Appendix C-2
Chemical Toxicity Summaries
For Tier 2 Toxic Air Contaminants

Arsenic and Arsenic Compounds

CAS Registry Numbers: Arsenic, As, 7440-38-2; Arsenious acid, As_2O_3, 1327-53-3; Arsenic oxide, As_2O_5, 1303-28-2; Arsenic acid disodium salt, Na_2HAsO_4, 7778-43-0; Arsenious chloride, $AsCl_3$, 7784-34-1

As

I. Physical and Chemical Properties

Description	As: Yellow, black or gray solid As_2O_3: White solid
Molecular formula	Arsenic As; Arsenious acid, As_2O_3; Arsenic oxide, As_2O_5; Arsenic acid disodium salt, Na_2HasO_4; Arsenious chloride, $AsCl_3$
Molecular weight	As, 74.92; As_2O_3, 197.82; As_2O_5, 229.82; Na_2HAsO_4, 185.91; $AsCl_3$, 181.28
Air concentration conversion	Not applicable for most compounds; $AsCl_3$: 1 ppm = 7.41 mg/m^3

II. Overview

There is some indication of differential effects of exposure to arsenic in human studies on birth weight and congenital malformations (Nordstrom et al., 1978, 1979ab; Beckman and Nordstrom, 1982) and on neurological development (IQ) (Siripitayakunkit et al.1999; Calderon et al., 2001). Studies in Chile comparing communities exposed to high or low arsenic in their drinking water have indicated an association arsenic exposure with elevated risks of fetal, neonatal, and postneonatal mortality (Hopenhayn-Rich et al., 2000).

Arsenic is a known human carcinogen by inhalation and oral routes of exposure. The principal sites of cancer formation are skin, lung and urinary bladder. Lesser sites include liver and kidney (IARC, 1987; NRC, 1999). The data of Smith et al. (1998) indicate that childhood exposures to arsenic in drinking water may be associated with a significant increase in lung cancer in younger men aged 30-39 years.

Arsenic is teratogenic in mice, rats, hamsters, rabbits, and chicks. Arsenite (As III) has been shown to cause reproductive and developmental effects at significantly lower doses than arsenate (As V). The

effects observed include increased fetal death, decreased fetal weight, and congenital anomalies. The anomalies most frequently reported include neural tube defects, eye defects, renal and gonadal agenesis, and skeletal malformations. Most studies have involved single high doses by gavage or injection. Maternal toxicity was often but not always observed in these studies (OEHHA, 1999a, 2000).

Calderon *et al*. (2001) studied two populations of six to nine year old children exposed to lead and higher or lower levels of arsenic in their drinking water. They concluded that increased arsenic exposure, as indicated by µg urinary arsenic excreted/g creatinine, was associated with an independent decrease in verbal IQ and long-term memory.

Concern for potential differential toxicity of arsenic compounds in children vs. adults is predicated on the carcinogenicity and developmental toxicity of arsenic compounds. The potential neurotoxicity of arsenic in children, possibly in combination with other environmental agents, is also a concern. Studies in mice (Meija et al., 1997) indicate combined effects of lead and arsenic on the central nervous system that were not observed with either metal alone.

III. Principal Sources of Exposure

Arsenic is ubiquitous and is found in low concentrations in soil and water and also in foods, particularly seafood. Ore refining processes, including the smelting of copper and lead, are the major sources by which arsenic dust and inorganic arsenic compounds are released. Arsenic trioxide (As_2O_3) is the most commonly produced form of arsenic. As_2O_3 is used as a raw material for the production of other inorganic arsenic compounds, alloys, and organic arsenic compounds. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 11,303 pounds of arsenic (CARB, 2000). The ambient air concentration in California in 1997 was 1.14 ng/m^3 (CARB, 1999). Concentrations of inorganic arsenic in drinking water can vary significantly. A 1995 survey (ACWA, 1995) covering 180 water agencies in California from 27 counties found the median value of 1500 samples to be 0.002 mg As/L (2 ppb). U.S. EPA (2001) concluded that 20-50 percent of public water systems in the western U.S. had arsenic levels greater than 5 ppb. Thus intake of arsenic from drinking water for an adult consuming 2L/day could be about 10 µg/d. Intake from food sources has been estimated at 50 µg As/d (adults, U.S.EPA, 1988). Thus the airborne contribution to total As intake from ambient air is probably very low (e.g.,1.14 ng/m^3 x 20 m^3/d = 23 ng/d; 0.023 x 100/60.023 = 0.4%).

IV. Potential for Differential Effects

A. Summary of Key Human Studies

A number of investigations were conducted in association with smelting operations in Ronnskar, Sweden and its impact on employees and the population in surrounding areas. The smelter produces copper and lead and a series of other products including gold, silver, zinc, arsenic trioxide, arsenic metal, selenium, nickel sulfate, and red lead. Emissions into the air and water of sulfur dioxide and heavy metals, notably lead, cadmium, mercury, and arsenic, have been studied in a series of investigations. Ronnskar arsenic emissions to air were reported to be 50 tons/year. Emissions of lead, cadmium, and mercury were 200, 5, and 1 ton/year, respectively (Beckman, 1978). Adverse effects on birth weight and increased incidence of congenital malformations were reported; however, arsenic exposures in the area were not well characterized and were confounded by other chemicals, particularly lead and cadmium (Nordstrom *et al*., 1978; 1979a,b; Beckman and Nordstrom, 1982).

The first publication examined the relationship of birth weight in infants of women working at the smelter between 1975 and 1976 and in four areas at varying distances from it (Nordstrom et al., 1978). The latter group consisted of 3688 pregnancies with 2700 single births from a separate region that served as a control (Umea). The average birth weight in offspring of the smelter employees (3391 g) was significantly lower than in Umea (3460 g, $p < 0.05$) and in the two regions farthest from the smelter (C and D; 3495 g and 3470 g, $p < 0.01$, $p < 0.05$, respectively). In the areas (A and B) closest to the smelter (< 10 km), birth weights were also significantly lower (3395 g and 3411 g) than the control ($p < 0.001$ and $p < 0.05$, respectively) and regions C and D ($p < 0.01$-0.05).

Nordstrom et al. (1979a) reports the results of a follow-up investigation of birth weights and birth order among female smelter employees at any time since it began operation in the early 1930s. Birth weight was examined in 881 employees born between 1930 through 1959 and in 2700 controls (Umea). The average birth weight of infants born to employees of the smelter was significantly lower than controls ($p < 0.01$). The decrease was mainly due to pregnancy orders greater than two ($p < 0.001$) and was most pronounced when the mother was employed during her pregnancy. Birth weight normally increases with birth order (as seen in the controls) so the authors note that this was an unusual and significant finding.

Nordstrom et al. (1979b) studied the frequency of congenital malformations in live born children registered at a hospital in the region close to the Ronnskar smelter (Skelleftea) during the period 1955 to 1976 and in children of female employees of the smelter. A total of 694 out of 24,018 live born children (2.9%) were found to have congenital malformations. There was no regional difference in rate or group of malformations in the areas served by the hospital. A temporal difference was observed: 1955-1966, 2.6%; 1966-1976, 3.3%, $p < 0.001$. A total of 39 of 1291 children of employees had malformations (3%). When the mother had worked at the smelter during pregnancy, 17 of 291 had malformations (5.8%) versus 22/1000 (2.2%) if the mother did not work. The difference between these two groups of children was statistically significant ($p< 0.005$). Six children had multiple malformations, and five of these had mothers who had worked at the smelter during pregnancy. For the

five children, the multiple malformations included cleft palate, malformed urethras, spina bifida, multiple bone malformations and multiple digestive and circulatory malformations.

Beckman and Nordstrom (1982) studied rates of congenital malformations and fetal death among wives of 764 smelter workers at the Ronnskar smelter. The reference group consisted of non exposed pregnancies within the same occupational group. The rate of congenital malformations was found not to be related to occupational exposure, smoking habits, or alcohol consumption. The rate of fetal deaths (spontaneous abortions and stillbirths combined) was significantly increased in pregnancies where the husbands were exposed, after adjustment for parental age and pregnancy order ($0.01<p<0.05$). The results suggest that fetal death is caused by germ cell damage through occupational exposure of fathers.

Calderon *et al*. (2001) conducted a cross-sectional study to examine the effects of chronic exposure to lead (Pb), arsenic (As), and nutrition on the neuropsychological development of children. Two populations of children aged six to nine years (N = 41, 39) with differing As exposure levels (63 v. 40 µg/g) but similar Pb exposures (8.9 v 9.7 µg Pb/dL blood, respectively) were compared using the Wechsler Intelligence Scale for Children (WISC) Revised Version for Mexico. After controlling for significant potential confounders, verbal IQ was observed to decrease with increasing urinary arsenic ($p < 0.01$). Language, verbal comprehension and long-term memory also appeared to be adversely affected by increasing arsenic exposure (concepts and knowledge factors, $p < 0.05$ each). Blood lead was significantly associated with a decrease in attention (sequential factor, $p < 0.05$). However since blood lead is an imprecise measure of lead burden there could be some residual confounding in this study.

The relationship between arsenic exposure via drinking water and neurological development as indicated by intelligence (IQ) was assessed in Thailand (Siripitayakunkit *et al*., 1999) in 529 children aged six to nine years using a cross-sectional design. Arsenic levels in hair were used to assess exposure and the WISC test for children was used to assess IQ. The range of arsenic concentrations in hair was 0.48 to 26.94 µg/g. The mean IQ of the study was 90.44 (range 54 to 123). Most of the IQs were classified as average (45.7%) or dull normal (31.6%). Approximately 14% and 3% of the children were in the borderline and mental defective groups, respectively. The percentage of children in the average IQ group decreased significantly from 57 percent to 40 percent with increasing arsenic exposure. The percentage in the lower IQ group increased with increasing As (23% to 38%) and in the low IQ group (zero to six percent). In a comparison of IQ between children with As hair levels ≤ 2 ppm or > 2 ppm, arsenic was found to explain 14 percent of the variance in IQ after controlling for father's occupation, mother's intelligence score, and family income. Arsenic levels in hair above 2 ppm were associated with a 0.75 point decrease in IQ below the grand mean, and As levels above 5 ppm with a two point decrease. Although the cross-sectional study design does not allow for establishment of the time precedence of exposure to arsenic, the investigators stated that the subjects of the study were born in a period of chronic arsenic poisoning and that this cohort has been continuously exposed since birth due to their non-mobility. The study suffers from small numbers of children exposed to low arsenic (hair arsenic ≤ 1 ppm) so this group could not be compared to the high arsenic children. Also the possible exposure to chemical confounders like lead is not discussed.

Hopenhayn-Rich et al. (2000) conducted a retrospective study of chronic arsenic exposure and risk of infant mortality in two areas of Chile: Antofagasta, with a documented history of As contaminated drinking water and Valparaiso, a comparable low-exposure city. Between 1950 and 1996, Antofagasta experienced an 86 percent decline in the late fetal mortality rate, an 81 percent decline in neonatal mortality rate, and a 92 percent decline in the post neonatal mortality rate. The declines in fetal and infant mortality rates in Valparaiso were 64, 77, and 92 percent, respectively. Despite the overall decline, rates for all outcomes increased in Antofagasta during 1958-1961 and declined thereafter. The increases and declines overall coincide with the period of higher arsenic levels in the drinking water. Results of a Poisson regression analysis of the rates of late fetal, neonatal and postneonatal mortality showed elevated relative risks for high arsenic exposure in association with each of the three mortality outcomes. The association between arsenic exposure and late fetal mortality was the strongest (RR = 1.72; CI, 1.54-1.93). Neonatal mortality (RR = 1.53; CI 1.40-1.66) and postneonatal mortality (RR = 1.26; CI, 1.18-1.34) were also elevated. These findings provide suggestive evidence for arsenic-related human developmental toxicity.

Ihrig et al. (1998) conducted a hospital-based case-control study of stillbirths and environmental arsenic exposure using an atmospheric dispersion model linked to a geographical information system. They collected data on 119 cases and 267 controls in a central Texas area including a facility with 60-year history of arsenic-based agricultural product manufacture. Four exposure groups were categorized (0, < 10 ng/m^3; 10-100 ng/m^3; and > 100 ng/m^3). For the period 1983-93 they fit a conditional logistic regression model including maternal age, race/ethnicity, parity, income group, exposure as a categorical variable, and exposure-race/ethnicity interaction. Effects were only seen in the Hispanic group with the medium exposure group having a prevalence odds ratio and 95% confidence interval of 1.9 (0.5-6.6) and the high exposure group 8.4(1.4-50.1). The authors postulate a possible influence of a genetic polymorphism affecting folate metabolism in Hispanic populations possibly leading to increased neural tube defects and stillbirths. This study is limited by small numbers, for example there were only seven cases in the high exposure group and five of these were Hispanic.

Smith et al (1998) studied lung and urinary bladder cancer mortality in a region of northern Chile (Antofagasta) where the residents were exposed to arsenic in their drinking water. Arsenic levels ranged from a population weighted average of 570 µg/L between 1955 and 1969 to 100 µg/L by 1980. The mortality ratios (observed/expected deaths) for bladder, kidney, liver, and skin cancers, and all other cancers combined, were not related to age in either sex. However, lung cancer mortality ratios were particularly high in younger men aged 30-39 yr (SMR = 11.7, 95 percent CI 6.4-19.6, $p < 0.001$). Also observed was a decreasing trend in chronic obstructive pulmonary disease deaths (COPD), with higher rates among younger men, particularly those aged 30-39. Four COPD deaths were reported among men (0.8 expected), and six deaths among women (0.1 expected). These ten individuals who died of COPD would have been young children at the time of peak arsenic water levels in 1955-1970. Additional evidence supporting a link between childhood arsenic exposure and subsequent lung disease comes from autopsies of children in the affected area. The results of five autopsies of children who died in 1968 and 1969 in Antofagasta showed evidence of arsenic poisoning including skin lesions; lung abnormalities were observed in four of the children whose lungs were examined. Two of these cases exhibited interstitial fibrosis (Rosenberg, 1974). Also, in a survey of

144 children in Antofagasta with skin pigmentation due to arsenic exposure, bronchopulmonary disease was 2.5-fold more frequent than in children with normal skin (15.9 vs. 6.2 percent, respectively) (Borgono et al. 1977).

B. Summary of Key Animal Studies

Nagymajtenyi *et al*. (1985) exposed pregnant CFLP mice (8-11 females/group) to As_2O_3 for 4 hours/day on gestational days 9-12 at concentrations of 0, 0.26, 2.9, or 28.5 mg As_2O_3/m^3 (~0.2, 2.2, and 21.6 mg As/m^3). A statistically significant decrease in fetal weight was observed in all the dose groups ($p < 0.05$), with a 3, 9, and 29% reduction in average fetal weight with increasing dose. Significantly increased fetal malformations were observed only in the highest dose group, primarily delayed ossification, with an apparent positive dose-related trend in the number of fetuses with malformations (3, 7, and 31, respectively). A similar dose-related trend in chromosome aberrations in liver cells was also observed in the number of cells with chromosomal damage: chromatid gaps, chromatid breaks, chromosome fragments, and chromosome breaks. Only the number of damaged cells and chromosome breaks at the high dose were significantly different from the control ($p < 0.05$).

Hood (1998) injected mice with 1200 or 1500 mg/kg-d methanearsonic acid (MMA) or with 800 or 1200 mg/kg-d dimethylarsinic acid (DMA) on gestation days 8 thru 14. MMA and DMA are the primary metabolites of inorganic arsenic in most animals and humans. Both arsenicals induced prenatal mortality and malformations in the developing offspring following maternal treatment on single gestation days. However, the doses employed were extremely high and "in the maternally toxic range".

Studies in mice exposed to sodium arsenite (11 or 13.8 mg/kg-d) and/or lead acetate (116 mg/kg-d) for 14 days have demonstrated both individual and combined effects on the central nervous system. Specifically arsenic was found to decrease norepinephrine levels in the hypothalamus and increase 5-hydroxyindole-3-acetic acid in the midbrain and striatum and to increase dopamine in the striatum. Treatment with lead and arsenic combined gave a 38 percent decrease of norepinephrine in the hippocampus that was not seen with either metal alone (Meija et al., 1997).

V. Additional Information

A. Other Respiratory Toxicity

Wulff *et al*. (1996) studied the risk of cancer in children born to women living in the vicinity of the Ronnskar smelter in Sweden. Thirteen cases of cancer were diagnosed in children born in the vicinity of the smelter compared to 6.7 cases expected based on national rates (Standardized Incidence Ratio (SIR) = 1.95, 95% C.I. 0.88-3.0) although this difference was not statistically significant. The SIR for the reference group was 1.0 (95% C.I. 0.7-1.30). Leukemia was the most common cancer in both

groups. Confounding exposures from other chemicals are still an important qualification as noted above for earlier studies of this site.

Bencko et al. (1977) analyzed hearing changes in a group of 56 10-year-old children residing near a power plant burning local coal of high arsenic content. The control group consisted of 51 children of the same age living outside the polluted area. Thresholds of hearing were examined at frequencies of 125-8000 Hz for air conduction and 125-4000 Hz for bone conduction. Hearing losses for air conduction were found in the exposed group at 125-1000 Hz and at 8000 Hz ($p = 0.0005$-0.05). For bone conduction hearing losses were seen at 125-500 Hz and 4000 Hz ($p = 0.0005$-0.05). Clinical examination showed a higher rate of enlarged tonsils and adenoids and concomitant phlegm-pus flow in the posterior nasopharynx in the exposed group versus the controls. However, history of more frequent nasopharyngeal and middle ear infections was lower in the exposed group compared to controls. The high statistical significance of the hearing impairments observed suggests that auditory damage, including total deafness, may be associated with arsenic exposure in these children. Auditory damage caused by arsenic poisoning has been observed in animal studies and in clinical experience (early German papers cited in Bencko et al., 1977).

B. Regulatory Background

An inhalation chronic REL of 0.03 $\mu g/m^3$ has been established by OEHHA based on reduction in fetal weight and increased incidence of growth retardation and skeletal malformations in mice (OEHHA, 2000). An inhalation acute REL of 0.19 $\mu g/m^3$ was set by OEHHA based on decreased fetal weight in mice (OEHHA, 1999a). Inorganic arsenic compounds are recognized as chemicals known to cause cancer and reproductive toxicity under Proposition 65 (Safe Drinking Water and Toxic Enforcement Act of 1986). The current inhalation cancer potency is 12 $(mg/kg\text{-}d)^{-1}$ or 3.3×10^{-3} $(\mu g/m^3)^{-1}$. The current oral cancer potency is that established by U.S. EPA of 1.5 $(mg/kg\text{-}d)^{-1}$ based on human skin cancer incidence (U.S.EPA, 1988; OEHHA, 1999b).

VI. Conclusions

Although there is evidence that infants and children may be more susceptible to arsenic exposure than adults, airborne exposures are very low and represent a small percentage of total exposure to arsenic. Thus arsenic has been placed in Tier 2. We may reconsider listing arsenic in future updates, particularly if evidence of significant exposures arise.

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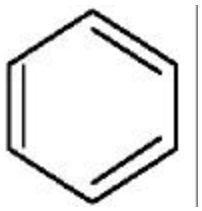
Benzene

CAS Registry Number 71-43-2



I. Physical and Chemical Properties

Description	clear, colorless liquid
Molecular formula	C_6H_6
Molecular weight	78.11 g/mol
Air concentration conversion	1 ppm = 3.19 mg/m^3 (at 25^{0}C)

II. Overview

Exposure to benzene is associated with increases in numerous adverse effects including bone marrow damage, changes in circulating blood cells, developmental and reproductive effects, alterations of the immune system, and cancer. Benzene is absorbed through all routes of exposure, and the metabolism and distribution does not appear to depend significantly on route of exposure (OEHHA, 2000a). In humans, the most sensitive responses to benzene are those related to the blood-forming organs.

In California, the acute Reference Exposure Level (REL, 6 hr) for benzene is 1300 µg/m^3, and the chronic Reference Exposure Level (REL) is 60 µg/m^3 (OEHHA, 1999a; 2000b). The acute and chronic REL values are based on hematotoxicity among benzene-exposed workers. The cancer potency factor is 0.1 (mg/kg-day)$^{-1}$, which corresponds to a unit risk factor of 2.9 x 10^{-5} (µg/m^3)$^{-1}$ (OEHHA, 2001). The California TAC value is based on increased rates of leukemia among benzene-exposed workers.

Benzene causes reproductive and developmental effects including reduced fetal weight, delayed ossification, fetal chromosomal damage, altered fetal hematopoiesis, and alterations to sperm. OEHHA

(1997) extensively reviewed the available literature on benzene's reproductive and developmental toxicity. Some of the conclusions of that document will be discussed below. Benzene was listed in 1997 as a reproductive and developmental toxicant by the State of California under Proposition 65, the Safe Drinking Water and Toxic Enforcement Act of 1986. OEHHA is currently developing a Maximum Allowable Daily Level (MADL) for the developmental effects of benzene as part the Proposition 65 Program.

However, since cancer is still likely to "drive" the TAC toxicity number for benzene, the focus of this summary will be to describe the scientific evidence on whether exposures to benzene early in life would result in a greater carcinogenic impact than exposures occurring later in life.

Summary of potential for differential effects

Benzene has been implicated as a potential risk factor for the development of childhood leukemia (OEHHA, 1997; Smith and Zhang, 1998; U.S. EPA, 1998; Reis et al., 1999). Some epidemiological studies have reported statistically significant associations of increases in childhood leukemia, especially acute non-lymphocytic leukemia, with maternal exposures during pregnancy or paternal exposures prior to conception to benzene or benzene-containing mixtures (Shu et al., 1988; Buckley et al., 1989; McKinney et al., 1991). These findings are consistent with evidence in animals that exposure to benzene induced DNA damage to sperm, transplacental genotoxicity, transplacental altered hematopoiesis and, possibly, transplacental carcinogenicity. However, other epidemiological studies did not find an association between occupational paternal exposure to benzene and childhood leukemias (Shaw et al., 1984; Kaatsch et al., 1998; Shu et al., 1999; Feychting et al., 2001).

Also, there is evidence in animals that exposures to benzene early in life and through adulthood resulted in a 2-fold higher increase in the incidences of cancer compared to exposures only as adults (Maltoni et al., 1989).

Since benzene has been associated with childhood leukemia in several epidemiological studies, and since early life exposures appear to differentially increase lifetime cancer risk in animal studies, benzene

is considered to be a priority chemical for evaluation of potential differential effects on infants and children.

III. Principal Sources of Exposure

Except for cigarette smoking, vaporization of gasoline and automobile exhaust are the primary sources of benzene exposure in the general population (Wallace, 1996). Past formulations of gasoline contained about one to two percent benzene; however, current formulations are required to contain no more than one percent benzene by volume.

The California Air Resources Board (CARB) routinely monitors ambient air concentrations of benzene throughout California through its air toxics network. In 1982, when the monitoring program began, estimates of the population-weighted annual concentration of benzene were roughly 5 ppb (16 $\mu g/m^3$) (CARB, 1984). These concentrations have declined steadily over time such that in 1994 average estimates across the state were approximately 1.2 ppb (3.8 $\mu g/m^3$) (CARB, 1995). The 12-month average ambient air concentration of benzene for California in 1997 to 1999 was 0.85 ppb (CARB, 2000). In addition to benzene emissions derived from mobile sources, there are significant emissions of benzene from stationary sources in California. These were estimated to be at least 870,000 pounds per year in 1997, based on data reported under the Air Toxics "Hot Spots" Program (AB 2588) (CARB, 1997).

In studies of human exposures to benzene, the primary sources of exposure among non-smokers were auto exhaust and gasoline vapor emissions. Most of the benzene in outdoor air comes from auto and gasoline vapor emissions; inhalation of ambient air accounts for a large percentage of an individual's total benzene exposure. Also, indoor air exposures due to intrusion of evaporative gasoline fumes in homes with attached garages and personal activities such as driving can contribute significantly to an individual's total exposure to benzene (Wallace, 1996). For example, a study sponsored by CARB found that benzene concentrations inside a vehicle was 3-to 7-fold higher than ambient levels nearby

(CARB, 1998). Other sources of exposure to benzene include contaminated drinking water, which can arise for example from contamination of water sources by leaking from underground fuel storage tanks.

IV. Potential for Differential Effects

The evidence presented here will be divided along two lines:1) evidence that suggests that benzene may be a transplacental and preconceptional carcinogen associated with childhood leukemia; and 2) evidence to suggest that children or the developing fetus exposed to benzene may exhibit a higher lifetime risk of cancer than equivalent exposures to adults.

A. Summary of Key Human Studies: Cancer

All major authoritative bodies classify benzene as a known human carcinogen, based on increased rates of leukemia among different benzene-exposed human populations. However, there is some evidence to suggest that benzene also causes childhood leukemia (see key human studies below), although a causal relationship would be difficult to establish at this time. Many researchers contend that childhood and adult leukemias are different diseases with different etiologies.

a) Childhood exposures resulting in increases in adult-onset leukemia

There are no human studies available that have examined childhood exposures to benzene and increases in lifetime risk of cancer.

b) Benzene and childhood leukemia

Benzene has been implicated as a potential risk factor for the development of childhood leukemia (OEHHA, 1997; Reis et al., 1999; Smith and Zhang, 1998; U.S. EPA, 1998). Some large epidemiological studies have reported increases in childhood leukemia associated with *in utero* exposures, and paternal exposure prior to conception to benzene. However, other studies do not suggest an association. The studies and their strengths and limitations are discussed below.

Childhood cancer is the second biggest killer of children (the first being accidents), and the most common form of childhood cancer is leukemia (Sandler and Ross, 1997). Moreover, childhood cases through 14 years of age account for 12 percent of all leukemia cases in the U.S. (Sandler and Ross, 1997). The incidence of leukemia among children younger than 15 years of age has remained fairly constant over the past 20 years except for a sharp increase from 1983 to 1984, which likely reflects a change in diagnosis or reporting (Linet et al., 1999). Deaths rates from childhood leukemia have declined steadily since 1975, which is believed to be due to increased survival from medical advances in treatment (Linet et al., 1999). Exposures to carcinogens during *in utero* development and in early childhood have been suggested as possible causal factors responsible for some of the increases in leukemia (Reis et al., 1999). In adults the most common leukemia types are myeloid and lymphatic, whereas the predominant type of leukemia in children is lymphatic. Benzene exposure in adults is most strongly associated with acute myelogenous leukemias (AML), although increased risks of non-AML leukemias are also reported (Crump, 1994; Hayes et al., 1997). Likewise, some epidemiological studies that have examined childhood leukemias by subtype with respect to paternal or maternal exposures to benzene have also found the strongest associations with acute myelogenous leukemias (Shu et al., 1988; Buckley et al., 1989).

c) Paternal or maternal exposure to benzene and childhood leukemia

A large case control study reported finding a statistically significant association, including a trend in exposure duration, between paternal benzene exposure and childhood acute non-lymphocytic leukemia among progeny (Buckley et al., 1989), while one study with a smaller number of cases did not (Kaatsch et al., 1998). Additionally, a study examining paternal benzene exposure and childhood leukemia (not separated by subtype) reported a positive association (McKinney et al., 1991). Two studies of paternal benzene exposure prior to conception and childhood leukemia (not separated by subtype) (Shaw et al., 1984; Feychting et al., 2001) or acute lymphocytic cases only (Shu et al., 1999) did not find an association. With respect to maternal exposure to benzene, high relative risk estimates have been reported for benzene exposure and childhood acute non-lymphocytic leukemia among progeny in one report (Shu et al., 1988), while a separate report did not find an association (Kaatsch et al., 1998).

Studies of childhood acute lymphocytic leukemia, the most common childhood leukemia subtype, did not find an association with maternal benzene exposure (Kaatsch et al., 1998; Shu et al., 1999). Other studies of parental exposures to childhood leukemia and benzene (among other agents) were also identified (Lowengart et al., 1987; Feingold et al., 1992) but the numbers of cases (or numbers of benzene-exposed parents) were too small to provide any meaningful information.

Shaw et al. (1984) examined the association between disease and risk factors including maternal age, birth order, socioeconomic status, and paternal occupation in a matched case-control study evaluating 255 cases of childhood leukemia reported to the California Tumor Registry. Controls (N=510) were matched by sex and county, by selecting the birth certificate immediately preceding and following the case's birth certificate. Exposure was determined from the occupation of the father as listed on the birth certificate. Occupational classifications determined by the NIOSH National Occupational Hazard Survey 1971-74 were used to classify fathers in the study as "potentially exposed" or "not exposed". This study found no association between paternal benzene exposure and childhood leukemia. As noted by the authors, it is possible that the failure to detect an association in this study is due to misclassification of exposure status. A limitation of the study is the likelihood of multiple chemical exposures confounding the data.

Shu et al. (1988) examined the association between maternal and paternal occupational exposures during pregnancy and childhood leukemia in a well-designed matched case-control interview study in Shanghai, China. Using a population registry, 309 childhood leukemia cases in China were compared to 618 control children. Exposure were ascertained through personal interviews with the parents which inquired about occupational exposures, and history x-rays, drug use, diseases and other potential risk factors. Paternal occupation during pregnancy was not associated with childhood leukemia, and exposures prior to conception were apparently not ascertained. These investigators found a statistically significant association between childhood leukemia and maternal occupation in the chemical industry (chemical processors and related workers, rubber and plastic products makers, leather workers, painters, and chemical analysts) (OR 3.3, 95 percent CI = 1.6 to 6.8). They found suggestions of increased risks associated with self-reported occupational exposure to benzene (OR 2.0, 95 percent CI

= 0.9 to 4.3) and gasoline (OR 1.6, 95 percent CI = 0.8 to 3.1). When childhood leukemia cases were separated by histopathological cell type, maternal benzene exposure was found to be associated with statistically significant increased risks of acute non-lymphocytic leukemia (OR 4.0, 95 percent CI = 1.8 to 9.3) but not with acute lymphocytic leukemia. Maternal gasoline exposure was associated with an increased risk of acute lymphocytic leukemia (OR 1.7, 95 percent CI = 1.0 to 3.0).

Buckley et al. (1989) conducted a case-control study of paternal and maternal occupational exposure to benzene of 204 children, aged 18 or less, in the U.S. with acute non-lymphocytic leukemia. 204 controls were also examined which were identified by random digit dialing and were matched by date of birth and race. Exposures were assessed through a one hour questionnaire with the mother and father. An elevated association between acute non-lymphocytic leukemia and occupational exposure of the fathers to solvents (including benzene) was observed. Odds ratios (OR) for childhood leukemia and paternal exposure to solvents relative to fathers with no solvent exposure were OR=2.6 (95 percent CI 1.3-5.5) for 1-1000 days exposed and OR=2.0 (95 percent CI 1.2-3.8) for fathers exposed for more than 1000 days. Similar associations were observed for childhood leukemia and paternal exposure to petroleum products (OR 2.4 for prolonged exposure, 95 percent CI = 1.3 to 4.1, p-value for trend = 0.002). This study is limited by the possibility of recall bias, although the authors believed that this was not likely to be occurring. Also, exposure groups included multiple chemicals of which benzene was only a part. One strength of the study for the question at hand is that it focused exclusively on acute non-lymphocytic leukemia, the subtype that is most strongly associated with adult exposures to benzene. Data on maternal exposure to solvents were not reported.

In another matched case-control study, McKinney et al. (1991) evaluated the associations between self-reported parental exposures to specific agents and childhood leukemia and non-Hodgkin's lymphoma in three areas of England with previously documented high rates of these diseases. Children diagnosed with leukemia or non-Hodgkin's lymphoma in the study area between 1974 and 1988 were included in the study. Cases occurring during this period included 113 cases of acute lymphoblastic leukemia (75 percent), 21 other cases of leukemia (14 percent), and 17 cases of non-Hodgkin's lymphoma (11 percent). Each case was matched to two controls by sex, date of birth, and health district of birth. Cases were included in the analysis if data were available for the case and at least one control. Exposure data were collected through face-to-face home interviews that asked questions specifically about parental exposure at work or through hobbies to a variety of suspected toxicants.

Twelve of 101 cases (12 percent) compared to six of 178 controls (three percent) had fathers who reported preconception exposure to benzene (OR 5.81, 95 percent CI = 1.67 to 26.44). Of all the specific agents examined, "the only independent contributions to risk in the preconceptional period were exposures to wood dust (odds ratio 3.00, 1.50 to 5.90), radiation (2.94, 1.13 to 7.63), and benzene (4.82, 1.24 to 18.84)." This study is limited by the possibility of recall bias.

Kaatsch et al. (1998), in a case-control study, examined the associations of various risk factors for 1037 cases of acute lymphocytic leukemia, 147 cases of non-lymphocytic leukemias and 234 cases of non-Hodgkin's lymphoma in Germany. Cases were identified through the German Childhood Cancer Registry. One control for each case was identified and recruited through local registration offices. Controls were matched by age, sex, and place of residence at the time of diagnosis. Exposure information (including benzene exposure) was obtained through self-administered questionnaires and subsequent telephone interviews by trained interviewers. Response rate for both the questionnaire and the telephone interview were different for cases and controls. For example, questionnaire response rates were 81.1 percent for cases and 66.6 percent for controls. Although no data were provided, the authors noted that they did not find any association between parental benzene exposure and childhood leukemias. The authors stated in their methods section that they analyzed the data by leukemia subtype using conditional logistic regression, but no benzene-related results by subtype were presented. Kaatsch et al. (1998) found no associations between exposure to ionizing radiation and childhood leukemia, and reported a significant negative association between maternal smoking and childhood leukemia. The study may be limited by response bias, which the authors did not address in the discussion of the findings. As with the other studies, this study is limited in that multiple chemical exposures of the parents are potential confounders. A strength of the study is the large number of subjects examined.

In a case-control study, Shu et al. (1999) examined 1984 case of child acute lymphocytic leukemia in the U.S. and Canada, identified through the Children's Cancer Group. Controls (N=1986) were selected by random digit dialing, matched by age, race and area code. Exposure information was obtained through a questionnaire and telephone interviews with the mother and also with the father if available. Paternal or maternal exposure to benzene or "petroleum products," either prior to

conception, during pregnancy or postnatal was not associated with childhood acute lymphocytic leukemia. As with the studies described above, this study is limited in that multiple chemical exposures of the parents are potential confounders. The numbers of cases assessed in the study is high; however, the study was limited to acute lymphocytic leukemias. Benzene is most commonly associated with acute non-lymphocytic leukemias from occupational exposures as adults (ATSDR, 1997). Also, Shu et al. (1988) suggested that benzene exposure *in utero* was more strongly associated with childhood non-lymphocytic leukemia than childhood lymphocytic leukemias. Thus, limiting the focus of the study to acute lymphocytic leukemias, as was done by Shu et al. (1999), may reduce the ability to observe an association with benzene exposure.

Feychting et al. (2001) examined 161 leukemia cases as part of a larger cohort study of Swedish children born to married couples in 1976-77 or 1981-82 (N=235,635 births). All children were followed through 15 years of age, and their vital status was determined through the Swedish Cause of Death Registry. Exposures to the father were obtained from the father's occupation as listed on the 1975 census (for births occurring in 1976 or 1977) or on the 1980 census (for births occurring in 1981 or 1982). The father's occupation was linked to a job-exposure matrix constructed as part of the study by industrial hygienists. Two occupational hygienists assessed the probability of exposure to different agents based on the type of industry and job title. Benzene was one of many specific compounds considered. Benzene was not significantly associated with paternal exposure prior to conception (RR = 1.23, 95 percent CI 0.39-3.85). The study strengths included the cohort study design which does not have the potential for recall bias and large numbers of children considered, although the total number of leukemia cases was moderate (N=161). Potential limitations of the study include significant possibility for exposure misclassification. Also, the study did not examine associations of benzene exposure with leukemia subtype.

Benzene is a component of gasoline and diesel fuels and engine exhaust; thus, workers in occupations closely related to motor vehicles are expected to be exposed to benzene. Researchers from the National Cancer Institute published a review of the epidemiological studies of childhood leukemia and paternal exposures via occupations involved with motor vehicles or exhaust gases (Colt and Blair,

1998). They summarized the evidence as follows: "There have been 12 studies of childhood leukemia and paternal employment in occupations related to motor vehicles or involving exposure to exhaust gases. Elevated risk was found in most of these studies, with statistically significant findings in six. Significant associations were found among diverse occupations such as motor vehicle or lorry drivers (12,32), mechanics and gas station attendants (17, 27, 33), and broader groups of motor vehicle-related occupations (18). In their review of leukemia, Linet and Cartwright (30) suggested that the link between motor vehicle occupations and adult leukemia may be due to benzene and other components in engine exhausts" (Colt and Blair, 1998). As with the case-control studies described above, this database provides suggestive evidence of an association between parental exposure to benzene and childhood leukemias.

Associations of paternal exposures and childhood leukemia are consistent with observations in animals that benzene induces DNA damage in sperm (see below). Also, associations of maternal exposures and childhood leukemia in humans are supported by observations in animal studies that indicate that benzene crosses the placenta and induces DNA damage in the fetus (see Section IV.B. Summary of the Key Animal Studies).

d) Other Critical Information from Human Studies

There are limited data on the effects of direct exposure of children to benzene. However, there is some indirect mechanistic evidence to suggest that children may be susceptible to benzene-induced childhood leukemia from *in utero* exposure. There is mounting evidence that key genetic events related to the development of childhood leukemia occur in the developing fetus. In both major forms of infant leukemia, acute lymphocytic leukemia and acute myelogenous leukemia, about 80 percent of *de novo* cases have rearrangements in the *MLL* gene at 11q23 (Ross et al., 1994). The *MLL* gene resembles a homeobox gene and is believed to help regulate the development of the organism and plays an important role in hematopoiesis (Ross et al., 1994). Studies of identical twins who develop leukemia have shown that the genetic change is acquired *in utero* and can be transferred from one twin to another, presumably from transfer of blood cells *in utero* from one twin to another. It has been suggested that

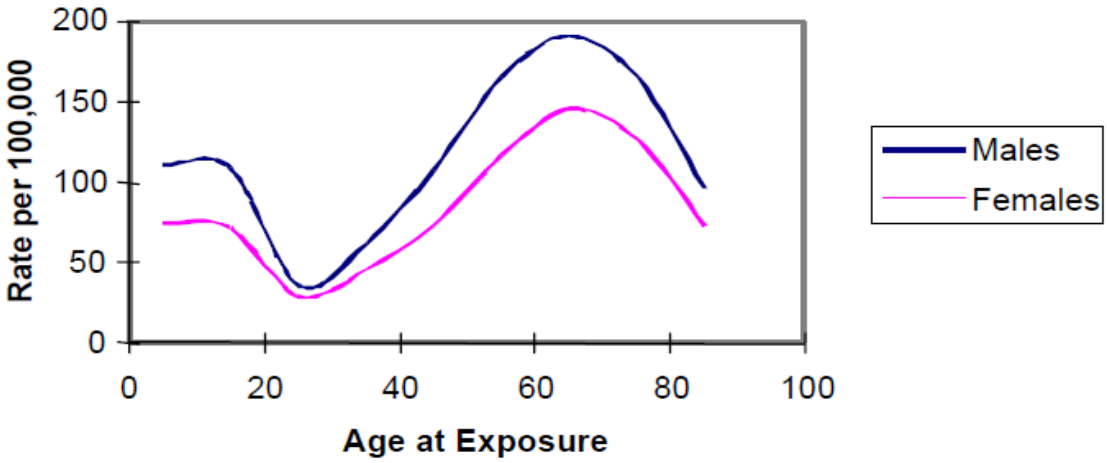
this genetic lesion in an appropriate stem cell is sufficient to cause infant leukemia (reviewed in Alexander et al., 2001). The *MLL* fusion product is rare in cases of *de novo* leukemia among older patients, except following treatment with topoisomerase II inhibiting chemotherapeutic drugs (Ross et al., 1994). Thus, it has been hypothesized that *in utero* exposures to topoisomerase II inhibiting compounds is relevant to childhood leukemia (Ross et al., 1994). Indeed, recent studies have found strong associations with infant leukemia and consumption of dietary topoisomerase II inhibitors (Ross et al., 1996), and with exposure to dipyrone ("Mexican aspirin") and propoxur (the insecticide, Baygon), two chemicals that are suspected to be topoisomerase II inhibitors based on their metabolism to phenolic compounds (Alexander et al., 2001). If this hypothesis proves to be correct, then benzene may also cause infant leukemia via this mechanism, since the major metabolites of benzene, namely phenol, catechol, hydroquinone, 1,2,4-benzenetriol, benzoquinone and trans,trans-muconaldehyde, are all topoisomerase II inhibitors (Hutt and Kalf, 1996; Franz et al., 1996). More research is needed.

Infants and children also may be vulnerable to leukemia induction from benzene because their hematopoietic cell populations are undergoing maturation and differentiation (U.S. EPA, 1998), although this difference may not be as pronounced as for other solid tissues since rapid cell turnover occurs throughout life in the bone marrow.

Our knowledge of radiation-induced leukemia may provide some insight as to the possible age-dependent patterns of leukemia arising from benzene exposures. Many commonalities have been observed between radiation-induced leukemia and benzene-induced leukemia. For example, the pattern of leukemia risk following exposure to ionizing radiation, benzene and chemotherapeutics is similar (OEHHA, 2000a; Finkelstein et al., 2000). Following exposure, leukemia rates rise rapidly within 5 to 15 years then decline to near background levels by about 30 years after exposure (NRC, 1990). Although we do not have information on benzene-induced leukemia for early life exposures, we do have such data from atomic bomb survivors and other radiation-exposed cohorts. This database may provide insight into the age-specific leukemia responses from DNA damage in the marrow. Interestingly, as shown in Figure 1 below, exposures that occur early in life and late in life confer greater excess risk than exposures between the ages of 20 and 45.

Figure 1: Excess Leukemia Mortality by Age at Exposure

Following Radiation Exposure (0.1 Sv)



Data from NRC (1990)

e) Mechanistic and metabolic data

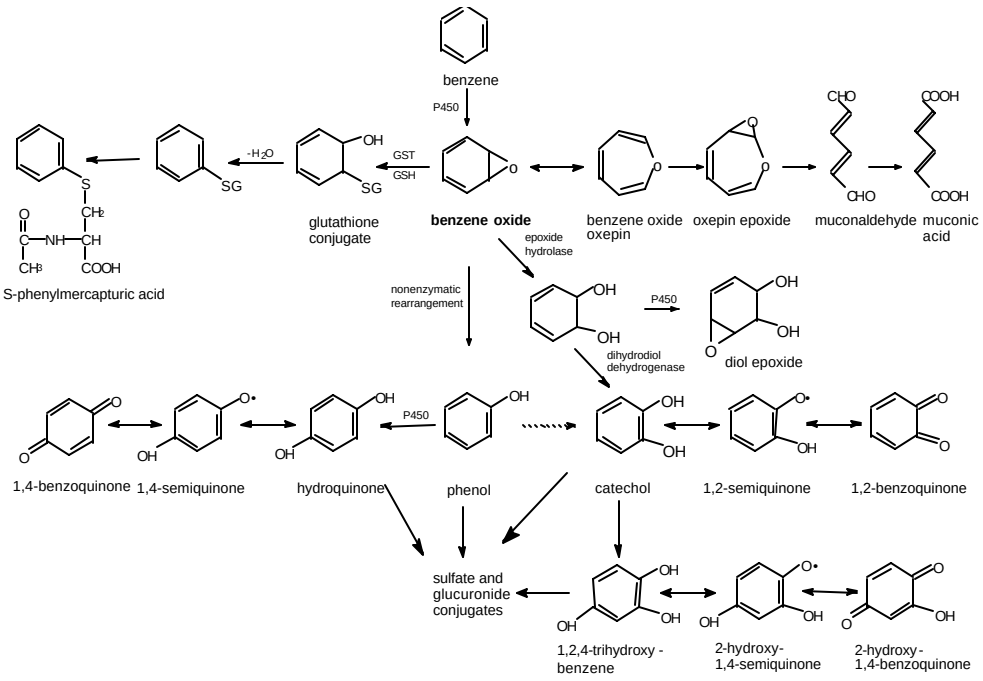
There is strong evidence that metabolism plays a critical role in benzene toxicity (Snyder and Hedli, 1996). For example, competitive inhibition of metabolism by toluene decreases benzene toxicity. Rodents given a partial hepatectomy (Sammett et al., 1979) or mice lacking the CYP2E1 gene (Valentine et al., 1996) had decreased metabolism of benzene and, correspondingly, decreased toxicity. There is no indication that the route of administration has a marked effect on the metabolites formed.

The metabolism of benzene is complex (Figure 2) and has been reviewed elsewhere (Snyder and Hedli, 1996; OEHHA, 2000a). To briefly summarize, benzene is metabolized primarily in the liver by cytochrome P450 2E1 and to a lesser degree by other P450 isozymes to form benzene oxide (or its oxepin) which spontaneously rearranges to phenol. Valentine et al. (1996) confirmed the central role of P450 2E1 by demonstrating that transgenic mice lacking CYP2E1 expression had decreased benzene metabolism, cytotoxicity, and genotoxicity compared to wild type mice.

CYP2E1, whose gene product is cytochrome P4502E1, is not highly expressed early in life (see introductory chapter of this report). Thus, the reduced expression of CYP2E1 in infants may infer a

reduced amount of toxic metabolites formed per unit exposure. However, currently it is unclear to what extent fetal isozymes of cytochrome P450 metabolize benzene. A detailed study of the expression of the other key enzymes in benzene metabolism (e.g., epoxide hydrolase, meyloperoxidase, phenol sulfatases, quinone reductases) would be needed to predict the possible impacts of fetal and infant exposures. In addition, over the course of the first several months of life, there is a maturation of the cytochrome P450 enzyme system and adult isoforms appear as neonatal forms regress. Thus, a young child is capable of producing toxic metabolites of benzene.

Figure 2. Benzene metabolism (from OEHHA, 2000a)



Benzene has been found in maternal and umbilical cord blood (OEHHA, 1997). It also appears likely that metabolites of benzene (formed from maternal metabolism) are transported to the fetus (OEHHA, 1997). Early life expression of important detoxification enzymes such as NADPH-dependent quinone oxidoreductase (NQO1) would likely be an important factor in any benzene-induced DNA damage from fetal- or maternal-formed reactive metabolites. OEHHA is not aware of any studies that have examined the fetal expression of NQO1 in humans, but in rodent liver NQO1 activity is very low in the fetus, but rises to adult levels a few weeks after birth (Hommes et al., 1978).

B. Summary of the Key Animal Studies

Benzene is a multisite carcinogen in rats and mice either by inhalation or oral routes of administration (reviewed in OEHHA, 2000a). Some information on transplacental carcinogenicity comes from studies in which rats were exposed to benzene (200 ppm in air) throughout gestation and lactation, and for an additional 85 weeks after weaning (Maltoni et al., 1983; 1985; 1989). Cancer rates in the offspring were compared to control animals and to their dams, who were exposed to the same concentration of benzene for the same period. Although no statistical analyses were reported, the authors stated that "an enhanced carcinogenic effect of benzene was observed in animals on which treatment was started during embryonal life" and that animals whose exposure began *in utero* had higher incidences of some tumor types than the breeders exposed only as adults (Maltoni et al., 1983; 1985; 1989) (Table 1). No carcinogenicity studies of benzene where exposure was limited to pregnancy were located.

As evident by the incidence data in Table 1, exposures *in utero*, through lactation and adulthood (total 104 wk) caused increased tumor incidences for some tumor type compared to maternal exposures for 85 wk. Among female offspring, Zymbal gland tumors (one of the more consistently responsive tumorigenic sites in rodents) exhibited a 2-fold increase in tumor incidences relative to incidences among the dams. Thus, an increase in overall exposure time by 20 percent resulted in a 2-fold increase in tumor response. It is unclear whether the increased rates are reflective of the increased overall exposure or due to differential susceptibility of the fetus and weanling. A detailed assessment would be required to determine if the increased rates among the rats exposed *in utero* and throughout life are greater than would be predicted by an equivalent 104 wk adult exposure. Although benzene administration to rodents generally does not result in the formation of leukemia, animal models are reasonable predictors of human risk, as cancer potency estimates from human and animal datasets using linear risk models are very similar in magnitude (OEHHA, 2000a).

a) Other Critical Information from Animal studies

(1) Sperm DNA damage

Associations of paternal exposures and childhood leukemia are supported by observations in animals that benzene induces DNA damage in sperm. Mice administered benzene via i.p. injection at seven doses ranging from 0.1 to 1.0 mL/kg-day on five successive days exhibited statistically significant dose-related increases in sperm head abnormalities in dose groups 0.4 mL/kg or higher with a peak effect at 0.6 mL/kg-day (Topham, 1980). Dose-related increases in chromosomal aberrations (breaks, fragments, exchanges) in the sperm were also observed in mice following administration of single oral doses of benzene at 0.25, 0.5 or 1.0 mL/kg relative to controls (Ciranni et al., 1991).

(2) Transplacental genotoxicity

Associations of maternal exposures and childhood leukemia in humans are supported by observations in animal studies that indicate that benzene crosses the placenta and induces DNA damage in the fetus. In mice, hematopoiesis is initiated in the fetal liver on gestational day 10, and peaks on gestational day 12 or 13, which is soon followed by the initiation of hematopoiesis in the bone marrow (OEHHA, 1997). Thus, gestational days 13 to 15 are considered a sensitive period for induction of hematopoietic genotoxicity in the fetal liver. For this reason, most of the relevant studies administered benzene on gestational days 13 to 15.

Increases in benzene-induced micronuclei were observed in fetal liver erythrocytes (polychromatic erythrocytes, PCE) in three studies (Ciranni et al. 1988; Ning et al. 1991; Xing et al. 1992) following exposure of the dams to benzene. A significant increase in fetal liver PCE micronuclei was found in mice given benzene by gavage on gestational day 13 (Ciranni et al. 1988) and on gestational day 14 or 15 (Ning et al. 1991; Xing et al. 1992), but not when given on gestational days 16 to 17 (Harper et al. 1989). Also, two studies reported increases in sister chromatid exchange in fetal cells after dams were administered benzene i.p. (Sharma et al. 1985; Xing et al. 1992).

Table 1. Summary of Carcinogenicity Studies Employing Early-life Exposure of Animals to Benzene

Species	Exposure Concentration	Dose Regimen	Tumor Type	Incidence: Dosed Group (Controls)	Reference
Sprague-Dawley rats (Breeders, 13 wk old)	200 to 300 ppm (see regimen)	200 ppm 4 h/d, 5 d/wk for 7 wk, then 7 h/d, 5 d/wk for 12 wk, then 300 ppm 7 h/d, 5 d/wk for 85 wk	Zymbal gland carcinoma Mammary (malignant and benign combined)	3/54 (1/60) 30/54 (24/60)	Maltoni et al., 1983, 1985, 1989
(Offspring)	200 to 300 ppm	See regimen for Breeders. Offspring exposed *in utero*, during lactation, and for 85 wk (104 wk total). Sacrifice at 150 wk.	Zymbal gland carcinoma Mammary (malignant and benign combined) Nasal carcinoma Hepatoma	14/75 (2/158) m[a] 8/65 (0/149) f[a] 6/75 (11/158) m 35/65 (84/149) f 1/75 (0/158) m 2/65 (0/149) f 2/75 (1/158) m 7/65 (0/149) f	
Sprague-Dawley rats	200 ppm (dose to the dams)	*In utero* from day 12 of gestation and during lactation. 4 hr/d, 5 d/wk for 7 wk, then 7 hr/d, 5 d/wk for 12 wk. Sacrifice 150 wk	Zymbal gland carcinoma Oral cavity carcinoma Hepatoma	4/70 (2/158) m 1/59 (0/149) f 2/70 (0/158) m 6/59 (0/149) f 2/70 (1/158) m 5/59 (0/149) f	Maltoni et al., 1983, 1985, 1989

[a] m, males; f, females

Most studies of transplacental genotoxicity compared effects in the fetus to those in the dam. Two studies found effects in the fetus (liver) and the dam (bone marrow) at similar doses (Sharma et al. 1985; Xing et al. 1992). Two other studies, both using i.p. administration (Ning et al. 1991; Xing et al. 1992) reported an effect in the fetus at a lower dose than in the dam. Thus, mouse fetuses appear to be susceptible to the genotoxic effects of benzene, but sensitivity relative to dams is unclear. Additionally, two studies using oral administration compared benzene-induced genotoxicity in the fetus, dam, non-pregnant female, and adult male. Cirrani et al. (1988) found similar increases in micronuclei of PCEs in virgin females as in pregnant dams and fetuses, but a larger effect in males. Harper et al. (1989) also reported a larger effect in males, a smaller effect in virgin females and, as mentioned above, no effect in pregnant dams or their fetuses, when exposed on gestational days 16 and 17.

(3) Transplacental alteration of hematopoiesis

Epigenetic mechanisms are likely involved in benzene-induced leukemia and include the alteration of hematopoiesis and clonal selection (OEHHA, 2000a). Evidence in animals suggests that exposure to benzene *in utero* alters maturation of lymphocytes, erythrocytes and granulocytes (OEHHA, 1997). The consequences of *in utero* exposure to benzene at air concentrations as low as 5 to 20 ppm can be detected as alterations in cell population numbers and functional properties that in several cases persist into adulthood (Keller and Snyder, 1986; Keller and Snyder, 1988; Corti and Snyder, 1996). Benzene-induced damage during the initial *in utero* stages of hematopoiesis appears to have lasting effects as has been demonstrated for a number of other toxicants (OEHHA, 1997).

V. Conclusions

OEHHA has placed benzene in Tier 2 due primarily to suggestive evidence of associations between benzene exposure and childhood leukemia supported by various lines of evidence from animal studies. Benzene exposures are ubiquitous due to its presence in fossil fuels. Benzene is a known human carcinogen, causing leukemia in worker populations. Although no human cancer studies of benzene-exposed children are available, there is good reason to believe that childhood exposure to benzene

would also contribute to adult-onset leukemias. Also, there is some evidence to suggest that exposure to benzene is associated with childhood leukemia. Paternal exposure to benzene prior to conception in humans has been associated in some studies with increased childhood leukemia, especially of the acute non-lymphocytic type, findings that are supported by observations in animals of benzene-induced DNA damage to sperm. Maternal exposure to benzene in humans also has been associated with increased incidences of childhood leukemia in some studies. These findings are supported by observations in animals of benzene-induced transplacental genotoxicity, altered hematopoiesis, and of carcinogenicity, following exposure *in utero* and continuing until weaning. However, it should be noted that other epidemiological studies that represented a large number of cases of various subtypes of leukemia (Kaatsch et al., 1998) or acute lymphocytic leukemia only (Shu et al., 1999) did not find an association with paternal benzene exposure. Thus, although there is suggestive evidence of an association between benzene and childhood leukemia, a causal relationship would be difficult to establish at this time.

It is difficult to predict whether postnatal exposures to benzene would be more or less likely to initiate leukemia than adult exposures. The impact of maternal-formed metabolites that cross the placenta needs to be considered in such comparisons. Expression of cytochrome P4502E1, the first metabolic step in benzene's metabolism and key to benzene's toxicity, is low in infants suggesting reduced formation of toxic metabolites. However, at several months of age the expression of cytochrome P4502E1 is equivalent to that of an adult. Thus young children are able to metabolize benzene to toxic intermediates. Also, detoxification enzymes such as NQO1 may not be functioning at an early age.

Studies of rodents exposed to benzene *in utero*, through weaning and adulthood exhibited 2-fold higher incidences of tumors of various sites than animals exposed only as adults. Since the two exposure groups received different total doses of benzene, a critical analysis is needed to determine whether the tumor rates observed in the rodents exposed early in life are greater than what would be predicted based on the rates from adult-only exposures. There is no human cancer study available that examines childhood exposures to benzene and lifetime leukemia risk. However, studies of radiation-induced leukemia show that early-life exposures result in greater lifetime risk of leukemia compared to adult exposures. It is not unreasonable to suggest that benzene exposure would cause a similar phenomenon.

Current ambient air levels of benzene (0.85 ppb) (CARB, 2000) are associated with a significant, upper-bound cancer risk in the California population (79 cancer cases per one million exposed), based on the current TAC unit risk factor for benzene. This calculation does not take into account emissions from "hot spots", which would result in additional risk in the immediate vicinity of the source. Thus, it is very important that Cal/EPA assess whether the measures taken under the TAC program to reduce benzene exposure are adequately protective of all major subpopulations, including children.

It should be noted that the current cancer potency values for benzene used in California (and by the U.S. EPA) are based on methods that included children, but do not account for any potential differences in susceptibility (i.e., response). Specifically, cancer potency estimates from the occupational cohort studies are used to estimate the cancer potency of benzene in the general population from continuous exposure. This extrapolation is accomplished using life table methods (NRC, 1990; OEHHA, 2000a). The worker-based potency estimate is applied to the age-specific leukemia rates in the general population starting at 0-5 years of age (which by definition includes childhood and adult leukemias). As has been observed for radiation-induced leukemias, there is evidence to suggest that benzene exposure early in life elicits a stronger carcinogenic response than equivalent exposures of working-age adults. Thus, the current estimate of the cancer potency for benzene may underpredict the risk from early life exposures, and standards based on this potency would not be adequately protective of children.

OEHHA may revisit listing benzene under SB25, particularly if more information on potential differential toxicity between children and adults becomes available.

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Carbon disulfide

75-15-0

S = C = S

I. Physical and Chemical Properties

Description	Clear, colorless or faintly yellow liquid
Molecular formula	CS_2
Molecular weight	76.14
Air concentration conversion	3.1 mg/m^3 per ppm at 25^{o}C

II. Overview

Neurotoxicity is one of the key toxicological endpoints of concern for infants and children. A primary target of carbon disulfide (CS_2) toxicity is the nervous system. The major neurotoxic actions of CS_2 measured in occupationally-exposed adults are the acute development of mental disturbances and the chronic development of neurophysiological and neuropathology changes (decreased peripheral nerve impulse conduction, motor and/or sensory neuropathies, cerebral or cerebellar atrophy, and neuropsychological organic changes) (Aaserud *et al.* 1988, 1990, 1992; Foa *et al.*, 1976; Hirata *et al.* 1992; Ruijten *et al.* 1990, 1993). Neuropathy and cardiovascular effects occur with chronic occupational exposures of 10 to 20 mg/m^3 (Johnson *et al.*, 1983; Vanhoorne et al., 1992; 1995). Animal studies have also shown that CS_2 is a neurotoxicant, primarily indicated by pathological changes in the nervous system. Transient delays in behavioral development have been reported in young animals exposed to as low as 10 mg/m^3 (3 ppm) CS_2 (Tabacova and Balabaeva, 1980).

There is some evidence of increased sensitivity to acutely lethal CS_2 exposures and lower detoxification rates of CS_2 in newborn animals.

There is some evidence of teratogenic and delayed developmental effects following exposure to CS_2 in animal studies. Developmental toxicity is another key toxicological endpoint for infants and children. Damage sustained during *in utero* exposure poses a risk of adverse postnatal health effects and, as such, it is necessary to consider developmental toxicity following prenatal exposure. The teratogenic findings among available studies do not present a consistent pattern of specific effects.

III. Principal Sources of Exposure

The most prominent industrial use of and source of occupational exposures to CS_2 has been in the production of viscose rayon fibers. There are no such facilities in California. Other uses of CS_2 include in the production of carbon tetrachloride and cellophane, and as a solvent for rubber, sulfur, oils, resins and waxes. In the past, CS_2 was used in soil fumigation and insect control in stored grain. Industrial

processes that produce CS_2 as a by-product include coal blast furnaces and oil refining (HSDB, 1995). The annual statewide emissions from facilities reporting under the Air Toxics Hot Spots Program in California based on the most recent inventory were estimated to be 1561 pounds of CS_2 (CARB, 2000), largely as fugitive emissions from refineries. CS_2 is not routinely monitored in ambient air in California.

IV. Potential for Differential Effects

A. Summary of Key Human Studies

There are no human studies that directly address age-related susceptibility. While occupationally exposed workers have been examined in several studies, there are no studies of children exposed to CS_2.

In an abstract of an epidemiological study of birth defects among female workers occupationally exposed to CS_2, Bao et al. (1991) reported an increased rate of birth defects (2.6% vs. 1.3%) among 682 exposed women compared to 745 women in the control group. The most common defects were congenital heart defects, inguinal hernia, and CNS defects. However, there was no significant difference in birth defects between those with estimated exposures greater than 10 mg/m^3 compared to those with lower exposures. There were no differences in rates of stillbirth, low birth weight, or neonatal or perinatal deaths among any of the groups.

A primary target of CS_2 toxicity is the nervous system. Neurotoxicity is one of the toxicological endpoints of concern for infants and children. The major neurotoxic action of CS_2 measured in adults in occupational settings is the development of mental disturbances, such as change of personality, irritability, and forgetfulness. These are often accompanied by neurophysiological and neuropathological changes after prolonged exposure; decreased peripheral nerve impulse conduction, motor and/or sensory neuropathies, cerebral or cerebellar atrophy, and neuropsychological organic changes (Aaserud *et al.* 1988, 1990, 1992; Foa *et al.*, 1976; Hirata *et al.* 1992; Ruijten *et al.* 1990, 1993). Alterations in behavioral indices measured in adults have been historically associated with high levels of CS_2, often in the excess of 20 ppm (Foa *et al.* 1976; Hannien *et al.*, 1978).

Studies have identified alterations in the nerve conduction of workers chronically exposed to lower CS_2 levels (Hirata *et al.*, 1992; Johnson *et al.*, 1983; Ruijten *et al.*, 1990, 1993). A cross-sectional study of Japanese spinning workers identified alterations in the central nervous system as measured by brain stem auditory evoked potential (BAEP) (Hirata *et al.*, 1992). The latencies of the three main BAEP components increased significantly in the CS_2 exposed workers (more than 20 years duration) when compared to controls. CS_2 exposures ranged from 3.3 to 8.2 ppm (mean 4.76 ppm). Ruijten *et al.* (1993) identified mild presymptomatic nerve impairment (decreased conduction velocities and response amplitudes) in 44 CS_2-exposed workers with an average cumulative exposure ranging from 192 to 213 ppm-year (mean duration 26.1 years).

In an occupational study evaluating the effects of CS_2 exposure on the peripheral nervous system, Johnson *et al*. (1983) identified a significant dose related reduction in the motor nerve conduction velocities in the calves and ankles of workers exposed to high (median 7.6 ppm) CS_2 levels versus a comparison group (median 0.2 ppm). Since the motor nerve conduction velocity, although reduced, was still within normal values, the authors considered the measured difference an indication of minimal neurotoxicity. The mean exposure concentration for all exposed workers (n = 145) ranged from 0.6 to 16 ppm (mean 7.3 ppm) with a mean 12.1 year duration. This study established a chronic LOAEL of 7.6 ppm for minor neurological effects (decreased peroneal nerve conduction velocity and sural nerve conduction velocity).

A second epidemiological study of interest has been partially reported by Vanhoorne and colleagues (1995). A group of 111 Belgian viscose rayon factory workers were exposed to 4 to 112 mg/m^3 CS_2 (time-weighted average 1 to 40 mg/m^3). Among four categories of cumulative exposure (0, 1 to 300, 301 to 600, and greater than 600 mg/m^3 years), a clear dose-response effect was observed for reduced mean peroneal motor nerve conduction velocities in both fast and slow fibers. Unfortunately, the data are incompletely reported, and the mean duration of exposure is not given. The lowest exposure group (1 to 300 mg/m^3 year; 0.36 to 11 mg/m^3 year TWA) may be associated with a significant reduction (approximately 5 to 10%) in peroneal motor nerve conduction velocity. Assuming the exposure duration was similar to that of the Johnson study (12.1 years), the equivalent TWA concentrations associated with 300 or 600 mg/m^3 year exposure are 2.8 and 5.6 ppm, respectively.

B. Summary of Key Animal Studies

The 24-hr lethal ip LD_{50} values for CS_2 were estimated in 1-, 5-, 10-, 20-, 30- and 40-day-old rats (sample size not specified) (Green and Hunter, 1985). 1-day-old rats (LD_{50} 583 mg/kg, ip) were about 3-times more susceptible than 20-day-old rats (LD_{50} 1545 mg/kg, ip).

^{14}C- and ^{35}S-labelled CS_2 was given ip to 1-, 5-, 10-, 20-, 30-, and 40-day-old rats (Snyderwine and Hunter, 1987). Thirty- and forty-day-old rats (sample size not reported) metabolized significantly more CS_2 to CO_2 and expired significantly less CS_2 than 1- to 20-day-old rats. Twenty-four hr after administration, up to 13 times more ^{35}S -label (radioactivity per g of tissue) were present in organs from 1-day-old rats than in similar organs from 40-day-old rats. The study does not specifically address the toxicological implications of the metabolic differences, and did not include fully mature animals. However, inability to detoxify CS_2 would lead to higher tissue concentrations and thus, potentially, increased toxicity.

New Zealand white rabbits (24 per group) inhaled 0, 60, 100, 300, 600 or 1200 ppm CS_2 for 6 h/d on gestation days 6 to 18 (Pathology Associates, 1991). Developmental toxicity (NOAEL = 300 ppm; 930 mg/m^3) was noted at concentrations lower than those associated with significant maternal toxicity (NOAEL = 600 ppm; 1860 mg/ m^3) (Pathology Associates, 1991). The adults did have some slight hematological changes at the 600 ppm level, but the authors questioned the biological significance of these marginal findings. Reduced fetal body weights were noted at 600 and 1200 ppm. Cumulative malformations were increased in the 1200 (3720 mg/m^3) but not 600 ppm group, though there were no

significant increases in any specific malformation in any group. Maternal effects at 1200 ppm were decreased body weight, ataxia, wheezing, and tremors. In an initial range-finding study, exposure to 3000 ppm was associated with significant lethality.

Rats were exposed to 100 mg/m^3 (32 ppm) for 4 hr/d on gestation days 7 and 8, and the embryos explanted to culture medium at day 9.5. Growth of explants of 10 treated and 17 control embryos was monitored for 44 hours. CS_2 at this concentration induced growth retardation in treated embryos relative to controls (Zhao et al., 1997).

In a two-generation study, Tabacova et al. (1983) exposed pregnant Albino rats (30-32 pregnant females per group) to CS_2 (0.03, 10, 100, or 200 mg/m^3). The two highest dose levels were both teratogenic and maternally neurotoxic. There were no significant adverse effects in the F1 generation at the 2 low dose levels. However, significant increases in teratogenicity were found in the F2 generation at 10 mg/m^3, as well as increased postnatal neurological effects including hypoactivity, mild ataxia and gait disturbances, hind-limb weakness, spinning and tremor (Tabacova et al., 1983). While the overall rate of malformations (club foot, hydrocephalus, microcephalus, generalized edema) exhibited a dose-response trend, with increased effects in the F2 generation, the specific malformations exhibited a less-consistent pattern. For example, while club foot was the predominant malformation in the F1 fetuses (occurring at 100 and 200 mg/m^3); much lower rates of club foot were noted in the F2 generation (including none in the 200 mg/m^3 group). Limitations of the study include a lack of information on chemical purity and exposure methods, lack of concurrent controls, lack of clear dose-response trend, and incomplete reporting on the statistical significance of reported behavioral effects.

Wistar albino rats (32 animals per group) were exposed to 50, 100, or 200 mg/m^3 CS_2 for 8 hours per days throughout gestation. There were no statistically significant results in the 50 mg/m^3 group. In the 100 and 200 mg/m^3 groups, there were statistically significant increases in reduced fetal body weights, and reduced post natal body weights for 21 days, which subsequently disappeared. There was an increase in external malformations (hydrocephalus, club foot, and tail deformations) at the two higher doses (Tabacova et al., 1978).

Behavioral effects were examined in the offspring of Lati:CFY rats (8 per group) exposed to CS_2 (0, 10, 700, or 2000 mg/m^3) for 6 hours per days over days 7 to 15 of gestation. The two high doses caused significant perinatal mortality. Avoidance conditioning was tested using a bell as a conditional stimulus prior to an electric shock. The animals learned to avoid the shock by jumping onto a pole at the sound of the bell. The latency to jump onto the pole and errors were measured as a means to evaluate avoidance conditioning in the treated versus control animals. The authors reported that there was a dose-related change in avoidance conditioning among male pups over the first 15 days (Lehotsky et al., 1985). While the magnitude of the effect on avoidance conditioning was greater at all doses relative to controls, and at 2000 mg/m^3 compared with 700 mg/m^3, the effect was virtually identical between the 10 and 700 mg/m^3. This lack of dose-response effect raises some question about the significance of this finding.

Effects of low (0.03 and 10 mg/m^3) prenatal exposures (8 hours per day throughout gestation) of CS_2 were studied in Wistar albino rats. No congenital malformations or significant prenatal effects were found in the 9-11 litters evaluated at each dose. Mortality during postnatal days 10 through 21 was increased in the 10 mg/m^3 group. Delays in the development of visual and auditory function were reported in the higher dose group (Tabacova and Balabaeva, 1980). There was no mention of maternal toxicity in this study.

Several other studies yielded either no teratogenic effects or effects only at maternally toxic exposures. Saillenfait et al. (1989) exposed rats via inhalation to 0, 100, 200, 400, or 800 ppm CS_2 for 6h/d during days 6-20 of gestation. Lower exposures (100 or 200 ppm; 310 or 620 mg/m^3) were not associated with maternal toxicity or adverse effects on the developing embryo or fetus. Higher concentrations (400 or 800 ppm; 1240 or 2480 mg/m^3) yielded a significant reduction of maternal weight gain as well as reductions of fetal body weight and a low incidence of club foot. Significant increases in unossified sternebrae were reported following 800 ppm (2480 mg/m^3) exposures. Nemec et al. (1993) reported no teratogenicity or maternal, developmental, or reproductive toxicity among pregnant CD rats and their offspring following exposure to 125 or 250 ppm (388 or 775 mg/m^3) from 2 weeks prior to mating through gestation day 19. At 500 ppm, dams had decreased body weight gain and food consumption; decreased litter viability but no teratogenic effects were also noted. CS_2 was not found to be teratogenic or embryotoxic following intraperitoneal administration to rats on days 1-15 of gestation (Beliles et al., 1980; Hardin et al., 1981). No significant effects were noted in animal inhalation exposures (20 to 40 ppm; 62 to 125 mg/m^3 CS_2) with either rats on days 1-19 of gestation or rabbits on days 1-24 of gestation.

Animal studies have also shown that CS_2 is a neurotoxicant not only in the developmental studies but also in adult animals. The neuropathologic changes consistently observed in rodents following CS_2 exposure include axonal swelling, demyelination, swelling at neuromuscular junctions, muscle atrophy and degeneration, damage to terminal axons, and nerve fiber breakdown (Clerici and Fechter, 1991; Colombi *et al.* 1981; Eskin *et al.*, 1988; Jirmanova and Lukas, 1984; Maroni *et al.*, 1979; Szendzikowski *et al.*, 1973). These adverse effects have been observed over a range of doses (250 to 800 ppm; 775 to 2480 mg/m^3), but few studies have attempted to establish a dose response for this CS_2-induced neurotoxicity.

In a 90 day subchronic inhalation study, Sprague Dawley and Fisher 344 rats exposed discontinuously (6 hours/day, 5 days/week) to CS_2 developed morphological alterations in nerves including axonal swelling and myelin degradation (Gottfried *et al.*, 1985). This study established a subchronic NOAEL of 50 ppm (155 mg/m^3) and a LOAEL of 300 ppm (930 mg/m^3) for morphological changes in nerves. A longer inhalation study in Wistar rats observed impairment in the conduction velocity of the sciatic and tibial nerves after 6 and 12 months of intermittent exposure to 289 ppm CS_2 (LOAEL of 289 ppm, 895 mg/m^3) (Knobloch *et al.*, 1979).

V. Additional Information

A. Other Toxicity

Although cardiovascular toxicity has not been singled out as an effect of concern for infants and children, there is little information on impacts of such toxicants on children's health. It is worth noting that CS_2 is associated with significant cardiovascular disease in occupational settings. Vascular atherosclerotic changes are also considered a major effect of chronic CS_2 exposure. Several occupational studies have demonstrated an increase in the mortality from ischemic heart disease in CS_2 exposed workers (Hernberg *et al.*, 1970; MacMahon and Monson, 1988; Tiller *et al.*, 1968; Tolonen *et al.*, 1979). A 2.5-fold excess in mortality from coronary heart disease in workers exposed to CS_2 was first reported by Tiller *et al.* (1968). A subsequent prospective study by Hernberg *et al.* (1970) found a 5.6-fold increased risk in coronary heart disease mortality and a 3-fold increased risk of a first nonfatal myocardial infarction in CS_2 exposed workers.

Egeland *et al.* (1992) and Vanhoorne *et al.* (1992) have reported that human exposure to CS_2 for more than one year causes changes in diastolic blood pressure, low density lipoprotein cholesterol, and apolipoproteins A1 and B. Egeland *et al.* (1992) used cross sectional data on 165 CS_2-exposed workers (245 controls) collected in 1979 by Fajen *et al.* (1981). Workers were exposed for at least 1 year in a viscose rayon factory to an estimated median TWA (8-hour) of 7.6 ppm. The Egeland *et al.* (1992) study indicated that modest CS_2 exposure (range 3.4 to 5.1 ppm, median 4.1 ppm (12.7 mg/m^3)) was associated with increased low density lipoprotein cholesterol (LDLc), which has been associated with atherosclerotic heart disease. No significant differences were seen between controls and the low CS_2 exposed group (range 0.04 to 1.02 ppm, median 1.00 ppm). This study indicates a chronic NOAEL of 1.00 ppm (3.1 mg/m^3) and a LOAEL of 4.1 ppm (12.7 mg/m^3) for increased LDLc and diastolic blood pressure. Vanhoorne *et al.* (1992) identified increased LDL-cholesterol, apolipoprotein B, systolic and diastolic blood pressure indicative of an increased coronary risk in workers from a Belgium viscose rayon factory (115 exposed and 76 controls). CS_2 concentrations ranged from 1 to 36 ppm (3.1 to 112 mg/m^3). Duration of exposure was not indicated. Even though these biochemical changes were observed, no significant increases in mild cardiovascular disease, such as angina, myocardial infarction, or ischemia were determined by ECG changes.

Wronska-Nofer (1973) showed a positive relationship between the level of triglycerides, the rate of cholesterol synthesis, and CS_2 exposure in Wistar rats exposed to 0, 73.8, 160, 321 or 546 ppm CS_2 for 5 hours/day, 6 days/week over 8 months. This study found a subchronic LOAEL of 73.8 ppm (229 mg/m^3) for disturbances in lipid metabolism (increase in serum cholesterol and serum triglycerides).

B. Regulatory Background - Brief description of RELs and URF

OEHHA is currently developing a chronic REL for CS_2 that is tentatively based on benchmark concentration modeling of neuropathy following chronic occupational exposure to carbon disulfide. A US EPA reference concentration (RfC) based on benchmark concentration (BMC) modeling (10% response) of neurotoxicity in occupationally exposed workers reported by Johnson and colleagues

(1983) was 0.7 mg/m^3 (0.2 ppm) (U. S. EPA, 1995). ATSDR, also using a LOAEL of 7.6 ppm from the Johnson data developed a chronic-duration inhalation MRL of 0.3 ppm. Environment Canada, using a BMC for 5% response derived from Johnson study, derived a Tolerable Concentration (TC) for CS_2 of 0.1 mg/m^3 (0.03 ppm) (Environment Canada, 2000).

VI. Conclusions

Carbon disulfide is a neurotoxicant in humans and animals. Neurotoxicity is a key toxicological endpoint of concern for infants and children. There is some evidence of developmental toxicity in animals, although the picture is inconsistent in terms of a clear dose-response relationship. Developmental toxicity is a key toxicological endpoint for infants and children, and damage sustained from prenatal exposure poses adverse health impacts postnatally. Exposures in ambient air in California are generally quite low as there are few emissions sources. Thus, OEHHA placed carbon disulfide into Tier 2. Should information arise indicating that localized exposures are significant, OEHHA may revisit listing carbon disulfide under SB 25.

VII. References

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Chlorine

CAS Registry Number: 7782-50-5

Cl-Cl

I. Physical and Chemical Properties

Description	Yellow/green gas
Molecular formula	Cl_2
Molecular weight	70.096 g/mol
Air concentration conversion	1 ppm = 2.9 mg/m^3 @ 25° C

II. Overview

Chlorine is a direct acting irritant; exposure results in irritation of the respiratory tract, eyes, and skin. There are no direct data indicating that children may be more susceptible to the toxicological effects associated with chlorine than adults. Acute controlled exposure studies in adults to known levels of chlorine have been carried out, while children have been exposed during accidental releases to high, but unknown concentrations. There are no known chronic exposures in children comparable to the long-term occupational exposures to chlorine in adults.

Chlorine may exacerbate asthma since it is a powerful respiratory irritant. As noted in the Introduction in Section III, OEHHA considers asthma to impact children more than adults and thus chemicals that may exacerbate or induce asthma should be considered for listing under SB 25. Since irritants exacerbate asthma, children may be more impacted by chlorine toxicity than adults. In addition, one study (D'Alessandro *et al*., 1996) indicates that adults with hyperresponsive airways (e.g., asthmatics) respond more to chlorine than adults with normal airway responsiveness.

III. Principal Sources of Exposure

In an industrial setting, chlorine is widely used as an oxidizing agent in water treatment and chemical processes. Chlorine is also used to disinfect swimming pool water. Chlorine gas is sometimes used at large public swimming pools, while household pools typically use hypochlorite solutions. Chlorine in the past was used to bleach wood pulp in pulp mills, although chlorine dioxide or ozonation is replacing this use of chlorine. Chlorine as sodium hypochlorite is commonly used as a household cleaner and disinfectant. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 244,955 pounds of chlorine (CARB, 1999). Since 1990 the ARB has monitored for particulate chloride, which includes chloride salts. In 1998, the last year for which complete data have been analyzed, the mean annual statewide concentration of particulate chloride was 1.49 µg/m^3. Ambient chlorine levels are not routinely monitored in California due to reactivity.

IV. Potential for Differential Effects

A. Summary of Key Human Studies

1) Acute exposure / effects in adults

D'Alessandro *et al*. (1996) exposed 10 subjects (age range 18-50), five with and five without airway hyperresponsiveness (HR), to 1.0 ppm chlorine for 1 hour by mouth-breathing facial mask. In addition, the five people with the HR were exposed to 0.4 ppm chlorine. After inhalation of 1.0 ppm, FEV_1 immediately fell significantly in both types of subjects; the decrease was greater among the HR subjects compared with the normals ($p = 0.04$). Specific airway resistance (SR_{aw}) increased more among the HR group compared with normals ($p = 0.04$). Among all 10 people, the proportional change in FEV_1 after exposure to 1.0 ppm chlorine correlated with baseline reactivity (Spearman rank correlation (r) = 0.64, $p < 0.05$). No significant chlorine-related pulmonary function deficits persisted 24 hours after exposure. Exposure of the 5 persons with HR to 0.4 ppm chlorine did not significantly affect pulmonary function. The authors concluded that persons with hyperreactive airways show a clinically significant, exaggerated airway response to 1.0 ppm chlorine, but not 0.4 ppm.

Rotman *et al*. (1983) studied clinically significant changes in pulmonary function tests (PFTs) following controlled chlorine exposures. Using a group of 9 volunteers (8 normal volunteers (ages 19-33) plus 1 volunteer with allergic rhinitis), data were collected on several PFTs following 4- and 8-hour exposures to 0, 0.5, and 1.0 ppm (0, 1.45, and 2.9 mg/m^3) chlorine. The subject with allergic rhinitis was excluded from the final group mean statistical analysis due to the severity of his response to chlorine exposure. Although 8-hour exposure to 1 ppm chlorine resulted in clinically significant decreases in FEV_1 (4 subjects) and clinically significant increases in specific airway resistance (SR_{aw}) (4 subjects), there were no reports of respiratory distress among the normal subjects (Rotman *et al*., 1983). The one subject with allergic rhinitis developed shortness of breath and wheezing following a 4-hour exposure to 1 ppm chlorine and left the exposure chamber (Rotman *et al*., 1983). Pulmonary function tests showed that this subject had a clinically significant increase in pulmonary SR_{aw} and a clinically significant decrease in FEV_1 when compared to sham exposure of 8 healthy subjects and when compared to the subject's own sham control values. The subject also had compromised lung function relative to the 8 healthy subjects during sham exposures. The pulmonary tests under sham control conditions also showed that exposure of the sensitive subject to 0.5 ppm chlorine for 8 hours, but not 4 hours, resulted in a clinically significant, greater than 100% increase in SR_{aw} and clinically significant, greater than 20% decrease in FEV_1. However, no clinical symptoms in the sensitive individual and no apparent indication of bronchoconstriction were reported at 0.5 ppm.

A concentration- and time-dependent severity of irritation to the eyes and throat was shown by exposure of "up to" 29 volunteer subjects (ages 20-33) to chlorine (Anglen, 1981). Volunteers were exposed for 4 or 8 hours to 0, 0.5, 1.0, and 2.0 (4 hour exposures only) ppm chlorine. Severity of irritation was subjectively measured by questionnaires from the subjects every 15-60 minutes, and was

divided into 5 categories, which ranged from barely perceptible to clearly objectionable. A statistically significant decrease in mean FEV_1 (−15.3%) for the group was observed following 8-hour exposure to 1.0 ppm chlorine. A consistent, statistically significant increase in throat irritation in subjects exposed to 1.0 ppm chlorine began at 1 hour into exposure. A NOAEL of 1 ppm was determined for a 30 minute exposure where no effects were reported. Consistent throat irritation was not observed in subjects during a 4-hour exposure to 0.5 ppm. However, 0.5 ppm chlorine produced throat irritation and an urge to cough after a 4-hour exposure.

Two earlier human studies suggest that some test subjects develop respiratory distress at concentrations of chlorine similar to that in Rotman *et al*. (1983). Rupp and Henschler (1967) gradually increased the concentration of chlorine was from 0 to 1.3 ppm over a 50 minute period. One subject developed shortness of breath and a severe headache following exposure to 1.0 to 1.3 ppm chlorine for 35 to 50 minutes. NIOSH (1976) suggested that this subject was sensitive to the irritant effects of chlorine. In a study by Beck (1959), one subject (out of 10) judged a 20 minute exposure to 1 ppm chlorine as unbearable due to sensory skin and conjunctival irritation, headache, and slight respiratory distress. It was not indicated in the study if this was a "sensitive" individual and it was unclear if clinical symptoms indicative of bronchoconstriction had actually occurred.

In a human poisoning case, a 20 year old male with a questionable history of asthma was exposed to 0.05 ounce/1,000 ft^3 ($^1/_{20}$ ounce per 1,000 cubic feet (equivalent to 19 ppm)) of chlorine for several minutes (Monto and Woodall, 1944). Immediately following exposure, the patient did not complain of any unusual irritation or shortness of breath. Several hours later, however, the subject was hospitalized with dyspnea and wheezing, with rales over the chest area. The diagnosis was pulmonary edema. The patient's past history included one questionable asthmatic attack in which he was subsequently told that he was sensitive to dust.

2) Acute exposure/effects in children

Children have been exposed to chlorine gas from leaking tanks while they were in swimming pools. In none of the exposure incidents in children was the exposure concentration measured or estimated. Thus it is difficult to compare effects with acute adult exposures to known concentrations of chlorine.

Decker (1988) reported that children exposed to chlorine gas had acute respiratory distress and eye irritation. Wood *et al*. (1987) reported that two boys, 3- and 7-years old, exposed to a high concentration of chlorine gas, had acute respiratory effects and were hospitalized. Sexton and Pronchik (1998) studied 13 children aged 6-18 years exposed by inhalation to chlorine gas at two community swimming pools. The patients had eye and throat irritation, chest pain, anxiety, shortness of breath, wheezing, and chest tightness, and most had occasional expiratory wheezing. Five children were admitted to the hospital due to hypoxia; four of these also had mild carbon dioxide retention.

Pulmonary function tests (PFT) were performed on 84 children (ages 9-17) from a school, near a plant manufacturing chemicals in Chembur, a suburb of Bombay, from which large amounts of chlorine gas leaked out two weeks previously (Pherwani *et al*., 1989). Only 20 had normal PFTs; 56 (66.7%)

showed an obstructive pattern and 8 (9.5%) showed a restrictive pattern of PFTs. The lower PFTs might be due to the incident, but the children also live in a polluted area of Bombay and a second incident occurred during the PFT administration.

3) Acute exposure/effects simultaneously in children and adults.

During an accident caused by a malfunction of the water chlorinating system in a community pool in Rome in 1998, 282 people, including 134 children under 14, inhaled hydrogen chloride and sodium hypochlorite and their reaction products (Agabiti *et al*., 2001). Acute respiratory symptoms occurred among 66.7% of adults and 71.6% of children. The incidences were highest among those who had chronic respiratory disease and had a longer duration of exposure. In about 30%, respiratory symptoms persisted for 15-30 days. Both in children and in adults, lung function levels were lower in those who reported a high intensity of exposure.

4) Exposure/effects in competitive swimmers.

An interesting subset of people exposed to chlorine is competitive swimmers and other young people in swimming pools, especially enclosed ones. Measurements of the chlorine concentration at the breathing level of swimmers (< 10 cm) obtained randomly during five nonconsecutive days in four different enclosed swimming pools in Spain yielded a mean chlorine level in all the pools of 0.42 ± 0.24 mg/m^3 (Drobnic *et al*., 1996). The authors noted that the mean value is below the TLV of 1.45 mg/m^3 (0.5 ppm) for an eight-hour workday. However, they estimate that a swimmer might inhale 4-6 g chlorine in a daily training session of 2 h, while a worker would inhale 4-7 g in 8 hours if working at the TLV.

Competitive swimming often starts early, at age 6 or so. Competitive swimmers inhale and "microaspirate" large amounts of air that floats above the water surface, which means exposure to chlorine and to chlorine derivatives from swimming pool disinfectants. The risk of asthma is especially increased among competitive swimmers, of which 36% to 79% show bronchial hyperresponsiveness to methacholine or histamine (Helenius and Haahtela, 2000). Mild eosinophilic airway inflammation is often seen.

B. Summary of the Key Animal Studies

In order to develop an animal model of the asthma-like abnormality known as reactive airways dysfunction syndrome (RADS; acute, irritant-induced asthma), Demnati *et al*. (1995) evaluated the effects of chlorine exposure on airway mucosa and lung parenchyma. Seventy-four Sprague-Dawley rats were exposed to air (controls) or to 50, 100, 200, 500, and 1,500 ppm of chlorine for 2 to 10 minutes. Exposure to 500 ppm did not induce significant histological changes. Exposure to 1,500 ppm for 2 minutes induced perivascular edema and the appearance of focal mild inflammation. Exposure to 1,500 ppm for 10 minutes caused profound histological changes. These included (1) airspace and interstitial edema associated with bronchial epithelial sloughing at 1 hour; (2) decreased edema and the

appearance of mucosal polymorphonuclear leukocytes at 6 to 24 hours (maximal at 12 hours); and (3) epithelial regeneration, manifested by hyperplasia and goblet cell metaplasia, at 72 hours.

Winternitz *et al*. (1920) reported severe lung edema and desquamation of the trachea and bronchial epithelium in dogs exposed to chlorine gas at lethal concentrations (concentration not reported). Bronchial constriction from the irritant properties was noted.

V. Additional Information

A. Other Toxicity

Exposure to 3-6 ppm (9-17 mg/m³) chlorine results in stinging or burning sensations from irritation and corrosion of mucous membranes including the eyes, skin, and the respiratory system (Baxter *et al*., 1989; Wither and Lees, 1985). At high concentrations, inhalation may result in necrosis of the tracheal and bronchial epithelium as well as in pulmonary edema. Delayed pulmonary edema may also develop up to 24 hours following acute exposure. Death at high exposure (400-1000 ppm) is mainly from respiratory failure or cardiac arrest due to toxic pulmonary edema. Bronchopneumonia may be a common and potentially lethal complication of pulmonary edema.

Shi (1990) evaluated workers (age range = 23-52 years) from a plant producing chlorine using diaphragm cells who were exposed to a range of 2.60-11.0 mg/m^3 (0.37-1.75 ppm) chlorine. Increased upper airway complaints and significant decrements in lung function were noted in chlorine-exposed workers.

Kennedy *et al.* (1991) compared pulp mill workers (including some exposed to chlorine or chlorine dioxide "gassings") to an unexposed control group of rail yard workers and found a significantly higher prevalence of wheezing in pulp mill workers (both smokers and nonsmokers) who had reported more than one episode of chlorine "gassing." The data suggested that chronic respiratory health impairment is associated with exposure to chlorine and/or chlorine dioxide.

In a study of Quebec pulp mill workers Bherer *et al*. (1994) found a 91% incidence of respiratory symptoms in workers who had experienced high accidental exposures. Twenty-three percent of the 58 workers still experienced bronchial obstruction and 41% continued to have bronchial hyper-responsiveness. Lower baseline FEV_1 was seen in those with a lower PC_{20}, and 52% of these workers showed an FEV_1 < 80% predicted. Thus high chlorine exposure can result in Reactive Airways Dysfunction Syndrome (RADS).

Ventilation was affected by chlorine inhalation, as indicated by a decrease in the maximal midexpiratory flow (MMF) in chlorine gas workers exposed to <1 ppm Cl_2 with occasional excursions (Chester *et al.*, 1969).

Exposure of rats and mice to 9-11 ppm chlorine for 6 hours produced severe lesions in specific locations in both olfactory and respiratory epithelia of the nasal passages with a widespread loss of cilia (Jiang *et al*., 1983).

Wolf *et al*. (1995) exposed male and female B6C3F1 mice and F344 rats (70 per group, 280 per sex per species) to chlorine gas at 0 ppm, 0.4 ppm, 1.0 ppm, and 2.5 ppm intermittently for 2 years. Exposure began when the animals were 55 days old. Statistically significant damage to olfactory epithelium occurred in all exposed rats and female mice and also in the 1.0 and 2.5 ppm exposed groups of male mice. A LOAEL of 0.4 ppm was determined for upper respiratory epithelial lesions.

Klonne *et al*. (1987) exposed 32 male and female rhesus monkeys to chlorine gas 6 hours/day, 5 days/week for one year to 0, 0.1, 0.5, and 2.3 ppm Cl_2. The animals have a 35-year life-span and were exposed while they were still growing. Pulmonary function evaluations yielded a statistically significant trend for increasing pulmonary diffusing capacity and distribution of ventilation values for males and females in the 2.3 ppm exposure group. Both males and females exhibited significantly increased respiratory epithelial hyperplasia at 2.3 ppm. A mild form of the lesions was also seen at 0.5 ppm, 0.1 ppm (females only), and in one control male.

B. Regulatory Background

Chlorine is a federal hazardous air pollutant (HAP) and was identified as a toxic air contaminant (TAC) in California in April 1993 under AB 2728.

OEHHA Health Guidance Values

Acute Reference Exposure Level (REL)	210 µg/m^3 (0.07 ppm)
Chronic Reference Exposure Level (REL)	0.2 µg/m^3 (0.08 ppb)
Cancer Potency	Not known to be carcinogenic
Proposition 65	Not listed

OEHHA (1999) adopted an acute Reference Exposure Level (REL) for chlorine of 0.07 ppm (210 µg/m³) based on a NOAEL of 1 ppm for a 30 minute exposure (Anglen, 1981). OEHHA (2000) adopted a chronic REL for chlorine of 0.08 ppb (0.20 µg/m^3) based on a Benchmark Concentration (BMC_{05}) of 0.14 ppm determined for rats (Wolf *et al*., 1995).

VI. Conclusions

Chlorine is a respiratory irritant that may exacerbate asthma. D'Alessandro *et al*. (1996) showed that people with hyperreactive airways were more affected by 1.0 ppm chlorine than non-hyperreactives, but this difference was not noted at 0.4 ppm. General ambient exposures are very small and most chlorine exposures occur after accidental releases. Due to limited evidence of a differential effect and low potential for exposure, OEHHA placed chlorine in Tier 2.

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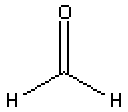
Formaldehyde

CAS Registry Number 50-00-0



I. Physical and Chemical Properties

Description	Colorless irritant gas
Molecular formula	CH_2O
Molecular weight	30.03
Air concentration conversion	1 ppb = 1.23 $\mu g/m^3$

II. Overview

Human studies suggest that children are more sensitive than adults to formaldehyde toxicity. In a study examining effects in both children and adults, Krzyzanowski *et al.* (1990) reported that increasing formaldehyde levels appeared to be associated with greater impacts on lung function in children than in adults in the same household. Krzyzanowski *et al.* (1990) found that formaldehyde in the home affects lung function in children at concentrations as low as 30 ppb, especially in asthmatics, as measured by peak expiratory flow rate. Adults in the same homes appeared to be less affected. This study also found that in homes with greater formaldehyde exposures (between 60 and 120 ppb), there was a greater prevalence of diagnosed asthma and chronic bronchitis in children, but not adults.

While Krzyzanowski *et al.* (1990) is the only study that directly compares the effects of formaldehyde in adults and children, there are several other studies that indicate that children are more sensitive to formaldehyde toxicity than adults. Of the numerous studies in adults (primarily occupational studies) the NOAEL and LOAEL are 32 $\mu g/m^3$ (26 ppb) and 92 $\mu g/m^3$ (75 ppb), respectively, after adjustment for exposure continuity. (These data are based on nasal and eye irritation observed in Wilhelmsson and Holstrom (1992), and histological lesions in the nasal cavity documented in Edling *et al*. (1988), and form the basis of the chronic REL, described in detail in OEHHA (2000)). However, studies in children, including the Krzyzanowski study above, indicate adverse health impacts in children at concentrations as low as 30 ppb. Wantke *et al.* (1996) reported that formaldehyde-specific IgE and respiratory symptoms were reduced when children transferred from schools with formaldehyde concentrations of 43 to 75 ppb to schools with concentrations of 23 to 29 ppb. Garrett *et al.* (1999) reported increased sensitization associated with the formaldehyde level in children's homes which had a median value of 15.8 $\mu g/m^3$ (12.6 ppb). And Franklin *et al.* (2000) reported significantly higher

exhaled nitric oxide, an indicator of airway inflammation, in the breath of children living in homes with formaldehyde concentrations greater than 50 ppb than in the breath of those children living in homes with formaldehyde levels below 50 ppb. These human studies are not entirely consistent with each other, and there is potential for confounding in each. Nevertheless, taken together, they suggest that children may be more sensitive to formaldehyde toxicity than adults.

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. While chamber studies in adults have not been convincing that formaldehyde exposure exacerbates asthma, the studies in adults may not be applicable to allergic asthma in children. As previously noted, Krzyzanowski *et al*. (1990) found that asthmatic children were more affected by formaldehyde than non-asthmatic children. In addition, allergic sensitization, as measured by elevated levels of formaldehyde-specific IgE, has been noted in two studies of children exposed to environmental levels of formaldehyde (Wantke *et al.*, 1996; Garrett *et al.*, 1999). The allergic sensitization may make children more sensitive to development of serious conditions such as asthma, although this has not been studied for formaldehyde. In addition to the data in children, animal data provide support for the contention that formaldehyde exposure may exacerbate asthma. Amdur (1960) showed that formaldehyde has a marked effect on airway resistance and compliance in guinea pigs. More importantly, Sweicechowski *et al*. (1993) showed that duration of exposure is important to the induction of airway hyperreactivity from formaldehyde. In this latter study, an 8-hour exposure to 1 ppm formaldehyde produced greater than expected effects on airway constriction compared to a 2-hour exposure at higher concentrations, suggesting that prolonged, low-level formaldehyde exposures may generate abnormal physiologic responses in the airways not detectable after acute exposures.

In addition to the human and animal studies of formaldehyde toxicity, OEHHA also considered exposure. Typical urban ambient air levels and indoor air levels can exceed the chronic REL of 2 ppb. Moreover, children are frequently exposed to levels of formaldehyde exceeding the chronic REL in indoor air of classrooms. A compilation of monitored California classrooms showed that children were exposed to a mean of 21 ppb and a maximum of 98 ppb (CARB, 2001, interdepartmental transmission). For these reasons, formaldehyde is considered a priority chemical for evaluation of potential differential effects on infants and children.

III. Principal Sources of Exposure

A. Ambient Air

Formaldehyde is released to outdoor air from both natural and industrial sources. Formaldehyde is formed naturally in the atmosphere during the oxidation of hydrocarbons, which react with hydroxyl radicals and ozone to form formaldehyde and other aldehydes. Outdoor air concentrations in urban environments ranged from 1-20 $\mu g/m^3$ and depend on local conditions (WHO, 1989; IARC, 1995). In 1998 and 1999 in California, the ambient mean formaldehyde concentration was 3.6 $\mu g/m^3$ (2.9 ppb) with a maximum of 14.3 $\mu g/m^3$ (11.5 ppb) (see Table 1 below). A major source of formaldehyde in urban air is incomplete combustion of hydrocarbon fuels, especially from vehicle emissions. Urban air

concentrations in heavy traffic or during severe inversions can range up to 100 $\mu g/m^3$ (WHO, 1989). Gaffney *et al.* (1997) found that in urban areas the introduction of oxygenated fuels led to increased anthropogenic emissions of formaldehyde during the winter, the season these fuels are used. Formaldehyde in vehicle emissions in 1994 were found to increase by 13% within 2 months after the average oxygen content of fuels sold in the San Francisco Bay area increased from 0.3 to 2.0% by weight (Kirchstetter *et al.*, 1996). In the Los Angeles area, the contribution of photochemical production of formaldehyde to levels in the air predominates over direct vehicular emissions (Grosjean and Wright, 1983). Numerous manufacturing processes also contribute to formaldehyde levels in the atmosphere (see below).

B. Indoor air

The emission of formaldehyde from common household products can be very high. In chamber studies simulating typical home conditions (70°F, 50% RH, 1.0 air exchange per hour) bare urea-formaldehyde wood products (particleboard, and plywood; product loading 0.46 m^2/m^3) have emission rates from a high of 1580 to a low of 9 $\mu g/m^2/hr$ (CARB, 1996). Wet products like latex paints, wallpaper, fingernail hardener, nail polish, and commercially applied floor finish also emit formaldehyde. Commercially applied floor finish and fingernail hardener has very high initial rate of formaldehyde emission. Other indoor sources such as wood and gas stoves, kerosene heaters, and cigarettes contribute intermittently to indoor formaldehyde levels. In general, indoor environments consistently have higher concentrations than outdoor environments, because many building materials, consumer products, and fabrics emit formaldehyde (Cal/EPA, 1992).

A recent survey measured formaldehyde concentrations inside conventional California residences. The mean value was 11 $\mu g/m^3$ (9 ppb) and the maximum was 39 $\mu g/m^3$ (31 ppb) (Avol, 1996; footnote "a" in Table 1). Current manufactured home concentrations are estimated to be 49 percent of the 1984-85 California Department of Health Services' (DHS) manufactured home study results. This reduction is based on the reduction of formaldehyde emissions from building materials that has occurred since the DHS manufactured home study was conducted (see footnote "b" in Table 1). The resulting mean concentration is 45 $\mu g/m^3$ (36 ppb) and the maximum is 282 $\mu g/m^3$ (227 ppb). A U.S. EPA study reported concentrations in public and commercial buildings. The mean value was 16 $\mu g/m^3$ (13 ppb) and the maximum was 32 $\mu g/m^3$ (26 ppb). Measurements inside schools have obtained a considerable range of formaldehyde concentrations. In 104 classrooms monitored in California, the mean concentration was 26 $\mu g/m^3$ (21 ppb) and the maximum was 122 $\mu g/m^3$ (98 ppb) (see Table 1).

TABLE 1. Formaldehyde Concentrations for Various Locations

Location	Mean µg/m^3 (ppb)	Max µg/m^3 (ppb)	Source for Values
Conventional homes	11.3 (9.1)	38.8 (31.3)	USC study, 99 Southern California homes[a]
Manufactured homes	45 (36.3)	282 (227)	Approx. 600 mobile homes from study conducted by DHS in 1984 & 1985. The average of summer and winter values was reduced 49% of '84 and '85 levels. Based on known reductions in building material emissions.[b]
Public & Commercial Buildings	16 (12.9)	32 (25.8)	EPA BASE Study, 100 buildings.[c] Used office building concentration data for Offices and Public Buildings, Restaurants & Lounges, and Other Indoor Spaces.
Industrial Plant	16 (12.9)	32 (25.8)	Used Office building data concentration (no data for Industrial Plant)
School	26.2 (21.1)	121.5 (98.0)	Data from several schools, 104 classrooms monitored by ARB. Includes northern and southern California.
In-Vehicle (Sacramento)	9.3 (7.5)	18.5 (14.9)	ARB, estimated average of Sacramento runs[d]
In-Vehicle (Los Angeles)	15.3 (12.3)	23.6 (19.0	ARB, estimated average of LA runs[d]
Outdoor	3.6 (2.9)	14.3 (11.5)	ARB, average of 1998 and 1999 statewide ambient means and maxima.

Conversion factor: 1 ppb =1.24 µg/m^3 @ 25 ° C

a Avol, E. (1996), "Residential Microenvironmental and Personal Sampling Project for Exposure Classification", draft final report to ARB, contract no. 92-317.

b Emission rates from Pickrell and Kelly were compared. Particleboard emissions are 92% of what they were in 1983, interior plywood emissions are 15% of emissions in 1983, and paneling emissions are 39% of 1983 emissions. If one takes a straight average of these reductions in emissions, building emissions today are estimated to be 49% lower than emissions in the early 1980's.

Pickerell, J., et al (1983), " Formaldehyde Release Rate Coefficients from Selected Consumer Products", Environmental Science and Technology 17(12): 753-757.

Kelly, T., et al (1999), "Emission Rates of Formaldehyde from Materials and Consumer Products Found in California Homes", Environmental Science and Technology 33 (1): 81-88.

c More information on the US EPA Building Assessment Survey and Evaluation (BASE) Study can be found at http://www.epa.gov/iaq/base/index.html.

d Rodes, C., et al (1998), "Measuring Concentrations of Selected Air Pollutants Inside California Vehicles", final report to ARB, contract no. 95-339.

In-vehicle studies have found formaldehyde concentrations to be similar to concentrations measured outdoors. A southern California study found an average in-vehicle formaldehyde concentration of 15.3 $\mu g/m^3$ (12.5 ppb) and a maximum concentration of 35.3 $\mu g/ m^3$ (28.8 ppb) during the summer of 1987 and winter of 1988 (Shikiya *et al.*, 1989). A study in Boston, Massachusetts, measured a mean formaldehyde concentration of 5.1 $\mu g/m^3$ (4.2 ppb) and a maximum concentration of 19.7 $\mu g/m^3$ (16.1 ppb) (Chan *et al.*, 1991a,b). A recent California study by Rodes *et al.* (1989; footnote "d" in Table 1) found concentrations in vehicles to be higher in Los Angeles and lower in Sacramento than found in the 1989 California study.

C. Emissions

Formaldehyde is released into the atmosphere from various manufacturing processes including: formaldehyde and resin manufacturing plants, plywood and particle-board mills, furniture factories and other wood product plants, paper and textile mills, garment factories, foundries, man-made mineral fiber plants, plastic production, and other miscellaneous processes such as photographic film manufacturing and development, embalming in funeral homes, and disinfecting in hospitals (IARC, 1995). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 1,589,810 pounds of formaldehyde (CARB, 1999a). In 1997, the population-weighted annual average exposure in the South Coast Air Basin was estimated to be 4.7 ppb formaldehyde (CARB, 1999b). The statewide ambient air concentration for 1999 was calculated by ARB to be 3.2 ppb (4.0 $\mu g/m^3$).

IV. Potential for Differential Effects

A. Summary of Key Human Studies

One study was found in the literature that directly compares the effects of formaldehyde in adults and children (Krzyzanowski *et al.* (1990). Results of this study show that children are more sensitive than adults to formaldehyde toxicity. In addition, three other studies found respiratory effects in children at concentrations lower than generally found in adults (Wantke *et al.*, 1996; Garrett *et al.*, 1999; Franklin *et al.*, 2000). These four studies are summarized here. While they are not entirely consistent with each other and there is potential for confounding in each, nevertheless taken together they suggest that children may be more sensitive to formaldehyde toxicity than adults.

Krzyzanowski *et al.* (1990) studied the relationship of formaldehyde to chronic respiratory symptoms and pulmonary function in children and adults. The sample consisted of 298 children (5-15 years of age) and 613 adults in 202 households in Tuscon, Arizona. Formaldehyde measurements were made with passive samplers in homes during two 1-week periods. The investigation also obtained data on tobacco usage and nitrogen dioxide levels in the home, as well as parents' education and ethnicity. Data on chronic cough, chronic phlegm, wheeze, attacks of breathlessness, and doctor diagnoses of chronic bronchitis and of asthma were collected from self-completed questionnaires. Peak expiratory flow rates

(PEFR) were obtained during the evenings and mornings for up to 14 consecutive days for each individual. The average formaldehyde concentration was 26 ppb. In a few cases the concentration exceeded 90 ppb, with a maximum value of 140 ppb.

The analyses of the symptoms and diagnosed disease used three exposure groups, <40 ppb, 40-60 ppb, >60 ppb. Log-linear analyses controlled for possible confounding by current smoking, environmental tobacco smoke (ETS) in nonsmokers or children, economic status using educational level of adults, and ethnicity. In children but not adults, the study found significantly greater prevalence rates of asthma and chronic bronchitis in homes with formaldehyde levels of 60-120 ppb than in those less exposed, especially using kitchen levels. The trend was highly significant for chronic bronchitis ($p<0.001$) and significant for asthma ($p<0.03$). The trend disappeared with the exclusion of children also exposed to environmental tobacco smoke. The prevalence rates of chronic respiratory symptoms were not related to formaldehyde exposures in either children or adults.

A longitudinal random-effects analysis of PEFR used constant covariates, asthma status, tobacco status, and socioeconomic status. Time-dependent covariates were episodes of acute respiratory illness, time of day and nitrogen dioxide levels. In children, levels of PEFR decreased linearly with formaldehyde exposure. Exposure to 60 ppb formaldehyde reduced PEFR 22% relative to unexposed children. Similarly, exposure to 30 ppb formaldehyde decreased PEFR 10% relative to unexposed children. The effects in asthmatic children exposed to formaldehyde below 50 ppb were greater than in healthy ones. The effects in adults were less evident: decrements in PEFR due to formaldehyde over 40 ppb were seen only in the morning, and mainly in smokers. These regressions are controlled for the possible effect of confounders.

These results suggest that formaldehyde has a greater effect on several measures of respiratory health in children than in adults. It is noteworthy that statistically significant results were obtained in children but not adults, even though the number of adults was greater than that of children, thus increasing the statistical power to detect an effect. However, the paper does not report any direct statistical analysis to test for a difference. In children the effects on PEFR appear to occur at least as low as 26 ppb, which was the mean value for the study.

Wantke *et al*. (1996) evaluated whether IgE-mediated sensitization and symptoms in children were associated with formaldehyde exposure at school. They studied 62 8-year olds attending primary school in Vienna. None of the children had asthma or wheezy bronchitis. Indoor formaldehyde concentrations were measured in classrooms of two buildings, one frame construction with particleboard used extensively as paneling and the other a brick building. A radioallergosorbent test (RAST) was used to assess the specific IgE to formaldehyde in all children while attending the paneled classrooms and 3 months after transfer to the brick building. In all children symptoms were evaluated by questionnaire before and 3 months after changing classrooms.

Elevated formaldehyde-specific IgE were detected in children whose classroom formaldehyde levels ranged from 43 to 75 ppb. Two of the three children with pathologically high RAST readings (>2.0) were in classrooms with 75 ppb formaldehyde. An additional 21 children had elevated RAST readings

(1.3-1.9). Symptoms found in the affected children were headache, nose bleeding, rhinitis, fatigue, cough, dry nasal mucosa and burning eyes. There was a good correlation between symptoms and the formaldehyde concentrations in the classrooms. However, elevated IgE levels to formaldehyde did not correlate with symptoms. After transferring to the brick building (formaldehyde ranged from 23 to 29 ppb) IgE levels in 20 children with elevated values dropped significantly ($p<0.002$). Symptoms also declined significantly: headache ($p<0.02$), nose bleeding ($p<0.001$), rhinitis ($p<0.01$), fatigue ($p<0.01$), cough ($p<0.10$), dry nasal mucosa ($p<0.05$) but not burning eyes.

These results appear to show a remarkable drop in symptoms after the move to a different building. The reduction in formaldehyde-specific RAST suggests that at least a part of the drop in symptoms after only 3 months may have been influenced by the decline of formaldehyde concentration of 36 ppb on average, from 63 ppb to 26 ppb. However, potential confounders, such as seasonal effect from December to March, were not ruled out. These concentrations of formaldehyde are only for the school week; effective continuous equivalent concentrations would be lower.

Garrett *et al*. (1999) investigated the relationship of formaldehyde to chronic respiratory symptoms and allergic response to aeroallergens in children. A total of 148 children 7-14 years of age were included in the study, of whom 53 were asthmatic, distributed among 43 homes. Formaldehyde levels were measured with passive samplers on four occasions over the course of a year in 80 homes in the Latrobe Valley, Victoria, Australia. At the last visit a respiratory questionnaire was completed for eight symptoms: cough, cough in the morning, shortness of breath, waking due to shortness of breath, wheeze/whistling, asthma attacks, chest tightness, and chest tightness in the morning. During the middle of the year, skin prick tests were performed with 12 environmental allergens, including four applications of fungi.

The median indoor formaldehyde level was 15.8 $\mu g/m^3$ (12.6 ppb), with a maximum of 139 $\mu g/m^3$ (111 ppb). The mean outdoor level was 0.7 $\mu g/m^3$, with a range of 0.3 to 15.3 $\mu g/m^3$. There was no significant association between formaldehyde and other contaminants.

There was an association between formaldehyde exposure and atopy in children. Parental asthma and parental allergy were found to be associated with current formaldehyde; so the children's association of atopy and formaldehyde was confirmed in those homes with parents having no asthma history. A logistic regression including an adjustment for gender and parental asthma found an odds ratio for atopy of 1.40 (0.98-2.00, 95% CI) associated with an increase in bedroom formaldehyde levels of 10 $\mu g/m^3$. There was a similar odds ratio for an increase of 20 $\mu g/m^3$ at the highest recorded level in the home. Furthermore, more severe allergic sensitization was demonstrated with increasing formaldehyde exposure. Also there was a marked jump in positive skin prick tests for the 20-50 $\mu g/m^3$ (16- 41 ppb) and the over-50 $\mu g/m^3$ groups in comparison to the less-than-20 $\mu g/m^3$ group, all these concentrations representing the highest recorded level in the home. The authors reported that there was no significant increase in the adjusted odds ratio of asthma or respiratory symptoms with formaldehyde exposure. However, among children suffering from respiratory symptoms, higher symptom scores were noted in those exposed to higher formaldehyde levels after adjustment for parental asthma status. There also

was a marked jump in respiratory symptom scores for the 20-50 $\mu g/m^3$ and over 50 $\mu g/m^3$ groups in comparison to the less than 20 $\mu g/m^3$ group, all at the highest recorded level in the home.

These results suggest that low-level exposure to indoor formaldehyde may increase the risk of allergic sensitization to common aeroallergens in children. The lack of a positive adjusted risk for asthma prevents the positive adjusted risk for atopy from being linked to respiratory disease in this study.

In children the effect of formaldehyde on atopy appears to rise abruptly between the lowest and the middle exposure groups, suggesting an effect occurring no higher than the range of 16 to 41 $\mu g/m^3$ which represented the highest recorded formaldehyde levels in the home. This jump is consistent with the 34% rise in atopy for an increase of 16 $\mu g/m^3$ in the highest recorded formaldehyde level.

TABLE 2. Distribution of atopic and nonatopic children by highest formaldehyde exposure

Formaldehyde exposure group	N	Nonatopic N=57	Atopic N=88	Proportion atopic
<20 $\mu g/m^3$	30	20	10	0.33
20-50 $\mu g/m^3$	75	27	48	0.64
>50 $\mu g/m^3$	40	10	30	0.75

Linear trend $p<0.001$
Source: Garrett *et al.* (1999)

Franklin *et al.* (2000) investigated possible inflammatory effects of formaldehyde at levels typically found in the home. The study recruited 224 healthy children 6 to 13 years of age (116 girls). Formaldehyde in homes was monitored using a passive sampling technique. As an indicator of respiratory inflammation, exhaled nitric oxide was measured directly into a fast response chemiluminescence nitric oxide analyzer. Lung function (spirometry) tests and skin prick tests for seven common allergens were conducted on the children. Housing factors were obtained from a questionnaire. The exhaled nitric oxide data and the spirometric data were each analyzed by multilinear regression after including housing factors found to be of marginal relationship in a bivariate screening analysis. The model also included the child's age and atopic status, because of a previously established relationship.

The formaldehyde levels measured in homes were found not to be related to either spirometric outcome, forced vital capacity or forced expiratory volume in one minute. However, exhaled nitric oxide levels were significantly elevated in children living in homes with average formaldehyde levels ≥ 50 ppb ($p=0.02$). Exhaled nitric oxide levels (geometric mean) were 15.5 ppb (95% CI: 10.5 to 22.9 ppb) for children from homes with formaldehyde concentrations ≥ 50 ppb compared with 8.7 ppb (7.9 to 9.6)

for children from homes with formaldehyde concentrations < 50 ppb. After using a multiple regression to control for other variables, the result became highly significant (p=0.002).

In view of apparent precautions taken to account for potential confounders, these results suggest that exposure to formaldehyde in homes may invoke a subclinical inflammatory response in the airways of healthy children. In their discussion, the author's further suggest that such an inflammatory response may explain some of the observed associations among formaldehyde exposure, respiratory morbidity, and immunologic responses. Their tentative hypothesis was that the reported immune response to formaldehyde exposure could result from damage to the airway epithelium, causing increased airway permeability and other inflammatory changes that would allow easier penetration of inhaled allergens to cells of the immune system. The association between formaldehyde concentrations and exhaled nitric oxide levels in this study occurred in children with no previous airway damage and was independent of atopy.

B. Summary of Key Animal Studies

No animal studies are available comparing effects of formaldehyde exposure early in life versus later in life. However, data from animal studies indicate that formaldehyde exposure may exacerbate asthma. 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 (see also Section II of the Introduction.) Animal studies bearing on the issue of induction and exacerbation of asthma are summarized here.

Amdur (1960) exposed groups of 4 to 18 guinea pigs to 0.05, 0.31, 0.58, 1.22, 3.6, 11.0, or 49 ppm formaldehyde for one hour. At the end of exposure and one hour later, the investigator measured intrapleural pressure, tidal volume, and rate of flow to the lungs, and calculated resistance to flow and lung compliance. Resistance and compliance were significantly different from the control level for the 0.31 ppm exposure ($p<0.05$) and increasingly significant at higher concentrations. One hour later only the 49 ppm exposure remained significant ($p<0.01$). Amdur (1960) also cannulated the tracheas of groups of 6 to 10 guinea pigs and exposed them for one hour to 0.90, 5.2, 20, or 50 ppm formaldehyde, and 1.14 or 3.6 ppm formaldehyde with 10 mg/m^3 sodium chloride. With the protective effect of the trachea bypassed, the resistance and compliance changed substantially and the addition of sodium chloride further enhanced the effect, including a significant effect after one hour for the 1.14 ppm formaldehyde exposure. These results show that formaldehyde that reaches the lungs has a marked effect on airways resistance and compliance, in addition to an effect on the upper airways.

Swiecechowski *et al*. (1993) exposed groups of five to seven guinea pigs to 0.86, 3.4, 9.4, or 31.1 ppm (1.1, 4.2, 11.6, or 38.6 mg/m^3) formaldehyde for 2 hours, or to 0.11, 0.31, 0.59, or 1.05 ppm (0.14, 0.38, 0.73, 1.30 mg/m^3) formaldehyde for 8 hours. An 8-hour exposure to $\geq$ 0.3 ppm ($\geq$ 0.4 mg/m^3) formaldehyde was sufficient to produce a significant increase in airway reactivity. Similar effects occurred after > 9 ppm (> 11 mg/m^3) formaldehyde for the 2-hour exposure group. Formaldehyde exposure also heightened airway smooth muscle responsiveness to acetylcholine (or carbachol) *ex vivo*. No inflammation or epithelial damage was seen up to 4 days post exposure.

The researchers suggest that duration of exposure is important to the induction of airway hyperreactivity from formaldehyde, and that prolonged (8-hour), low-level exposures may generate abnormal physiologic responses in the airways not detectable after acute (2-hour) exposures.

Riedel *et al.* (1996) studied the influence of formaldehyde exposure on allergic sensitization in the guinea pig. They exposed three groups of guinea pigs (12/group), to clean air or two different formaldehyde concentrations (0.13 and 0.25 ppm) over 5 consecutive days. Following exposure they sensitized the animals with allergen by inhalation of 0.5% ovalbumin (OA). Three weeks later, they performed specific bronchial provocation with OA using a body plethysmographic measurement of compressed air. Also they determined specific anti-OA-IgGl antibodies in serum. In another group of six animals, they examined the respiratory tract histologically for signs of inflammation directly after the end of formaldehyde or clean air exposure. In the group exposed to 0.25 ppm formaldehyde, 10/12 animals were found to be sensitized to OA (positive reaction on specific provocation) vs. 3/12 animals in the control group ($P < 0.01$). Furthermore, compressed air measurements of specific bronchial provocation and serum anti-OA-antibodies were significantly higher in the 0.25 ppm formaldehyde group than in controls. The median compressed air measurement was 0.35 ml for the formaldehyde-exposed group vs. 0.09 ml for the controls ($p< 0.01$), indicating increased bronchial obstruction. The median anti-OA-IgGl measured in the formaldehyde-exposed group was 13 vs. less than 10 EU in the controls ($p < 0.05$), indicating enhanced sensitization. In the group exposed to 0.13 ppm formaldehyde, no significant difference was found compared to the control group. Histological examination found edema of the bronchial mucosa, but there was no sign of inflammation of the lower airways in formaldehyde-exposed guinea pigs. The investigators concluded that short-term exposure to a low concentration of formaldehyde (0.25 ppm) can significantly enhance sensitization to inhaled allergens in the guinea pig.

V. Additional Information

A. Respiratory Effects in Adults

Numerous controlled and occupational human exposure studies have been conducted with both asthmatic and normal subjects to investigate formaldehyde's irritative and pulmonary effects in adults. These studies are discussed in detail in OEHHA (1999 and 2000). While the limited data available in children indicate adverse health effects of formaldehyde at concentrations as low as 30 ppb, the most sensitive studies in adults indicate health impacts at higher concentrations.

In a recent review of the medical and toxicological literature, OEHHA (2000) found a NOAEL and LOAEL of 32 $\mu g/m^3$ (26 ppb) and 92 $\mu g/m^3$ (75 ppb), respectively, after adjustment for exposure continuity. These were based on nasal and eye irritation, nasal obstruction, and lower airway discomfort, as well as histological nasal lesions (including rhinitis, squamous metaplasia and dysplasia) in chemical plant workers (Wilhelmsson and Holstrom, 1992; Edling *et al.*, 1988). Formaldehyde concentrations of 0.2 –2 ppm in a variety of workplace settings have been associated with significant decreases in lung function (measurements include FVC, FEV_1, FEV_1/FVC, FEF_{25-75} and FEF_{75-85}), respiratory irritation, eye and nose discomfort, deep airway discomfort, diminished olfactory ability, and

delayed mucociliary clearance (Alexandersson and Hedenstierna, 1989; Kilburn *et al.*, 1989; Malaka and Kodama, 1990; Holmstrom and Wilhelmsson, 1988). Alexandersson *et al.* (1982) reported eye and throat irritation as well as significant reductions in lung function in workers exposed to a mean formaldehyde concentration of 0.36 ppm (range = 0.04 - 1.25 ppm). Symptoms reported in residential setting include concentration-related eye, nose and throat irritation and headaches in persons living in mobile homes (concentrations greater than 0.1 ppm; Ritchie and Lehnen, 1987) or homes insulated with urea-formaldehyde foam (at 0.043 ppm; Border *et al.*, 1988), and exacerbation of chronic respiratory and allergy problems (0.09 ppm; Liu *et al.*, 1991).

B. Formaldehyde, Asthma, and Adults

It is unclear from the literature whether or not formaldehyde can induce or exacerbate asthma in adults. It appears that the effects of formaldehyde on asthmatics may be dependent on previous, repeated exposure to formaldehyde. Burge *et al.* (1985) found that 3 out of 15 occupationally exposed workers challenged with formaldehyde vapors (1.5 to 20.6 ppm for brief durations) exhibited late asthmatic reactions. Six other subjects had immediate asthmatic reactions likely due to irritant effects. Asthmatic responses (decreased PEF, FVC, and FEV_1) were observed in 12 occupationally-exposed workers challenged with 1.67 ppm (2.5 mg/m^3) formaldehyde (Nordman *et al.*, 1985). Similarly, asthmatic responses were observed in 5 of 28 hemodialysis workers occupationally exposed to formalin and challenged with formaldehyde vapors (concentration not measured) (Hendrick and Lane, 1977). In asthmatics not occupationally exposed to formaldehyde, Sheppard *et al.* (1984) found that a 10-minute challenge with 3 ppm formaldehyde coupled with moderate exercise did not induce significant changes in airway resistance or thoracic gas volume. Other studies of asthmatics and previously exposed workers did not find statistically significant effects on lung function measurements from challenges of formaldehyde exposure in the range of 0.4 to 3 ppm for up to four hours (ATSDR, 1999). The National Academy of Sciences' Institute of Medicine notes in their report "Clearing the Air. Asthma and Indoor Air Exposures" (NAS, 2000), that there is suggestive evidence of formaldehyde exposure and wheezing or respiratory symptoms but insufficient evidence to determine whether or not an association exists between formaldehyde exposure and asthma development.

These studies demonstrate a wide range of asthmatic responses, suggesting that formaldehyde may have an important effect in some adults but not others. In addition to different cellular responses, the uptake in the mucous lining of the airways may also differ among the study populations. Another factor in some of these results is different durations of exposure. The results of Swiecechowski *et al.* (1993) in guinea pigs suggests that the longer exposures at lower concentrations of formaldehyde may amplify the effect to be the same as shorter exposures at higher concentrations. Although there are no comparable studies for children, their immature immune systems are likely to produce responses that are more varied as well as shifted toward sensitization to formaldehyde exposure.

While these studies have not been convincing that formaldehyde exposure exacerbates asthma in adults, the studies in adults may not be applicable to children to allergic asthma in children. As previously noted, Krzyzanowski *et al.* (1990) found that asthmatic children were more affected by formaldehyde than non-asthmatic children. In addition, allergic sensitization, as measured by elevated levels of

formaldehyde-specific IgE, has been noted in two studies of children exposed to environmental levels of formaldehyde (Wantke *et al.*, 1996; Garrett *et al.*, 1999). The allergic sensitization may make children more sensitive to development of serious conditions such as asthma, although this has not been studied for formaldehyde. In addition to the data in children, animal data provide support for the contention that formaldehyde exposure may exacerbate asthma. Amdur (1960) showed that formaldehyde has a marked effect on airway resistance and compliance in guinea pigs. More importantly, Sweicechowski *et al.* (1993) showed that duration of exposure is important to the induction of airway hyperreactivity from formaldehyde. In this latter study, an 8-hour exposure to 1 ppm formaldehyde produced greater than expected effects on airway constriction compared to a 2-hour exposure at higher concentrations, suggesting that prolonged, low-level formaldehyde exposures may generate abnormal physiologic responses in the airways not detectable after acute exposures.

C. Regulatory Background

The California Environmental Protection Agency (Cal/EPA) in 1992 identified formaldehyde as a Toxic Air Contaminant. OEHHA's health effects assessment focused primarily on carcinogenicity and the development of the cancer potency factor of 5.0×10^{-6} $(\mu g/m^3)^{-1}$ (7×10^{-6} ppb^{-1}) for a 70 year lifetime. This value was based primarily on nasal cancers in rats, using a metabolic model. The International Agency for Research on Cancer (IARC) in 1987 and again in 1995 found the evidence of carcinogenicity of formaldehyde to be limited in humans and sufficient in animals, and classified formaldehyde as a probable human carcinogen, Category 2A (IARC, 1995). The U.S. Environmental Protection Agency (U.S. EPA) in 1987 classified formaldehyde in Group B-1, a probable human carcinogen, and determined a cancer potency factor of 6.5×10^{-5} $(\mu g/m^3)^{-1}$, based on nasal cancers in rats. This value was modified in 1991 to 1.3×10^{-5} $(\mu g/m^3)^{-1}$ for the Integrated Risk Information System (IRIS). The Occupational Safety and Health Administration (OSHA) in 1987 concluded that formaldehyde should be regarded as an occupational carcinogen.

The chronic reference exposure level (REL) for formaldehyde is 3 $\mu g/m^3$ (2 ppb) (OEHHA, 2000). This value was based on a NOAEL of 32 $\mu g/m^3$ (26 ppb) for symptoms of irritation in workers. The acute REL for formaldehyde is 74 ppb based on irritation of asthmatics. The World Health Organization (WHO) in 1989 determined a threshold value of 60 $\mu g/m^3$ (50 ppb). The National Institute of Occupational Safety and Health (NIOSH, 1992) in 1988 determined a REL-TWA (Time-Weighted Average) of 20 $\mu g/m^3$ (16 ppb), based on the threshold of reliable measurement at that time. The Occupational Safety and Health Administration (OSHA) in 1992 established a PEL-TWA (Permissible Exposure Level) of 920 $\mu g/m^3$ (750 ppb), based on reducing risk due to cancer and eye, nose and throat irritation and sensitization. The American Conference of Governmental Industrial Hygienists (ACGIH) assigned a Threshold Limit Value (TLV) of 360 $\mu g/m^3$ (300 ppb), based on irritation in less sensitive workers but not protecting the most sensitive workers. The Agency for Toxic Substances and Disease Registry (ATSDR) in 1999 derived a chronic Minimal Risk Level (MRL) of 10 $\mu g/m^3$ (8 ppb) based on a study of changes in nasal tissue in workers.

VI. Conclusions

Formaldehyde is a respiratory irritant. Formaldehyde exposure is associated with decrements in lung function and elevated respiratory symptoms in children. One study that evaluated both children and adults found that children appear to be more sensitive than adults (Krzyzanowski et al., 1990) although the effect was seen primarily in children also exposed to ETS. There is extensive exposure to formaldehyde particularly indoors. Although there is some evidence indicating children may be more sensitive to formaldehyde respiratory toxicity, at this time, OEHHA has placed formaldehyde in Tier 2. OEHHA may revisit listing formaldehyde in the future, particularly if more information becomes available regarding differential toxicity between children and adults.

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Ethylene Glycol Ethers (EGME, EGEE, EGMEA, EGEEA)

I. Physical and Chemical Properties

Ethylene glycol ethyl ether (EGEE; 2-ethoxyethanol)

CAS Registry Number	110-80-5
Description	Colorless liquid
Molecular formula	$C_4H_{10}O_2$
Molecular weight	90.12 g/mol
Air concentration conversion	3.69 µg/m^3 per ppb at 25ºC

Ethylene glycol ethyl ether acetate (EGEEA; 2-ethoxyethanol acetate)

CAS Registry Number	111-15-9
Description	Colorless liquid
Molecular formula	$C_6H_{12}O_3$
Molecular weight	132.16 g/mol
Air concentration conversion	5.41 µg/m^3 per ppb at 25ºC

Ethylene glycol methyl ether (EGME; 2-methoxyethanol)

CAS Registry Number	109-86-4
Description	Colorless liquid
Molecular formula	$C_3H_8O_2$
Molecular weight	76.09 g/mol
Air concentration conversion	3.1 µg/m^3 per ppb at 25ºC

Ethylene glycol methyl ether acetate (EGMEA; 2-methoxyethanol acetate)

CAS Registry Number	110-49-6
Description	Colorless liquid
Molecular formula	$C_5H_{10}O_3$
Molecular weight	118.3 g/mol
Air concentration conversion	4.83 µg/m^3 per ppb at 25ºC

II. Overview

Developmental toxicity is one of the key toxicological endpoints of concern for impacts on infants and children. The developing fetus is susceptible to certain glycol ethers and their acetates. These are ethylene glycol ethers with alkyl chains of one or two carbon atoms: EGME, EGMEA, EGEE, and EGEEA. The developing fetus appears to be susceptible at levels lower than those associated with maternal toxicity. The effects of EGME, EGEE, and their acetates are considered severe because they include teratogenicity, testicular toxicity, and fetotoxicity in rabbits.

Bolt and Golka (1990) report that a woman, who was exposed occupationally to EGMEA, bore in successive pregnancies two sons with penile hypospadia. Recent epidemiologic studies from Europe suggest an association of major congenital malformations with exposure to glycol ethers during the first trimester of pregnancy.

These chemicals are fetotoxic and teratogenic in animals. Pregnant animals exposed during development to either EGEE or EGME by inhalation had more malformed or dead fetuses than unexposed controls (Tinston *et a*l., 1983a; Doe, 1984; Hanley *et al*., 1984). Inhaled EGME and EGEE tend to cause skeletal anomalies and developmental neurotoxicity at exposure concentrations below those causing toxicity to mature animals. EGEE, EGEEA, EGME, and EGMEA appear to be more toxic to the developing human than to humans at later stages of life. Therefore, EGEE and EGME (and their acetates) are considered priority chemicals for evaluation of potential differential effects on infants and children.

It is difficult to estimate the magnitude of risk that would occur at concentrations typical of California urban ambient air, since no monitoring data are available. Point source emissions of total glycol ethers for the facilities reporting emissions under the Air Toxics Hot Spots Program appear to be substantial (Table 1 below). These include significant amounts of ethylene glycol ethyl ethers. Data are not readily available to determine to what extent the emissions of unspecified glycol ethers represent methyl or ethyl ethers or their acetates.

III. Principal Sources of Exposure

Table 1. Air Toxics Hot Spots Emissions of Various and Total Glycol Ethers

Glycol ether	California emissions	Ambient air levels	SCAQMD
EGEE	443,748 pounds	Not monitored	Not monitored
EGEEA	66,851 pounds	Not monitored	Not monitored
EGME	7,398 pounds	Not monitored	Not monitored
EGMEA	3,060 pounds	Not monitored	Not monitored
Glycol ethers (not speciated)	2,922,744 pounds	Not monitored	Not monitored

EGEE. Ethylene glycol monoethyl ether is a widely used solvent for nitrocellulose, dyes, inks, resins, lacquers, paints, and varnishes. EGEE is also a component of many cleaning agents, epoxy coatings, paints, hydraulic fluid, and is an anti-icing fuel additive in aviation. EGEE is also a chemical intermediate in the production of another solvent, ethylene glycol monoethyl ether acetate (EGEEA). The specific annual statewide industrial emissions of EGEE from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 443,748 pounds (CARB, 2000), plus some proportion of the non-speciated glycol ethers.

EGEEA. Ethylene glycol monoethyl ether acetate is used in automobile lacquers where it retards "blushing" and evaporation and imparts a high gloss (HSDB, 1996). It is also used as a solvent for nitrocellulose, oils, and resins and as a component of varnish removers and wood stains. EGEEA is

also used in the treatment of textiles and leather. The annual specific statewide industrial emissions of EGEEA from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 66,851 pounds (CARB, 2000).

EGME. Ethylene glycol monomethyl ether (EGME) is used as a solvent for cellulose acetate and resins as well as a solvent in the semiconductor industry. It is also used in dyeing leather and in the manufacture of photographic film. EGME is used as an anti-freeze in jet fuels. Quick drying varnishes, enamels, nail polishes, and wood stains may also contain EGME. The specific annual statewide industrial emissions of EGME from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 7398 pounds (CARB, 2000), plus some proportion of the non-speciated glycol ethers.

EGMEA. Ethylene glycol monomethyl ether acetate is used as a solvent for nitrocellulose, cellulose acetate, and various other gums, resins, waxes, and oils. It is also used in the semiconductor industry and in textile printing, photographic films, lacquers, and silk-screening inks. The annual specific statewide industrial emissions of EGMEA from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 3,060 pounds (CARB, 2000).

The Air Resources Board does not monitor routinely for glycol ethers. Much of the emissions of glycol ethers (nearly 3 million pounds) is not speciated, so we do not know how much additional toxic ethylene glycol ethers are emitted. The concern for exposure is in the vicinity of specific facilities that emit large amounts of these ethylene glycol ethers (Hot Spots) and produce high concentrations in the local area.

IV. Potential for Differential Effects

A. Summary of the Key Human Studies

Congenital malformations may be induced in humans. In a case report Bolt and Golka (1990) describe a woman, exposed occupationally to EGMEA during pregnancy, who bore sons with penile hypospadia in successive pregnancies. Saavedra *et al.* (1997) described facial malformations and varying degrees of mental retardation in 44 offspring of mothers who were exposed occupationally to EGME and ethylene glycol at the factory of an American company producing capacitors in Mexico.

Using 6 registers in the European Registration of Congenital Anomalies (EUROCAT), a large group of investigators studied major congenital malformations reported between 1989 and 1992 (Ha *et al*., 1996; Cordier *et al*., 1997; Lorente *et al*., 2000). Preliminary results (Ha *et al*., 1996) among mothers who were working during pregnancy found excesses of oral clefts (OR = 2.0; 95% CI = 1.1-4.1), central nervous system malformations (OR = 1.8; 95% CI = 1.1-3.3), and musculoskeletal malformations (OR 1.6; 95% CI = 0.9-2.8) among glycol ether exposed mothers. However, only one mother was exposed to "group I glycol ethers" (a classification defined in the report as EGME, EGMEA, EGEE, EGEEA, "and some polyethylenic compounds").

More definitive results were reported by Cordier *et al*. (1997). The entire study comprised 984 cases with major congenital malformations and 1,134 matched controls. After adjustment for several potential

confounding exposures (other solvents, anesthetic gases, pesticides, lead), the overall odds ratio (OR) of congenital malformation associated with glycol ether exposure was 1.44 [95% CI = 1.10-1.90]. This OR was based on the exposure to glycol ethers of 158 of the 648 cases occurring among mothers who worked during the first trimester. The association with exposure to glycol ethers appeared particularly strong for neural tube defects (94 cases, 28 exposed to glycol ethers, OR = 1.94; 95% CI = 1.16-3.24), multiple anomalies (114 cases, 34 exposed, OR = 2.00; 95% CI = 1.24-3.23), and cleft lip (64 cases, 23 exposed, OR = 2.03; 95% CI = 1.11-3.73). However, this study did not discuss sub-grouping of glycol ethers.

The relationship between occupational exposures of 851 women (100 mothers of babies with orofacial clefts and 751 mothers of healthy referents) who worked during the first trimester of pregnancy and orofacial clefts (Lorente *et al*., 2000) was evaluated. This analysis suggested that occupational exposure to glycol ethers was associated with orofacial clefts (OR 1.7, 95% CI 0.9-3.3 for cleft lip with or without cleft palate).

EGME is metabolized by an alcohol dehydrogenase to methoxyacetaldehyde, which is then metabolized by aldehyde dehydrogenase to methoxyacetic acid. EGEE is metabolized to ethoxyacetaldehyde, then to ethoxyacetic acid. The alkoxyacids are considered to be more toxic than their parent glycol ethers. EGMEA and EGEEA are hydrolyzed to acetate and the respective ethylene glycol ethers, which are then dehydrogenated.

B. Summary of the Key Animal Studies

EGEE. Exposure to EGEE induces malformations in offspring in the absence of significant maternal toxicity. Nelson *et al*. (1981) reported changes in brain chemistry in the offspring of Sprague-Dawley rats (n=14-15) exposed to 100 ppm (369 mg/m^3) EGEE for 7 h/day on gestational days (gd) 7-13 or 14-20. The only effect observed in the dams was slightly prolonged gestation in those exposed on gd 14-20 ($p < 0.001$). Six neurobehavioral tests were used to assess CNS functioning at various stages of development. In the pups exposed during gd 7-13, a decreased rotorod speed and an increased latency period for leaving the central area of an open field were observed. The activity of the offspring of rats exposed during gd 14-20 decreased on the activity wheel, and avoidance conditioning, begun on day 60 of age, revealed that these pups received an increased number and duration of shocks. Whole brain norepinephrine levels in the newborns of the exposed dams from both exposure groups (7-13 and 14-20 days) decreased. At age 21 days, norepinephrine was increased in the cerebrum, brain stem and midbrain of 7- to 13-day exposed pups only. Increased dopamine levels were found in the cerebrum only of pups from both exposure periods, while serotonin was increased in the 14-20 day exposure group. The midbrains of pups exposed on gd 7-13 had protein levels that exceeded the controls. (Gross teratogenic anomalies (terata) were not detected in this study, possibly due to either insufficient numbers of animals or inadequate procedures.)

Specific skeletal defects were reported in the progeny of pregnant rats exposed to 10, 50, and 250 ppm (40, 200, and 920 mg/m^3) EGEE 6 hours per day on days 6-15 of gestation (Tinston *et a*l., 1983a). Maternal toxicity, as indicated by reduced hemoglobin, hematocrit, and mean cell volume in

red blood cells, was observed in rats exposed to 250 ppm EGEE. A significant reduction in the number of live fetuses was observed in rats exposed to 10 and 250 ppm, and a reduction in total litter weight was observed in rats exposed to 10 ppm and 50 ppm. Intergroup comparison showed significantly increased incidence of total minor skeletal defects in fetuses in the 250 ppm dose group; delayed ossification was the most common abnormality observed at this dose. Specific skeletal defects, including delayed ossification of the cervical vertebrae and sternebrae and the presence of extra ribs, were significantly increased in not only the 250 ppm dose group but also in the 50 ppm dose group where there was no apparent maternal toxicity.

EGEEA. EGEEA is fetotoxic and teratogenic at concentrations below that necessary to induce maternal toxicity. Pregnant rabbits (24 or 25/group) were exposed to 0, 25, 100, or 400 ppm EGEEA by inhalation for 6 hours/day on gd 6-18 (Tinston *et al.*, 1983b; reviewed in Doe, 1984) and were killed on gd 29. Maternal effects (decreased weight gain, decreased food consumption, decreased hemoglobin) were observed at 400 ppm. The number of rabbits with total fetal resorptions was increased in the 400 ppm dose group, accompanied by a decrease in weight in surviving fetuses. A reduction in average fetal weight was also observed at 100 ppm EGEEA, but this effect may relate to the increased litter size among dams in this dose group. Evidence of teratogenicity was observed in the 400 ppm dose group, with increased major malformations of the vertebral column. Both 400 and 100 ppm EGEEA were found to be fetotoxic as indicated by retarded ossification. No statistically significant effects were observed in the 25 ppm dose group. (A single case of a major defect (kidney agenesis) was observed in both the 25 and 400 ppm EGEEA dose groups.)

EGME. EGME is fetotoxic and teratogenic at concentrations below that necessary to induce maternal toxicity. Hanley *et al*. (1984) exposed pregnant rats and rabbits to 3, 10, or 50 ppm (9.6, 32, or 160 mg/m^3) EGME for 6 hours per day on gd 6-15 (rats) or gd 6-18 (rabbits). Pregnant mice were exposed to 10 or 50 ppm (32 or 160 mg/m^3) EGME for 6 hours per day on gd 6-15. Transient decreases in maternal body weight gain among rats, mice, and rabbits exposed to 50 ppm were the only consistent signs of maternal effects. A statistically significant increase in the incidence of skeletal variations was observed in rats and mice following maternal exposure to 50 ppm EGME. Gross soft tissue (cardiovascular) and skeletal teratogenic effects and significantly decreased fetal body weights were observed in rabbits following maternal exposure to 50 ppm EGME. In rabbits, a significant increase in the rate of fetal resorption was observed in the 10 ppm exposure group (Table 2). Thus 10 ppm was considered a LOAEL for increased resorptions and 3 ppm a NOAEL. Although the authors attribute the statistical significance of this effect to an unusually low rate of resorptions in controls compared to historical controls, historical control data were not presented.

Table 2. EGME Rabbit Teratology: Selected Observations from Hanley et al. (1984)

	0 ppm	3 ppm	10 ppm	50 ppm
No. litters	23	24	24	24
Live fetuses/litter	8 ± 2	7 ± 3	8 ± 3	6 ± 3
Implantations resorbed	4% (7/180)	8% (14/186)	11% (23/210)*	24% (46/191)*
Litters with resorptions	22% (5/23)	42% (10/24)	58% (14/24)*	67% (16/24)*
Fetal bw (g)	39.57 ± 5.48	39.13 ± 6.24	38.83 ± 4.54	35.88 ± 3.79*
Limb defects	0	1 fetus	1 fetus	55 fetuses in 16 litters*
Cardiovascular defects	0	0	0	34 fetuses in 15 litters*

* $p<0.05$ vs. the control value

EGME may cause changes in brain chemistry when exposure occurs during development. Nelson *et al*. (1984) exposed 18 Sprague-Dawley male rats to 25 ppm EGME (78 mg/m^3) 7 hours/day, 7 days/week for 6 weeks prior to mating with unexposed females. The brains of 21-day-old offspring had neurochemical changes, especially in the brainstem and cerebrum (e.g., dopamine, norepinephrine). They showed no behavioral effects as indicated by neuromotor function, activity, and simple learning ability. The offspring of pregnant females (15 animals/group) in both groups exposed to 25 ppm EGME during either gestation days 7-13 (group 1) or 14-20 (group 2) had similar neurochemical changes (e.g., decreased levels of acetylcholine and increased levels of cerebral dopamine at 21 days post-partum). There was a significant difference in avoidance conditioning in the offspring of the group exposed during gestation days 7-13. The concentration of 25 ppm is a LOAEL for developmental toxicity in this study.

V. Additional Information

A. Other Toxicity

EGEE, and EGME and their respective alkoxy metabolites all cause toxicity to 9.5-day-old rat embryos cultured in vitro. Based on a comparison done with this assay the potency for teratogenicity for the ethylene glycol ethers (and for their alkoxyacid metabolites) is EGME > EGEE > EGPE (ethylene glycol propyl ether) > EGBE (ethylene glycol butyl ether) or the shorter the chain, the greater the potency (Rawlings *et al*., 1985)

The glycol ethers cause damage to the developing fetus at exposure levels below those that cause maternal toxicity. Toxicity to the bone marrow and thymus at higher doses in adult animals indicate the possibility of enhanced risk to developing hematopoietic and immune systems.

B. Regulatory Background

EGME, EGMEA, EGEE, and EGEEA are federal hazardous air pollutants (HAPs) and were identified as toxic air contaminants (TACs) in California in April 1993 under AB 2728. The acute and chronic

RELs are tabulated below (Table 3). The 4 chemicals are listed under Proposition 65 as developmental toxicants and as male reproductive toxicants.

Table 3. OEHHA Health Guidance Values and Proposition 65 Status

Glycol ether	Acute REL	Chronic REL	Cancer Potency	Prop 65
EGEE	370 μg/m^3 (100 ppb) (6 h avg time)	70 μg/m^3 (20 ppb)	None	Developmental and male repro toxicant
EGEEA	140 μg/m^3 (25 ppb) (6 h avg time)	300 μg/m^3 (60 ppb)	None	Developmental and male repro toxicant
EGME	93 μg/m^3 (30 ppb) (6 h avg time)	60 μg/m^3 (20 ppb)	None	Developmental and male repro toxicant
EGMEA	None	90 μg/m^3 (20 ppb)	None	Developmental and male repro toxicant

C. Description of RELs

EGEE. The acute REL for EGEE of 370 μg/m^3 (OEHHA, 1999) is based on specific skeletal defects, including delayed ossification of the cervical vertebrae and sternebrae and extra ribs, seen in the fetuses from pregnant rats exposed by inhalation 6 hours per day on days 6-15 of gestation (Tinston *et al*., 1983a; Doe, 1984). The chronic REL for EGEE of 70 μg/m^3 (OEHHA, 2000) is based on testicular degeneration and decreased hemoglobin in rabbits as reported by Barbee *et al*. (1984).

EGEEA. The acute REL for EGEEA of 140 μg/m^3 (OEHHA, 1999) is based on teratogenicity and fetotoxicity in rabbits as determined by Tinston *et al*. (1983b). The chronic REL for EGEEA of 300 μg/m^3 (OEHHA, 2000) is based on the same study.

EGME and EGMEA. The acute REL for EGME of 93 μg/m^3 (OEHHA, 1999) is based on teratogenic effects in rabbits as reported by Hanley *et al*. (1984). The chronic REL for EGME of 60 μg/m^3 (OEHHA, 2000) is based on testicular toxicity (reproductive system) in rabbits as determined by Miller *et al*. (1983). The chronic REL for EGMEA of 90 μg/m^3 (OEHHA, 2000) is also based on the Miller *et al*. study.

The most sensitive toxic endpoints associated with EGEE, EGEEA, EGME, and EGMEA are developmental toxicity and male reproductive toxicity. These glycol ethers appear to be more toxic to the developing human than to humans at later stages of life. However, based on current risk assessment methodology, the existing health criteria for glycol ethers should be adequately protective of children because they are based on developmental endpoints in animals. The lowest developmental NOAEL reported is 3 ppm for EGME and the acute REL of 30 ppb is 100-fold lower than the lowest NOAEL while the chronic REL of 20 ppb is 150-fold lower.

VI. Conclusions

There is evidence in both humans and animals that exposure to specific glycol ethers can result in developmental toxicity. Developmental toxicity is one of the endpoints of concern for impacts on infants and children. Exposures to glycol ethers are not well characterized, but may occur near sources of industrial emissions. Thus, glycol ethers have been placed in Tier 2. Should evidence become available that exposures to glycol ethers, especially EGEE and EGME and their acetates, are significant near emissions sources, OEHHA may consider listing glycol ethers in a future update.

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Manganese and Compounds

7439-96-5

Mn

I. Physical and Chemical Properties

Description	Lustrous, gray-pink metal (Mn); green (MnO), black (MnO_2) or pink ($MnCl_2$) crystals; brownish-black powder (Mn_3O_4)
Molecular formula	See above
Molecular weight	54.9 g/mol
Air concentration conversion	Not applicable

II. Overview

There is evidence from human studies and animal experiments that manganese exposure can lead to neurodevelopmental and behavioral effects. Neurotoxicity and developmental toxicity are two of the key toxicological endpoints of concern to infants and children. Animal studies show that newborns are particularly susceptible possibly because manganese homeostasis is not established until around the time of weaning (Miller et al., 1975). No direct human studies were found that would tell us whether human newborns would be especially vulnerable, but children do appear to be more susceptible to manganese poisoning during total parenteral nutrition (Komaki *et al*, 1999). Manganese appears to have the potential to differentially impact infants and children, however there is a low potential for manganese exposure. Methylcyclopentadienyl manganese tricarbonyl (MMT), an organic manganese compound, has been used as a gasoline additive in some locations, but its use is prohibited in California (California Code of Regulations, Title 13, Section 2254).

III. Principal Sources of Exposure

Metallic manganese is used in the manufacture of steel, stainless steel, and other metal alloys (HSDB, 1999). The most recent average ambient air level of manganese in California is 21 ng/m^3 (CARB, 1999). The annual statewide emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 105,000 lbs in 1998 (CARB, 1999). Thus, there is some concern about near-source exposures.

Individuals may be exposed to manganese dust by inhalation, or to manganese salts dissolved in drinking water sources. Foods (particularly nuts and grains) are a source of essential manganese. Adequate Intake (AI) for children 1 to 3 years of age is 1.2 mg Mn/day. AI is 2.3 mg/day for men, and 1.8 mg/day for women (Institute of Medicine, 2001). At the statewide average concentration, ambient

exposure would account for about one five-thousandth of the daily exposure. There is evidence in rats that inhaled manganese can be absorbed directly through the olfactory bulbs (Brenneman *et al*., 2000). This could theoretically increase the effect of inhaled manganese on the brain, but more research will be needed to address this question.

IV. Potential for Differential Effects

A. Summary of Key Human Studies

Studies of the effects of manganese exposure in both adults and children demonstrate the potential for neurological effects. As noted in the Introduction, neurotoxicity is one of the key toxicological endpoints of concern for infants and children. Male workers (n=92, plus 101 matched controls) in an alkaline battery plant in Belgium exposed to manganese dioxide dust were the subjects of a cross-sectional epidemiological study (Roels *et al*., 1992). The subjects were evaluated for neurobehavioral function, lung function, hematological parameters and by urinalysis. Significant decrements in performance on tests for visual reaction time, eye-hand coordination, and hand tremor were found in exposed workers relative to controls. Lifetime integrated respirable dust levels (LIRD) ranged from 0.04 to 4.43 mg Mn $\times$ years /m^3, with a geometric mean of 0.793 mg Mn $\times$ yrs/m^3. Average exposure time was 5.3 years (0.2 to 17.7 years). Similar results had been reported in an earlier study by the same group of investigators (Roels *et al*., 1987). There are no directly comparable studies in children. Neurobehavioral effects are likely to affect children differentially because children's nervous systems are developing, as discussed in the introduction.

Kilburn (1987) studied the aboriginal natives of Groote Eylandt, Australia. This island off the coast of Australia has long been a source of manganese ore. It had been informally observed that drinking water on the island is often discolored by manganese. Also, there had been reports of high incidence of birth defects among children born on the island. Kilburn compared the natives of this island with a group of mainland natives. He found that stillbirths were higher among the islanders (42 percent versus 29.5 percent) but did not give a statistical analysis of significance. During the study period of 1975 to 1985 there were 293 children born to the islanders. He reported on a number of congenital abnormalities, but admitted that the numbers were not sufficient for a statistical analysis of significance. He also reported on neurological disorders manifest as "problems of weakness, gait, coordination and ocular movements." Again, there is no statistical analysis. The results of this study are inconclusive though perhaps suggestive of adverse effects from manganese exposure.

Children receiving total parenteral nutrition (TPN) are at increased risk for hypermanganesemia, cholestasis, and basal ganglia damage (Fell *et al*., 1996). TPN appears to bypass the normal homeostatic controls on blood manganese levels. The normal "reference range" for manganese in the blood is 72 to 210 nmol/L. Eleven child patients on TPN who were identified as having hypermanganesemia and cholestasis had blood manganese levels of 615 to 1840 nmol/L. It is difficult to establish the order of causality, but these investigators are of the opinion that manganese may contribute to cholestasis. Cholestatic disease generally improved concomitant with blood manganese decline after reduction or withdrawal of manganese supplementation. The patients had been receiving

the usual recommended manganese dose of 1.0 µmol/kg per day for those less than 10 kg body weight, and 0.8 µmol/kg per day for those over 10 kg body weight. The authors recommend decreasing this to not more than 0.018 µmol/kg per day together with regular examinations of the nervous system (yearly cranial magnetic resonance imaging).

There are more recent reports of manganese causing problems for pediatric TPN patients (Kelly, 1998; Komaki *et al*., 1999). Kelly found that liver disease develops in 40 to 60% of infants who require long-term TPN for intestinal failure. Kelly points out that although there is clearly a relationship between manganesemia and liver disease, it is not clear which one causes the other. Cholestasis may contribute to hypermanganesemia because bile is the main route of excretion of manganese. Komaki *et al*. (1999) reported on a two-year-old female patient on TPN who experienced tremor and seizures resulting from accumulation of manganese in her brain. This patient had received 1.1 mg Mn per day (82 µg/kg per day). This would be equivalent to 1.5 µmol/kg per day, which is higher than the "recommended dose" discussed in the paper by Fell et al. (1996).

Collip *et al*. (1983) reported elevated hair manganese in children with learning disabilities (hyperactivity). Hair manganese levels in eight-year-old children were 0.268 µg/g in normal children and 0.434 µg/g in learning disabled children. Similarly, Barlow (1983) reported a mean level of 0.84 µg Mn/g hair in 68 hyperactive children compared to a mean of 0.68 µg/g in control children. It is not known whether this is due to higher exposure to manganese or to a lower elimination rate of manganese in children. More work will be needed to determine if manganese exposure causes learning disabilities in exposed children, and, if so, at what exposure levels this effect would occur. No discussion of lead as a confounder was presented in this paper.

Collip *et al*. (1983) also found that formula-fed infants had significantly greater increases in hair manganese than breast-fed infants. The amount of manganese in infant formulas may be greater than optimal. Collip *et al*. (1983) reported that whereas human milk had only 10 µg Mn per quart, commercial infant formulas generally ranged from 200 to 1,000 µg Mn per quart.

B. Summary of Key Animal Studies

Animal experiments demonstrate that exposure to excess manganese can lead to accumulation of manganese in critical areas of the brain. This in turn can lead to changes in brain chemistry and neurological dysfunction. Neonatal animals appear to be particularly vulnerable to accumulation of manganese in critical brain areas because manganese homeostasis is not established until later.

Newborn mice are unable to excrete injected manganese for the first 17 days of life (Miller *et al*, 1975). During this period manganese accumulates in the tissues, particularly in the liver and brain. After this period manganese is excreted and homeostasis of manganese is maintained. This inability to control manganese levels in tissues by excretion might make neonatal animals more susceptible to manganese poisoning.

Experiments in farm animals and laboratory animals (rats) indicate that manganese homeostasis is "suspended" during pregnancy and lactation (reviewed by Cawte, 1985). Suspension of homeostasis allows for higher levels of manganese in blood and in tissues including brain.

In discussing studies with mice (their own and others), Webster and Valois (1987) state, "The neonate is clearly the stage of human development potentially at the greatest risk from environmental manganese. The high intestinal absorption, absent excretory mechanism and ready accumulation in the developing brain all combine to make this a period of concern." The neonate appears to be more vulnerable than the fetus (Webster and Valois, 1987) because of the inability of the neonate to maintain manganese homeostasis. Kontur and Fechter (1985) also state that, "Neonatal animals show differential absorption, accumulation, and excretion of manganese relative to adults."

Dorman *et al*. (2000) studied the relative sensitivity of neonatal and adult CD rats to manganese-induced neurotoxicity. The rats were administered $MnCl_2$ orally at doses of 0, 25, or 50 mg/kg. These doses were given to neonatal rats during lactation, and to adult rats for 21 consecutive days. All rats were evaluated in behavioral and neurochemical tests. Increased pulse-elicited acoustic startle response amplitude was observed in neonates from both manganese chloride treatment groups on post natal day (PND) 21. Increased striatal, hippocampal, hindbrain and cortical manganese concentrations were observed in all manganese-exposed neonates on PND 21. Increased striatal, cerebellar and brain residue manganese concentrations were observed in adult rats from the high dose group. The authors conclude, "neonates may be at greater risk for manganese-induced neurotoxicity when compared to adults receiving similar high oral levels of manganese."

Young rats (starting at 21 days of age) showed neuronal degeneration (characterized by fewer nuclei per unit area with less staining per nucleus) in cerebral and cerebellar cortex after 30 days of administration of 50 µg $MnCl_2$ per day (Chandra and Shukla, 1978). Similar neuronal degeneration appeared in adult rats only after 120 days of Mn administration at the same exposure level.

Pappas et al. (1997) exposed female rats and their litters to manganese in drinking water from conception until PND 30. The concentrations of manganese in the water were 0, 2, and 10 mg/ml. Body weight gain was decreased significantly in the high dose group, but not in the low dose group. Cortical levels of manganese were 35% higher than controls in the low dose group (not statistically significant) and 150% higher in the high dose group. Behavioral testing of the pups showed significantly increased locomotor activity and rearing in the high dose, but not the low dose group. Other behavioral tests (radial arm maze, Morris water maze) showed no significant differences between exposed and control rats. There was a significant decrease in cortical thickness in pups of both exposed groups relative to the controls. The investigators were not able to determine the exact cause of the cortical thinning (decreased cell number, reduction in cell size, reduced arborization, etc.). They speculated that it may have been due to malnutrition, as it was seen only in rats with significantly reduced body weight gain. Brain cytochemistry tests on the rats included choline acetyltransferase, glial fibrillary acidic protein, tyrosine hydroxylase and mesencephalic dopamine. None of these cytochemical tests showed any significant differences between exposed and control rats.

Lown *et al*. (1984) found significantly increased activity in pups of mice exposed to MnO_2 dust by inhalation during and after pregnancy. Brenneman *et al*. (1999) observed hyperactivity in PND 21 pups of CD rats exposed to $MnCl_2$ in drinking water at a dose of 50 mg/kg bw.

Deskin, Bursian and Edens (1980) exposed male rats from birth to 24 days to 1, 10 and 20 µg/g bw $MgCl_2$ by oral gavage. Body weight gain was unaffected at all doses. Dopamine (but not norepinephrine) was reduced in the hypothalamic areas of rats exposed to 10 and 20 µg/g $MnCl_2$. The depletion of dopamine induced by alpha-methyl-p-tyrosine (a tyrosine hydroxylase inhibitor) was less in the hypothalamic areas of chronic manganese-treated rats, suggesting that dopamine turnover was reduced.

Not all the studies of neurotoxicity from early-in-life exposures have been positive. Kontur and Fechter (1985) exposed pregnant rats to 0, 5, 10, or 20 mg/ml of manganese chloride ($MnCl_2$) in drinking water. They found that the higher doses (10 and 20 mg/ml) were sufficient to cause a significant decrease in maternal weight gain. The highest dose caused a significant decrease in average litter size. The investigators showed that manganese crossed the placenta and accumulated in the fore- and hindbrains of the pups. However, the accumulation of manganese in the brains of the rat pups was limited; increasing the exposure beyond 5 mg/ml did not result in increased accumulation of manganese in the brains. The investigators looked at catecholamine turnover and development of acoustic startle response in newborn pups that had been exposed to manganese *in utero*. They found that neither of these parameters was significantly affected by manganese exposure. The authors conclude that although manganese exposure in utero causes reduced maternal weight gain and reduced litter size it is not specifically neurotoxic to newborn rats exposed *in utero* (Kontur and Fechter, 1985).

Webster and Valois (1987) injected pregnant mice i.p. with 12.5, 25 and 50 mg manganese sulfate/kg. An increase in fetal death and decrease in fetal weights were observed in all dose groups. Exencephaly was induced with 25 mg/kg on gestation day (GD) 8. The higher dose (50 mg/kg) induced fetal deaths, but no exencephaly. The authors state that the exencephaly observed at 25 mg/kg is "probably of little significance in view of the high dose necessary for teratogenesis." Colomina *et al*. (1996) investigated the relationship between day of exposure and embryo/fetotoxicity in mice injected s.c. with 50 mg manganese chloride/kg on days 9, 10, 11 and 12 of gestation. No teratogenic effects were observed in this study. Days 9 and 10 of gestation were determined to be the most sensitive for embryotoxicity evidenced by significant increases in late resorptions and increased incidence of postimplantation loss. Days 9 and 10 were also the most sensitive for fetotoxicity (reduced fetal body weight and increased incidence of skeletal defects).

Treinen *et al*. (1995) exposed pregnant Sprague-Dawley rats to manganese chloride via tail-vein injection on GD 6-17. Dosages included 5, 20 or 40 µmol/kg. At the two higher doses they observed specific skeletal variations: wavy ribs and reduced ossification.

In a study involving subcutaneous injection of pregnant Swiss albino mice, Sanchez *et al*. (1993) reported a NOAEL of 2 mg/kg/day $MnCl_2$ for embryotoxicity and skeletal variations.

V. Additional Information

A. Regulatory Background

The California chronic Reference Exposure Level (REL) for manganese is 0.2 μg/m^3. It is based on a human study (discussed above) showing neurobehavioral effects, by Roels *et al*. (1992). In this study, workers were classified according to their integrated lifetime exposure to manganese dioxide dust (ranging from < 0.6 to >1.2 mg Mn × yrs/m^3). The geometric mean for all exposed workers of the integrated lifetime occupational exposure, divided by the mean occupational exposure duration, was used as a LOAEL (0.15 mg Mn/m^3) to calculate the REL.

VI. Conclusions

There is evidence from both human studies and animal experiments that manganese may exert a differential toxic effect on infants and children. Human studies show that hyperactive children and children with learning disabilities may have higher hair levels of manganese than normal children (Collip, Chen and Maitinsky, 1983). This suggests that manganese may act as a neurodevelopmental toxicant on young children. Animal studies show that newborn animals are unable to maintain homeostasis of manganese (Miller et al., 1975) and that as a result manganese accumulates in the brains of animals exposed at young ages (Dorman et al., 2000). There is also evidence that manganese exposure to young animals can cause neurodegenerative changes such as neuronal degeneration and cortical thinning (Chandra and Shula, 1978; Pappas et al., 1997).

Manganese does not appear to be a major hazard from the point of view of widespread air exposures. Although ambient air levels may be higher near sources, the general statewide ambient levels are low (21 ng/m^3). Assuming a child breathes 10 m^3 of air daily, the amount of manganese to which the child would be exposed by air at this ambient level would be about one five-thousandth of the amount needed in the diet to fulfill the adequate intake level (1.2 mg Mn/day). OEHHA has placed manganese in Tier 2 primarily because of current low potential for airborne exposures. Should new information become available indicating significant exposures, manganese should be reconsidered for listing under SB25.

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Mercury

CAS Registry Number 7439-97-6

I. Physical and Chemical Properties

Mercury is found in the environment in the metallic (Hg^0), inorganic (Hg_2^{+2}, Hg^{+2}), and organic (alkyl) forms.

Description	Silvery, odorless heavy liquid
Molecular formula	Hg
Molecular weight	200.59 g/mol
Air concentration conversion	Not applicable for mercury salts; 1 ppm = 8.34 mg/m^3

II. Overview

The brain and the kidneys are the primary targets of mercury toxicity with the central nervous system more sensitive than other organs. There is good evidence from human low-dose chronic exposures that fetuses exposed *in utero* are more sensitive to the toxic effects of mercury than adults. Children are potentially more susceptible than adults due to differences in the stages of brain development and organ growth that occur during the fetal, infant, and childhood developmental periods. Elemental and methylmercury readily cross the placental barrier; mercuric species, which do not readily cross the adult blood-brain barrier, are able to cross into the brain in neonatal stages due to the incompletely formed blood-brain barrier. In the brain, elemental mercury and methylmercury are slowly transformed to mercuric ion, which tends to remain at the site of formation. The toxic effects associated with exposure to elemental mercury are believed to be due to the mercuric ion.

III. Principal Sources of Exposure

The principal sources of exposure to mercury in the general population are ingestion and inhalation of mercury compounds from dental amalgams, and ingestion of fish (fresh water and marine) and seafood which contain mercury, primarily as methylmercury. Adults and children may also be exposed to small amounts of elemental mercury from broken thermometers, barometers, fluorescent lights, electric switches and inhalation of atmospheric mercury.

In the environment, mercury comes from natural and anthropogenic sources. Mercury can be released into the air through geothermal activity and the weathering of mercury ore-containing rock. Anthropogenic sources of mercury, primarily from the combustion of fossil fuels, and incineration of medical and municipal waste, contribute greater than 80% of the mercury emitted from point sources

according to the Agency for Toxic Substances Disease Registry (ATSDR, 1999) and 25% of overall (natural and anthropogenic) mercury emissions to the atmosphere (U.S. EPA, 1997). Based on the most recent inventory by the California Air Resources Board (CARB, 2000), annual statewide industrial emissions of mercury in 1998 were 9,714 lbs, and ambient air levels were 1.6 ng/m^3.

Once in the environment, interconversion between the different forms of mercury can occur. Conversion of inorganic mercury to methylmercury occurs primarily in microorganisms especially in aquatic systems. In the methylated form, mercury bioaccumulates up the food chain and can reach very high concentrations in some fish, many of which are consumed by humans.

The majority of exposure to mercury is dietary. The 50^{th} percentile of mercury ingestion for the U.S. population is 1.4 µg/day (NRC, 2000) while exposures via inhalation are on the order of 30 to 40 ng/day (1.6 ng/m^3 X 10 to 20 m^3/day), based on the average ambient concentration. The ratio of the average statewide ambient concentration to OEHHA's chronic Reference Exposure Level is 0.02. Exposures near sources of emissions may be higher.

IV. Potential for Differential Effects

There is a considerable body of evidence from human poisoning episodes that exposure *in utero* and postnatally results in developmental neurotoxicity. Thus, infants and children are susceptible subpopulations for adverse health effects from mercury exposure. These effects fall into several general categories: 1) effects on neurological status; 2) age at which developmental milestones are achieved; 3) infant and preschool development; 4) childhood development (age 6 and above); and 5) sensory or neurophysiological effects (U.S. EPA, 2000).

Whereas methylmercury and elemental mercury readily cross the blood-brain barrier and the placental barrier, the mercuric ion (Hg^{2+}) does not readily cross these barriers. However, in fetuses and neonates mercuric species concentrate more in the brain because the blood-brain barrier is incompletely formed. Methylmercury and elemental mercury are lipophilic and are distributed throughout the body. In adults mercuric species accumulate more in the kidney. However, in neonates mercuric species do not concentrate in the kidneys but are more widely distributed to other tissues (National Research Council, NRC, 2000). It is possible that the increased distribution of mercuric species to the brain in fetuses and neonates accounts for some of the sensitivity of the brain to mercury during these developmental periods. The sensitivity of the fetal brain might also be due to the high proportion of dividing cells during neuronal development in the fetal and neonatal periods. These dividing cells may be more sensitive to damaging effects of mercury-protein complexes.

In addition to prenatal and postnatal dietary exposure, neonates may receive added postnatal dietary exposure to mercuric species and methylmercury from breast milk (Drexler and Schaller, 1998; Sundberg *et al*., 1999). School children can be accidentally exposed to elemental mercury which is a

curiosity and an attractive nuisance (George *et al*., 1996; Lowry *et al*., 1999). Younger children may also be exposed when elemental mercury is spilled on floors and carpets where they are more active.

A. Summary of Key Human Studies

The effects of low-dose *in utero* exposure to mercury on neurological development in school-age children were studied by Grandjean *et al*. (1997) in 917 mother-infant pairs in the Faroe Islands. Neurobehavioral tests were administered at birth and at 1 and 7 years to follow the effects of pre- and postnatal exposure to dietary methylmercury. Families in the Faroe Islands consume a diet high in protein from fish and marine mammals (whales). Mercury in maternal hair, children's hair, and cord blood was measured. Levels of mercury in the mothers' hair and in cord blood at birth were significant predictors of neuropsychological dysfunction in the children at seven years of age. Cord blood mercury was a significant predictor of dysfunctions in several tests intended to measure domain-specific neuropsychological effects: finger tapping, preferred hand; continuous performance test; mean reaction time, WISC-R digit span; Boston Naming Test (with and without cues); and California Learning Test (short-term and long-term reproduction). Maternal hair mercury was also a predictor of deficits in several tests, but most test scores were more strongly associated with mercury levels in cord-blood. These effects were seen at mercury levels at which there were no signs of toxicity in the mothers.

In the 1950s and 60s neurological disease was noted in many people living around Minamata Bay in Japan. People of all ages were affected, but effects were most severe in infants and children. The disease was traced to methylmercury pollution in the bay that accumulated to high levels in fish (10-40 ppm). Families were chronically exposed to methylmercury because fish were a major source of dietary protein. The neurological effects in children were often not recognized until they were several months old. Children with severe clinical neurological problems were born to mothers without clinical symptoms or with less severe symptoms. The clinical effects in prenatally exposed children included microcephaly, cerebral palsy, seizures, and mental retardation (Harada, 1995). Other neurological effects of mercury poisoning in this population included paresthesia, ataxia, visual defects, dysarthria, hearing effects and death. The collection of observed effects in children and adults was called Minamata disease. Takeuchi T. (1968) described three progressive stages of neuropathology for Minamata disease for fetal, infant, and adult exposures that demonstrate differential susceptibilities. The fetal pathologies were most severe showing disorganized cell layers and misoriented cells throughout the brain. Adult pathologies were least severe and more localized. Rogan (1995) noted that some children exposed to methylmercury in Minamata suffered profound effects while there were no symptoms in their mothers. All these studies suggest that children are more sensitive to the neurodevelopmental effects of mercury than are adults.

There is also some concern that subtle neurobehavioral effects may not manifest themselves until later in life following mercury exposures. Harada (1995) divided patients with non-acute Minamata disease into three types based on the time course of the appearance of mercury poisoning symptoms: gradually progressive type, escalating progressive type and delayed onset type. An example of delayed onset includes cases of constriction of the visual field (a common symptom of Minamata disease) which started two to three years after cessation of fish consumption.

In older adults the majority of mercury in the brain is mercuric ion. It is not clear whether the mercuric ion or methylmercury is the proximate toxicant responsible for toxic effects in the brain. Adult autopsies of poisoning patients and those with Minamata disease showed an increase in mercuric ion and focal lesions in the cerebrum and cerebellum which are responsible for coordination, balance and sensations (Clarkson, 1997; Davis *et al*., 1994). Prenatal exposures produced damage throughout the brain in children with clinical symptoms very much like cerebral palsy (Harada, 1995).

Similar clinical effects were observed following mercury poisoning incidents in Iraq in 1971 and 1972 (Marsh *et al*., 1981). In Iraq, families were acutely exposed to high levels of mercury when they ate bread made from methylmercury-treated seed grain. These exposures occurred over a two to three month period and children were exposed *in utero*. Marsh *et al*. (1981; 1987) identified 84 mother infant pairs exposed to methylmercury during pregnancy. Mercury levels were determined in sucessive 1 cm segments of maternal hair and peak mercury levels were correlated with the results of neurological examinations of the children and surveys of maternal symptoms. Clinical symptoms of Minamata disease were documented; parasthesis in exposed adults and neurological deficits in children. Neurological effects in children included altered muscle tone, increased deep tendon reflexes, delays in developmental milestones (i.e., walking or talking), and seizures. Mild effects were seen in children whose mothers' peak hair mercury was 68-180 ppm while the most severe effects were seen when peak hair mercury levels were 165-320 ppm.

McKeown-Eyssen *et al*. (1983) found that abnormalities of muscle tone (increased and decreased) and decreased reflexes were significantly ($p=0.05$) associated with an index of prenatal mercury exposure in boys but not girls in a study in Quebec. There was no consistent dose-response relationship in this study.

Effects in very young children (less than 5 years) are difficult to measure reproducibly and reported effects of mercury are subject to differences in age at examination, the test used, scoring criteria and other problems. In a prospective study Kjellstrom *et al*. (1986) examined a cohort of New Zealand children for whom prenatal exposure to methylmercury was estimated based on maternal hair samples. The children of 73 women whose hair mercury levels exceeded 6 ppm (high-mercury group) were matched with reference children on the basis of maternal ethnicity and age, hospital of birth and child age. In follow-up evaluations at four years of age, 52% of the high-mercury group had abnormal or questionable scores on the Denver Developmental Screening Test (DDST) compared with 17% of the control group ($p<0.05$). However, the DDST has been criticized for being insensitive to variations within the range of normal performance and therefore not particularly useful for neurobehavioral toxicology studies (Dietrich and Bellinger, 1994).

Kjellstrom *et al*. (1989) followed a cohort of 237 children up to six years of age in New Zealand. Children were placed in a high-mercury group based on mercury in maternal hair. Multiple controls were matched to each high-mercury child and 26 psychological and scholastic tests were administered to test general intelligence, language development, fine and gross motor coordination, academic attainment, and social adjustment. In the initial analysis a significant association was found between

maternal hair mercury level and domain tests for: full-scale IQ; language development; visual-spatial skills; and gross motor skills.

Davidson *et al*. (1998) tested 711 children in the Seychelles Islands at 66 months of age using a battery of standardized neurodevelopmental tests. Mothers of these children consumed about 12 fish meals per week. Hair mercury was measured in mothers and in test children. The tests used in this study provided more global scores of neurodevelopment that integrate performance over multiple separate neuropsychological domains. Some of the tests in this study overlap those in the Faroe and New Zealand studies but were given at a slightly earlier age, 5.5 years. No adverse effects of prenatal or postnatal mercury exposure were found for the six primary domain endpoints: cognitive ability, expressive and receptive language, letter and word recognition, reading and arithmatic achievement, visual and spatial ability, and social and adaptive behavior. The only significant association ($p = 0.05$) was an apparent enhanced performance on four of these six measures among children with increased exposure to methylmercury. The battery of tests used in this study have been criticized as being less sensitive to the subtle domain specific changes found in the Faroe study. Also children in this study were tested at 5.5 years of age which is a period of rapid developmental change when test assessments are less sensitive due to individual differences in the rate of cognitive maturation (NRC, 2000).

In studies of 6-7-year-old children in a fishing village in Madeira, Murata *et al*. (1999) found that the brainstem auditory evoked potentials increased by 0.058 ± 0.048 ms ($p=0.23$) and 0.128 ± 0.058 ms ($p=0.03$) at 20 and 40 Hz, respectively, when maternal hair mercury concentrations exceeded 10 µg/g. At these maternal hair mercury levels, visual evoked potentials increased by 3.16 ± 1.57 ms ($p=0.05$) and 0.62 ± 1.55 ms ($p=0.69$) at 15 and 30 minutes of arc, respectively. Although the overall effect of these changes may be small they are consistent with mercury effects on sensory and neurological function.

B. Summary of Key Animal Studies

Animal studies support the human study observations that damage to the central nervous system and kidneys are the primary toxic effects of mercury following sub-acute and chronic exposures.

Pregnant Sprague-Dawley rats were exposed on gestation days 11-14 and 17-20 to elemental mercury vapors (1.8 mg/m^3) for one or three hours per day (Danielsson *et al*., 1993). There was no difference among treatment groups for maternal weight gain and no obvious mercury toxicity in the dams. Offspring exposed *in utero* did not differ from controls by several measures including body weight, clinical signs, pinna unfolding, righting reflex (measured daily from day 2 postpartum) and negative geotaxis (at days 7, 8 and 9). However, at 3 months of age exposed males but not females showed significant decrements in four measures of spontaneous motor activity measured on three consecutive days: locomotion, rearing, rearing time and total activity measured by the interuption of infrared light beams in two grids at different levels in an activity chamber. By 14 months, the high dose animals showed hyperactivity in these same tests. At 4 months, treated males had significantly higher latency in a maze-learning test while at 15 months there was no difference between treated and control animals in a circular swim maze test. A significant difference was seen between treated and control males at 7

months in a test of habituation to novel environments. Habituation was measured as the ratio of spontaneous motor activity during the second 30 min in a test chamber to that in the first 30 min.

In a study designed to simulate the natural course of human fetal-type Minamata disease, rat fetuses were conceived in female Wistar rats pre-exposed to low oral doses of methylmercury chloride (1, 2 or 3 mg/kg/day) for 5 or 12 days prior to mating and during gestation. The effects of mercury on the fetal brain were determined by histological examination of the brains at one time point, embryonic day 22, from at least 20 fetuses from three maternal rats of each treatment group and eight individuals from two controls. The fetuses showed varying degrees of neuronal degeneration in the brain stem, cingulate cortex, thalamus and hypothalamus (Kakita *et al*., 2000). This pattern of damage was different from that seen in rats treated with methylmercury chloride in the postnatal or adult stages. In rats treated for 10 days from postnatal day 15, widespread degeneration was found in the cerebral cortex, striatum and red nucleus, while in adults treated starting on postnatal day 60, severe lesions were seen in the cerebellum and dorsal root ganglia.

In humans rapid brain growth occurs primarily during the third trimester, whereas in rats it occurs after parturition. To model the effects of methylmercury exposure during this time of rapid neuronal development, Sakamoto *et al*. (1998) orally exposed six neonatal Wistar rats to methylmercury chloride (5 mg/kg/day) starting with postnatal day 1. Body weight was monitored daily and weight loss was observed in the treated group starting on day 26. Neuropathological effects were examined in two rats, which showed severe nervous symptoms on days 32 and 34. Histologically, widespread neuronal degeneration was observed on days 32 and 34 within the cerebral neocortex, neostriatum, red nucleus, brainstem, cerebellum and spinal sensory ganglia, compared to four control rats sacrificed on day 31. Although limited in size, this study suggests that the developing nervous system is sensitive to methylmercury.

V. Additional Information

A. Regulatory Background

Neurotoxicity is the most sensitive effect of mercury exposure. In California, the acute reference exposure level for mercury is 1.8 $\mu g/m^3$, and the chronic Reference Exposure Level is 0.09 $\mu g/m^3$ (0.01 ppb) for the critical effects: hand tremor, memory disturbances, neurobehavioral and autonomic dysfunction (OEHHA, 1999; 2000). The Environmental Protection Agency (U.S. EPA, 1997; 2000) has calculated a reference dose of 0.1 µg/kg-day. This RfD was based on the combined incidence of neurological effects (i.e., age at walking and score on a neurological examination) in children exposed *in utero* to methylmercury in the maternal diet reported by Marsh *et al*. (1987) for 81 mother-infant pairs. The RfD was derived from a benchmark dose limit on the 95% lower confidence limit on the dose at a 10% risk level using a Weibull model. This level was calculated as 11 ppm in maternal hair and 44 µg/L in maternal blood. A composite uncertainty factor of ten was applied to derive an RfD of 0.1 µg/kg-day.

VI. Conclusions

Mercury is a neurotoxic substance with substantial human evidence indicating infants and children are susceptible subpopulations for this effect. However, exposures via inhalation are relatively small in California. Thus, mercury was placed in Tier 2. If evidence becomes available that localized exposures may be significant, OEHHA may revisit listing mercury under SB 25.

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Methyl Bromide

H_3C-Br

74-83-9

I. Physical and Chemical Properties

Description	Colorless gas
Molecular formula	CH_3Br
Molecular weight	94.95
Air concentration conversion	1 ppm = 3.89 mg/m^3 @ 25°C

II. Overview

Some evidence of teratogenicity and reduced birth weight has been observed after methyl bromide exposure in rabbits and rats. Developmental toxicity is a key toxicological endpoint of concern for infants and children. Damage sustained following prenatal exposure poses health concerns postnatally, and, thus should be considered in evaluating impacts on infants and children. Neurotoxicity due to methyl bromide (MeBr) exposure has been reported in occupational settings, accidental exposures, and in animal studies. Neurotoxicity is also an endpoint of concern for infants and children, primarily due to the prolonged period of development of the nervous system (e.g., through adolescence). However, exposures to MeBr from non-agricultural stationary sources are not widespread. Therefore, MeBr was proposed for listing in Tier 2 in the initial prioritization of TACs (see Table 1 of the Introduction).

III. Principal Sources of Exposure

Current uses of MeBr include the fumigation of homes and other structures for termites and other pests. Methyl bromide is also used to fumigate soil before planting, and fruits and vegetables after harvest. In 1981, 6.3 million pounds of MeBr were reported to have been used in California (Alexeeff and Kilgore, 1983). In 1991, its use had grown to 18.7 million pounds in the state (Cal/EPA, 1993). It should be noted that only stationary source emissions of methyl bromide can be regulated by the Air Resources Board and the local air districts. No data were available to characterize the quantity of methyl bromide emissions from such sources.

IV. Potential for Differential Effects

A. Summary of Key Human Studies

Methyl bromide is neurotoxic in both humans and animals. Neurotoxicity is one of the key toxicological endpoints for infants and children. Human studies reporting neurotoxicity are described in the following paragraphs.

During a two-week manufacturing operation, 90 persons were exposed to concentrations of MeBr generally less than 35 ppm (136 mg/m^3) (Watrous, 1942). Symptoms of toxicity developed sometime during the workshift, for example, following a few hours of exposure. In others, the symptoms were delayed and did not develop until several hours following the shift. The symptoms occurred in 33 of the 90 workers and were described as mild systemic symptoms primarily of anorexia, nausea and headache. Anorexia (reported by 25 of the 90 workers) was a common symptom and in some cases lasted for a week or more post-exposure, but without marked weight-loss. In some cases, the symptoms progressed to vomiting. Headache was a fairly common symptom (16 of 90) which disappeared when exposure ceased. While exposure was measured in a crude fashion using a "Frigidaire Leak Detector" (measures halides by color of flame), extensive monitoring was conducted throughout the manufacturing operation. In general, concentrations were at or below the limit of detection of 35 ppm.

Workers (n = 32) exposed to MeBr during fumigation of soil or structures were compared to a referent group of 29 workers not exposed to MeBr, but exposed to other fumigants (Anger *et al.*, 1986). Exposures to MeBr were not quantified. It was found that workers exposed to MeBr had a higher rate of neurological symptoms and performed less well on several behavioral tests. Several confounding factors were present in this study, including lack of adjustments for age, alcohol consumption, prescription medication, illegal drugs, education, or ethnic group between the exposed and the referent groups.

Nine Dutch greenhouse workers were exposed to methyl bromide from an adjacent fumigated area via poor seals around a door and open pipes (Hustinx *et al*., 1993). Methyl bromide used (200 g/m^3) was 5 times the legal amount. Some workers had been previously exposed to methyl bromide and had experienced symptoms (nausea, vomiting, dizziness, and poor memory). On the first day of fumigation, the methyl bromide level was 25 ppm in the nonfumigated side. On the second day when the workers were poisoned, the methyl bromide levels ranged from 150 to 200 ppm. All, except one worker, experienced extreme nausea, repeated vomiting, and dizziness. The other one felt only a burning sensation in the throat. Two workers later developed seizures. Others complained of headache, nausea, ataxia, slurred speech, and a sensation described as "floating." The serum bromide levels ranged from 51 to 363 mg/L. Most of the workers serum bromide levels (5-119 mg/L) remained elevated 19 days after exposure. The severity of the symptoms did not correlate with the bromide levels, but was associated with known previous exposures to methyl bromide.

A study by Garnier *et al*. (1996) found that two workers similarly exposed to high concentrations of MeBr (about 17,000 mg/m^3) exhibited substantially different symptoms. Glutathione-S-transferase (GST) activity was measured in the erythrocytes of both patients. GST activity was apparent in the patient with severe poisoning who was, therefore, considered a conjugator. The second patient who exhibited only mild symptoms lacked measurable GST activity in the erythrocytes and was therefore classed as a nonconjugator. Conjugators appear to be homozygous or heterozygous bearers of the gene for GST, which is not restircted to the erythrocytes. As cited by Garnier *et al*. (1996), the gene is lacking in 20.4 % of whites, 21.8% of African-Americans, 64.6% of Chinese-Americans, 60.2% of Korean-Americans, and 9.7% of Mexican-Americans. Thus, conjugation of MeBr with glutathione may be a toxifying step for neurotoxicity and the ability to conjugate may reflect susceptibility to neurotoxicity. However, conjugation apparently protects against the cytogenetic effects of MeBr (Hallier *et al*., 1993). These latter investigators note that about one-quarter of the human population does not possess GST activity in erythrocytes, and that this enzyme is not found in erythrocytes of laboratory animals (rats and mice). For this reason, studies in laboratory rodents may underestimate the neurotoxicity of MeBr in humans.

B. Summary of Key Animal Studies

A number of studies have demonstrated that methyl bromide is neurotoxic in animals. Although studies that evaluated differential effects in immature and mature animals are not available, neurotoxicants are a general concern because the nervous system develops over a prolonged period of time into adolescence. Neurotoxic effects observed in several studies are summarized below.

Kato *et al*. (1986) observed focal lesions in the brain in rats (10-12 per group) after inhalation of 150 ppm (585 mg/m^3) MeBr 4 hours/day, 5 days/week for 11 weeks. In another experiment, rats were exposed to 0, 200, 300, or 400 ppm (0, 777, 1160, or 1550 mg/m^3) MeBr 4 hours/day, 5 days/week for 6 weeks. Exposures of 300 ppm or greater resulted in neurological dysfunction, including ataxia and paralysis.

Anger *et al*. (1981) determined that rabbits are more sensitive than rats to MeBr-induced neurotoxicity. In this study, rats or rabbits were exposed to 0 or 65 ppm (0 or 254 mg/m^3) MeBr for 7.5 hours/day, 4 days/week, for 4 weeks. Nerve conduction velocity and eyeblink reflex were impaired in rabbits but not rats exposed to 65 ppm MeBr. Similarly, rats did not exhibit neurological signs after exposure to 55 ppm (215 mg/m^3) MeBr for 36 weeks. Rabbits exposed to 26.6 ppm (104 mg/m^3) 7.5 hr/day, 25 total hours per week for 30 weeks did not display any neurological effects (Russo *et al.*, 1984).

The National Toxicology Program (NTP) conducted 14-day, 6-week, 13-week and 103 week wxposures to study the toxicology and carcinogenesis of MeBr in rats and mice (NTP, 1992). Neurotoxic effects were noted in all the studies. In the 14-day study, groups of fove B6C3F1 mice of each sex were exposed via inhalation to 0, 12, 25, 50, 100, or 200 ppm MeBr 6 hours/day, 5 days/week for 2 weeks. All MeBr exposed groups exhibited signs of neurotoxicity including trembling, jumpiness, and paralysis, which were more pronounced and severe in the animals exposed to 50 ppm and higher. In the 6-week study, rats and mice (5 animals/sex/group) were exposed to 0 or 160 ppm (0

or 624 mg/m^3). Animals in the 160 ppm dose group showed high mortality rates, loss in body weight and histological changes in multiple organ systems including brain, kidney, nasal cavity, heart, adrenal gland, liver, and testes (NTP, 1992). Brain lesions included necrosis in the thalamus, hippocampus, and cerebellum.

In the 13-week study, 18 rats/sex/group were exposed to 0, 30, 60, or 120 ppm (0, 117, 233, or 466 mg/m^3) MeBr for 6 hours/day, 5 days/week. The mice were exposed to 0, 10, 20, 40, 80, or 120 ppm (0, 39, 78, 155, 311, or 466 mg/m^3) 6 hours/day, 5 days/week. Pseudocholinesterase activity and neurobehavioral tests were conducted in the mice. Serious effects, including 58% body weight loss, 17% mortality and severe curling and crossing of the hindlimbs were observed in mice exposed to 120 ppm MeBr. There were no changes in pseudocholinesterase activity.

A carcinogenesis bioassay was also conducted by NTP. Mice (86 animals/group) were exposed to 0, 10, 33, or 100 ppm (0, 38.8, 128, or 388 mg/m^3) MeBr for 6 hours/day, 5 days/week, for 103 weeks (NTP, 1992). In this study, high mortality rates in both males and females in the 100 ppm group resulted in a discontinuation of exposure after 20 weeks. A low incidence of sternal dysplasia and a significant decrease in locomotor activity at some time points (but not other later time points) were noted in the 10 ppm group. No differences were consistently observed in neurobehavioral tests between controls and the 10 or 33 ppm dose groups. Statistically significant quantitative behavioral changes were noted in the high dose males at 3 months. The animals had a heightened response to sound compared to controls and were less active than controls. Hindlimb grip was impaired in the high-dose male mice, and they showed a longer latency than controls in the hot plate test. After 6 months, female mice in the 100 ppm group were significantly less active than controls but their previous heightened response to startle had disappeared. At 9 months, there were no differences between treatment group females, but when tested at 24 months, lower activity and heightened startle response were again apparent in the 100 ppm females. Treatment-related cerebellar and cerebral degeneration was observed in the high-dose males and females. The authors note that degenerative changes in the cerebellum and cerebrum were noted more often in animals that died early possibly indicating an association between these toxic effects and mortality.

Neurotoxicity was also noted in other studies of short-term exposures. A 5-day exposure of rats (10 animals/group) to 0, 90, 175, 250, or 325 ppm (0, 350, 680, 971, or 1260 mg/m^3) resulted in lesions in the nasal olfactory sensory cells, and the cerebellum beginning at 175 ppm (Hurtt *et al.*, 1987). Hurtt and Working (1988) later observed severe histological damage to the nasal epithelium following a single exposure to 90 or 200 ppm (351 or 780 mg/m^3) MeBr. Olfactory function, measured by the ability to locate buried food, was impaired at the 200 ppm exposure.

Sikov *et al.* (1981) in evaluating the teratogenic potential of MeBr in rats and rabbits exposed to 0, 20, or 70 ppm (0, 78, or 272 mg/m^3) 7 hours/day, 5 days/week for 3 weeks during days 1-19 (rats) or 1-24 (rabbits) of gestation, noted no maternal or fetal effects in the rats; however, severe maternal neurotoxic effects were observed in the rabbits that resulted in 24/25 deaths. In this study, no significant maternal or fetal effects were observed at a concentration of 20 ppm.

Methyl bromide exposure is associated with developmental toxicity in some animal studies. Developmental toxicity is a key toxicological endpoint for infants and children. As discussed in the Introduction to the report, fetal damage sustained as a result of exposure to toxicants is a source of adverse health impacts postnatally, and therefore falls within the scope of this report.

MeBr (99.9% pure) was administered to Sprague Dawley rats of both sexes by whole-body inhalation 6 hours per day and 5 days per week at the nominal concentrations of 0, 3, 30, or 90 ppm (American Biogenics Corp., 1986; Hardisty, 1992; Busey, 1993). Parental animals were exposed for about 40 or 55 days and 90 to 105 days before their first and second matings, respectively, and were exposed for a total of 132-145 days before they were sacrificed. There was no exposure to MeBr between gestation day 21 and lactation day 4 in any of the four birthing periods. The pups were not directly exposed to MeBr until after weaning (postnatal day 28). Body weights (91-95% of control values) and body weight gain (76% of control values) during the pre-mating periods were significantly decreased only in the F_0 males of the 90 ppm group. For the 30 and 90 ppm F_1 groups, the body weights at pre-mating and during reproduction were consistently lower than those of controls. The absolute brain (wet) weights of the F_0 males, F_1 males, and F_1 females in the 90 ppm groups were significantly ($p > 0.05$) decreased by 5%, 6%, and 6%, respectively, compared with controls. The brain weight of the F_1 females of the 30 ppm group was also reduced by 5% ($p > 0.05$). These findings are interesting in light of the known neurotoxic effects observed in mature animals.

At birth, the pup body weights of the treated groups were either higher or not significantly different from controls; the only exception was the lowered body weight of the F_{2a} 90 ppm group. During lactation, the F_{1a} and F_{1b} pups of the 30 and 90 ppm groups showed significantly reduced body weights on lactation days 14 to 28. The F_{2a} 90 ppm pup body weights were lower at birth than the controls and remained reduced throughout lactation. The F_{2a} 30 ppm pup body weights showed significant reduction on lactation days 14 to 28. The F_{2b} 30 and 90 ppm pup body weights were decreased, starting as early as 4 days after birth. The reduction in body weight was greater in the F_{2a} and F_{2b} progeny (reduction of 9 to 21 % at 90 ppm) compared respectively to the F_{1a} and F_{1b} pups (reduction of 5 to 11 % at 90 ppm). Since the pups were not exposed to MeBr during the lactation period, except perhaps via the maternal milk, the finding of reduced body weights suggested that growth retardation might be an effect due to the 14 to 15 days of *in utero* exposure.

For the female F_{2b} progeny from the 90 ppm group, the absolute weights of the brain, heart, kidneys, and liver were reduced significantly ($p \leq 0.05$) by 7%, 15%, 18%, and 23% when compared to control values, respectively. The absolute weights of the kidneys, liver, and testes of the corresponding male progeny were also reduced, though to a lesser degree. The organ to body weight ratios were generally not significantly different from control values.

Histology data showed a decrease in the width of the cerebral cortex of the F_1 90 ppm groups (both sexes), which can be construed as developmental neurotoxicity. The reduced brain (fixed) weights for the F_1 30 ppm females suggested that the LOAEL for the reduced cerebral-cortex width was 30 ppm. Quantitative histological parameters were not affected in the F_0 90 ppm adults which suggested that the effects in the F_1 animals were the result of *in utero* and/or lactational exposure.

The parental NOAEL was 3 ppm based on reduced fertility. The progeny NOEL was 3 ppm based on the decreased pup body weights and organ weights, reduced F_1 brain weight and reduced cerebral cortex width at 30 ppm.

Exposure of pregnant New Zealand white rabbits to MeBr at 80 ppm from gestation days 7 to 19 resulted in maternal neurotoxicity and decreased body weight (Breslin *et al*., 1990). Developmental effects observed in the fetuses of the 80 ppm group included omphalocele, retro-esophageal right subclavian artery, gall bladder agenesis, fused sternebrae, and decreased fetal body weight. No effects on the fetuses and does were observed at 40 ppm (155 mg/m^3). The gall bladder agenesis was considered a significant finding due to the very low incidence in historical controls. Thirteen fetuses from 5 does had no gall bladder. Three of the does did not exhibit symptoms of toxicity; the gall bladder agenesis thus occurred at doses lower than those necessary for significant maternal toxicity.

In summary, MeBr has been demonstrated to induce neurotoxicity in adult animals; observed effects include brain and olfactory sensory cell histological lesions, motor and sensory dysfunction. Developmental toxicity endpoints noted include decreased pup body and organ weights and organ and skeletal abnormalities, including reduced cerebral cortex widths. This last effect combined with the neurotoxicity data from adult animals suggests that fetal or neonatal animals may be at increased risk of developmental neurotoxicity from MeBr exposure compared to adult animals.

V. Additional Information

A. Genotoxicity and Carcinogenicity

MeBr is genotoxic in *in vitro* and *in vivo* mutation assays (reviewed by IARC, 1999). It is a direct-acting mutagen in Salmonella mutation assays and the mouse lymphoma L5178Y assay, causes increased micronuclei formation in mouse bone marrow and peripheral blood cells and increased frequencies of sister chromatid exchange (SCE) in CHO cells and in mouse bone marrow cells *in vivo*. DNA alkylation was detected in both rats and mice after *in vivo* exposure by the oral, intraperitoneal, or inhalation routes, and DNA damage was found in the germ cells of rats after inhalation exposure. Occupational studies suggest that MeBr may also be genotoxic to humans. Increased lymphocyte SCE frequencies and blood protein (S-methylcysteine) adducts were observed in soil fumigators (Hallier *et al.*, 1993). Structural fumigation workers have been found to have increased incidences of micronuclei and hypoxanthine-guanine phosphoribosyl transferase (*hprt*) mutations in lymphocytes and oropharyngeal cells (Calvert *et al.*, 1998a,b).

In contrast, MeBr has not been demonstrated to be carcinogenic in animal bioassays. Tumor incidence was not increased in male and female Wistar rats exposed to MeBr by whole-body inhalation at concentrations of 0, 3, 30 or 90 ppm (0, 12, 117 or 350 mg/m^3) for 6 hours/day, five days/week for 29 months (Reuzel *et al.*, 1991). MeBr did not induce significant increases in any tumor types in the 2-year mouse inhalation exposure study described above (NTP, 1992). Gotoh et al. (1984) report no

treatment-related increases in incidence of tumors in male and female BDFI mice exposed to MeBr by whole-body inhalation at concentrations of 0, 4, 16 or 64 ppm (0, 16, 62 or 249 mg/m^3) for 6 hours/day, five days/week for 104 weeks. The incidence of pituitary gland adenomas was significantly increased in male Fischer 344/DuCrj rats (whole body MeBr inhalation exposure at 0, 4, 20 or 100 ppm (0, 16, 78 or 389 mg/m^3) for 6 hours/day, five days/week for 104 weeks) when compared to controls (16/50, 23/50, 19/50 and 30/50 in control, 4, 20 and 100 ppm groups, respectively; $p < 0.01$) (Gotoh *et al.*, 1994). However, no increase in tumor incidence was noted in female rats exposed in the same study to the same MeBr concentrations as the male rats. The IARC and US EPA classifications for MeBr are Group 3 (not classifiable as to its carcinogenicity in humans) (IARC, 1999) and Class D (not classifiable as to human carcinogenicity) (US EPA, 2001), respectively.

B. Regulatory Background

Methyl bromide is a Federal Hazardous Air Pollutant (HAP) and was identified as a Toxic Air Contaminant (TAC) in California in April 1993 under AB 2728. OEHHA has adopted an acute non-cancer Reference Exposure Level (REL) of 3900 µg/m^3 (1 ppm) and a chronic REL of 5 µg/m^3 (1 ppb) for methyl bromide (OEHHA, 1999a; OEHHA, 2001). Methyl bromide as a structural fumigant is listed under Proposition 65 as being known to cause developmental toxicity (OEHHA, 1999b).

VI. Conclusions

Methyl bromide is a neurotoxicant in both humans and animals. Neurotoxicity is a key toxicological endpoint for infants and children, primarily because of the prolonged period of development of the nervous system. Methyl bromide also induces developmental toxicity, another key toxicological endpoint for infants and children's health. Although there is concern for the toxicity of methyl bromide, exposures from stationary sources are not widespread and relatively low. Thus, OEHHA placed methyl bromide in Tier 2. Should evidence arise of significant exposures in the vicinity of stationary sources, OEHHA may revisit listing methyl bromide under SB 25.

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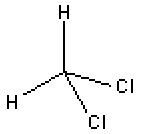
Methylene Chloride

CAS Registry Number: 75-9-2



I. Physical and Chemical Properties (HSDB, 1999)

Description	Colorless, volatile liquid
Molecular formula	CH_2Cl_2
Molecular weight	84.93
Air concentration conversion	1 ppm = 3.47 mg/m^3 @ 25^o C

II. Overview

The effects of methylene chloride have not been studied in children and there are no studies showing that exposure to methylene chloride *per se* differentially affects children. However methylene chloride is metabolized to carbon monoxide which is bound with higher affinity by fetal vs adult hemoglobin (affinity constant m = 213 vs 208; Di Cera *et al*., 1989). Thus the neurotoxic and cardiovascular effects of CO metabolically produced from methylene chloride following relatively high exposures may be exacerbated in fetuses and in infants with higher residual levels of fetal hemoglobin. Maternal exposure to high levels of CO during gestation has resulted in elevated levels of carboxyhemoglobin in the fetus and lowered birth weights in humans and animals. In animals, altered neurobehavioral adaptation has been attributed to prenatal maternal methylene chloride exposure. OEHHA's chronic Reference Exposure Level is based on significant elevation of carboxyhemoglobin (>2%; DiVincenzo and Kaplan, 1981) in humans exposed occupationally with a LOAEL of 40 ppm (14 ppm time adjusted to a 24 hour day). It includes an intraspecies uncertainty factor of 10 and a LOAEL uncertainty factor of 10 for a total uncertainty factor of 100. It should be noted that typical ambient concentrations are almost three orders of magnitude lower than our chronic REL.

Methylene chloride vapors are heavier than air (vapor density = 2.93) and tend to concentrate near the ground. Because of their shorter stature, children may be more at risk of exposure than adults during accidental spills or through the use of methylene chloride in unventilated areas. The higher ventilation rates in children compared to adults mean that children may receive a higher dose of methylene chloride than adults during inhalation exposures.

III. Principal Sources of Exposure

Methylene chloride is used in paint and varnish remover, in aerosols as a co-solvent or vapor pressure depressant, and in solvent degreasing and metal cleaning. It is used in plastics processing and in

extraction of fats and oils from food products. The removal of methylene chloride from such consumer products as hair sprays and some paint removers has reduced the opportunity for non-occupational exposure. Statewide median and maximal monitored ambient concentrations were 0.50 and 4.8 ppb in 1999. Of 16,332,000 lbs released in California in 2000, 5,350,000 lbs were in Los Angeles County (CARB, 2001). In the South Coast Air Basin, the range of median and maximal values in 1999 were 0.5-1.1 and 1.4-4.5 ppb, respectively, as reported by the California Ambient Toxics Monitoring Network (CARB, 1999).

IV. Potential for Differential Effects

A. Summary of Key Human Studies

To study the biotransformation of inhaled methylene chloride, COHb formation was measured in the blood of eleven resting non-smokers following methylene chloride exposure to 50, 100, 150 and 200 ppm methylene chloride for 7.5 hrs on 5 consecutive days. All exposure levels produced elevated COHb and CO in exhaled air. The peak blood COHb saturation was 1.9, 3.4, 5.3, and 6.8%, respectively, for the 50, 100, 150, and 200 ppm groups (DiVincenzo and Kaplan, 1981).

Low birth weights have been associated with maternal exposure to elevated CO during the last trimester. Exposure to >5.5 ppm of CO during the last trimester of pregnancy was associated with an increased risk of low birth weight (odds ratio 1.22, 95% CI 1.03-1.44) among 125,573 children born to women living in Los Angeles (Ritz and Yu, 1999). It is important to note that COHb was not determined in mothers or newborns and CO was only one of numerous air pollutants to which the mothers were exposed.

Similarly Koren *et al*. (1991) studied the effects of CO poisoning from various sources (including from exposure to methylene chloride) during gestation on pregnancy outcomes in 38 women. There was a significant association ($p<0.001$) between severe maternal CO toxicity and adverse pregnancy outcomes including cerebral palsy. While this study underscores the potential impact of CO poisoning during pregnancy, the specific outcomes of the three methylene chloride exposures were not reported.

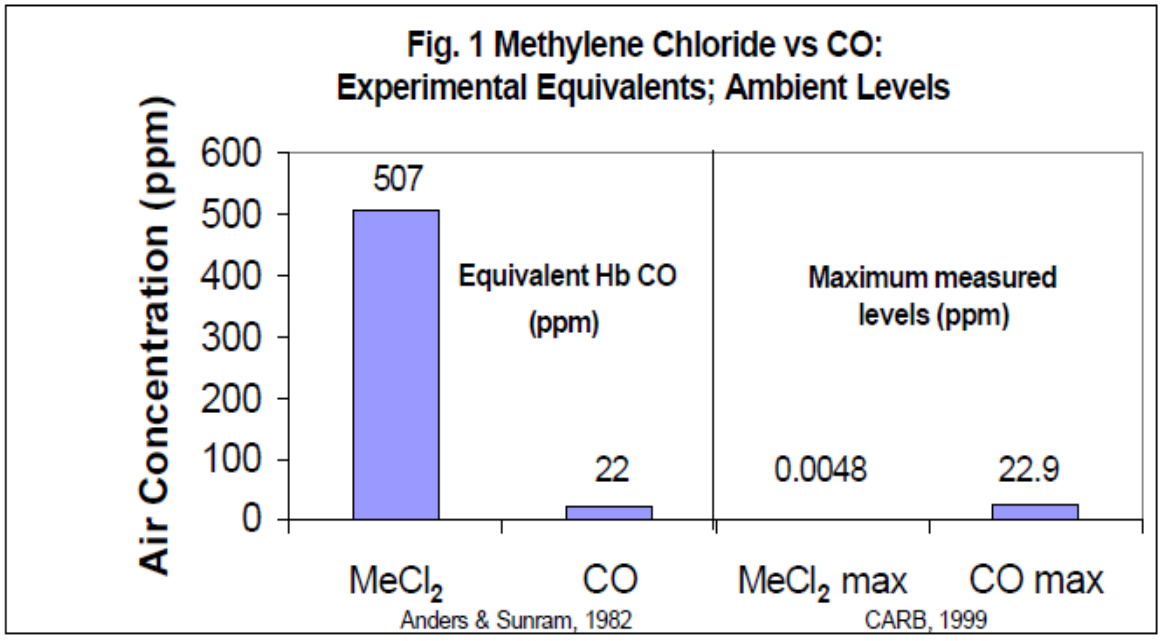
The information on potential developmental effects in humans is limited. A retrospective study of female pharmaceutical workers exposed to a variety of organic solvents indicated that solvent exposure, and particularly methylene chloride exposure, resulted in an increase in spontaneous abortions (Taskinen *et al*., 1986). In all, 1,795 pregnancies were followed with 142 spontaneous abortions occurring. The odds ratio for methylene chloride exposure was 1.0 to 5.7 (average = 2.3; $p<0.06$). There was a significant effect of exposure to four or more solvents compared with age-matched controls ($p<0.05$). The concentrations of methylene chloride were not reported in the study.

B. Summary of Key Animal Studies

No studies were identified which provided evidence of differential susceptibility from postnatal exposures of young animals to methylene chloride. However there is some suggestion that gestational exposure to very high levels may result in persistent neurobehavioral alterations in the offspring.

Bornschein *et al*. (1980) exposed rats before and/or during pregnancy to 0 and 4500 ppm methylene chloride. The exposed pups showed significantly slower rates of behavioral habituation to novel environments at 10 and 15 days of age ($p<0.01$). By 150 days of age, exposed male rats still demonstrated significantly altered activity levels compared to controls ($p<0.02$). In this exploratory study, levels of carboxyhemoglobin were not determined to distinguish the effects of CO versus the parent compound, methylene chloride.

Anders and Sunram (1982) exposed pregnant rats (gestation day 21) to methylene chloride at 507 ppm or CO at 22 ppm for 1 hour and monitored the fetal and maternal blood concentrations of methylene chloride and/or CO. Exposure to 22 ppm CO gave approximately the same fetal blood CO levels as those achieved following the 23-fold higher exposure to methylene chloride at 507 ppm (157 vs 160 nmol/ml) (Fig. 1). Whereas the level of methylene chloride in fetal blood was significantly lower than in maternal blood, the levels of fetal CO were comparable to maternal CO and thought to be due to equilibration of CO between maternal and fetal circulation.



V. Additional Information

A. Other Toxicity

Methylene chloride is rapidly absorbed through the lungs into the systemic circulation and excreted via the lungs in exhaled air. At high concentrations most of the absorbed methylene chloride is exhaled unchanged with the remainder metabolized to carbon monoxide (CO), carbon dioxide (CO_2) and inorganic chloride. The main toxic effects of methylene chloride are reversible central nervous system (CNS) depression and carboxyhemoglobin (COHb) formation. The neurotoxicity of methylene chloride

is thought to be related to its lipophilicity, which allows it to enter nerve cell membranes and interfere with signal propagation, and to the hypoxia associated with the formation of COHb. COHb formation above 2% is considered the critical effect in humans. In addition to the CNS, the liver, heart, kidneys and lungs may be adversely affected at high doses.

The health effects of methylene chloride derive from the lipophilicity of the parent compound and the toxicity of its metabolites, formaldehyde and CO. The available data suggest that there are two pathways by which methylene chloride is metabolized. One pathway utilizes cytochrome P-450 2E1 (CYP2E1) and produces CO and CO_2. The second involves glutathione transferase T1-1 (GSTT1-1) and leads via formaldehyde to CO_2 (Mainwaring *et al*., 1996). However, at low exposure levels methylene chloride is thought to be metabolized predominately by the P-450s to CO (Stewart *et al*., 1972).

B. Regulatory Background

The acute inhalation reference exposure level is 14,000 $\mu g/m^3$ and a chronic inhalation reference exposure level of 400 $\mu g/m^3$ (100 ppb) was developed (OEHHA, 2000) based on occupational exposure (DiVincenzo and Kaplan, 1981). Methylene chloride is classified as a probable human carcinogen (B2) based on sufficient evidence of carcinogenicity in animals and inadequate human data. The cancer inhalation unit risk was established at 1 $x10^{-6}$ $(\mu g/m^3)^{-1}$ by the California Department of Health Services (CDHS, 1987).

VI. Conclusions

Methylene chloride is metabolized to carbon monoxide, which has a higher affinity for fetal hemoglobin than adult hemoglobin. This is therefore a basis for differential toxicity beween infants and adults. The chronic Reference Exposure Level (cREL) OEHHA developed for methylene chloride is based on the formation of carboxyhemoglobin in adults. The ratio of the ambient air concentrations (statewide average) to the cREL is 0.005. Thus, general exposures are well-below levels that would result in significant carboxyhemoglobin formation even in the neonate. Thus, OEHHA has placed methylene chloride in Tier 2. There are, however, significant emissions statewide of methylene chloride from facilities in the Air Toxics Hot Spots program, and so local concentrations may be higher than the statewide average. Should information indicating locally significant concentrations become available, OEHHA may revisit listing methylene chloride under SB 25.

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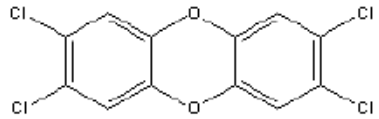
Polychlorinated Dibenzo-*p*-dioxins (PCDDs), Dibenzofurans (PCDFs) and Biphenyls (PCBs)

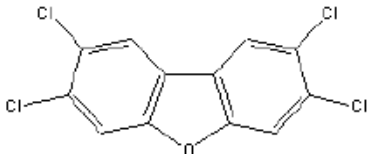
Including: all 2,3,7,8-chlorinated PCDDs and PCDFs, and PCBs

I. Selected structures:

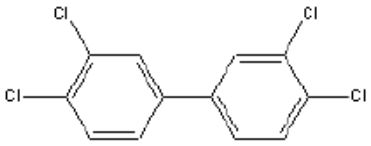
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD)



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)

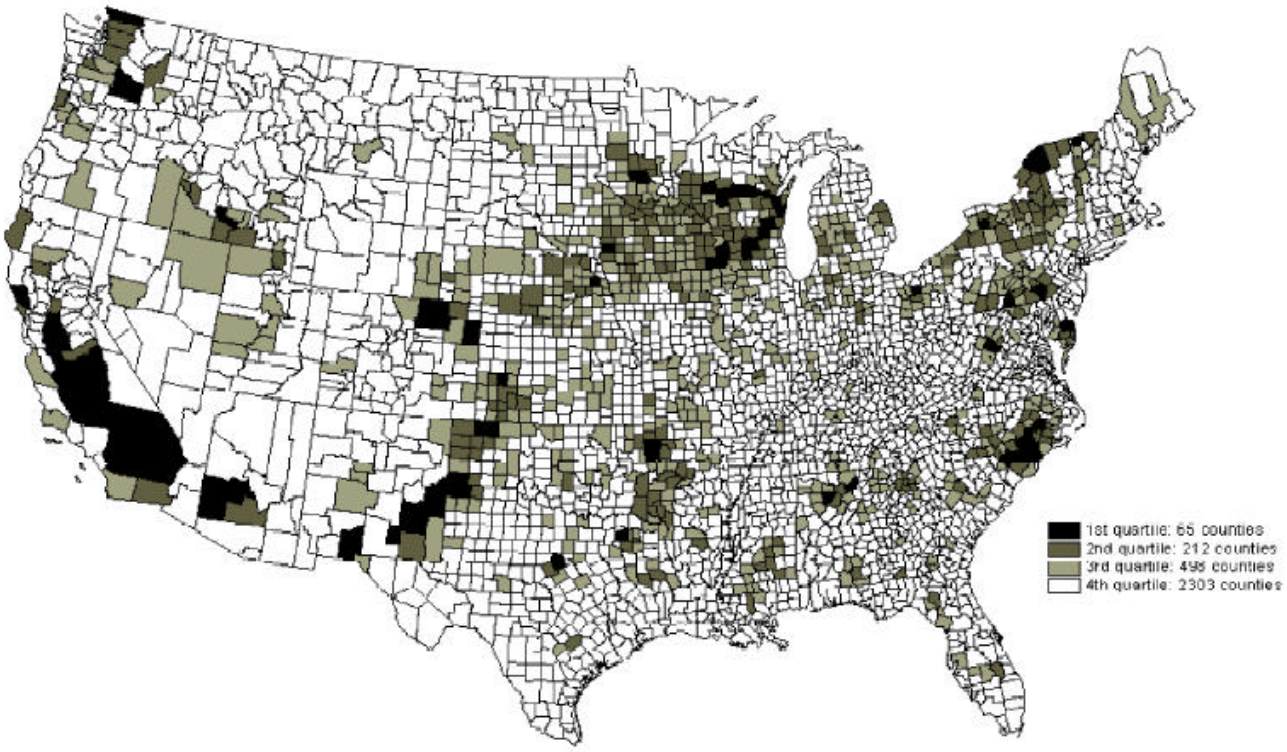
Concerns about dioxins and dioxin-like compounds are justified not only because of toxicity but also because of their very long biological and environmental persistence. These toxic air pollutants settle in grass and feed, which are then eaten and become a source of contamination for humans in livestock, 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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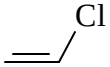
Vinyl Chloride

75-01-4



I. Physical and Chemical Properties

Description	Colorless gas or liquid
Molecular formula	CH_2=CHCl
Molecular weight	62.5
Air concentration conversion	1 ppm = 2.56 mg/m^3

II. Overview

Vinyl chloride is a known human carcinogen (reviewed by Kielhorn et al., 2000) that has been shown experimentally to be more carcinogenic in young animals than in older animals (Maltoni et al., 1981; Drew et al., 1983; Maltoni and Cotti, 1988; Cogliano et al., 1996). It is also a transplacental carcinogen in laboratory animals (Maltoni et al., 1984). It is therefore reasonable to expect that there may be a differential impact on infants and children who are exposed to this chemical.

Rodent experiments by Maltoni et al. (1981) and Drew et al. (1983) showed that animals exposed to vinyl chloride by inhalation before weaning developed more tumors and different types of tumors and with a shorter latency than animals exposed later in life. This suggests that infants and children may be more sensitive to the carcinogenic effects of vinyl chloride than are adults. For this reason vinyl chloride was considered to be a priority chemical for evaluation of potential differential effects on infants and children.

III. Principal Sources of Exposure

In 1993, the production of vinyl chloride in the United States was nearly 14 billion pounds (U.S. EPA, 1999). Vinyl chloride is used in the manufacture of numerous polyvinyl chloride products used in construction such as electrical wire and cable insulation, piping, industrial and household equipment, and medical supplies. There is heavy demand from the automobile, rubber, paper and glass industries.

Vinyl chloride has not been detected in the ambient air of California at or above a detection limit of 0.5 ppb, except for measurements taken adjacent to vinyl chloride-related industries and landfills (California Air Resources Board, 1990). For example, ambient measurements of vinyl chloride were found to range from 2 ppb to 15 ppb at 24 out of 251 landfills tested (California Air Resources Board, 1990). Ambient air exposure to vinyl chloride is expected to occur from the discharge of exhaust gasses from factories that use or manufacture the chemical, or from evaporation from areas at which chemical wastes

are stored (U.S. EPA, 1999). Under calm conditions, with a vapor density of 2.15, concentrated vinyl chloride vapor may disperse slowly and flow along the ground, accumulating in low spots (Agency for Toxic Substances and Disease Registry, 1992).

IV. Potential for Differential Effects

A. Summary of Key Animal Studies

a) Mutagenicity

Vinyl chloride is mutagenic in most major short-term tests. Its activity is enhanced in the presence of exogenous or endogenous metabolic activation, suggesting that a metabolite may be more mutagenic than the vinyl chloride molecule itself (California Department of Health Services, 1990).

Following exposure via inhalation to 600 ppm vinyl chloride (4 hours per day for 5 days) 10-day-old rat pups yielded almost four times the amount of DNA adducts 7-(2'oxoethyl) guanine (OEG) and 3-ethenoguanine (EG) as did lactating adult rats (see Table 1, below) (Swenberg et al., 1992). The authors suggested that the increased relative levels of 3-ethenoguanine DNA adduct, which is a highly efficient mutagen causing G→A transitions, leads to a greater susceptibility of newborn rats compared with adult rats to vinyl chloride induced carcinogenesis.

Table 1: 7-(2'oxoethyl) guanine (OEG) and 3-ethenoguanine (EG) concentrations in rat tissue DNA measured immediately after exposure to 600 ppm vinyl chloride (based on Swenberg et al., 1992).

Tissue	OEG (pmol/μmol guanine)	EG (pmol/μmol guanine)
Pups (10 day old)		
Liver	162 ± 36	1.81 ± 0.25
Lung	20 ± 7	0.21 ± 0.08
Kidney	29 ± 1	0.31 ± 0.02
Adults (lactating females)		
Liver	43 ± 7	0.47 ± 0.14
Lung	20 ± 5	0.27 ± 0.03
Kidney	Not analyzed	< 0.12

b) Carcinogenicity

Maltoni et al. (1981) reported the results of one of the most extensive bioassays ever performed by a single institution on a single compound. This study involved nearly 7,000 animals. The design of the study was to test the effects of a wide range of variables on the carcinogenicity of vinyl chloride in

rodents. The variables investigated included species (rats, mice and hamsters), strains, age and sex, routes of exposure (inhalation, ingestion, injection), doses (1 ppm to 30,000 ppm by inhalation), and schedules of treatment (early in life exposure versus later in life exposure).

Several of the experiments within the Maltoni study provide data that can address the question of the effect of age at exposure. Cogliano et al. (1996) evaluated the Maltoni data to characterize quantitatively the effects of age at exposure. For example the results for Sprague-Dawley rats exposed by inhalation to concentrations of vinyl chloride in air ranging from 0 to 12,000 ppm showed that rats exposed for five weeks beginning at 1 day old had almost a 50% incidence of angiosarcoma, whereas rats exposed for five weeks beginning at 13 weeks of age exhibited an incidence of less than 10% (see Figure 2 in Cogliano et al., 1996). Even rats exposed for 25 weeks beginning at 13 weeks of age showed an incidence of angiosarcoma of less than 10%. Sprague-Dawley rats exposed for 52 weeks beginning at 13 weeks of age had a lower incidence of angiosarcoma than rats exposed for 5 weeks beginning at 1 day of age (see Table 2 below and Figure 3 in Cogliano et al., 1996). Exposures to newborns produced hepatomas, a tumor not seen in rats exposed for 52 weeks starting at 13 weeks of age (Maltoni et al., 1981). In later experiments, Maltoni et al. (1984, 1988) demonstrated that rat fetuses and neonates had enhanced susceptibility to angiosarcomas, hepatocellular carcinomas and neuroblastomas.

Table 2: Incidence of Angiosarcomas in Rats Exposed to Vinyl Chloride by Inhalation (from Maltoni et al., 1996)

Group and concentration	Liver Angiosarcomas per 100 animals
Adults (start at 13 weeks of age)	
No treatment (control)	0 (0/60)
50 ppm	1.7 (1/60)
250 ppm	5.1 (3/59)
500 ppm	10.0 (6/60)
2500 ppm	21.7 (13/60)
6,000 ppm	22.0 (13/59)
10,000 ppm	11.7 (7/60)
Newborns	
6,000 ppm	40.5 (17/42)
10,000 ppm	34.1 (15/44)

note: Adults were exposed for weeks 14 to 65; newborns were exposed for weeks 1 to 5.
See also Cogliano et al., 1996, Figure 3.

Cogliano et al. (1996) reviewed earlier experimental studies by Drew et al. (1983) and Maltoni et al. (1981). They conclude, "A study of partial-lifetime exposures in these animal species suggests that the

lifetime risk of cancer depends on the age at exposure, with higher lifetime risks attributable to exposures at younger ages. Studies of newborn animal exposures further demonstrate that a brief exposure in newborns can, by the end of life, induce a higher incidence of tumors compared to long-term exposure occurring later in life, including tumor types not induced by exposure later in life."

Drew et al. (1983) looked at the effect of age and exposure duration on vinyl chloride oncogenicity in females of several different species of rodents. Groups of female CD-1 Swiss mice, B6C3F1 mice, Fischer 344 rats, and Golden Syrian hamsters (N = 54 for mice, N = 56 for rats and hamsters) were exposed to vinyl chloride for six hours/day, five days/week for six, 12, 18, or 24 months, beginning at eight weeks of age, and observed for their lifespans. Other groups were held until six or 12 months of age, exposed for six or 12 months, and then observed for the remainder of their lifespans. The exposures were conducted at a single dose level for each species; mice, rats and hamsters were administered 50, 100, and 200 ppm, respectively. All animals exposed to vinyl chloride at age eight weeks (the start of the experiment) exhibited decreased survival relative to controls (Drew et al., 1983). B6C3F1 mice experienced the most significant shortening of lifespan regardless of the age at which exposure was begun. No significant decrease in survival was observed in rats, hamsters, or Swiss mice initially exposed after six months of age. Other clinical signs of vinyl chloride toxicity were not evident and liver necrosis was not observed.

In rats, exposure to vinyl chloride (100 ppm) was associated with hemangiosarcomas, mammary gland adenocarcinomas and adenomas, and hepatocellular carcinomas (Drew et al., 1983). The incidence of hemangiosarcomas was a function of the duration of exposure; the longer the exposure period the greater the incidence of hemangiosarcomas. A six-month exposure produced a low incidence of hemangiosarcomas and hepatocellular carcinomas only if begun early in life. No hemangiosarcomas and only one hepatocellular carcinoma were produced when six-month exposure was started in 12 or 18-month-old animals. One-year exposures produced a significant incidence of tumors, especially if begun early in life. The incidence of mammary gland adenocarcinomas and fibroadenomas was not always related to exposure duration, but the incidence was higher in rats whose exposure began at eight weeks of age. Hepatocellular carcinomas were induced in a dose-related manner in rats when exposures began at eight weeks.

In hamsters, hemangiosarcomas, mammary gland carcinomas, stomach adenomas, and skin carcinomas were associated with exposure to 200 ppm vinyl chloride (Drew et al., 1983). The highest incidence of hemangiosarcomas and stomach adenomas occurred in animals exposed early in life for only six months. The highest incidence of mammary gland carcinomas was seen in animals exposed at an early age for up to twelve months. Exposure beginning at or after eight months of age resulted in a markedly lower tumor incidence, probably because the lifespans of chronically exposed hamsters were significantly reduced to the point that late-appearing tumors would not be expressed.

Mice appeared to be the species most sensitive to the carcinogenic effects of vinyl chloride (50 ppm) (Drew et al., 1983). Hemangiosarcomas and mammary gland carcinomas in B6C3F1 and Swiss mice, and lung carcinomas in Swiss mice only were associated with vinyl chloride exposure. In B6C3F1 mice, exposure to vinyl chloride for six months resulted in 60-70 percent incidence of

hemangiosarcomas, regardless of the age at exposure initiation. The incidence of mammary gland carcinomas in B6C3F1 mice was greatest when the animals were exposed early in life. Lower incidences of this tumor were seen when initial exposure occurred at a later age. In Swiss mice, exposure to vinyl chloride at an early age resulted in the highest incidence of hemangiosarcomas, mammary gland carcinomas, and lung carcinomas, regardless of duration of exposure. Lower incidences of all tumors were observed in animals exposed later in life.

In vinyl chloride-induced rat liver angiosarcomas, Ki-ras mutations were not observed (as they were in vinyl chloride induced angiosarcomas in humans) but 44 percent did have p53 mutations (Froment et al., 1994). The mutations in liver tumors caused by vinyl chloride are distinct from those detected in sporadic liver cancers. The data available at present suggest that etheno adducts may initiate the oncogenic process following exposure to vinyl chloride. Animal studies indicate that young animals are more sensitive than adults to the formation of these adducts (Swenberg et al., 1992).

The animal experiments of Drew et al. (1983) and Maltoni et al. (1981) clearly indicate that exposure to vinyl chloride early in life has a more potent effect than exposure later in life or exposure distributed throughout the lifetime of the animal. Applying these results to humans would suggest that exposure to infants and children might have a significantly greater carcinogenic effect than exposure to older people. At present this increased potential susceptibility of children to the carcinogenic effects of vinyl chloride has not been incorporated directly into the formula for calculating cancer risk for vinyl chloride. In determining a cancer risk value for vinyl chloride, California Department of Health Services (CDHS, 1990) acknowledged that newborn animals showed greater sensitivity to the carcinogenic effects of vinyl chloride than older animals. CDHS used this as a rationale for choosing a value for cancer unit risk (2×10^{-4} ppb^{-1} or 7.8×10^{-5} $m^3/\mu g$) that was at the top of a range of values calculated from human and animals studies (2.5×10^{-5} to 2×10^{-4} ppb^{-1}).

c) Reproductive and Developmental Toxicity

John et al. (1977) tested for effects of maternally inhaled vinyl chloride on embryonic and fetal development in rodents. Pregnant CF-1 mice, Sprague-Dawley rats and New Zealand white rabbits were exposed to 500 ppm of vinyl chloride for seven hours per day during the period of major gestational organogenesis. Other groups of mice and rabbits were exposed to vinyl chloride concentrations of 50 and 2500 ppm. Fetotoxicity occurred in mice at 500 ppm, and the effects included increased fetal resorption, decreased fetal body weight, reduced litter size, and retarded cranial and sternebral ossification. Rat offspring showed decreased body weight at 500 ppm maternal exposure and dilated ureters at maternal exposure to 2500 ppm. No sign of maternal or developmental toxicity was observed in rabbits at either concentration (John *et al.*, 1977).

B. Summary of Key Human Studies

a) Reproductive and Developmental Toxicity

Several epidemiological studies have been conducted to assess potential reproductive and developmental effects in the families of vinyl chloride workers (California Department of Health

Services, 1990). Edmonds et al. (1975, 1978) conducted two case-control studies evaluating central nervous system malformations among offspring of vinyl chloride workers and families living near polyvinyl chloride facilities in Indiana and West Virginia. More cases than controls lived within three miles of the polyvinyl chloride plants ($p<0.02$). Mothers living in Ohio communities with PVC production facilities gave birth to an excess number of children with congenital malformations as compared to the expected number based on the state average or based on the experience in the balance of the counties in which these cities are located (Infante, 1976). In a review of epidemiological studies related to vinyl chloride exposure, Hemminki and Vineis (1985) concluded that there was inadequate evidence linking environmental or paternal exposures to vinyl chloride with birth defects.

b) Carcinogenicity

Creech and Johnson (1974) described three cases of liver angiosarcoma in workers at a Kentucky rubber plant. Because liver angiosarcoma is a rare tumor (20 to 25 cases per year in the U.S.), the clustering of three cases in one facility indicated an abnormally high incidence of this cancer. Based on this report, and animal studies, multiple studies of workers exposed to vinyl chloride were conducted. By 1999 there had been over twenty epidemiological studies relating vinyl chloride to various cancers.

The association between vinyl chloride exposure and increased risk for other cancers is not as clear as that for liver cancer. Some evidence associates exposure to vinyl chloride with increased mortality ratios for brain cancer, lung cancer, and lymphoma. Since these cancers appear more commonly in the general population than liver angiosarcoma, it becomes more difficult to demonstrate increased risk due to exposure (California Department of Health Services, 1990).

There is some indication that workers exposed to vinyl chloride may be at greater risk for brain cancer. The studies that relate to this question are summarized in Table 14 of the Public Health Goal document (OEHHA, 2000a). Five studies found a statistically significant positive association between brain cancer and vinyl chloride exposure ($p<0.05$) (Byren et al., 1976; Waxweiler et al., 1976; Equitable Environmental Health, 1978; Weber et al., 1981; Cooper, 1981). Brain cancer incidence increased an average of four-fold above that expected for the general population in these five studies. Other studies found no association (OEHHA, 2000a, Table 14). Some later papers included in Table 14 re-examined the data from the earlier studies. The question of the association, if any, between vinyl chloride exposure and brain tumors as well as other non-liver cancers should be the subject of a future meta-analysis.

The evidence linking vinyl chloride exposure to lung cancer remains inconclusive. Analyses of SMRs for cancer of the lung were performed in 12 studies. Of these, seven studies showed an increased risk for lung cancer, but only one was statistically significant at the 5 percent level (Buffler et al., 1979). This increased risk persisted after adjusting for personal smoking habits (for this particular cohort). However, this cohort was small and the study was unable to demonstrate an increased risk for any other cancer.

An association between vinyl chloride exposure and lymphoma has not been established. Five studies evaluated the risk of lymphoma development among workers occupationally exposed to vinyl chloride.

Four of the studies showed a positive trend for lymphoma among vinyl chloride workers, but only Weber et al. (1981) noted statistical significance. However, the statistical power in all of these studies was less than 80 percent to demonstrate a relative risk of two, and less than 40 percent to show a relative risk of 1.5 (California Department of Health Services, 1990).

In human cases of angiosarcoma of the liver induced by vinyl chloride, mutations have been found in the p53 and Ki-ras genes (reviewed by Kielhorn et al., 2000). Similar studies have been conducted in animals (see below under "mutagenicity" and "carcinogenicity").

There is ample evidence that vinyl chloride is carcinogenic in humans. However, there is no direct human evidence indicating that infants or children would be more sensitive than adults. There is evidence for a differential effect on young animals from the Maltoni and Drew studies (above).

V. Regulatory Background

An acute Reference Exposure Level (REL) for vinyl chloride of 180,000 $\mu g/m^3$ has been adopted by OEHHA (OEHHA, 1999). This REL is based on subjective reports of mild headaches and dryness of eyes and nose in human volunteers (Baretta et al., 1969). OEHHA has developed a Public Health Goal (PHG) of 0.05 mg/L (or ppb) for vinyl chloride in drinking water (OEHHA, 2000a). The PHG is based on carcinogenic effects observed in an inhalation study by Drew et al. (1983), wherein the authors observed an increase in lung carcinoma incidence in female Swiss mice exposed to vinyl chloride. The PHG was calculated from a cancer slope factor of 0.27 $(mg/kg\text{-}day)^{-1}$ developed under the Toxic Air Contaminant Program by the California Department of Health Services (1990).

Vinyl chloride is listed under the California Safe Drinking Water and Toxics Enforcement Act of 1986 (Proposition 65) as a chemical known to the State to cause cancer (OEHHA, 2000b). It is not listed as a developmental or reproductive toxicant.

VI. Conclusions

Vinyl chloride may be more carcinogenic to infants and children than to adults, based on the results of animal studies by Drew et al. (1983) and Maltoni et al. (1981). These studies showed that animals that were exposed to vinyl chloride pre-weaning developed more tumors with a shorter latency than animals that were exposed later in life, and that they developed types of tumors not seen in the later-exposed animals. This was not due simply to longer time for tumor development in the younger mice as evidenced by the analysis of Cogliano et al. (1996) and Maltoni's own analyses (Maltoni et al., 1981, 1984 and 1988).

While measurements of vinyl chloride in ambient air suggest levels below the limit of detection, there may be localized exposures due to emissions from PVC manufacturing or landfills. Thus, while not a general ambient air concern, vinyl chloride emissions from local hotspots may be higher. Should information become available indicating that local exposures are significant, OEHHA may revisit listing vinyl chloride under SB 25.

VII. References

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