (0.39) (p = 0.01)	-1.06 (0.48) (p = 0.03)	-1.28 (0.98) (p = 0.20)
Reading	-0.99 (0.19) (p < 0.001)	-1.44 (0.30) (p < 0.001)	-1.53 (0.31) (p < 0.001)	-1.66 (0.36) (p < 0.001)	-1.71 (0.93) (p = 0.07)
Block Design	-0.10 (0.04) (p = 0.009)	-0.13 (0.06) (p = 0.03)	-0.11 (0.06) (p = 0.04)	-0.05 (0.07) (p = 0.45)	-0.08 (0.22) (p = 0.72)
Digit Span	-0.05 (0.22) (p = 0.04)	-0.08 (0.04) (p = 0.03)	-0.09 (0.05) (p = 0.11)	-0.09 (0.07) (p = 0.20)	-0.25 (0.17) (p = 0.17)

Adjusted for gender, ethnicity, poverty index, parent education and iron status.

Source: *Lanphear et al. (2000) Public Health Rep. 115;521-9.*

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Particulate Emissions from Diesel-Fueled Engines

I. Physical and Chemical Properties

Diesel exhaust is a complex mixture of thousands of gases, vapors, and fine particles. At least 40 components are listed by the Air Resources Board (ARB) as toxic air contaminants (see Table 1).

Table 1 - Toxic Air Contaminants Found in Diesel Exhaust

Acetaldehyde	Chlorobenzene	Methanol
Acrolein	Chromium compounds	Methyl ethyl ketone
Aniline	Cobalt compounds	Naphthalene
Antimony compounds	Cresol	Nickel
Arsenic	Cyanide compounds	4-Nitrobiphenyl
Benzene	Dibenzofuran	Phenol
Beryllium compounds	Dibutylphthalate	Phosphorus
Biphenyl	Ethyl benzene	*POM (including PAHs)
bis [2-Ethylhexyl]phthalate	Formaldehyde	Propionaldehyde
1,3-Butadiene	Hexane	Selenium compounds
Cadmium	Lead compounds	Styrene
Chlorinated dioxins and dibenzofurans	Manganese compounds	Toluene
Chlorine	Mercury compounds	Xylene isomers & mixtures

* Polycyclic Organic Matter (including Polycyclic Aromatic Hydrocarbons)

Particulate emissions from diesel-fueled engines (hereinafter referred to as diesel exhaust particulate matter or DEPM) was identified by the Air Resources Board as a Toxic Air Contaminant (TAC) in 1998. Typical diesel exhaust particles have mass-median aerodynamic diameters ranging from 0.1 to 0.25 micrometers (µm) (Groblicki and Begeman, 1979; Dolan et al., 1980; NRC, 1982; Williams, 1982). More than 90 percent of the particles are smaller than 1 µm (Pierson et al., 1983; Cal/EPA, 1998), and are mainly aggregates of spherical elemental carbon particles coated with organic and inorganic substances. The particles have a sponge-like structure and large surface area which attracts compounds of low volatility to the inside or surface of the particles. The primary organic compounds associated with the particles include aliphatic hydrocarbons, polycyclic aromatic hydrocarbons (PAHs), and PAH-derivatives (Zielinska, 1990). Methylated PAHs appear to be the most abundant PAH derivatives, and more than 50 nitro-PAHs have been identified in diesel exhaust (Cal/EPA, 1998; Part

A, Appendix III). Limited data indicate PAHs and PAH derivatives are about 1% by weight of diesel exhaust particles (CE-CERT, 1997).

II. Overview

There are several lines of evidence indicating that infants and children may be disproportionately affected by diesel exhaust particulate. This includes direct evidence, such as the enhancement of allergic response and some of the truck-traffic studies evaluating respiratory health, and indirect evidence, such as health effects associated with particulate matter.

- Diesel exhaust particulate matter (DEPM) enhances allergic responses in the nasal and airway epithelium. DEPM has been reported to facilitate the development of new allergy to aeroallergens. Accumulating evidence suggests that immunological memory and the development of an allergic phenotype are established within the first few years of life, so that exposures occurring in early childhood may determine whether a child will develop allergies or not. DEPM appears to potentiate allergic responses in susceptible individuals, though the impact of DEPM on the development of an allergic diathesis has not been established. Most childhood asthma, a condition characterized by chronic airway inflammation, is associated with allergy. Airway inflammation has been demonstrated in humans after controlled exposures to diesel exhaust and DEPM. Animal studies provide strong support for the enhancement of allergic responses, and airway inflammation seen in human studies. Experimental animals also develop airway hyperresponsiveness, the functional hallmark of asthma, after exposure to DEPM.

- Several epidemiological studies conducted in Europe have reported associations between truck traffic density (largely diesel-powered) and adverse respiratory symptoms, including atopy, in children living along busy roadways. In one study examining the relationships between general traffic density and respiratory health, children appeared to be more sensitive to traffic-related pollution (e.g., cars, trucks, and buses) than adults.

- Diesel exhaust particles contribute to ambient airborne particulate matter (PM, specifically measured as PM_{10} – particles 10 microns or smaller in mean aerodynamic diameter – and $PM_{2.5,}$ a subfraction of PM_{10}). PM_{10} and $PM_{2.5}$ have been associated in numerous studies with adverse respiratory health effects in children, including exacerbation of asthma, bronchitis, cough, and wheeze. As a constituent of PM, DEPM would be expected to contribute to PM-associated health effects, although the extent of the contribution has not been directly studied and would likely vary with concentration. To the extent that asthma is more common among children than adults and that, because age-related anatomic and physiological differences can render children's airways more quickly susceptible to obstruction, one may characterize PM-associated asthma attacks as disproportionately affecting children. In some circumstances in which DEPM constitutes a significant fraction of PM (e.g., on school buses), this observation could be extended to DEPM as well. Recent epidemiological studies have also provided some evidence that exposure to PM_{10} is associated with increased infant mortality, and DEPM may contribute to this effect.

- Diesel exhaust contains polycyclic aromatic hydrocarbons (PAHs). Immunosuppressive effects have been reported in animals as a result of PAH exposure, and have been found to be more severe and to occur at lower doses early in life, especially *in utero*. Analogous effects may also occur in exposed infants and children. In addition, human fetal exposure to PAHs results in elevated levels of PAH-DNA adducts in the neonate. In experimental animals, neonates are more sensitive to such genotoxicity than their mothers. Exposure early in life to genotoxic carcinogens, which are important constituents of diesel exhaust particles, may result in higher cancer risks than when exposure occurs later in life. This represents another mechanism by which diesel exhaust particles may disproportionately impact children. PAHs have also been associated with intrauterine growth retardation and low birth weight in animals and humans. PAHs in diesel exhaust may contribute to these effects.

- Children experience higher particle doses per lung surface area than adults in the same environmental setting (see Introduction Section III.A.1.). In addition, in young children hand-to-mouth activity results in greater consumption of household dust and dirt, which in an urban setting would be contaminated by settled traffic particulates, than occurs in adults. Thus, oral exposures to PAHs (and other constituents of diesel exhaust particles) are also higher in children than adults. Differential exposure patterns are one of the criteria evaluated to prioritize the TACs for listing under SB 25.

Key studies are discussed in Section IV below.

III. Principal Sources of Exposure

Diesel exhaust PM is emitted from diesel-fueled mobile sources (on-road vehicles and off-road mobile sources), stationary area sources, and stationary point sources. Based on the 1995 emissions inventory, on-road diesel vehicles contribute approximately 58 percent (15,680 tons per year (TPY)) of California's diesel exhaust PM. Other mobile sources contribute about 37 percent (9820 TPY), and stationary area and point sources contribute the remaining amount (5% or 1400 TPY). Stationary area sources of diesel exhaust include shipyards, warehouses, heavy equipment repair yards, and oil and gas production operations where exhaust emissions result from multiple locations within the site (Cal/EPA, 1998). The primary stationary sources that have reported emissions of diesel exhaust are heavy construction (except highway), electrical services, and crude petroleum and natural gas extraction (Cal/EPA, 1998).

The total emissions of DEPM from stationary sources in California reporting under the Air Toxics Hot Spots program are estimated to be at least 31,000 pounds per year (ARB, 1997).

Emissions of DEPM from on-road mobile sources in California are expected to decline by approximately 50 percent from 1990 until about 2010 as a result of mobile source standards and regulations adopted by the ARB through 1998. The expected reduction is mainly due to diesel vehicle emission and fuel regulations, even though both the number and vehicle miles traveled (VMT) of heavy-duty trucks are expected to increase during this period (Cal/EPA, 1998). Additional efforts to reduce

DEPM emissions include the replacement of diesel-powered heavy- and medium-duty vehicles with clean-fuel alternatives (such as those using methanol or compressed natural gas), and the use of particle traps and other pollution reduction technologies on new and existing diesel engines. In September 2000, ARB approved a Risk Reduction Plan to Reduce Particulate Matter Emissions from Diesel-fueled Engines and Vehicles (Diesel RRP). The Diesel RRP includes developing regulations that will substantially reduce emissions from diesel-fueled engines.

The ARB has conducted a preliminary estimation of diesel exhaust concentrations for California's 15 air basins using a PM-based exposure method. This method used the ARB emissions inventory's database for $PM_{10,}$ ambient PM_{10} monitoring network data, and the results from several studies, in which chemical speciation of ambient data was performed, along with receptor modeling techniques, to estimate statewide outdoor concentrations of diesel exhaust PM_{10}. The 1995 outdoor statewide population-weighted average diesel exhaust PM concentration is estimated to be 2.2 micrograms per cubic meter ($\mu g/m^3$). The basin-wide average diesel exhaust PM estimates ranged from 0.1 (in the Great Basin Valley) to 2.7 $\mu g/m^3$ (in the South Coast Air Basin) (Cal/EPA, 1998). Estimates for the year 2000 are slightly lower, ranging from 0.1 $\mu g/m^3$ in the Great Basin to 2.4 $\mu g/m^3$ in the South Coast basin, on average. These statewide or basin-wide averages underestimate exposures on a smaller scale near sources of diesel exhaust emissions. For example, the ARB measured DEPM next to a freeway, reporting up to 10 $\mu g/m^3$ five meters from the freeway fence line (Cal/EPA, 1998). In addition, ARB reports a range of 3.3 to 22.9 $\mu g/m^3$ of black carbon soot measured inside a vehicle in Los Angeles under varying driving conditions (ARB, 1998). The measure of soot was strongly influenced by the presence of diesel vehicles in front of the test vehicle. Examining the PM10 measurements taken inside the vehicle under the same driving circumstances, it appears that diesel particles, measured as soot, constitute between 10 and 30 % of the PM10 mass measured inside the vehicle.

IV. Potential for Differential Effects

Several lines of evidence suggest that DEPM has the potential to differentially impact infants and children. In particular, DEPM enhances allergic responses to aeroallergens and may facilitate the development of new allergies in susceptible individuals. This may have implications for the development and exacerbation of pediatric asthma, which is considered to be related to allergy in about 80% of cases (Pearce et al. 1998).

During the past few years substantial evidence has accumulated suggesting that the development of an allergic phenotype (atopy), which can render one susceptible to asthma, allergic rhinitis, and eczema, may be determined by exposures occurring in early childhood (Brehler et al. 1999). People who develop atopy have a genetic predisposition to produce IgE (immunoglobulin E) antibodies against inhaled or ingested environmental antigens. Whether atopy actually develops appears to depend, at least in part, on early-life exposures to certain allergens, particularly those found indoors. Development of sensitivity to aeroallergens is a complex process requiring an orchestrated coordination of antigen-presenting cells (macrophages or dendritic cells in the airways), T-lymphocytes and B-lymphocytes, which produce IgE antibodies against specific antigens. IgE-mediated hypersensitivity to aeroallergens is a key ingredient in the evolution of the chronic inflammation observed in asthma. While the role of

DEPM in the development of atopy is undefined, numerous studies suggest that DEPM exposure enhances IgE-mediated and other responses to aeroallergens in atopic individuals, including worsening symptoms of allergic rhinitis. In addition, to the extent that DEPM exposure may facilitate the development of allergic sensitization, it may enlarge the spectrum of aeroallergens that may cause symptoms in any given individual with allergic rhinitis or asthma.

DEPM is a variable component of ambient PM_{10}, which has been shown in epidemiological studies to exacerbate asthma, and is associated with elevated risk of cough, chronic bronchitis and wheeze. Traffic studies also contribute to the evidence that DEPM affects respiratory function, and one study examining impacts of traffic-related pollution on respiratory function indicates that children were impacted to a greater degree than adults in the same households. In addition, PM_{10} is associated with increased infant mortality in a number of studies, including in areas where diesel exhaust is a major component of urban PM_{10}. The lines of evidence are discussed in the following sections.

A. Summary of Key Human Studies

a) Immunological and Respiratory Effects of Diesel Exhaust PM

There is growing scientific evidence that diesel PM enhances allergic responses to pollen and other allergens. Enhancement of allergic responses in the nasal and airway epithelium may increase rates of allergic rhinitis and possibly asthma in children. Japanese researchers evaluating the prevalence of Japanese cedar allergy found that pollinosis (as manifested by symptoms of allergic rhinitis isolated to the cedar pollen season, and confirmed in individuals seeking medical care by allergy skin and blood tests) was higher among residents living near heavily trafficked roads lined with cedars than in residents living in cedar forests without traffic congestion (Ishizaki 1987). Though these results are striking, they should be considered suggestive, as the researchers didn't control for some important confounders. A number of studies indicate that DEPM can induce immunological allergic reactions as well as localized inflammatory responses in humans (Diaz-Sanchez et al., 1994, 1996, 1997, 2000; Terada et al., 1997, Takenaka et al., 1995). Diaz-Sanchez et al. (1997, 1999) found that in allergic human subjects, nasal IgE antibody response and allergic inflammation were enhanced by co-exposure to diesel particulate matter. They also demonstrated that DEPM can induce sensitization in atopic individuals to a new allergen. Both *in vivo* and *in vitro* studies exploring the mechanisms of enhanced response to allergen have been conducted. Casillas et al (1999) suggest that DEPM may enhance allergic inflammation through increased formation of reactive oxygen species by DEPM-associated PAHs engulfed by phagocytic cells. This in turn may activate intracellular signaling pathways that result in the production and secretion of biochemical messengers (chemokines and cytokines) involved in allergic inflammation. Key studies demonstrating the enhancement of allergic responses by diesel exhaust are discussed below.

(1) Intranasal instillation studies

Diaz-Sanchez et al. (1997) challenged thirteen subjects who had a positive skin test to short ragweed intranasally with ragweed (RW) pollen alone or with diesel exhaust particulate matter (DEPM) (0.3 mg). Nasal lavage fluids were examined for immunoglobulin (Ig) secreting cells, levels of various Igs and

cytokine profile. The number of IgE-secreting cells was significantly elevated in nasal washes (day 4) following exposure to RW alone or RW+DEPM, while the number of IgA-secreting cells was not. Challenge with RW plus DEPM significantly increased the levels of total and RW-specific IgG4 and of RW-specific IgE in nasal lavage fluids compared to RW alone. IgE represents antibodies associated with allergy, while the gene coding for IgG4 is located in close proximity to that coding for IgE; thus, changes in levels of these Igs often occur in concert. On day one, RW-specific IgE levels were 6 times higher in RW + DEPM subjects (62.7 $\pm$ 55.1 Units/ml) compared with RW alone (10.2 $\pm$ 10.2 U/ml). By day 4 the RW-specific levels were 16 times higher in the RW + DEPM subjects (up to 180 U/ml) than in the RW-alone lavage fluids (up to 12 U/ml). These differences were highly significant ($p < 0.005$ and $p < 0.001$ for days 1 and 4 post-challenge, respectively). Levels of mRNA 18 hours post-challenge for the Th-2-type cytokines IL-4, IL-5, IL-6 and for IL-10 and IL-13 were significantly greater ($p < 0.01$ to $p < 0.05$) in cells recovered from the nasal lavage fluid of those challenged with RW + DEPM relative to RW alone. ("Th-2" refers to a subset of white blood cells [lymphocytes] associated with allergic-type responses, in contrast to Th-1 lymphocytes considered to be associated with nonallergic immune responses.) The authors note that the sentinel Th-1-type cytokine interferon gamma (IFN- γ) was significantly decreased by exposure to DEPM +RW but not by RW alone. These results indicate that the activation of Th-2 cells by allergen exposure can be markedly augmented by exposure to DEPM, with striking effects in the production of antigen-specific IgE, which may in turn increase the likelihood of respiratory symptoms. In another study, these investigators found that the messenger RNA (mRNA) for pro-inflammatory cytokines in macrophages and nasal epithelial cells were significantly increased in human volunteers following intranasal instillation of diesel particles (Diaz-Sanchez et al., 1996).

Nasal challenge with DEPM induced sensitization to a neoallergen in atopic individuals (Diaz-Sanchez et al., 1999). In this study, ten atopic (i.e., allergic) subjects were administered a solution of keyhole limpet hemocyanin (KLH) or KLH + DEPM intranasally three times at two-week intervals. IgE anti-KLH antibodies were present in the nasal lavage fluids at 28 and 32 days for those administered KLH + DEPM, but not in the KLH alone group. Anti-KLH IgG4 levels in lavage fluid were higher in the KLH + DEPM group relative to the KLH group ($p < 0.01$). There was no change in IFN-γ pre-challenge and post-challenge in either group, but challenge with KLH + DEPM greatly increased the levels of IL-4 (a cytokine that has a major influence on the synthesis of IgE by B-lymphocytes) in the nasal lavage fluid, whereas there was no increase in the KLH-only group. This study demonstrates that DEPM can induce sensitization in atopic individuals to a new allergen (as measured by production of KLH-specific IgE) under conditions where administration of the allergen alone does not induce sensitization. Production of IgE antibodies in response to inhaled antigens is the hallmark of sensitization. The authors note that exposure to airborne DEPM may cause atopic individuals to become sensitized to materials to which they would not otherwise respond.

In addition, DEPM administration has been shown to potentiate allergic rhinitis symptoms in volunteers (Diaz-Sanchez et al., 2000). In this study, 11 volunteers allergic to dust mite were administered dust mite allergen intranasally to stimulate an allergic response. A single-blind, placebo-controlled cross-over study design was implemented with subjects randomly receiving 0.3 mg DEPM, 0.3 mg carbon black (CB), or saline intranasally at intervals separated by at least 6 weeks. Immediately following

DEPM, CB, or saline administration, a dust mite allergen challenge was performed and the dose necessary to obtain a pre-designated symptom score was determined. (Symptoms included sneezing, nasal itching and congestion, and rhinorrhea.) Co-administration of DEPM markedly increased the symptom scores after nasal challenge. Only about 1/5 the amount of allergen was needed to provoke the same symptom score when given with DEPM as compared with allergen alone. Carbon black co-administration with allergen did not increase the symptom score relative to saline plus allergen. In a second phase of the study, subjects were given allergen alone, allergen plus saline, allergen plus DEPM, or allergen plus CB intranasally. Histamine (a vasoactive amine associated with allergic symptoms and bronchoconstriction) in nasal lavage supernatant was measured. Higher histamine levels were measured in the supernatant of the nasal lavage fluid when DEPM was co-administered with allergen compared to allergen administration with saline or CB. In addition, the investigators showed potentiation of histamine release by DEPM extract in a murine mast cell line. This investigation demonstrates that DEPM can affect mast cells (containing pre-formed histamine) to augment immediate responses to allergen, and can elicit symptoms of allergic rhinitis in circumstances in which exposure to allergen alone would not.

(2) In vitro cell studies

In experiments *in vitro*, extracts of PAHs from DEPMs (PAH-DEPM) enhanced the production of IgE in purified human B cells prepared from blood mononuclear or tonsil cells (Takenaka et al., 1995). Terada et al. (1997) found that DEPM extracts enhanced human eosinophil adhesion to nasal epithelial cells, an event necessary for the infiltration of inflammatory cells from the circulating pool in the blood to tissues, and caused eosinophil degranulation, which plays an important role in nasal allergy. (Eosinophils are a class of white blood cells involved in allergic inflammation and asthma, as well as other conditions.) These same investigators showed that incubation of human nasal epithelial cells and mucosal microvascular endothelial cells with DEPM extract caused an increase in the expression of mRNA for the histamine-1 receptor (HR-1), and increased the amounts of the cytokines interleukin-8 (IL-8 – a chemoattractant or chemokine for neutrophils, white blood cells involved in acute inflammatory reactions) and granulocyte macrophage colony stimulating factor (GM-CSF – a growth factor promoting the development of two types of immune/inflammatory cells) in the incubation medium (Terada et al., 1999). In this study the epithelial and endothelial cells were incubated in the presence or absence of DEPM extract, 0.5 to 50 ng/ml, for 6 to 24 hours. HR-1 mRNA expression was significantly increased at 50 ng DEPM extract/ml relative to controls. Histamine-induced secretion of IL-8 and GM-CSF into the culture medium was significantly increased at 50 ng extract/ml relative to controls. The authors conclude that DEPM enhances inflammation by upregulation of the HR-1 receptor as well as increasing the production of the inflammatory cytokine IL-8 and GM-CSF.

In a similar vein, Bayram et al.(1998) observed that exposure of human bronchial epithelial cells *in vitro* results in the release of the pro-inflammatory cytokine IL-8, GM-CSF, and intercellular adhesion factor molecule-1 (ICAM-1) from these cells, which could influence the development of airway disease. The study utilized cultured bronchial cells recovered from lung cancer patients (from cancer-free areas), and exposed the cells to varying concentrations of suspended DEPM (50 to 100 µg/ml). Analysis of the cell culture medium after 24-hour incubation indicated a dose-dependent release of IL-8 ($p < 0.05$), which was significantly greater than when cells were incubated in the presence of similar concentrations of

activated charcoal or controls. Both ICAM-1 and GM-CSF were increased but only at the middle dose (50 µg/ml). The increase at the high dose or 100 g/ml DEPM (100 µg/ml DEPM) was not significant at $p < 0.05$. Upregulation of ICAM-1 has been observed in human bronchial epithelial cells in culture by others (Takizawa et al., 2000). In another in vitro study, an organic extract of DEPM was evaluated for its ability to modulate production of chemotactic cytokines (chemokines) by peripheral blood mononuclear cells from healthy human donors (Fahy et al., 1999). Polycyclic aromatic hydrocarbons extracted from DEPM were incubated with PBMC at concentrations ranging from 0.5 to 50 ng/ml up to 48 hours. Concentrations of chemokines (IL-8, RANTES [**R**egulated on **A**ctivation, **N**ormal **T**-cell **E**xpressed and **S**ecreted], and MCP-1) were quantified in the supernatant, and mRNA for the chemokines was evaluated in the cells. Dose-dependent increases in IL-8 and RANTES were observed from 2 to 48 hours; while MCP-1 decreased. The same pattern of mRNA expression (increased for IL-8 and RANTES, decreased for MCP-1) was observed in the cells measured at 24 hrs, indicating that the DEPM-PAH extracts act at the transcriptional level. The authors note that this modulation of chemokine production may favor preferential recruitment of neutrophils (by IL-8), eosinophils and memory T cells (by RANTES) but not monocytes and macrophages (by MCP-1) upon exposure to diesel exhaust.

All these studies *in vitro* support the inflammatory reactions and modulation of inflammatory cells and cytokines observed after intranasal instillation of DEPM and inhalation of whole DE, and provide some potential mechanisms by which DEPM may exacerbate allergies and asthma.

(3) Exposure to diesel exhaust via inhalation

Exposures of humans to whole diesel exhaust via inhalation have also demonstrated inflammatory responses. Salvi et al. (1999) exposed 15 healthy nonatopic volunteers to whole diesel exhaust (0.3 mg/m^3 as particulate) or air on two different occasions for one hour with 15 minute exercise periods. Lung function measurements including peak expiratory flow rate (PEFR), forced vital capacity (FVC), forced expiratory flow in 1 second (FEV1), and forced expiratory flow mid range (FEF25-75%) were taken immediately before and after exposures. Endobronchial biopsy, bronchial wash (BW), and bronchoalveolar lavage (BAL) were also performed 6 hours after exposure. BW and BAL fluids were centrifuged and a differential cell count conducted on the pellet. The supernatant was analyzed for albumin, protein, lactate dehydrogenase (LDH), IL-8, ICAM-1, fibronectin, methylhistamine, and other indicators of injury. There were no differences in lung function measurements between DE-exposed and control subjects. However, a marked inflammatory response was observed in DE-exposed subjects manifested as increased numbers of neutrophils in the BW, and increases in B- lymphocytes, methylhistamine levels, and fibronectin in the BAL, as well as increases in the number of neutrophils in the submucosa (3-fold; $p < 0.003$) and epithelium (4-fold, $p < 0.03$) of the bronchial biopsies. There were also statistically significant increases in mast cell numbers ($p < 0.002$), CD4+ and CD8+ cells (T lymphocytes) ($p < 0.04$) in the submucosa and/or epithelium of the bronchi of DE-exposed subjects. Marked increases in immunostaining for the endothelial adhesion molecules ICAM-1 and VCAM-1, as well as increased numbers of cells expressing ligand for ICAM-1, VCAM-1 ($p < 0.007$ and $p < 0.03$, respectively) and LFA-1 (a leukocyte adhesion molecule) ($p < 0.001$) were observed in the epithelium and submucosa. These adhesion molecules facilitate transfer of circulating inflammatory cells into

tissues. Peripheral blood samples 6 hr after DE exposure showed a significant increase in neutrophils and platelets following DE exposure. This study shows clearly that diesel exhaust exposure results in an acute inflammatory response and that lung function tests, which have traditionally been used to assess responses to environmental insults such as diesel exhaust exposure, may still be normal in the presence of lung injury and inflammation. In another study by the same investigators (Rudell et al., 1999), bronchoalveolar lavage was performed on 10 healthy subjects following a 1-hour exposure to air, diesel exhaust or filtered diesel exhaust. Each subject received each exposure in random order at 3-week intervals. Differential cell counts were performed on the BAL fluid and phagocytosis was measured. As in the previous study, diesel exhaust exposure resulted in a recruitment of inflammatory cells into the airway. The particle filter did not reduce the effect observed, although it should be noted that the particle filtering efficiency was only 50%, and as such may not have been sufficient to separate effects of particles from vapor phase constituents.

Nightingale et al. (2000) also studied airway responses in healthy volunteers following a 2-hr exposure to 0.2 mg DEPM/m^3. As in the study by Salvi et al (1999), there were no measurable changes in lung function in DEPM-exposed subjects, but an inflammatory response was observed, as measured by increases in neutrophil count and myeloperoxidase levels in induced sputum and by increased exhaled CO levels (a measure of oxidative stress).

(4) Epidemiological studies – traffic-related respiratory effects

Several environmental epidemiological studies provide evidence supporting the notion that exposure to diesel exhaust may contribute to the expression of allergic disease, including asthma. Reports also suggest associations between exposure to truck traffic, which consisted largely of heavy-duty diesel trucks, and a variety of adverse respiratory outcomes, including symptoms of asthma and allergic rhinitis. These endpoints are particularly important to children's health, and the studies summarized below specifically included health evaluations of exposed children.

There are observations in children of atopic sensitization (Kramer et al., 2000), asthma and allergic rhinitis (Edwards et al. 1994, Duhme et al., 1996), decreased lung function (Wjst et al., 1993) and various chronic respiratory symptoms (van Vliet et al., 1997, Oosterlee et al., 1996) associated with increased traffic density. In the study by Duhme et al. (1996), truck traffic specifically was associated with wheezing (OR 2.47, 95% CI 1.74 – 3.52 for constant truck traffic on street of residence relative to no truck traffic) and allergic rhinitis (OR 1.96, 95% CI 1.40 – 2.76 for constant truck traffic) in children aged 12 - 15. The more recent report by Ciccone et al. (1998) also found associations of adverse respiratory health impacts in children with heavy vehicular (diesel powered) traffic. In this study, children living in metropolitan centers along streets with frequent truck traffic (in relation to no truck traffic) showed significantly increased risks for bronchiolitis (OR 1.74, 95% CI 1.09 – 2.77), pneumonia (OR 1.84, 95% CI 1.27 – 2.65) and for multiple episodes of bronchitis (OR 1.69, 95% CI 1.24 – 2.30) early in life (0-2 years). There were also increased risks of current respiratory symptoms, including wheeze and cough, in children living along streets with frequent truck traffic compared with those children living along streets with the no truck traffic.

Studies from Holland indicate that diesel particulate is associated with respiratory impacts (Brunekreef et al., 1997; van Vliet et al., 1997). These investigators not only used traffic counts, but also measured carbonaceous particulate material in indoor air in schools, and found a strong association of decreased lung function with black particle exposure and truck traffic density (Table 2). In this cross-sectional study, over 1000 children ages 7-12 years living in six areas in the Netherlands with homes located near major roadways (carrying between 80,000 and 152,000 total vehicles per day, including between about 8,100 to 17,600 trucks/day) were evaluated with respiratory questionnaires and lung function tests. Measures used to assess exposure to traffic-related pollutants included: 1) distance of the children's homes and schools from major roadways; 2) traffic density counts; 3) and indoor measurements of PM_{10} (with conversion to Black Smoke (BS), representing largely soot particles) and NO_2. Multiple linear regression was used to investigate associations between lung function and pollution measures, adjusting for age, height, weight, gender, parental respiratory symptoms, smoking in the home, pets, dampness or mold in home, ethnicity, number of persons in the home, gas cooking, gas-fired unvented heaters, and socioeconomic status (parental education). Truck traffic density was related to forced expiratory volume in 1 second (FEV1), and peak expiratory flow (PEF) for children living within 1000 m of a major roadway. Decrements in lung function were strongly associated with truck traffic density rather than with automobile traffic density. The effect of truck traffic density on FEV1 increased when the analysis was restricted to children living within 300 m of a roadway, enhancing the likelihood of a causal association. For girls, the effects were stronger than for boys. A clear dose-response relationship was observed between truck traffic density and forced expiratory volume in one second (FEV_1) in children living <300 meters from a roadway (see Figure 1 in Brunekreef et al, 1997).

Respiratory symptoms from the same cross-sectional study were reported by van Vliet et al. (1997), based on parental responses to questionnaires for 1068 children. More symptoms were reported for children living within 100 m of the roadway than for those living within 1000 m of a major roadway. BS concentrations decreased with increasing distance from the freeway. The strongest associations between pollution and respiratory symptoms were observed in girls: chronic cough and wheeze were significantly elevated for girls living within 100 m of a major roadway relative to those living between 100 and 1000 m away (OR = 2.45, 95% CI = 1.16-5.16 for chronic cough, and OR=3.05, 95% CI = 1.11-8.41 for wheeze). Van Vliet et al. (1997) also reported substantially elevated point estimates for odds ratios relating density of truck traffic and BS concentrations measured in schools to these symptoms as well as to asthma attacks, rhinitis, and bronchitis in the past year (ORs ranged from 1.93 to 4.34 in girls only, but were not statistically significant).

Table 2. Lung function decrements as percentage changes (95% CI in parentheses) in forced expiratory volume in 1 second (FEV1) and peak expiratory flow rate (PEFR) in children living near major roadways by measures of vehicle-related air pollution

Population and pollution indicator	FEV1 per 10,000 trucks	FEV1per 10 mg BS/m^3	PEFR per 10,000 trucks	PEFR per 10 mg BS/ m^3
All children living within 1000 m of roadway	-2.5 (-5.3, 0.4)	-1.2 (-3.5, 1.5)	-8.0 (-12.2, -3.6)	-2.6 (-6.7, 1.6)
All children living within 300 m of roadway	-4.1 (-7.9, -0.1)	-3.7 (-7.2, -0.2)	-7.7 (-13.4, -1.7)	-5.8 (-11.1, -0.2)
Boys living within 300 m or roadway	-1.8 (-7.5, 4.2)	1.9 (-3.8, 8.0)	-9.7 (-17.8, -0.7)	-2.5 (-11.2, 7.0)
Girls living within 300 m of roadway	-6.2 (-11.5, -0.6)	-8.3 (-13.0, -3.4)	-6.1 (-13.9, 2.4)	-7.8 (-14.7, -0.3)

Source: Brunekreef et al (1997)

Oosterlee et al. (1996) present data indicating that children may be disproportionately impacted by traffic-related pollutants. In this study, the CAR ("**C**alculation of **A**ir pollution by **R**oad traffic") model was used to estimate pollution levels for each street in the city of Haarlem, the Netherlands. The model accounts for kind of vehicles (car, bus, truck, gas-fueled, diesel-fueled), mean traffic density, emissions rates for each type of vehicle, local topography including buildings, background concentrations, and meteorology to estimate air pollution levels. Validation measures showed a mean error of 6% (SD=9%) for peak NO_2 concentrations. Respiratory symptoms in children (up to 15 yrs of age) living along streets with estimated traffic density of 10,000 to 30,000 vehicles/day (estimated NO_2 concentrations of 62 to 80 ppb) were compared to those of children living in the same neighborhoods on streets with little traffic. Symptoms were ascertained by questionnaires completed by the subjects' parents, who provided information about chronic cough, episodes of cough with phlegm, wheeze, dyspnea, attacks of dyspnea with wheeze, doctor-diagnosed asthma, medication use, allergy, school absenteeism, doctor visits, for 106 children living on busy streets and 185 "control" children. Adjusted odds ratios for girls were significant for wheeze ever, wheeze in the past year, dyspnea with wheeze ever, dyspnea with wheeze in the past year, and respiratory medication use (Table 3). In contrast to the strong effects in girls, none of the symptoms appeared elevated in boys. This is interesting and not easily explained, but consistent with reports by Brunekreef et al. (1997) and van Vliet et al. (1997). Current asthma medication use was significant for all children, boys and girls combined, living along busy streets (OR 2.2 (95% CI = 1.1-4.6)), but not for adults. The authors conclude that the apparent lack of effect

in adults relative to the children indicates a difference in susceptibility to ambient traffic related air pollution between children and adults. While this study did not directly measure DEPM, diesel exhaust is a major component of traffic-related pollution in the Netherlands, including NO_2, the pollutant modeled in this study.

Table 3. Respiratory symptoms in girls (0-15 years of age) living along busy streets (relative to girls living on streets with little traffic).

Symptom	Adjusted OR (95% CI) for girls
Wheeze-ever	4.4 (1.4-13.6)
Wheeze in past year	5.3 (1.1-25.0)
Dyspnea w/ wheeze ever	4.8 (1.3-17.7)
Dyspnea w/ wheeze in past year	15.8 (1.4-17.7)
Respiratory medication	2.9 (1.1-7.9)
Doctor diagnosed allergy	2.5 (0.7-9.2)

Adjusted for age, maternal education, passive smoking, presence of unvented hot water heater, heating type, home humidity, pets, and crowding.
Source: Oosterlee et al., 1996.

b) Epidemiological studies - General Ambient PM_{10} Effects

Diesel exhaust particulate contributes to ambient particulate matter 10 μm in diameter or less (PM_{10}), especially in areas with heavy traffic. The extent of the contribution of diesel exhaust particulate to PM_{10} health effects has not been directly studied. However, health effects of PM_{10} may be, in part, attributable to the diesel exhaust particulate component. Exposure to PM_{10} in ambient air (to which diesel exhaust is a significant contributor in many urban areas) has been associated with a number of adverse effects on the respiratory system, particularly cough, phlegm and other symptoms of irritation, and decline in lung function. Reported adverse effects include both measures of morbidity (symptoms, increased hospital admissions), and increased mortality. These effects are particularly seen for asthmatics and those with other existing respiratory or cardiovascular disease, and the elderly (Thurston, 2000 as included in Cal/EPA, 2000). Although the contribution of diesel exhaust particulate to the statewide average PM_{10} is relatively small in California (5% or so), it is a more significant portion of PM_{10} and $PM_{2.5}$ in urban locations. In addition, in some cities outside the U.S. that have been studied for effects of PM_{10}, diesel is the major contributor to total PM_{10}. Ostro et al. (1996), in a mortality-PM time-series study in Santiago, Chile, cites Sandoval et al. (1985) which indicates approximately 74% of the PM_{10} in Santiago is from diesel sources. Diesel vehicles account for about 87% of black smoke emissions in London (QUARG, 1993). Effects of PM_{10} are quantitatively similar across different cities

throughout the world with varying constituents of PM_{10}. Thus, cardiovascular and respiratory morbidity and mortality that have been repeatedly linked to ambient PM appear to be an effect of small particles in general. There is no reason to expect a priori that particles from a diesel-fueled source would be selectively less toxic than those from other combustion sources which contribute to ambient PM. There certainly is not a protective effect for morbidity or mortality in the cities where diesel exhaust contributes a substantial portion of the PM_{10}.

(1) Effects of ambient PM_{10} pollution on infants and children

Numerous investigators have reported that respiratory symptoms in children can be exacerbated by exposure to airborne particles and sulfates. These effects have greater health implications in children with asthma, and can lead to an increased incidence of asthma attacks. Since the prevalence of asthma is higher among children than among adults (CDC, 1996a,b), PM-related exacerbations may put proportionately more children at higher risk. Diesel exhaust particles contribute to ambient air $PM_{10,}$ particularly in urban areas with heavy vehicular traffic. In the Thurston et al. (1997) study of children with asthma at a summer camp, prescription medication use, as prescribed in physician-verified cases of asthma exacerbation, was a metric of severe air pollution effects associated with sulfate (a measure of particle pollution). Ostro et al. (1995) in a paper published in a peer-reviewed proceedings, found significant associations between PM_{10} and asthma symptoms in 7-12 year old Los Angeles residents. This finding was confirmed and strengthened in a subsequent study (Ostro et al., 2001). Delfino et al. (1998) found significant associations between asthma symptoms in children 9-17 years of age with both 1-hour and 8-hour PM_{10} measurements. In an earlier study, Delfino and colleagues found a significant association between PM_{10} and bronchodilator use in asthmatic children (Delfino et al. 1997). In a re-analysis of published studies, Hoek et al. (1998) noted significant reductions in peak expiratory flow rates in children associated with PM_{10} measurements. Pulmonary function has also been associated with measurements of particulate matter 5 microns or smaller in a study in school children (Linn et al., 1996). In a large study in 12 Southern California communities, asthma, bronchitis, cough, wheeze and lung function decrements were associated with PM_{10} pollution, as was deficits in lung function growth in children, though because of pollutant covariation, these effects could not be ascribed exclusively to PM (Peters et al., 1999a,b; Gauderman et al., 2000). In any case, the published literature is replete with findings that PM air pollution can adversely affect children's respiratory health.

Burnett et al. (1994) note that the largest percent increase in hospital admissions associated with PM_{10} is in the 0-1 year old age group of children in Ontario, suggesting that infants may be especially susceptible (see also Introduction section III). Infant and child mortality has also been associated with acute exposures to PM_{10} pollution in time-series studies in Mexico (Loomis et al., 1999), Delhi (Cropper et al., 1997) and Bangkok (Ostro et al., 1998) and in a case-control study in the Czech Republic (Bobak and Leon, 1999). The time-series study design allows the investigator to account for the potential effects of other co-varying pollutants on infant and child mortality, and these studies were able to ascribe the effects to PM_{10} pollution. Other cross-sectional studies have linked infant mortality in the U.S. (Woodruff et al., 1997) and the Czech Republic (Bobak and Leon, 1992) to longer-term exposures to PM, although the influence of other pollutants in these cross-sectional studies can't be easily characterized and thus confounding may exist.

Children are also exposed to more PM per unit body weight and per lung surface area than adults by virtue of their higher breathing rates (see Section III in the Introduction). The studies showing infant and child morbidity and mortality, combined with higher doses due to higher breathing rates of children, as well as higher rates of asthma in children, indicate that children may be disproportionately impacted by PM relative to adults. This conclusion was supported by the review of our prioritization of the Criteria Air Pollutants (CAP) under SB 25 by the Air Quality Advisory Committee. Indeed, PM_{10} was determined to be the highest priority CAP for re-review of the standard (Cal/EPA, 2000).

c) Carcinogenicity

The epidemiological studies of the relationship between human exposure to diesel exhaust and lung cancer involve occupational situations that necessarily involve adults but not children, so direct evidence of differential effects on infants or children is not available from this source. There are a considerable number of studies of adult human exposure to diesel exhaust in an occupational setting and associations with cancer. Lipsett and Campleman (1999) identified 39 independent estimates of relative risk among 30 studies of diesel exhaust and lung cancer, while HEI (1995) summarized more than 35 epidemiologic studies (16 cohort and 19 case-control) of occupational exposure to diesel emissions. Meta-analyses from the existing studies support the observed association between occupational exposure to diesel exhaust and increased risk of lung cancer. These analyses also found that the relationship between occupational diesel exhaust exposure and lung cancer could not be attributed to potential confounding by cigarette smoking (Bhatia et al., 1998, HEI, 1995; Lipsett and Campleman, 1999). The available studies were reviewed in the TAC document *Health Risk Assessment For Diesel Exhaust* (OEHHA, 1998).

It has been noted in the introductory chapter of this report that cancer risks may generally be more severe when exposure occurs *in utero* or early in postnatal life. Additionally, it is known that diesel exhaust particulate contains PAHs and PAH derivatives. These are suspected of being substantial contributors to the observed carcinogenicity of diesel exhaust. In a separate section of this report, evidence is presented that benzo[*a*]pyrene and other PAHs are not only carcinogenic to adults, but present a significantly greater hazard of carcinogenicity to the fetus and to infants and children. This evidence includes data on DNA adducts following human exposure to PAHs. In addition, developmental toxicity has been observed following PAH exposure *in utero*, including reduced birth weight and dysmorphogenesis. The evidence in this case also is primarily for exposure to PAHs in tobacco smoke and ambient or indoor air pollution. Thus, the presence of PAHs in diesel exhaust poses another concern for disproportionate impacts on infants and children. The reader is referred to the summary of differential toxicity for benzo[*a*]pyrene and PAHs in this report for further details of these effects.

B. Summary of Key Animal Studies.

Animal studies support the enhancement of allergic and inflammatory responses seen in human studies. These studies provide evidence that diesel exhaust can potentiate responses to aeroallergens, which

could facilitate the exacerbation and possibly the evolution of allergic diseases, including asthma and allergic rhinitis.

a) Immunological Toxicity

Studies in mice have shown that intranasal or intraperitoneal co-administration of DEPM and allergen and inhalation of DEPM enhances IgE production in response to Japanese cedar pollen and ovalbumin (Muranaka et al., 1986; Takafuji et al., 1987; Takano et al., 1997; Miyabara et al., 1998). These studies were initially undertaken because of an increase in prevalence of allergic rhinitis caused by pollens in Japan that corresponded to an increase in the number of diesel-fueled cars in Japan, and the higher rates in polluted urban areas relative to nonpolluted areas (Muranaka et al., 1986; Takafuji et al., 1987; Miyamoto, 1997). DEPM can enhance antigen-induced airway inflammation in a mouse model (Takano et al., 1997). Male ICR mice were treated intranasally with either ovalbumin (OVA), DEPM, OVA + DEPM, or vehicle weekly over 6- or 9-week periods. Bronchoalveolar lavage (BAL) and differential cell counts of the lavage fluid, and lung histology were conducted 24 hours after the last treatment. Measurements of eosinophils, lymphocytes, and neutrophils in BAL fluid as well as in the lung tissue were conducted. In addition, the investigators quantified cytokine levels in BAL and lung tissue supernatants. OVA-specific IgE, IgG1, and IgG2a were measured. A 20-fold greater increase in the eosinophil content of BAL fluid was observed in mice instilled with OVA + DEPM relative to OVA alone. The vehicle group showed no eosinophils in the BAL fluid. DEPM co-administration also produced a 10-fold increase in neutrophils and a 4-fold increase in lymphocytes in the BAL fluid relative to OVA alone. A marked infiltration of eosinophils and lymphocytes was noted in the bronchioles and bronchi of DEPM + OVA treated mice compared to OVA or DEPM alone. Goblet (mucous-secreting) cell number in the airway epithelium was increased 13-fold in the OVA + DEPM group relative to the OVA group alone. These results were all statistically significant ($p < 0.05$ to $p < 0.001$). The Th2 cytokine IL-5, which is involved in the pathogenesis of allergic reactions through enhancement of differentiation, recruitment and activation of eosinophils, was highly elevated in BAL fluid and lung tissue supernatants of OVA + DEPM mice relative to those for OVA or DEPM alone. Antigen-specific immunoglobulins IgE, IgG1, and IgG2a were all significantly elevated in the DEPM + OVA group compared to the OVA group alone after 9 weeks of treatment. The authors note that these cellular and cytokine changes demonstrate enhanced allergic airway inflammation from exposure to DEPM, and that the co-administration of DEPM and antigen "established the fundamental traits of asthma, including eosinophilic airway inflammation, airway hyperresponsiveness, and mucus hypersecretion…"

Exposure of OVA-sensitized C3H/HeN mice via inhalation (2-3 mg/m^3) for five weeks also resulted in enhanced eosinophil numbers in the BAL fluid and lung tissue, as well as increased goblet cell counts in the airway epithelium following exposure to OVA aerosol relative to air exposure plus OVA aerosol alone (Miyabara et al., 1998). Diesel exhaust exposure also increased the production of OVA-specific IgE and IgG1, measured in the serum, and the expression of IL-5 in lung tissue. The same investigators evaluated longer term (40-week) exposures to diesel exhaust at 0.3, 1.0, or 3.0 mg/m^3 in OVA-sensitized ICR mice to study the effects on allergen-related airway inflammation (Takano et al., 1998). As in the earlier study, diesel exhaust inhalation exposure enhanced eosinophil recruitment and airway

hyperresponsiveness in mice following challenge with OVA. DE exposure caused a dose-dependent increase in the cellularity of the BAL fluid, including increases in macrophages and neutrophils compared to the clean-air controls. Eosinophilic infiltration into the airways was observed with OVA challenge in the DE exposed mice but not the clean-air controls. DE inhalation also produced a dose-dependent increase in IL-5 in the BAL and lung tissue supernatants, and of GM-CSF in lung tissue supernatants.

Other investigators have evaluated inhalation of diesel exhaust or instillation of DEPM and production of asthma-like symptoms or allergic responses in animal models. Sagai et al (1996) instilled 0.1-0.2 mg DEPM intratracheally to ICR and W/W^{-} mice once per week for 5, 8, 11, or 16 weeks. Controls received buffered saline or 0.2 mg activated charcoal. Bronchoalveolar lavage fluid obtained at sacrifice was centrifuged and differential cell counts conducted on the pellet. Lung tissue was examined for eosinophils and neutrophils. Airway hyperresponsiveness in response to acetylcholine was assessed in some of the mice. Proliferation of mucus cells, mucus secretion into the bronchioles, and edematous changes in the submucosa were observed after 11 or more instillations. In addition, hyperplasia of goblet cells and thickening of the bronchiolar wall were noted. Neutrophils, eosinophils, and lymphocyte infiltration into the lamina propria of the bronchi and bronchioles and accumulation of eosinophils in the bronchi, bronchioles and alveoli were evident ($p < 0.05$ to $p < 0.001$). Eosinophils reached maximal levels at the 11th week and remained elevated thereafter. Degranulation of eosinophils was also observed by transmission electron microscopy. Sialic acid (an indicator of mucus hypersecretion), and the numbers of neutrophils and eosinophils were significantly elevated in the BAL fluid of the 0.2 mg treatment group relative to controls or charcoal-treated animals ($p < 0.05$ to 0.001). Airway hyperresponsiveness in response to acetylcholine challenge was enhanced in a dose-dependent fashion by intra-tracheal instillation of DEPM. In mice instilled with 0.2 mg DEPM, airway resistance increased with only 10% of the acetylcholine required to induce a response in the control animals ($p < 0.001$). These changes were suppressed when superoxide dismutase bound to polyethylene particles was instilled, indicating a role of reactive oxygen species in the airway inflammatory response and the etiology of hyperresponsiveness.

Kobayashi et al. (1997) used a guinea pig model of rhinitis to evaluate the effects of inhaled diesel exhaust on nasal mucosal hyperresponsiveness to histamine. Guinea pigs were exposed either to air or diesel exhaust (1 or 3.2 mg DEPM/m^3), and intranasal pressure, nasal secretion and sneezing in response to histamine dripped into the nostril were measured. Effects of DEPM or carbon particles on sneezing were also assessed following intranasal instillation. DEPM instilled intranasally increased the frequency of histamine-induced sneezing compared to saline administration ($p < 0.05$), but carbon particles did not. Diesel exhaust inhalation at the higher dose increased histamine-induced sneezing three-fold over air exposed controls ($p < 0.05$), and induced nasal secretion significantly ($p < 0.05$). Intranasal pressure was not significantly different after inhalation exposure to diesel exhaust in this study. This study demonstrates that intranasally instilled DEPM and short-term high inhalation exposures to diesel exhaust can aggravate nasal rhinitis in an animal model.

Kobayashi (2000) demonstrated that longer-term inhalation exposure to diesel exhaust (0.3 or 1 mg DEPM/m^3 for five weeks), with intranasal instillation of ovalbumin (OVA) once per week, augmented the sneezing response to OVA challenge and increased the production of anti-OVA IgG and IgE.

Sneezes were counted for 20 minutes after each OVA administration. By the 3rd week, the high dose DE-exposed animals showed increased sneezing in response to OVA challenge relative to controls, and the response increased in the 4th to 6th weeks. By the 4th administration the low-dose group showed an augmented response ($p < 0.05$), which increased in significance in the following weeks. DE exposure increased the titers of anti-OVA IgG and IgE in the systemic circulation several-fold relative to air-exposed controls. DE exposure also increased the number of eosinophils in the nasal epithelium and submucosa, a hallmark of nasal allergic reaction. These studies show enhancement of allergic reactions by diesel exhaust in animal models and support the human studies indicating the same.

b) Developmental and Reproductive Effects

Developmental toxicity as a result of maternal exposure to diesel exhaust has been reported (Watanabe and Kurita, 2001). The anogenital distance was significantly longer in both male and female fetuses following exposure to diesel exhaust from gestation days 7 to 20. This endpoint is indicative of developmental toxicity on the reproductive tract. Developmental toxicity is one of the endpoints of concern for children's health, as noted in the Introduction Section II.B.1. Although exposure resulted in some changes in maternal hormone levels relative to controls, the authors concluded that the effects observed were the result of exposure-induced changes in the fetus and its interaction with the maternal endocrine system, rather than maternal toxicity or adaptation.

Taneda et al. (2000) report anti-estrogenic activity of diesel particle extracts in a model yeast system. In a study by Meek (1998), diesel particle extracts activated the aryl hydrocarbon receptor (as does dioxin) and induced transcription of reporter genes regulated by the AhR and estrogen receptors. These two studies indicate potential endocrine disruption activity by chemicals bound to diesel exhaust particles. Endocrine disruption is noted in the introduction as a toxicological endpoint of concern for children.

The summary of PAH effects in this report lists extensive developmental effects of PAH exposure, including teratogenesis. It is plausible that such effects would result also from exposure to diesel exhaust due to its PAH content. However, it does not appear that the endpoints observed for PAH developmental toxicity have been adequately evaluated for diesel exhaust exposure.

V. Additional Information

A. Mutagenicity

Diesel exhaust particles have been shown to be mutagenic in a variety of assays *in vitro*, including the *Salmonella* reverse mutation assay and various assays in mammalian cells. Induction of chromosome aberrations *in vitro*, and of sister chromatid exchanges and micronuclei both *in vitro* and *in vivo*, has also been observed. Formation of DNA adducts, in some cases identified as derived from PAHs and/or nitro-PAHs, has been reported both *in vivo* and *in vitro*. Genetic toxicity of diesel exhaust and of particulate material derived therefrom was reviewed in detail by OEHHA (1998). Note that levels of PAH-DNA adducts have been associated with low birth weight in humans (see PAH summary).

B. Regulatory Background

Diesel exhaust PM is listed as a toxic air contaminant under California's air toxics program (AB 1807). OEHHA (1998) reported the following cancer potency estimates:

Unit Risk Factor: 1.3 E-4 - 1.5 E-3 $(\mu g/m^3)^{-1}$ (measured as particulate matter)
[Scientific Review Panel unit risk "reasonable estimate" = 3.0 E-4 $(\mu g/m^3)^{-1}$.]

Slope Factor: 1.1 E+0 $(mg/kg\text{-}day)^{-1}$

This estimate was based on human occupational exposure lung tumor incidence in studies of US railroad workers (Garshick *et al*. (1987, 1988), using estimated exposure concentrations (Woskie *et al*., 1988a,b) and a relative risk model (OEHHA, 1998). Additional analyses by others support this range of risk (Harris, 1983; Steenland et al., 1998).

Diesel exhaust is listed as a carcinogen under Proposition 65, and IARC (1989) lists diesel exhaust as a probable human carcinogen (Group 2A).

OEHHA has adopted a chronic Reference Exposure Level of 5 $\mu g/m^3$. The REL is based primarily on two studies. Ishinishi et al. (1988) showed histological changes in the lung in rats exposed to diesel exhaust at concentrations of 0.96 mg particulate/$m^3$and higher, and Mauderly et al. (1988) showed inflammatory, histological, and biochemical changes in the lung of rats exposed to 3.47 mg diesel exhaust particulate/m^3. The NOAEL, after adjustment for dosimetry used in estimating the REL, was 155 $\mu g/m^3$ for the Ishinishi study; application of a cumulative uncertainty factor of 30 led to an REL of 5 $\mu g/m^3$. Statewide average ambient concentrations are about one-half of the REL, while concentrations can exceed that in urban areas and inside vehicles.

VI. Conclusions

From the above discussion, it is apparent that diesel exhaust has the potential to disproportionately impact infants and children, and OEHHA has, therefore, placed diesel exhaust particulate in Tier 1. The development of atopy, a major risk factor for childhood asthma, occurs as a result of exposures in early childhood among individuals with a genetic predisposition to produce IgE. Enhanced allergic and inflammatory responses have been observed in both animals and humans exposed intranasally or via inhalation to diesel exhaust particulate. Numerous studies indicate that exposure to diesel exhaust particles can:

- Increase total IgE and IgG4 in humans and animals.
- Potentiate responses to allergen, producing marked increases in allergen-specific IgE.
- Favor the activation of Th-2 over Th-1 lymphocytes, enhancing the production of Th2 cytokines, which are associated with allergic inflammation.

- Cause degranulation of eosinophils, with increased release of histamine, and worsen symptoms of allergic rhinitis in humans.
- Cause marked acute airway inflammation in humans after controlled exposures.
- In animal models, cause chronic eosinophilic inflammation and airway hyperreponsiveness, two of the principal characteristics of asthma.
- Facilitate the development of allergy in the presence of diesel exhaust particulate where exposure to the allergen alone was insufficient to induce an allergic response.

Studies of DEPM-associated PAHs suggest plausible mechanisms by which diesel exhaust may exert this suite of immunological effects. The adjuvant effects of diesel exhaust exposure may influence exacerbations of, and perhaps the development of atopic conditions, including allergic rhinitis and asthma. While the precise role (if any) of diesel exposure in the development of atopy has not been defined, evidence has accumulated over the past decade that exposures to a variety of agents that impact the immune system during the first few years of life are critical in determining whether an individual will develop an allergic diathesis.

In addition to the mechanistic evidence from human and animal studies, several studies have shown that exposure to ambient PM_{10} leads to exacerbation of asthma, lung function decrements and increased cough, wheeze, and chronic bronchitis in children. Diesel exhaust is a component of PM_{10}. The extent of the contribution of diesel exhaust particulate to PM_{10} health effects in children and adults has not been directly studied. However, exacerbation of asthma and other adverse health impacts from PM_{10} may, in part, be attributable to DEPM exposure. More to the point, a range of adverse respiratory impacts in children has been associated with traffic density, and especially heavy-duty diesel-fueled truck traffic density and measures of black smoke (sooty fine particles). One of these traffic studies suggests that children are more affected by traffic-related pollutants than adults in the same household. Respiratory impacts may be greater in very young children because their developing lung and immune systems are especially vulnerable to chemical insult. Asthma is potentially more serious in children, especially very young children, because of their smaller airways (see Introduction Section II). Thus, decrements in lung function, exacerbation of asthma and possibly induction of allergic asthma facilitated by diesel exhaust particulate exposure may contribute to disproportionate impacts of diesel exhaust on children.

Epidemiological studies have also provided evidence that exposure to PM_{10} is associated with increased infant mortality. Diesel exhaust is a major contributor to PM_{10} in some of the areas studied (e.g., Bangkok, Mexico, New Delhi), but is not as significant in the U.S. Nonetheless, diesel exhaust particulate contributes to PM_{10} and thus may, in part, be responsible for this observed effect; certainly, there is not a protective effect in the cities where diesel exhaust contributes a substantial portion of the PM_{10}.

Diesel exhaust particulate contains approximately 1% PAHs. Several non-carcinogenic effects have been observed following exposure *in utero* to PAHs or to mixtures containing them, and are described in the PAH summary in this report. These included teratogenesis, low birth weight in humans and

rodents, immunotoxicity, loss of fertility in rodents exposed to benzo[a] pyrene *in utero*, and disruption of lymphocyte maturation and hematopoiesis. These occurred at doses at which maternal toxicity (other than long-term effects such as carcinogenesis) is minimal or absent. In several cases the effects observed after exposure *in utero* parallel toxic effects in the adult (e.g. immunotoxicity, reproductive toxicity, myelotoxicity), but whereas the effects are reversible after exposure of the adult, exposure of the fetus results in an irreversible effect. There are many carcinogenic PAHs and several studies showing that potency increases when exposure occurs perinatally. Thus, the presence of PAHs on diesel exhaust particles is another reason to consider that DEPM disproportionately impacts children.

Children have higher exposures to airborne particles than do adults at the same particle concentration due to their higher breathing rates (see Introduction Section III.). In addition, higher particle doses are experienced by children per unit of alveolar surface area due to particle deposition dynamics. Thus, exposures to diesel exhaust particles in the same environmental settings may be greater for children than for adults.

With regard to diesel exhaust carcinogenesis, the evidence indicating that infants and children may be more susceptible than adults is mainly indirect, since studies of this endpoint in children are not available. However, the more extensive data on PAHs and carcinogenesis reported in the summary for PAHs tend to support the expectation that higher risk might be incurred by exposure to diesel exhaust as infants and children than adults.

VII. References

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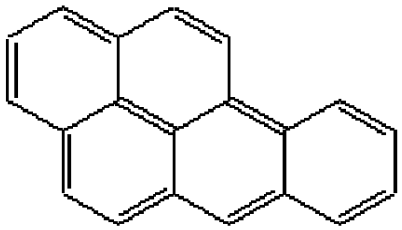
Polycyclic Organic Matter

(including, but not limited to: benzo[*a*]pyrene, benzo[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, benzo[*k*]fluoranthene, dibenz[*a,j*]acridine, dibenz[*a,h*]acridine, 7H-dibenzo[*c,g*]carbazole, dibenzo[*a,e*]pyrene, dibenzo[*a,h*]pyrene, dibenzo[*a,i*]pyrene, dibenzo[*a,l*]pyrene, fluoranthene, 2-methyl fluoranthene, 3-methyl fluoranthene, indeno[1,2,3-*cd*]pyrene, 5-methylchrysene, naphthalene, 1-nitropyrene, 4-nitropyrene, 1,6-dinitropyrene, 1,8-dinitropyrene, 6-nitrochrysene, 2-nitrofluorene, chrysene, dibenz[*a*,h]anthracene, 7,12-dimethylbenzanthracene, 3-methylcholanthrene, 5-nitroacenaphene)



Benzo[*a*]pyrene: 50-32-8

I. Physical and Chemical Properties - Benzo[*a*]pyrene

Description	Yellow crystalline solid: volatile at elevated temperatures.
Molecular formula	$C_{20}H_{12}$
Molecular weight	252.3
Air concentration conversion	1 ppm = 10.3 mg/m^3

The compound benzo[*a*]pyrene is one of the most extensively studied members of the class of Polycyclic Aromatic Hydrocarbons (PAHs). This class, along with various PAH derivatives important as environmental pollutants, is also described as Polycyclic Organic Matter (POM). POM has been identified as a Toxic Air Contaminant by the Air Resources Board. POM consists of over 100 identified compounds, and is defined by the Federal Clean Air Act as organic compounds with more than one benzene ring that have a boiling point greater than or equal to 100^{o}C. The usual definition of a PAH specifies that the compounds are hydrocarbons with no hetero-atom substitutents or ring members, and that the compound include at least two (or, according to some authors, three) concatenated aromatic (usually benzene-like) rings. Although benzo[*a*]pyrene is one of the more abundant members of the class, it comprises no more than 5 percent of the total PAHs present in the atmosphere (Ronia *et al*., 1983). PAHs, and derivatives such as nitro-PAHs and PAH quinones, are included under the TAC category of POM. Additionally, although naphthalene is included within the Federal Clean Air act definition of POM, it is also separately listed as a Federal hazardous air pollutant and thus as a California TAC. In this summary, the toxicity of PAHs and nitro-PAHs is reviewed for

potential impacts on the health of children and infants, with specific reference to those PAHs identified or evaluated as carcinogens by U.S. EPA, IARC, and the California TAC program (see Section V.C.) Additional specific compounds, and mixtures containing PAHs, were considered where data indicate carcinogenic or other biological activities similar to those seen for the identified carcinogenic PAHs, especially where these data include evidence of differential impacts on infants and children.

Most of the POM occurring as air pollution is attached to particulate matter. Compounds with two rings (e.g., naphthalene), although solid at room temperature in bulk, are sufficiently volatile that as air pollutants they occur in the vapor phase. Compounds with three to four rings (e.g., pyrene) occur either in the vapor phase or bound to particles, depending on the temperature and pressure. Compounds with five rings (e.g., dibenzo[*a*,*h*]anthracene, benzo[*a*]pyrene) exist as particles in the atmosphere (Atkinson, 1995). PAHs may also exist as solids in soil or sediment (ATSDR, 1993). PAH-derivatives include nitro-PAHs, amino-PAHs, oxygenated PAHs (phenols, quinones), and heterocyclic aromatic compounds containing nitrogen, sulfur and oxygen (Finlayson-Pitts and Pitts, 1986).

II. Overview

There are a number of toxicological endpoints associated with PAHs to which infants and children may be especially susceptible.

- There is a general concern, based on mechanistic arguments and experimental and epidemiological data for a number of carcinogens, that exposure to a carcinogen early in life may have a greater overall impact than a similar exposure to an adult. Numerous investigators have used neonatal exposures in rodents, which have generally been found to show greater sensitivity to carcinogenesis (i.e. the number of compounds showing a statistically significant effect, the incidence and latency of tumors, and the sites affected), relative to adults (Vesselinovitch *et al*., 1979). In addition, specific data are available showing increased sensitivity to PAHs and derivatives in young animals. A number of PAHs, PAH derivatives and mixtures containing PAHs have been identified as carcinogens in animals or humans.

- Comparative studies of the relative susceptibility to carcinogenesis at different ages have been reported. Intraperitoneal injections of benzo[*a*]pyrene to infant (day 1 or day 15) or young adult (day 42) mice produced greater lifetime incidence of lung and liver tumors in the mice treated at younger ages (Vesselinovitch *et al*., 1975). When 3 doses of fluoranthene, 2- or 3-methylfluoranthene were injected into newborn CD-1 mice, there were high incidences of liver and lung tumors following a short latency period (Lavoie *et al*., 1994). When 8 doses of 1-nitropyrene, 1,3-dinitropyrene, 1,6-dinitropyrene or 1,8-dinitropyrene were injected into newborn CD rats, there were increases in various tumors, some of which occurred after a very short latency period (Imaida *et al*. 1995). In addition, 1,6-dinitropyrene or 1,8-dinitropyrene produced an early increase in leukemia. These experiments are described in detail in a later section of this summary.

- Several studies in animals and humans indicate that prenatal exposure to PAHs results in serious or irreversible effects in the fetus, including cancer, teratogenesis and low birthweight. As discussed in the introductory section of this report, fetal damage sustained as a result of exposure to environmental toxicants is a source of adverse postnatal health impacts, and therefore falls within the scope of this report.

- Transplacental carcinogenesis by PAHs is a well-known phenomenon (Sram *et al*., 1998; Anderson *et al*., 1995). In one experiment, this was associated with induction of a specific mutation in the Ha-*ras* proto-oncogene in the fetus, which can be expressed post-natally with appropriate promotion (Yamasaki *et al*., 1987). Thus, it appears that the mechanisms underlying transplacental carcinogenesis are similar to those for carcinogenesis after postnatal exposure, but the sites of tumor appearance are frequently more diverse, and the sensitivity is greater (Nikonova, 1977).

- Several non-carcinogenic effects have been observed following exposure *in utero* to PAHs or to mixtures containing them. These included teratogenesis (Shum *et al*., 1979; Feuston and Mackerer, 1996), low birth weight in humans (Perera *et al*., 1998; Dejmek *et al*., 2000) and rodents (McKee *et al*., 1987b), immunotoxicity (Urso *et al*., 1992), loss of fertility in rodents exposed to benzo[a] pyrene *in utero* (Mackenzie and Angevine, 1981), human transplacental exposure resulting in hemolytic anemia (Zinkharn and Childs, 1958; Anziulewicz *et al*., 1959) and disruption of lymphocyte maturation and hematopoiesis (Holladay and Smith, 1994). These occurred at doses at which maternal toxicity (other than long-term effects such as carcinogenesis) is minimal or absent. In several cases the effects observed after exposure *in utero* parallel toxic effects in the adult (e.g. immunotoxicity, reproductive toxicity, myelotoxicity), but whereas the effects are reversible after exposure of the adult, exposure of the fetus results in an irreversible effect.

- In addition to differential sensitivity to toxic effects in young animals and humans, there is greater exposure of children to environmental PAHs (Chuang et al., 1999). Children's daily doses of PAHs (per kg of body weight) were generally higher from all routes of exposure than those of adults in the same household (Chuang et al., 1999) or city (Ptashekas et al., 1996). PAH-DNA adducts have been detected in the human placenta (Everson et al., 1986, Weston et al., 1989), the umbilical cord blood of newborns (Whyatt et al., 1989), and the fetuses of experimental animals (Withey et al., 1992; 1993).

- Based on these observations, studies in humans and animals suggest that children may be more sensitive to the toxic effects of PAHs and PAH derivatives (including, but not limited to, benzo[*a*]pyrene, benzo[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, benzo[*k*]fluoranthene, dibenz[*a,j*]acridine, dibenz[*a,h*]acridine, 7H-dibenzo[*c,g*]carbazole, dibenzo[*a,e*]pyrene, dibenzo[*a,h*]pyrene, dibenzo[*a,i*]pyrene, dibenzo[*a,l*]pyrene, fluoranthene, 2-methyl fluoranthene, 3-methyl fluoranthene, indeno[1,2,3-*cd*]pyrene, 5-methylchrysene, naphthalene, 1-nitropyrene, 4-nitropyrene, 1,6-dinitropyrene, 1,8-dinitropyrene, 6-nitrochrysene, 2-nitrofluorene, chrysene, dibenz[*a*,h]anthracene,

7,12-dimethylbenzanthracene, 3-methylcholanthrene, 5-nitroacenaphthene), and mixtures containing PAHs.

III. Principal Sources of Exposure

PAHs and other POM components are produced by the incomplete combustion of any carbon-containing materials, including fossil fuels and vegetable matter. PAHs have been detected in exhaust from both gasoline and diesel powered motor vehicles, smoke from residential wood combustion, and fly ash from coal-fired electric generating plants (Finlayson-Pitts and Pitts, 1986). Burning of vegetable materials and other waste is responsible for 50 percent of the total statewide emissions of benzo[*a*]pyrene. Other sources of benzo[*a*]pyrene, such as residential wood combustion, coal combustion, and residual oil combustion, are responsible for about 15 percent of the total statewide emissions. Some sources of PAHs and other POM components are non-anthropogenic; these can be formed during any naturally occurring combustion, such as forest fires (U.S. EPA, 1994). Benzo[*a*]pyrene and other PAHs occur in crude oils, shale oils, and coal tars, and are emitted with gases and fly ash from active volcanoes (HSDB, 1995). In spite of these natural sources, the most important contributors to air pollution by PAHs are usually anthropogenic.

A. Mobile sources

In California, mobile sources contribute more than 35 percent of the total benzo[*a*]pyrene emissions. (Benzo[*a*]pyrene in air is quantifiable by standardized methods and is frequently used as a marker for emissions of a range of PAHs and other POM components.) Within the mobile source category, light duty vehicles are responsible for 30 percent of benzo[*a*]pyrene emissions, while heavy duty vehicles contribute approximately 10 percent (OEHHA, 1993). Before the introduction of catalytic converters (around 1974), mobile sources were the major contributor of benzo[*a*]pyrene emissions. The decreasing number of older, more polluting vehicles, and the introduction of low and even zero emission vehicles and clean fuels as part of California's Motor Vehicle Program has led to a significant and continuing reduction of POM emissions (including benzo[*a*]pyrene and other PAHs) from light-duty vehicles. Emissions from on-road mobile source diesel exhaust PM_{10} in California are expected to decline by approximately 50 percent from 1990 until about 2010 as a result of mobile source standards and regulations adopted by the ARB through 1996 (ARB, 1998). However, the proportional reductions in emissions per mile from heavy-duty (primarily diesel powered) vehicles have so far been less dramatic than those from gasoline-powered vehicles. This has focused attention on the role of diesel exhaust as an important source of air pollutants, including both volatile and particulate POM, especially in metropolitan areas (SCAQMD, 2000). Stationary sources using diesel engines are also significant sources in some areas. Additional efforts to address this issue include the replacement of diesel powered heavy and medium-duty vehicles with clean-fuel alternatives (such as those using methanol or compressed natural gas), and the use of particle traps and other pollution reduction technologies on new and existing diesel engines. However, the impact of these changes has so far been limited.

B. Stationary Sources

The primary stationary sources that have reported emissions of benzo[*a*]pyrene or other PAHs in California are paper mills, manufacturers of miscellaneous wood products, industrial machinery manufacturers, petroleum refining and the wholesale trade in petroleum and petroleum products (ARB, 1997). Estimated total emissions of PAHs from stationary sources in California are about 370,000 pounds per year, based on data reported under the Air Toxics "Hot Spots" Program (AB 2588). Table 1 lists the emissions of some individual PAHs. In addition there are approximately 2,600 pounds of unspecified POM and 250,000 pounds of unspecified PAHs reported as emissions from Hot Spots facilities. (ARB, 1997).

Table 1: California Emissions for Individual PAHs

Compound	Emissions (pounds/year)
Acenaphthene	6
Acenaphthylene	27
Anthracene	285
Benzo[*a*]pyrene*	304
Benz[*a*]anthracene*	175
Benzo[*b*]fluoranthene*	175
Benzo[*k*]fluoranthene*	181
Benzo[*g,h,i*]perylene	10
Chrysene*	275
Dibenz[*a,h*]anthracene*	211
Dibenz[*a,e*]pyrene*	3
Fluoranthene	23
Fluorene	32
Indeno[1,2,3-*cd*]pyrene*	204
Naphthalene	360,000
Phenanthrene	63
Pyrene	42

(Data from ARB, 1997)
* Chemicals for which PEFs are available (OEHHA, 1993)

OEHHA reviews risk assessments submitted under the Air Toxics "Hot Spots" Program (AB 2588). Of the risk assessments reviewed as of April 1996, PAHs were a major contributor to the overall cancer risk in 43 of the approximately 550 risk assessments reporting a total cancer risk equal to or greater than 1 in 1 million, and contributed to the total cancer risk in 166 of these risk assessments. PAHs also were the major contributor to overall cancer risk in 8 of the approximately 130 risk assessments reporting a total cancer risk equal to or greater than 10 in 1 million, and contributed to the total cancer risk in 54 of these risk assessments (OEHHA, 1996).

C. Ambient Concentrations

California Air Resources Board's air toxics network monitors several PAHs routinely. Table 2 gives the network's mean concentration of various PAHs from January 1996 through December 1996 (ARB, 1998). The population-weighted annual ambient concentration of benzo[*a*]pyrene in California was estimated as 0.53 ng/m^3 based on 1988 to 1989 monitoring data (ARB, 1997). There are no Air Resources Board ambient measurements of naphthalene. However, Atkinson (1995) measured 12 hour average ambient concentrations of naphthalene in Redlands, California in October 1994. The levels observed ranged from 348 to 715 ng/m^3. The overall mean concentration for POM from several study areas throughout the United States during 1984-91 was 8.4 ng/m^3 (U.S. EPA, 1993).

Table 2: California Ambient Concentrations of PAHs

PAH Compound	Mean Concentration (ng/m^3)
Benzo[*a*]pyrene	0.194
Benzo[*b*]fluoranthene	0.245
Benzo[*g,h,i*]perylene	0.619
Benzo[*k*]fluoranthene	0.100
Dibenz[*a,h*]anthracene	0.031
Indeno[1,2,3-*cd*]pyrene	0.327

(Data from ARB, 1997)

D. Indoor Air

Benzo[*a*]pyrene and other POM components are significant as indoor air pollutants. According to two large field studies conducted in California, the major sources of indoor PAHs are tobacco smoking, wood burning in fireplaces and wood stoves, and infiltration of polluted outdoor air (ARB, 1992; Sheldon *et al*., 1993). The largest field study was conducted in northern California, in which 13 PAHs were measured inside 280 homes during the winter. Concurrent outdoor samples were collected at each home for 24 hours. The homes were selected based upon the occupants' use of tobacco, fireplaces, wood stoves, and gas heat. Table 3 lists the average indoor concentrations for some PAHs for each type of combustion source.

Average indoor PAH levels ranged from about one-fourth to 6 times the average of outdoor levels. When compared to concentrations inside homes with no obvious combustion sources ("no source"), substantially higher concentrations of all 13 PAHs were measured inside homes where smoking occurred. In addition, wood burning in fireplaces and wood stoves appeared to cause slight to moderate increases in indoor concentrations of benzo[*a*]anthracene, chrysene, benzofluoranthenes, and benzo[*a*]pyrene. Investigators estimated that infiltration of polluted outdoor air was also a major contributor to indoor concentrations of PAHs, particularly outdoor air polluted by wood smoke (Sheldon *et al*., 1993).

Table 3: Average PAH Concentrations in Northern California Homes (ng/m^3)

PAH Compound(s)	Smoking	Fireplace	Woodstove	Gas Heat	No Source
Benzo[*a*]pyrene	2.2	1.0	1.2	0.41	0.83
Benzo[*e*]pyrene	1.1	0.49	0.55	0.25	0.42
Indeno[1,2,3-*cd*]pyrene	2.8	1.7	1.9	0.92	1.4
Benzo[*ghi*]perylene	2.0	1.4	1.5	0.78	1.3
Pyrene	4.1	2.0	2.5	1.6	1.8
Chrysene	2.0	0.56	0.61	0.24	0.4
Fluoranthene	4.5	1.9	2.3	1.4	1.6
Benzo[*a*]anthracene	1.3	0.43	0.55	0.17	0.32
Benzofluoranthenes	3.7	1.6	2.0	0.81	1.5

(Data from Sheldon et al., 1993)

Another field study measured 12 PAH compounds inside 125 southern California homes during a relatively warm fall season. At each home, two consecutive 12-hour samples were collected. Concurrent samples were also collected outside 65 of those homes. Average indoor PAH concentrations ranged from about one-half to two times the corresponding outdoor levels. Table 4 shows average concentrations (combined daytime/nighttime) for some PAHs. Levels of most PAHs were significantly higher in homes where smoking occurred than in nonsmokers' homes. As in the northern California study, investigators estimated that infiltration of polluted outdoor air was a major source of PAHs indoors (ARB, 1992).

Table 4: Average PAH Concentrations in Southern California Homes (ng/m^3)

PAH Compound(s)	Indoor Average	Outdoor Average
Benzo[*a*]pyrene	0.70	0.30
Benzo[*e*]pyrene	0.39	0.28
Indeno[ghi]perylene	1.1	0.51
Benzo[ghi]perylene	2.4	1.0
Pyrene	2.8	2.2
Chrysene	0.30	0.39
Fluoranthene	2.2	2.5
Benzo[*a*]anthracene	0.16	0.18

(Data from ARB, 1992)

E. Exposures to Children

There is evidence that children are more heavily exposed to PAHs than adults (on a body-weight adjusted basis), and thus may suffer disproportionately from their impact, whether or not they are more susceptible to PAH toxicity. This is a result of the body size and activity patterns of children, higher breathing rates (on a body weight adjusted basis, especially for infants), and their propensity for greater dust and soil contact than adults. In particular, children in low-income families may have high exposures to PAHs. Such exposures could result from household proximity to heavy traffic or industrial sources, environmental tobacco smoke, contaminated house dust or soil, among others.

A series of studies (Chuang *et al*., 1999) in Durham, NC and adjacent rural areas were conducted to estimate total PAH exposure of children in low-income families, and the relative importance of the environmental pathways for PAH exposure (Table 5).

Table 5: Potential daily dose of carcinogenic (US EPA B2) PAHs (ng/kg b.w./day)

Pathway	Average	S.D.	Minimum	Maximum
Adults				
Inhalation	1.77	3.29	0.12	15.3
Non-diet ingestion	1.28	0.91	0.38	4.24
Diet	16.3	16.7	1.61	60.9
Total	19.4	16.8	4.12	62.0
Children				
Inhalation	3.93	4.88	0.37	19.6
Non-diet ingestion	8.88	6.21	2.62	29.2
Diet	24.8	23.7	1.35	97.0
Total	37.6	23.7	12.2	107

(Data from Chuang et al., 1999)

Higher indoor PAH levels were observed in the smokers' homes compared to nonsmokers' homes. Higher outdoor PAH levels were found in the inner city versus rural areas. Airborne PAHs deposit on soil and household surfaces, thus contributing to PAH exposures from dust (via ingestion, and inhalation of re-suspended dust) and food. The relative concentration trend for PAH in dust and soil was: house dust > entryway dust > pathway soil. The PAH concentrations were generally higher in adults' than in children's food samples. Children's potential daily doses of PAH were higher than those of adults in the same household, when intakes were normalized to body weights. Inhalation is an important pathway for children's exposure to total PAH because of the high levels of naphthalene present in both indoor and outdoor air. Ingestion pathways became more important for children's exposure to the subset of PAHs ranked as B2 (probable human carcinogens) by the U.S. EPA (see Section V.C.), most of which are of low volatility. These PAHs are included in our proposed listing. The analysis of variance results showed that inner city participants had higher total exposure to B2 PAHs than did rural participants.

Such differences between children and adults may be even more noticeable in highly contaminated environments. A monitoring program (Ptashekas *et al*., 1996) which included benzo[*a*]pyrene was carried out in two Lithuanian cities, Vilnius, the capital of the country, and Siauliai during 1991 to 1995. Higher amounts of benzo[*a*]pyrene were found in the urine of children compared to adults, in both the control and high-risk zones. Both this and the previous study indicate that environmental PAH exposures of children result in higher body burdens than for adults in the same environments.

Crawford *et al*. (1994) examined biomarkers of environmental tobacco smoke in preschool children and their mothers. There were increases in the biologically effective dose of the carcinogenic (PAH) components of ETS in children exposed to ETS, as assessed by levels of PAH-albumin adducts. Tang *et al*. (1999) confirmed these findings in further studies of molecular and genetic damage from environmental tobacco smoke in young children, and found not only increases in protein adducts with PAHs and 4-aminobiphenyl, but also of sister-chromatid exchanges in peripheral lymphocytes of Hispanic and African-American children with home exposure to environmental tobacco smoke. Thus children exposed to ETS show increases not only in general tobacco-related biomarkers such as cotinine, but in biomarkers specific for PAHs and in genetic damage.

Airborne PAHs may contribute to exposure of children by non-inhalation routes. For example, breast milk is a route of exposure in infancy. Few studies of PAH occurrence in breast milk have been carried out, but Somogyi and Beck (1993) described a study conducted in the Federal Republic of Germany that found a number of single PAH compounds at concentrations of 5-15 ng/kg milk and, among these, benzo[*a*]pyrene, was detected at a concentration of 6.5 ng/kg. This is consistent with the findings of West and Horton (1976), who observed transfer of polycyclic hydrocarbons from the diet to milk in rats, rabbits, and sheep. Interestingly, this was more extensive in the rat (an omnivore like humans) than in the herbivorous rabbit and sheep. Lavoie *et al*. (1987) also observed transfer of benzo[*a*]pyrene and two tobacco-specific carcinogens into the milk of lactating rats.

F. Exposures in utero

Exposure of the fetus to airborne PAHs can occur via transplacental transfer from the mother. This contributes to the body burden of PAH and PAH-DNA adducts in the child. A study (Klopov, 1998) of pregnant women in the Russian Arctic region found substantial maternal exposure to PAHs, and detected PAHs in most samples of cord blood and placenta analyzed. The results support the barrier role of the placenta, because levels of many PAH compounds in cord blood were lower than in maternal blood. Nevertheless, these compounds were passing through the placental barrier, at least partially, with the concentrations of some PAHs (anthracene and benzo[e]pyrene) higher in cord blood than in maternal blood. Autrup *et al*. (1995) and Autrup and Vestergaard (1996) measured the polycyclic aromatic hydrocarbon-albumin adduct level in serum isolated from the mother and the umbilical cord. The median maternal/fetal adduct ratio was approximately 1.3 (maternal blood/umbilical cord blood) and a positive association between the adduct levels in the mother and umbilical cord blood was observed.

These observations are consistent with the observation of PAH compounds and their metabolites in the fetus after maternal exposure in experimental animal studies (Withey *et al*., 1993, Withey *et al*., 1992; Kelman and Springer, 1982; Kihlstrom, 1986). Howard *et al*. (1995) showed that 1-nitropyrene was transported both across the placenta and into milk in mice following oral or intraperitoneal dosing. 0.7% of the administered dose crossed the placenta as 1-nitropyrene and/or its metabolites, and accumulated in the fetuses and amniotic fluid, with both C-oxidized and nitro-reduced metabolites being detected.

Everson *et al*. (1986) reported detection of smoking-related covalent DNA adducts in human placenta. Weston *et al*. (1989) isolated PAH-DNA adducts specifically identified as r-7,t-8,t-9,c-10-tetrahydroxy-7,8,9,10-tetrahydroBaP residues from human placenta. Arnould *et al*. (1997) also reported detection of benzo[*a*]pyrene-DNA adducts in human placenta and umbilical cord blood. Determination of tobacco consumption by urinary cotinine established that among smokers, adducts were found in 13 placenta specimens (from 10 to 60 fmol/50 µg DNA) and 12 umbilical cord blood samples (from 10 to 22.15 fmol/50 µg DNA). Thus a mother's tobacco consumption is linked to the accumulation of benzo[*a*]pyrene-DNA adducts in the placenta and cord blood. These adducts are seen in smaller quantities in the umbilical cord blood, probably because of the metabolic capacity of the placenta which impacts transfer of benzo[*a*]pyrene from the mother to the fetus.

Zenzes *et al*. (1999) investigated whether benzo[*a*]pyrene diol epoxide-DNA adducts are detectable in pre-implantation embryos, and studied their relationship to parental smoking. Seventeen couples were classified by their smoking habits: (i) both smokers; (ii) wife non-smoker, husband smoker; and (iii) both non-smokers. Their 27 embryos were exposed to a monoclonal antibody that recognizes benzo[*a*]pyrene diol epoxide-DNA adducts. The proportion of stained blastomeres was higher for embryos of smokers than for non-smokers (0.723 versus 0.310). The mean intensity score was also higher for embryos of smokers (1.40±0.28) than for non-smokers (0.38±0.14; $P = 0.015$), but was similar for both types of smoking couples. The mean intensity score was positively correlated with the number of cigarettes smoked by fathers ($P = 0.02$). Increased mean immunostaining in embryos from smokers, relative to non-smokers, indicated a relationship with parental smoking. The similar levels of immunostaining in embryos from both types of smoking couples suggest that transmission of modified DNA is mainly through spermatozoa. Paternal transmission of modified DNA was confirmed by detection of benzo[*a*]pyrene diol epoxide-DNA adducts in spermatozoa of a smoker father and his embryo.

The results of Whyatt *et al*. (1998) indicate that PAH-induced DNA damage in mothers and newborns is increased by ambient air pollution. These investigators measured PAH-DNA adducts in maternal and umbilical white blood cells of 70 mothers and newborns from Krakow, Poland. The modulation of DNA adduct levels by genotypes previously linked to risk of lung cancer was also investigated. There was a dose-related increase in maternal and newborn adduct levels with ambient air pollution at the women's place of residence among subjects who were not employed away from home ($p=0.05$). Maternal smoking (active and passive) significantly increased maternal ($p<0.01$), but not newborn adduct levels. Neither the CYPIAI Mspl nor the GSTM1 polymorphism was associated with maternal adducts. However, adducts were significantly higher in newborns heterozygous or homozygous for the

CYP1A1 MspI RFLP compared to newborns without the RFLP (p=0.04). In the fetus, DNA damage appears to be enhanced by the CYP1A1 MspI polymorphism. A novel feature of this study was the measurement of PAH-DNA adduct levels in white blood cells of mother-newborn pairs. Transplacental exposures to PAHs are generally an order of magnitude lower than maternal exposure. The finding that levels of adducts in newborns were similar to those in mothers (in spite of the protective effects of metabolism by maternal and placental enzymes) suggests an enhanced susceptibility to DNA damage in a fetus compared to the mother.

In a separate analysis, Whyatt *et al*. (1998) determined PAH-DNA adduct levels in mother-new born pairs from Limanowa, a rural area outside Krakow where ambient pollution levels are lower but where home use of coal for heating is significantly greater. Among the 67 pairs analyzed, mean adduct levels in the newborns significantly exceeded those in the mothers. This suggests that adduct formation and the resulting DNA damage caused by maternal exposure to PAHs could be amplified in the fetus.

IV. Potential for Differential Effects

A. Summary of Key Human Studies

a) Carcinogenicity

Systematic studies addressing the relative sensitivity of infants and children to carcinogenesis by PAHs were not identified in the scientific literature. However, there is an enormous literature on the carcinogenicity of polycyclic hydrocarbons, and various mixtures containing them that may be encountered in the workplace or the general environment. Indeed, the observations of Pott (1775) on scrotal cancer in chimney sweeps (who were mostly children, and were exposed to soot from coal fires, a rich source of PAHs and other POM) are generally regarded as the first objective account of chemical carcinogenesis in humans.

b) Developmental Toxicity

Developmental toxicity has been identified as a process having impacts on children's health, as noted in the introductory chapter of this report. PAH effects reported in animals have included obvious anatomical abnormalities (see Section IV.B.b.1), but perhaps because of the less extreme exposures of humans in polluted environments compared to the animal toxicity experiments, these have not been clearly associated with human PAH exposures. However, more subtle changes such as morphometric abnormalities indicative of developmental delay, and intrauterine growth retardation (IUGR) leading to low birth weight infants have been reported both for populations living in polluted environments, and for mothers who smoke or are exposed to secondhand tobacco smoke. Recent studies of these effects are described below. While these exposures involve complex mixtures with many toxic components, the detailed associations shown, the finding of chemical-specific DNA adducts in affected offspring (see Section V.A) and the concordance with animal effects implicate PAHs as important causative toxicants for these effects. Low birth weight and developmental delay are associated with adverse experience of morbidity and mortality in childhood (and also with adverse health impacts later in life), so it is of

particular concern that well-documented reports of IUGR in humans exposed to PAH-polluted air have appeared.

Perera *et al*. (1998) studied developmental effects of fetal exposure to PAHs via ambient pollution. The study was carried out in an industrialized area of Poland with relatively high levels of PAH pollution from coal burning. PAH-DNA adducts in leukocytes and plasma cotinine were measured in umbilical cord blood, as dosimeters of transplacental PAH and cigarette smoke, respectively. The study subjects were 70 newborns from the industrialized city of Krakow and 90 newborns from Limanowa, a rural town with far greater use of coal for home heating. Newborns whose levels of PAH-DNA adducts were above the median ($3.85/10^8$ nucleotides) had a significantly decreased birth weight, birth length, and head circumference (Table 6). Cotinine was also significantly inversely associated with birth weight and length.

Table 6: Birth outcomes in Polish Newborns.

	Birth Weight (g)		**Birth Length (cm)**		**Head Circumference (cm)**	
Group	**Difference**	**P value**	**Difference**	**P value**	**Difference**	**P value**
Krakow	- 205	0.11	-1.8	0.02 *	-0.9	0.05 *
Limanowa	-129	0.16	-0.8	0.17	-1.2	0.0004 *
All	-147	0.05 *	-1.1	0.02 *	-0.9	0.0005 *

(Data from Perera et al., 1998)
Difference between those with high (above median) and low (below median) leukocyte levels of PAH-DNA adducts.

Dejmek *et al*. (2000) found that exposure to carcinogenic PAHs in air pollution during early pregnancy was associated with an increased adjusted odds ratio for low birth weight ("intrauterine growth retardation" - IUGR). Birth outcomes were studied over a four-year period in two towns in Bohemia (Czech Republic): Teplice (1100 births/yr) and Prachatice (450 births/year). Teplice is located in an industrialized area with surface mining of brown coal, chemical industry and large coal-fired power plants; in this area the level of general air pollution, including both particulate material (PM) and PAHs, is high. Prachatice on the other hand is located in a more rural and mountainous area without major industrial activity, and the general level of pollution (including PM) is much lower in this area. However, there is a single large point source of PAH emissions in the town. At both locations, levels of air pollution by PM and PAHs varied seasonally and over the duration of the study. Air pollution levels were measured continuously at both locations. Seven specific PAHs identified by IARC as potentially carcinogenic to humans (c-PAHs: chrysene, benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, dibenz[*a,h*]anthracene, indeno[1,2,3-*c,d*]pyrene) were measured. These PAHs are identified as carcinogenic in our TAC identification document (OEHHA, 1993).

IUGR was defined as birth weight below the 10^{th} percentile, by sex and gestational week, in the general Czech population. Of 3,349 pregnancies at Teplice, 322 (9.6%) exhibited IUGR. At Prachatice, 124

(8.2%) of 1505 pregnancies were affected. (The study cohorts were limited to full-term pregnancies of mothers of European origin). When births were categorized according to the exposures to PM_{10}, $PM_{2.5}$ and PAH, there was a significant association with exposure to air pollution, specifically to the carcinogenic PAHs. In Teplice the PAH and PM levels are highly correlated so the effects of these two pollutants could not be distinguished at this site alone. However, at Prachatice there is much less particulate pollution relative to the amount of PAH, and by comparing results at both sites it was concluded that the effect was associated with the PAH content of the pollution, not the $PM_{(10 \text{ or } 2.5)}$. Results relating incidence of IUGR to pollution experienced in the first gestational month are presented in Table 7 as adjusted odds ratios (AOR) for the medium and high exposure categories, relative to the low exposure category. These adjusted odds ratios were calculated using a logistic regression model in which adjustments were applied for the identified confounding variables: parity, maternal age and height, pre-pregnancy weight, education, marital status, month-specific maternal smoking, season, seasonal and long-term conception rate effects, and year of study.

The association between exposure to PAH and incidence of IUGR was only significant when exposure during the first month of gestation was considered (AOR for medium exposure = 1.63, 95% CI 0.87 - 3.06, $p < 0.13$; AOR for high exposure = 2.39, 95% CI 1.01 – 5.65, $p < 0.045$). Exposure at other times had no consistent effect, although a possible weak association with exposure during the eighth month was noted in Teplice only. This was interpreted as indicating that the induction of IUGR by PAH exposure resulted from an early developmental effect: this is consistent with other data suggesting that IUGR results from specific impairments of placental development soon after implantation (Zhang et al., 1995). Trends of IUGR vs. PAH exposure during the first month of exposure were highly significant in Teplice; each 10 ng increase in PAH exposure level resulted in an increase in AOR of 1.22 (95% CI 1.07 – 1.39, $p < 0.004$). A similar trend was observed in Prachatice, although it did not achieve statistical significance by this analysis.

The presence of other materials besides POM in air pollution and cigarette smoke makes it difficult to definitively state the impact of PAHs on birth weight and development. However, there was specific correlation of these outcomes with PAH-DNA adducts. There is extensive evidence from other studies showing PAH-DNA adducts in humans exposed to air pollution or cigarette smoke; and there are animal studies where developmental delay and low birth weight are seen after exposure to pure PAHs. This evidence makes a causal relationship between low birth weight and developmental changes and exposure to PAHs highly plausible.

c) Acute Toxicity in Children

Hemolysis has been reported in infants exposed to very high doses of naphthalene (Siegel and Wason, 1986). The effect appears to be caused by the metabolites (1- and 2- naphthol and naphthoquinones), which produce methemoglobinemia. This mode of action implies a potential for differential sensitivity of infants, due to their reduced capacity for methemoglobin reduction compared to adults. However, even in infants the doses required to produce this effect are much greater than could plausibly result from airborne levels of naphthalene; the case reports generally involved absorption by the dermal or oral routes. Zinkharn and Childs (1958) reported examining an infant exhibiting acute hemolytic anemia that

was only exposed during gestation. His mother had inhaled and ingested mothballs containing naphthalene during pregnancy (especially during the last trimester). However, it was not possible to provide an estimated infant exposure.

Table 7: Adjusted Odds Ratios of Intrauterine Growth Retardation by c-PAHs and PM_{10} in the first gestational month in Teplice and Prachatice.

Pollutant	Specification	District	Medium [a]		High [a]	
			AOR[b]	95% CI	AOR[b]	95% CI
PM_{10}	—	Teplice	1.44	(1.03–2.02)	2.14	(1.42–3.23)
	—	Prachatice	2.11	(1.03–4.33)	1.09	(0.49–2.46)
c-PAHs	—	Teplice	1.59	(1.06–2.39)	2.15	(1.27–3.63)
	—	Prachatice	1.49	(0.81–2.73)	1.26	(0.60–2.63)
	<7.5 km (n=551) [c]	Prachatice	1.89	(0.56–6.29)	2.44	(0.60–9.83)
	Lower cut-offs [d]	Prachatice	1.63	(0.87–3.06)	2.39	(1.01–5.65)

Data from Dejmek et al. (2000)

a. Cutoffs for PM_{10}: Low= < 40 µg/m^3, Medium = 40 to < 50 µg/m^3, and High = ≥50 µg/m^3.
Cutoffs for Carcinogenic PAHs (c-PAHs): Low = < 15 ng/m^3, Medium = 15 to < 30 ng/m^3, and High = ≥30 ng/m^3.
b. Medium to Low and High to Low, adjusted for parity, maternal age and height, pre-pregnancy weight, education, marital status, month-specific maternal smoking, season, seasonal and long-term conception rate effects, and year of the study.
c. Only mothers living up to 7 km from the monitor station in Prachatice region.
d. c-PAHs: Low = < 2 ng/m^3 ; Medium = 2 to < 20 ng/m^3 ; High = ≥20 ng/m^3 .

B. Summary of Key Animal Studies

a) Carcinogenicity

The carcinogenicity of PAHs and other POM constituents in animals is well known; the literature, which is very extensive, has been evaluated by IARC (1987, 1989). It is generally considered both on theoretical grounds and as a result of experimental evidence that exposures to carcinogens early in life may result in higher tumor yields. Shorter average times to tumor, and a wider range of sensitive sites, have also been reported following early in life exposures in some experiments. As discussed in the introduction to this report, there is concern that children may suffer adverse health consequences including enhanced rates of cancer from exposure to any carcinogen, due to the increased sensitivity *in utero* and early in life. There is particular concern where specific evidence of enhanced sensitivity at younger ages exists for the specific compound or class of compounds considered.

Although in the standard rodent bioassay protocol exposure begins at the young adult stage, neonatal or young rodents have often been used in carcinogenesis bioassays, including the early carcinogenesis

studies by Innes *et al*. (1969). It has generally been supposed that starting exposure at an early age would maximize the sensitivity of the assay, and this has been observed in practice (Vesselinovitch *et al*., 1979). In some cases, such as the widely used neonatal Strain A mouse lung adenoma assay (Stoner *et al*., 1984), the combination of neonatal exposure, a highly sensitive strain and selective endpoint definition has been used to produce a result in a matter of weeks. This contrasts with the two years usually required for a bioassay using the standard NTP protocol.

Vesselinovitch *et al*. (1975) studied the carcinogenicity of benzo[*a*]pyrene in two hybrid strains of mice exposed by a single intraperitoneal injection at 1, 15 or 42 days old. Tumors were observed at several sites, and the relationship between tumor incidence and age at exposure varied from site to site. In the case of liver and lung tumors, young mice were more sensitive than older mice, showing both higher tumor incidence and shorter time before appearance of tumors. Incidence of tumors was generally higher for liver and lung tumors than for other sites.

The strains used were C57BL/6J x C3HeB/FeJ F_1 ("B6C3F_1") and C3HeB/FeJ x A/J F_1 ("C3AF_1"); treated group sizes varied from 30 to 62 animals whereas control groups contained 98 to 100 animals. Doses of 75 or 150 µg benzo[*a*]pyrene were dissolved in trioctanoin. Control animals had low mortality; controls included two groups, in one of which survivors were necropsied at age 90 weeks and in the other survivors were necropsied at age 142 weeks. Incidences of all tumors in controls were low, except for lung tumors in the C3AF_1 mice (Males: 49/97 at 90 weeks; 60/100 at 142 weeks. Females: 26/100 at 90 weeks; 50/100 at 142 weeks). Treated animals were examined regularly for tumors throughout life; mortality immediately after dosing was virtually zero, but later survival was impacted by the appearance of lethal tumors.

Results are presented in Table 8. For liver tumors in both strains, the incidence was greater, and the average age of tumor appearance was lower, in animals treated at 1 day than at 15 days. This trend continued when comparing those treated at 42 days. The authors judged these differences to be significant ($P < 0.01$) using the χ^2 test for incidence comparisons and Student's t-test for comparing averages at which tumors were detected at autopsy. Similar trends were observed for lung tumors, although the high background incidence of this tumor in the C3AF_1 mice reduced the extent and statistical significance of the differences.

Lavoie *et al*. (1994) examined the tumorigenic activity of fluoranthene, 2-methylfluoranthene and 3-methylfluoranthene in newborn CD-1 mice. All three compounds were assayed at intraperitoneal doses of 3.46 and 17.3 µmol, given during the course of three injections on days 1, 8 and 15 after birth. Effective group sizes were between 16 and 34 (alive at 1 year: survival was about 50%). The bioassay was terminated when mice were 1 year old. Fluoranthene, a compound of interest as an air pollutant, is inactive as an initiator in the skin-painting assay (with phorbol ester promotion) in the adult mouse, which is often regarded as a particularly sensitive assay for the carcinogenic activity of PAHs. Among the five isomers of methylfluoranthene, only 2-methylfluoranthene (2-MeFA) and 3-methylfluoranthene (3-MeFA) are active as tumor initiators on adult mouse skin. But fluoranthene and 2-MeFA induced lung tumors in both male and female neonatal mice (Table 9).

Table 8: Incidence of lung and liver tumors in mice treated with benzo[*a*]pyrene at various ages

		Liver tumors				Lung tumors					
	Dose:	75mg/kg		150 mg/kg		75mg/kg			150 mg/kg		
***Strain*/ Sex**	**Age when dosed [a] (days)**	**% tumors [b]**	**Time to tumor [c] (weeks)**	**% tumors [b]**	**Time to tumor [c] (weeks)**	**% tumors [b]**	**Time to tumor [c] (weeks)**	**Multi-plicity [d]**	**% tumors [b]**	**Time to tumor [c] (weeks)**	**Multi-plicity [d]**
B6C3F$_1$											
Males	1	55	86	81	81	43	103	3	59	84	4
	15	60	93	58	81	25	103	2	36	82	2
	42	13	108	9	87	36	119	2	38	95	2
Females	1	7	129	18	121	49	126	3	62	112	4
	15	7	116	7	90	33	122	2	40	101	3
	42	0		0		26	131	2	17	118	3
C3AF$_1$											
Males	1	34	80	46	69	93	78	6	92	70	8
	15	27	90	23	77	93	87	5	94	75	6
	42	0		3	79	93	91	5	87	85	6
Females	1	2	91	2	70	93	82	7	93	73	7
	15	2	102	2	62	94	98	5	91	79	6
	42	0		0		87	93	5	90	83	6

(Data from Vesselinovitch et al., 1975)

a. Age (in days) at which animals received i.p. injections of BP at the stated dose level, dissolved in trioctanoin.
b. Number of mice bearing liver or lung tumors / effective number exposed, expressed as a percentage.
c. Average age (in weeks) at which tumors were observed.
d. Average number of grossly visible lung tumors per whole lung.

Table 9: Carcinogenicity of fluoranthenes in neonatal mice.

Compound	Dose	Newborn Mouse			Adult mouse results (skin bioassays)
		Sex	Lung Tumors %	Liver Tumors %	
Fluoranthene	17.3 µmol	F	86***	7	-ve
		M	65**	100***	
	3.46 µmol	F	35*	0	
		M	43*	64***	
2-Methyl Fluoranthene	17.3 µmol	F	69***	31***	+ve
		M	96***	92***	
	3.46 µmol	F	18	3	
		M	16	45*	
3- Methyl Fluoranthene	17.3 µmol	F	21	11*	+ve
		M	19	69***	
	3.46 µmol	F	15	0	
		M	25	33	
DMSO Control	17.3 µmol	F	12	6	
		M	17	17	

(Data from LaVoie et al., 1994)
P: *** < 0.001, ** < 0.005, *< 0.05

Fluoranthene, 2-MeFA and 3-MeFA when administered to newborn mice also induced a significant incidence of liver tumors among male mice, although only 2-MeFA was tumorigenic in the liver of female mice.

The findings in this study are typical of those obtained in neonatal rodent experiments, in that tumors appeared after a relatively short latency, at multiple sites. As in the case of other similar bioassays using the neonatal mouse model, it is notable that a high yield (up to 100% for some compound, site and sex combinations in the high-dose groups of this experiment) is obtained after only three injections early in life, with a very small total dose. While the testing of these compounds in adults is inadequate, the limited data available suggest that the carcinogenic response in adults is not so strong. In adult bioassays, dosing even with potent carcinogens must usually be continuous or repeated for several months, or else continued doses of promoters applied (as in the case of the skin studies reported for these fluoranthenes), before a statistically significant incidence of tumors is observed. LaVoie et al. (1994) also commented, in comparing the results of this study with those of earlier similar experiments, that although a substantial tumor yield was obtained with studies which were terminated earlier, this experiment showed increased appearance of tumors due to the continuation of the study into mid-life (1 year old). They also noted an apparent increase in the grade of the lesions (carcinomas vs. adenomas) in the longer experiment, which supports the concept that the lesions observed in the adult mice after

neonatal exposure are the result of progression of lesions created, but not necessarily evident, in infancy, soon after the exposure.

Another bioassay, reported by Imaida et al. (1995), illustrates a typical experimental design where the bioassay is started with neonatal rodents to maximize the sensitivity and shorten the duration of the experiment. This was performed with 1-nitropyrene, and 1,3-, 1,6- and 1,8-dinitropyrene; except for 1,3-dinitropyrene, these PAH derivatives have established cancer potency equivalency factors (OEHHA 1993; OEHHA, 1999). (In other experiments reported in the same paper, the effects of 4-nitropyrene, and phenolic metabolites of 1-nitropyrene were also described.) Newborn female CD rats received subcutaneous injections of 1-nitropyrene or dinitropyrenes at 8 weekly intervals starting on the day of birth (total dose = 6.3 µmol). Controls received injections of the solvent, dimethyl sulfoxide, only. The rats were killed at 67 weeks. As shown in Table 10, the dinitropyrenes induced malignant fibrous histiocytoma (MFH) at the site of injection. 1,6- and 1,8-dinitropyrene produced 100% incidences of this tumor, and also induced leukemia with incidences of around 20%. 1-nitropyrene induced a 33% incidence of mammary tumors.

Table 10: Induction by 1-NP and dinitropyrenes of MFH, mammary tumors and leukemia in newborn female CD rats by s.c. injection

Compound[a]	Effective no. of rats	No. of rats with MFH (%)	Average MFH induction period (d)	No. of rats with mammary tumors (%)	Average mammary tumor induction period (d)	No. of rats with leukemia (%)	Average survival period (d)
1-NP	49	0	-	16 (33)[b]	441	0	481
1,3-DNP	43	5 (12)[b]	247	9 (21)	472	0	468
1,6-DNP	46	46 (100)[d]	115	5 (11)	150	9 (20)[c]	149
1,8-DNP	37	37 (100)[d]	122	5 (14)	143	8 (22)[c]	164
DMSO	40	0	-	8 (20)	450	0	495[e]

(Data from Imaida et al., 1995)

a The animals received eight s.c. injections with nitropyrenes, at weekly intervals starting on the day of birth (total dose = 6.3 µmol). The rats were killed at 67 weeks.
b $p < 0.05$ as compared to the solvent control.
c $p < 0.005$.
d $p < 0.0001$.
e One animal had a carcinoma of the Zymbal gland.

MFH = malignant fibrous histiocytoma NP = nitropyrenes DNP = dinitropyrene DMSO = dimethyl sulfoxide.

In all cases the average time to tumor was short compared to a standard two-year bioassay, in which tumors are usually observed mostly towards the end of the experiment. This was particularly notable for 1,6- and 1,8-dinitropyrene, where the average time to tumor for both the MFH and the leukemia was between 100 and 150 days. (The leukemias were either fatal or, if incidental, observed at necropsy, so

the average survival time is taken as time to tumor in this case.) Wislocki et al. (1986) obtained similar results with regard to induction of lung tumors in neonatal mice for a number of nitroaromatics, including nitropyrenes. The total doses used were somewhat lower, but smaller incidences of tumors were observed. Also, the neonatal mouse lung adenoma bioassay is generally considered to be an even more sensitive test system than the neonatal rats used by Imaida et al. (1995). The experiments by Wislocki et al. (1986) were used by OEHHA (1993) in calculating potency equivalence factors for some nitropyrenes.

Since these PAH derivatives induce leukemia, a cancer to which children are known to be particularly susceptible, there may be further cause for anticipating a differential impact of these pollutants on children, although it is not known how, or if, these agents may interact with other causes of childhood leukemia. The possible differential impact of leukemogenic agents on infants and children is discussed in the introductory section of this report.

b) Developmental Toxicity

(1) Teratogenesis

As noted in the discussion of developmental toxicity in the introduction to this report, the induction of either anatomical or functional terata is considered an effect having an adverse effect on the health of infants and children. Similarly, where such effects are noted in animal experiments, this is supporting evidence of the potential for impacts on the health of infants and children. Postnatal mortality is clearly an effect within these terms of reference, but fetal mortality (usually identified as resorptions in animal experiments) is not. However, the occurrence of pre-natal mortality should be noted as an indicator of fetotoxicity, since many fetotoxins (including PAHs, as described below) produce a spectrum of effects. These include anatomical and functional teratogenesis, prenatal mortality, perinatal mortality (stillbirth), postnatal mortality, growth retardation and developmental delay. The combination of these outcomes observed in a particular experiment may depend on dose level and timing, test species used, and other experimental conditions.

Intraperitoneal benzo[*a*]pyrene, at doses between 50 and 300 mg 1 mg/kg body weight given at day 7 or 10 of gestation, causes *in utero* toxicity and teratogenicity in mice (Shum *et al.*, 1979). A reduction in the number of surviving offspring (resulting both from resorptions and stillbirths) was observed in all cases. The severity of the effect was correlated with the ability of the fetus and maternal systems to metabolize benzo[*a*]pyrene, which is influenced by induction of aryl hydrocarbon hydroxylase (AHH). Mice (and other mammals) are described as genetically "responsive" when AHH activity is induced by exposure to PAHs and other activators of the *Ah* receptor. A greater impact on pre-and post-natal mortality was observed in C57BL/6 mice which are responsive to AHH induction than in non-responsive AKR inbred mice. Malformations were noted in the responsive mice only; these included club foot, hemangioendothelioma, cleft palate and various other anomalies of the skeleton and soft tissues. Representative results from this study are shown in Table 11.

Table 11: Impact of benzo[*a*]pyrene treatment on fetal and newborn survival and malformations in responsive and non-responsive mice.

Dosed on Gestation Day	Strain	# Litters	# Implant-ations	# Stillborn	# Resorptions	# Malformed	% all effects
7	B6	7	48	2	19	17	79
	AK	6	43	0	9	0	21
10	B6	10	62	0	11	18	47
	AK	11	78	1	8	0	12
12	B6	7	47	1	20	3	51
	AK	5	45	0	6	0	13
Control	B6	31	187	2	33	0	19
	AK	12	107	0	6	0	6.5

(Data from Shum et al., 1979)
B6 = C57BL/6 mice, responsive to AHH induction.
AK = AKR mice, non-responsive to AHH induction.
Dose given was 200 mg/kg benzo[*a*]pyrene i.p

With the use of AKR x (C57BL/6) $(AKR)F_1$ and (C57BL/6) $(AKR)F_1$ x AKR backcrosses, it was shown that allelic differences at the *Ah locus* in the fetus could be correlated with dysmorphogenesis. If the mother is non-responsive *(Ah^d/Ah^d)*, the Ah^b/Ah^d genotype in the fetus is associated with more stillborns, and resorptions, decreased fetal weight, increased congenital anomalies, and enhanced P_1-450-mediated covalent binding of BP metabolites to fetal protein and DNA, when compared with the Ah^d/Ah^d genotype in the fetus from the same uterus. If the mother is responsive *(Ah^b/Ah^d)*, however, none of these parameters can be distinguished between Ah^b/Ah^d and Ah^d/Ah^d individuals in the same uterus, presumably because enhanced BP metabolism in maternal tissues and placenta cancels out these differences between individual fetuses.

Other investigators have also shown teratogenesis as a result of exposure *in utero* to PAHs, including mixed materials such as are often encountered as environmental contaminants. Feuston and Mackerer (1996) administered clarified slurry oil (CSO), a refinery stream produced by processing crude oil, to pregnant Sprague-Dawley rats on gestation days [GD] 9-12, via dermal application, at doses of 0, 10, 100, and 1000 mg/kg. Maternal toxicity was evident in the dams exposed to CSO, but clear evidence of developmental toxicity was observed at 1000 mg/kg. The effects seen included increased embryolethality, decreased body weight, and anomalous development (cleft palate, brachydactyly, edema). A low incidence of abnormal fetal development was observed at 100 mg/kg. Three to seven-ring polycyclic aromatic compounds are present in CSO, and the authors considered that these PAHs were responsible for the developmental toxicity.

McKee *et al*. (1987a;b) reported reproductive and subchronic toxicity studies of liquids derived from liquefaction of coal. These materials were of interest as a potential novel route to liquid fuels, but the

products contain a number of polycyclic aromatic hydrocarbons, which are concentrated in the high-boiling fractions of the coal-derived liquids. Following treatment of pregnant Sprague-Dawley rats with 0.02, 0.1, 0.5 or 1 g/kg of coal-derived fuel oil or recycle solvent, significant fetal growth retardation was observed, as well as the induction of a small number of diverse skeletal and visceral terata. Results of an experiment with "EDS recycle solvent", a PAH-containing fraction from the coal liquefaction process, are shown in Table 12; significant reductions in number, crown-rump length and weight of fetuses were observed at 0.5 and 1.0 g/kg.

Table 12: Fetal measurements following exposure *in utero* to EDS recycle solvent

Measurement[a]	**Dosage group**			
	Control	Low (0.1 g/kg)	Mid (0.5 g/kg)	High (1.0 g/kg)
Number of male fetuses / litter	5.96 ± 2.05 (49)	5.79 ± 1.69 (24)	3.96* ± 2.37 (25)	0.13** ± 0.46 (23)
Number of female fetuses / litter	5.61 ± 2.06 (49)	5.67 ± 1.52 (24)	3.64* ± 2.23 (25)	0.35** ± 0.57 (23)
Crown-rump length: male fetuses (mm)	3.60 ± 0.16 (292)	3.58 ±0.15 (139)	3.37*** ± 0.17 (99)	3.06*** ± 0.28 (3)
Crown-rump length: female fetuses (mm)	3.52 ± 0.18 (275)	3.48 ± 0.19 (136)	3.27*** ± 0.20 (91)	3.07*** ± 0.19 (8)
Weight of male fetuses (g)	4.23 ± 0.32 (288)[b]	4.22 ± 0.30 (139)	3.82*** ± 0.41 (99)	2.79*** ± 0.45 (3)
Weight of female fetuses (g)	4.07 ± 0.33 (273)	3.96 ± 0.38 (136)	3.47*** ± 0.38 (91)	3.07*** ± 0.35 (8)

(Data from McKee et al., 1987)

a Results expressed as the group mean ± S.D. (n)

b There were 6 fetuses in 1 litter which were measured, but not weighed.

* $p < 0.05$ by ANOVA.

** $p < 0.01$ by ANOVA.

*** Significantly different from controls ($P < 0.05$) by standard nested analysis of variance.

(2) Developmental reproductive toxicity

Infertility was observed in CD-1 mice after exposure *in utero* to benzo[*a*]pyrene (Mackenzie and Angevine, 1981). Groups of 30 or 60 pregnant female mice were given doses of 10, 40 or 160 mg/kg/day benzo[*a*]pyrene in 0.2 ml corn oil on days 7 through 16 of gestation; controls received corn oil only. There was no maternal toxicity or embryolethality at any dose level, although pregnancy maintenance was impaired at 160 mg/kg/day. Mean pup weight was reduced in the litters of all treated dams, with the effect becoming more noticeable with age. As adults, offspring which were exposed to benzo[*a*]pyrene *in utero* showed loss of fertility in controlled breeding studies with untreated partners:

at the higher doses this included complete infertility, and histological abnormalities of the gonads. Treated pup weights, and results of the breeding studies with the F_1 mice are shown in Table 13.

Table 13: Pup weight and reproductive performance of male and female F1 mice exposed prenatally to benzo[*a*]pyrene

	Benzo[*a*]pyrene (mg/kg/day)[a]			
	0	**10**	**40**	**160**
Treated Pup Weight				
Mean pup weight at 4 days (g)	2.7 ± 0.02	2.8 ± 0.04	2.5 ± 0.02	2.2 ± 0.04
Mean pup weight at 20 days (g)	11.2 ± 0.1	11.6 ± 0.1	10.4 ± 0.1**	9.7 ± 0.2**
Mean pup weight at 42 days (g)	29.9 ± 0.2	28.2 ± 0.3**	28.0 ± 0.2**	26.8 ± 0.4**
F_1 Male breeding study				
Number of F_1 males tested[b]	45	25	45	20
Fertility index[c]	80.4	52.0*	4.7**	0.0**
Mean litter size	11.0 ± 0.1[d]	10.7 ± 0.2	10.8 ± 0.6	-
F_1 Female breeding study				
Number of F_1 females tested[e]	35	35	55	20
Fertility index	100.0	65.7**	0.0**	0.0**
Mean litter size	12.9 ± 0.2	10.4 ± 0.4**	-	-

(Data from MacKenzie and Angevine, 1981)

a Mice were exposed prenatally to benzo[a]pyrene on days 7 through 16 of gestation.

b Beginning at 7 weeks of age, each F_1 male was exposed to 10 untreated females over a period of 25 days.

c Fertility index: Females pregnant/females exposed to males x 100.

d Mean ± SEM.

e Beginning at 6 weeks of age, each F_1 female was cohabitated continuously with an untreated male for 6 months.

* Significantly different from controls ($P<0.05$).

** Significantly different from controls ($P<0.01$).

Thus, *in utero* exposure to benzo[*a*]pyrene interfered with the development of the reproductive organs. The severity of the effects seen in this experiment are notable: males exposed to 40 mg/kg benzo[*a*]pyrene showed severely atrophied and essentially aspermic seminiferous tubules. Exposed females showed hypoplastic ovaries with very few follicles or corpora lutea; most of the animals exposed to the higher doses had no identifiable ovaries or only remnants of ovarian tissue. The endocrine effects of such changes are likely to be substantial throughout postnatal growth and development as well as in the adult. The observation in this experiment of low pup weight as a trend of marginal significance immediately after birth, but becoming more noticeable and statically significant in older (20 or 42 day old) pups may be indicative of endocrine effects.

Similar reductions in fertility of female NMRI mice were observed by Kristensen *et al*. (1995) after exposure *in utero* to 10 mg/kg/day oral benzo[*a*]pyrene on days 7-16 of pregnancy.

(3) Developmental Immunotoxicity

As discussed in the introductory section of this report, the immune system is an important potential target for impacts on children's health. This is not only because of the prevalence of infections in children (e.g. otitis media, respiratory infections etc.) but because the immune system is far from mature in the neonate, and undergoes important structural and functional changes during infancy and childhood. In view of this continuing postnatal development phase it is very likely that there would be enhanced sensitivity to exposures to toxicants during infancy, and greater severity of the response, similar to the effects reported in this section.

Urso and Gengozian (1982), Urso and Johnson (1988) and Urso *et al*. (1992) reported a series of experiments in mice which demonstrated that a single exposure to benzo[*a*]pyrene during pregnancy results in immunosuppression in the offspring which is noticeable not only in the neonates but also later in life, and also changes in the maternal immune system which may impact the maintenance of pregnancy and the subsequent immunological status of the offspring. (They suggested that the effects in the offspring might be related to the later development of tumors at a large number of sites in these mice.) The immune responses were measured as the degree of anti-sheep erythrocyte plaque-forming response, mixed lymphocyte response of cultured lymphocytes, and measures of T-cell function. A typical experiment reported by Urso *et al*. (1992), in which mice were treated at mid-pregnancy with a single intraperitoneal injection of 150 mg/kg benzo[*a*]pyrene, is shown in Table 14.

Table 14: Progeny and maternal mixed lymphocyte response

	MLR expressed as % controls*			
	Progeny		**Maternal**	
Time after treatment	**Spleen**	**Thymus**	**Spleen**	**Thymus**
17 days gestation (G)		95	50	47
19 days G		105	51	15
1 day postnatal (P)		61		
3 days P		60		14
7 days P	55	26	40	8
4 weeks P	13			
20 weeks P	44			
53 weeks P	60			
104 weeks P	43			

Data from Urso et al. (1992)

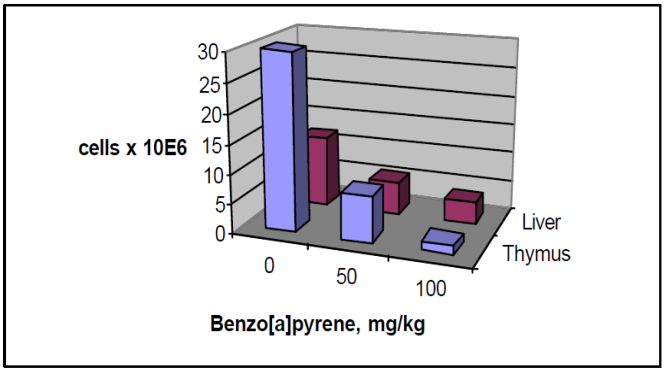
* MLR, mixed lymphocyte response by responder cells cultured for 4 days with allogeneic stimulator cells (mitomycin C inactivated) after a [^{3}H]-thymidine pulse. Each value represents 6-10 determinations;

fetal thymus value is a pool of tissue from fetuses (3-6) of one mother. Values above 95%, not significant; all others significant at $P < 0.05$ to $P < 0.0001$ (Student's t-test).

The maturation of the immune system involves a process of growth, differentiation and selection of various classes of lymphocyte, and also movement from initial locations in the fetal liver and thymus to the bone marrow and peripheral lymphoid tissues. The differentiation and selection processes result in different functional classes of T cells whose interactions are essential in establishing the characteristics of the adult immune system such as self-tolerance, recognition of foreign antigens and appropriate balance of humoral and cell-mediated responses. Disruption of these developmental processes can have diverse and deleterious effects, including autoimmune disease, atopy and immunosuppression.

Holladay and Smith (1994) demonstrated severe depletion of both thymus and liver cell of $B6C3F_1$ mice exposed to benzo[*a*]pyrene (maternal dose 50, 100 or 150 mg/kg/day) on days 13-17 of gestation. Numbers of thymocytes and fetal liver cells (obtained by mechanical disruption, resuspension and washing in lysing solution to remove erythrocytes) determined by Coulter counter are shown in Figure 1.

Figure 1: Fetal thymus and liver cellularity in $B6C3F_1$ mice exposed to benzo[*a*]pyrene *in utero*



(Data from Holladay and Smith, 1994.)

Differences in the proportions of various classes of surface antigens (CD4, CD8 and HSA) were also noted in perinatally isolated thymocytes. The authors concluded that the changes were suggestive of impaired maturation in the surviving thymocytes, and that these and other changes observed were consistent with the long-term immunosuppression seen in mice exposed to benzo[*a*]pyrene *in utero*. Similar effects of benzo[*a*]pyrene on development of T-cells, with long-term consequences for development of the immune system, have been reported by others, including Rodriguez *et al*. (1999).

c) Pulmonary Toxicity (naphthalene)

Naphthalene is a quantitatively important member of the PAH class, and shares a number of toxic effects (including carcinogenicity, as recently reported by NTP [2000]) with PAHs of greater molecular size and complexity.

Naphthalene causes damage to both ciliated and Clara cells of the bronchiolar epithelium in mice (Van Winkle et al., 1995; Plopper, 1992a,b). Neonatal mice were more sensitive to this damage than adult mice (Fanucchi et al, 1997). Swiss Webster Mice at post-natal day (PND) 7 or 14, or adults, received 25, 50 or 100 mg/kg naphthalene by intraperitoneal injection, and the lungs were prepared for histological examination. Both observational and morphometric evaluation showed dose-dependent damage to the bronchiolar epithelium. There was loss of both ciliated and non-ciliated (Clara) cells, as indicated by changes in total epithelial thickness and in volume fractions of the various cell types, and appearance of vacuolated (injured) cells. Effects were similar in adults and young mice, but whereas the adult mice showed a LOAEL of 100 mg/kg for most effects, the 7 and 14-day old mice showed LOAELs of 25-50 mg/kg. Although the doses in this experiment were given intraperitoneally, the effects appear to depend on metabolism of naphthalene in the target tissues and are therefore anticipated to occur regardless of the dose route. Mice, which have high cytochrome P-450 activity in the bronchiolar epithelium, are more sensitive to naphthalene than rats or hamsters where this activity is lower (Plopper et al 1992b). These data indicate that infants and children may be more susceptible to the effects of naphthalene than adults.

V. Additional Information

A. Metabolism of PAHs and formation of PAH adducts

We assume that the majority of the toxic end points described in this summary, in both animals and humans, are the result of the generation of reactive intermediates by metabolism, followed by reactions of these intermediates with sensitive sites in the cell, particularly DNA, as has been observed for benzo[*a*]pyrene and other PAHs in both adult and fetal tissues (Kleihues *et al*., 1980; Shugart and Matsunami, 1985; Bolognesi *et al*., 1985). Unless repaired, the adducts give rise to mutations, followed by cytotoxicity and/or cancer. Teratogenicity also apparently involves reactive intermediate toxicity (Wells and Winn, 1996). Some PAH metabolites, such as quinones, are highly reactive in redox reactions, and thus some additional mechanisms may include the action of reactive oxygen species with cellular components rather than direct reactions with PAH metabolites.

In the metabolism, and ultimately carcinogenicity, of PAHs, both Phase I (activation) enzymes and Phase II (detoxification and conjugation) enzymes are important. Enzymes of both phases are inducible by PAHs and by other related compounds. Both the structural genes determining the stability and activity of the enzymes, and the regulatory genes controlling the induction of the enzymes, are subject to important polymorphisms in both humans and animals. Thus determination of the metabolic capabilities present in the fetus and young animal helps to identify situations where these life stages might be more susceptible to the toxicity of PAHs.

Phase I metabolism of low-molecular-weight chemicals appears only near term in the rodent, and is poorly inducible transplacentally (Anderson *et al*., 1989); such agents are relatively ineffective as fetal carcinogens. Phase I metabolism of aromatic carcinogens (including PAHs) appears early in gestation and is highly inducible transplacentally in rodents by PAHs, resulting in dramatic proportional increases in enzyme activity. Phase II enzymes convert the Phase I metabolites of PAHs or other aromatic compounds to water-soluble forms, and, compared with Phase I enzymes, generally have higher constitutive activity, but a lower degree of inducibility in the fetus. In the mouse a single dominant gene, Ah, confers responsiveness to induction of PAH metabolism by cytochrome P-4501A1; the recessive allele is associated with non-responsiveness. Transplacental exposure to 3-methylcholanthrene also had a permanent imprinting effect, increasing the capacity of the livers to metabolize PAHs as adults 13 months after exposure.

The potential for differential effects is clearly presented by the existence of a highly inducible cytochrome P-4501A1 capable of PAH metabolism in the fetus and at subsequent developmental stages, coupled with a less inducible Phase II system following a different developmental timetable. Cresteil *et al*. (1986) examined cytochrome P-450 isoenzyme content in fetal and postnatal rat liver. In the untreated rat fetus, cytochrome P-450 was easily quantified spectrally, but no isoenzyme could be detected immunochemically. After birth, each isoenzyme develops independently in untreated animals. β-naphthoflavone and benzo[*a*]pyrene hydroxylase activities were significantly increased by 3-methylcholanthrene at any age, whereas the induction of the isosafrole isoenzyme was effective only after 2 weeks of age. Similarly, pretreatment with phenobarbital resulted in the induction of the phenobarbital-B and pregnenolone-16α-carbonitrile isoenzymes and their associated mono-oxygenase activities in fetal and neonatal rat liver, but at different times. Thus P-450 isoenzymes develop independently and different mechanisms regulate the temporal expression of P-450 genes. P-450s present in fetal and early neonatal rats are replaced in older animals by immunologically different isoenzymes. Pretreatment with 3-methylcholanthrene or phenobarbital induces identical isoenzymes in fetal, neonatal and adult rat livers *in vivo*. In addition, 3-methylcholanthrene and phenobarbital are potent inducers of the TCDD-binding protein in the fetal rat liver, where this is a rate-limiting step in induction of cytochrome P-450 (Marie *et al*., 1988). These reports present a complex picture for the Phase I activities of interest for PAH metabolism.

Neubert and Tapken (1988) found that three oral doses of 17.5 mg benzo[*a*]pyrene /kg body wt just significantly induce benzo[*a*]pyrene hydroxylase in maternal liver. In contrast, induction of benzo[*a*]pyrene hydroxylase was demonstrable in 9-12 day old embryos at tissue levels about one tenth those required for induction in maternal liver (0.3 – 1.1 μmol/kg wet weight in whole embryo vs. 5.9 – 1.1 μmol/kg in maternal liver). The benzo[*a*]pyrene tissue concentrations required to induce benzo[*a*]pyrene hydroxylase in fetal liver on day 18 of gestation were about one half of those necessary for induction in maternal liver (1.3 – 3.4 μmol/kg in whole embryo vs. 1.4 – 7 μmol/kg in maternal liver).

The preceding reports (complemented by other related findings, *e.g.* Sunouchi *et al.*, 1984; Lum *et al.*, 1985) show developmental changes in metabolism in the liver. Similar considerations apply to metabolism in other tissues.

Benzo[*a*]pyrene induces Phase II enzymes in the developing rodent. Administration of benzo[*a*]pyrene (50 mg/kg/d) to pregnant rats significantly increased glutathione-S-transferase (GST) activity in placental tissue-extract and total fetal tissue-extract (Cervello *et al*., 1992).

Rouet *et al*. (1984) studied developmental patterns of drug-metabolizing enzymes in the C57Bl/6 mouse brain, lung and liver. These could be divided into three stages: (I) at the end of intrauterine life, where an increase in activity was observed; (II) during the first days after birth, where a decrease was seen; and (III) from the 6th day until weaning, where there was a gradual increase, reaching adult values. Pulmonary benzo[*a*]pyrene hydroxylase activity showed an abrupt burst starting on day 6 of postnatal life, then decreased slowly to become steady, and finally increased again. The major metabolic pathways catalyzed by glutathione-S-transferase and epoxide hydrolase were operative in mouse fetal brain and lung, just as in liver. In addition, enzymatic systems were found to be inducible during fetal life by exogenous compounds such as β-naphthoflavone.

Sindhu *et al*. (1996) found that repeated exposure of both male and female juvenile ferrets to ETS results in an increased production, by lung tissue *in vitro,* of (+)-*anti*-benzo[*a*]pyrene diol epoxide (the ultimate carcinogen) from benzo[*a*]pyrene diol. They also observed increases in DNA binding in the assay.

Dvorchik and Hartman (1982) demonstrated that liver obtained from the fetal stump-tailed monkey (*Macaca arctoides*) is capable of catalyzing the hydroxylation of benzo[*a*]pyrene as early as midterm, and that the apparent K_m is similar to that obtained with human fetal microsomes. The rate of benzo[*a*]pyrene hydroxylation increased almost 100-fold between midterm and 2 weeks after birth.

B. Metabolism of PAHs in human fetus and placenta.

The liver of the human fetus is an active site for drug metabolism, and multiple P450s are present in fetal liver that can catalyze, albeit with lower activity, the same reactions as in adult human liver. Cresteil *et al*. (1982) measured P450 concentration, related mono-oxygenase activities, epoxide hydrolase, and glutathione-S-transferase activities in the liver of human fetuses aged from 15 to 38 wk and in adults. Aniline hydroxylase, benzphetamine demethylase, epoxide hydrolase, and glutathione-S-transferase activities reach about half of adult values as early as 15-25 wk of gestation. The metabolism of benzo[*a*]pyrene, ethoxycoumarin, and testosterone in position 6 beta is very low in the non-induced fetus.

The human placenta has metabolizing capabilities for various xenobiotics, including PAH. Blanck *et al*. (1983) demonstrated biotransformation of benzo[*a*]pyrene and 7-ethoxyresorufin in microsomes from human fetal liver and placenta. Pelkonen (1984) reviewed maternal, placental, and fetal xenobiotic metabolism and found evidence for extensive xenobiotic metabolism in both fetus and placenta. There was a very large inter-individual variation in enzyme activities. Manchester *et al*. (1984) found indications of first pass protection of the fetus by placental xenobiotic metabolism. Placental metabolism is generally considered to be protective of the fetus, by converting benzo[*a*]pyrene or other PAHs to deactivated metabolites before they can impact the fetus.

Barnea and Avigdor (1991) found that benzo[*a*]pyrene at 50 µM caused a significant increase in the AHH enzyme activity of first-trimester human placenta explants after an incubation of 6 h. In contrast, 50 µM 3-methylcholanthrene had no effect. Pasanen and Pelkonen (1994) reported that P450 enzymes in human placenta metabolize several xenobiotics, although compared with the liver the spectrum of substrates and metabolic activities is somewhat restricted. Maternal cigarette smoking increases the expression of CYP1A1. This induced activity results in greater activation of benzo[*a*]pyrene and formation of DNA adducts. Marker activities for CYP3A enzymes, the most abundant P450s in adult human liver and active in fetal liver, were not detectable in human placental microsomes.

Maternal smoking is known to induce AHH in the placenta. As one example, Huel *et al*. (1989) examined AHH in human placenta of both active and passive smokers and confirmed that smoking during pregnancy is associated with a marked increase in placental AHH. Placental AHH was related to the number of cigarettes smoked per day. Moreover, AHH was significantly higher in pregnant women passively exposed to tobacco smoke, relative to controls.

C. Regulatory Background

Polycyclic Organic Matter (POM) is a federal hazardous air pollutant, and this was identified as a toxic air contaminant in April 1993 under AB 2728. OEHHA prepared health risk assessment documents (OEHHA, 1993; 1999) for the California Toxic Air Contaminants program in which benzo[*a*]pyrene and various other PAHs were considered.

The U.S. EPA and IARC have classified benzo[*a*]pyrene as a probable human carcinogen (U.S. EPA, 1994; IARC, 1987). IARC (1987; 1989; 1996) has also identified a number of other specific PAHs as probable or possible human carcinogens (Table 15). The State of California has determined under Proposition 65 that several POM compounds (including benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, chrysene, indeno[1,2,3-*cd*]pyrene, 3,7-dinitrofluoranthene, and 3,9-dinitrofluoranthene) are carcinogens (California Code of Regulations (CCR), 1997).

An inhalation potency for benzo[*a*]pyrene was developed for the California Toxic Air Contaminants program (OEHHA, 1993) using a linearized multistage model applied to respiratory tract tumor incidence data from an inhalation bioassay in male hamsters (Thyssen *et al*., 1981). The cancer potency factor calculated was 3.9 $(mg/kg\text{-}day)^{-1}$, corresponding to a unit risk of 1.1×10^{-3} $(\mu g/m^3)^{-1}$. An oral potency factor of 12 $(mg/kg\ day)^{-1}$ for benzo[*a*]pyrene was also developed, based on the incidence of gastric tumors (papillomas and squamous cell carcinomas) in male and female mice (Neal and Rigdon, 1967; OEHHA, 1993). A number of PAHs were identified by IARC as carcinogens (Class 2B or above), but data were inadequate to permit calculation of specific inhalation cancer potency factors for these compounds. OEHHA (1993) assessed these, along with chrysene and some nitro-PAHs using a relative potency scheme (Collins *et al*., 1998) with benzo[*a*]pyrene as a reference compound (Table 16).

In addition to the listing of benzo[*a*]pyrene and other PAHs as a toxic air contaminant, it should be noted that diesel exhaust particulate matter has been listed as a toxic air contaminant, and diesel exhaust is listed as a carcinogen under Proposition 65. Both the volatile and particulate fractions of diesel

exhaust contain PAHs and nitro-PAHs, which are considered important, but not exclusive, contributors to the carcinogenicity and other adverse health effects of diesel exhaust.

Table 15: IARC groupings of PAHs, mixtures with PAHs, and derivatives.

Group 1	**Group 2A**	**Group 2B**
Coal-tar pitches	Benz[*a*]anthracene	Benzo[*b*]fluoranthene
Coal-tar	Benzo[*a*]pyrene	Benzo[*j*]fluoranthene
Coal gasification	Creosotes	Benzo[*k*]fluoranthene
Coke production	Dibenzo[*a,h*]anthracene	Carbon black extracts
Mineral oils	Diesel engine exhaust	Carbon black
Shale-oils		Dibenz[*a,h*]acridine
Soots		Dibenz[*a,j*]acridine
Tobacco smoke		7H-Dibenzo[*c,g*]carbazole
Smokeless tobacco products		Dibenzo[*a,e*]pyrene
		Dibenzo[*a,h*]pyrene
Aluminum production		Dibenzo[*a,i*]pyrene
		Dibenzo[*a,l]*pyrene
		Indeno[1,2,3-*cd*]pyrene
		5-Methylchrysene
		5-Nitroacenaphthene
		1-Nitropyrene
		4-Nitropyrene
		1,6-Dinitropyrene
		1,8-Dinitropyrene
		6-Nitrochrysene
		2-Nitrofluorene
		3,7-Dinitrofluoranthene
		3,9-Dinitrofluoranthene
		Gasoline engine exhaust

(Data from IARC Supplement 7, 1987, and IARC Volumes 46, 1989 and 65, 1996.)

Group1: carcinogenic to humans.
Group 2A: probably carcinogenic to humans.
Group 2B: possibly carcinogenic to humans.

Table 16: OEHHA Potency Equivalency Factors (PEF)

PAH or Derivative	CAS Number	Suggested PEF
benzo[*a*]pyrene	50-32-8	1.0 (Index compound)
benz[*a*]anthracene	56-55-3	0.1
benzo[*b*]fluoranthene	205-99-2	0.1
benzo[*j*]fluoranthene	205-82-3	0.1
benzo[*k*]fluoranthene	207-08-9	0.1
dibenz[*a,j*]acridine	224-42-0	0.1
dibenz[*a,h*]acridine	226-36-8	0.1
7H-dibenzo[*c,g*]carbazole	194-59-2	1.0
dibenzo[*a,e*]pyrene	192-65-4	1.0
dibenzo[*a,h*]pyrene	189-64-0	10
dibenzo[*a,i*]pyrene	189-55-9	10
dibenzo[*a,l*]pyrene	191-30-0	10
indeno[1,2,3-*cd*]pyrene	193-39-5	0.1
5-methylchrysene	3697-24-3	1.0
1-nitropyrene	5522-43-0	0.1
4-nitropyrene	57835-92-4	0.1
1,6-dinitropyrene	42397-64-8	10
1,8-dinitropyrene	42397-65-9	1.0
6-nitrochrysene	7496-02-8	10
2-nitrofluorene	607-57-8	0.01
Chrysene	218-01-9	0.01
dibenz[*a*,h]anthracene*	53-70-3	1.1
7,12-dimethylbenzanthracene*	57-97-6	65
3-methylcholanthrene*	56-49-5	5.7
5-nitroacenaphthene*	602-87-9	0.034

The nitro-PAHs are those listed as IARC class 2B. Although chrysene is an IARC class 3 carcinogen, the U.S. EPA classifies it as Group B2.

* Inhalation unit risks were calculated independently for these compounds by OEHHA; the PEF shown is the ratio of the calculated unit risks for these compounds to that for benzo[a]pyrene.

VI. Conclusions

This evaluation of POM as a toxic air contaminant causing health effects in infants and children was primarily based on the known health effects of PAHs (which are the major components of POM), and of related specific chemicals that occur as components of POM. Also considered were exposures to air pollutant mixtures from defined sources, of which POM is a known component and where health effects can be at least partly ascribed to this POM component or to specific PAHs identified in the mixture. These mixtures include diesel exhaust, environmental tobacco smoke, and air pollutants emitted by domestic or industrial combustion of solid fuels (coal etc.).

Many PAHs, PAH derivatives and pollutant mixtures containing them have been shown to be carcinogenic in animals and/or humans. There is a general concern that exposure to a carcinogen early in life may have a greater overall impact than a similar exposure to an adult. Additionally, there are experimental data showing that young animals are more sensitive to the carcinogenicity of certain PAHs and PAH derivatives.

Prenatal exposure to PAHs results in serious or irreversible effects in the fetus. Fetal damage sustained as a result of exposure to environmental toxicants is a source of adverse postnatal health impacts. For instance, PAHs are transplacental carcinogens. The mechanisms underlying transplacental carcinogenesis are apparently similar to those for carcinogenesis after postnatal exposure, but the sensitivity and diversity of tumor sites are often greater.

Fetotoxicity and teratogenesis have been observed following animal exposure *in utero* to PAHs or to mixtures containing them. Such exposures to PAHs, or to mixtures containing them, have also been found to result in related adverse effects, such as low birth weight in both humans and rodents. PAH exposures *in utero* have also been found to cause structural and functional disturbances of the immune and hematopoietic systems, and loss of fertility, in the offspring. These occurred at doses causing little or no concurrent maternal toxicity. Some of these effects observed after exposure *in utero* parallel toxic effects in the adult (e.g. immunotoxicity, reproductive toxicity, myelotoxicity), but whereas these effects are reversible in the adult, the effect of fetal exposure is irreversible.

There is greater exposure of children to environmental PAHs compared to adults. Children's daily doses of PAHs (per kg of body weight) were generally higher from all routes of exposure than those of adults in the same household. Biomarkers for direct impacts associated with adverse health outcomes, such as DNA adducts, are increased in children exposed to environmental pollution by PAHs and related POM components.

In view of this range of evidence for differential sensitivity of the fetus, infants and children to health effects induced by POM components such as PAHs, and for greater exposure of children to POM, OEHHA has placed POM in Tier 1 of the priority list.

VII. References

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