CHRONIC TOXICITY SUMMARY

DIETHANOLAMINE

(DEA; 2,2'-iminodiethanol; 2,2'-iminobisethanol; diethylolamine; 2,2'-aminodiethanol; 2,2'-dihydroxydiethylamine)

CAS Registry Number: 111-42-2

I. Chronic Toxicity Summary

Inhalation reference exposure level	**3 µg/m^3** (0.6 ppb)
Critical effect(s)	Laryngeal lesions in rats
Hazard index target(s)	Respiratory system; cardiovascular system

II. Physical and Chemical Properties (Melnick and Thomaszewski, 1990; Dow, 1980; CRC, 1994)

Description	Colorless crystals
Molecular formula	$C_4H_{11}NO_2$
Molecular weight	105.14 g/mol
Density	1.097 g/cm^3 @ 20°C
Boiling point	268.8°C
Melting point	28°C
Vapor pressure	0.00014 torr @ 25°C
Solubility	Soluble in alcohol, water, acetone
Conversion factor	1 ppm = 4.3 mg/m^3 @ 25°C

III. Major Uses and Sources

Diethanolamine is used in the formation of soaps, emulsifiers, thickeners, wetting agents, and detergents in cosmetic formulations (Melnick and Thomaszewski, 1990; Knaak *et al*., 1997). It is used as a dispersing agent in some agricultural chemicals, as an absorbent for acidic gases, as a humectant, as an intermediate in the synthesis of morpholine, as a corrosion inhibitor, and as a component in textile specialty agents (Beyer *et al*., 1983). Diethanolamine is permitted in articles intended for use in production, processing, or packaging of food (CFR, 1981; cited in Melnick and Thomaszewski, 1990). It is also found in adhesives, sealants, and cutting fluids (Melnick and Thomaszewski, 1990). 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 1520 pounds of diethanolamine (CARB, 2000).

IV. Effects of Chronic Exposures to Humans

There have been no controlled or epidemiological studies of chronic diethanolamine exposure in humans. There is a single case report of occupational asthma determined to be due to the patient's handling of a cutting fluid containing diethanolamine (Piipari *et al*., 1998). Specific bronchial provocation tests were done with the cutting fluid containing DEA and with DEA aerosol at two concentrations (0.75 mg/m^3 and 1.0 mg/m^3) below the occupational limit of 2.0 mg/m^3. DEA caused asthmatic airway obstruction at both concentrations, but IgE-antibodies specific for DEA were not found.

V. Effects of Exposures in Animals

Diethanolamine replaces choline in phospholipids (Blum *et al.*, 1972). DEA also reversibly inhibits phosphatidylcholine synthesis by blocking choline uptake and competing for utilization in the CDP-choline pathway (Lehman-McKeeman and Gamsky, 1999). Systemic toxicity occurs in many tissue types including the nervous system, liver, kidney, and blood system.

Gamer *et al*. (1996*)* exposed groups of 26 Wistar rats (13 male and 13 female) head-nose to a liquid aerosol of DEA for six hours per working day for 90 days at target concentrations of 15, 150, and 400 mg/m^3. Three of each sex were used for whole animal perfusion studies and the remaining 20 animals were examined for pathology. The study found no functional or morphological evidence of neurotoxicity. Retardation of body weight increase was observed in animals exposed to high concentrations. No systemic effects occurred at the low dose, but systemic effects in the liver, kidney, male reproductive system, and red blood cell occurred in the high concentration dose group. In the mid-dose group, mild liver and kidney effects were present. Local irritation of the larynx and trachea was found in the high and mid dose groups; irritating laryngeal effects were also detected in the low dose group. Based on this study 15 mg/m^3 is a NOAEL for liver and kidney effects and a LOAEL for irritation of the larynx. The equivalent continuous exposure at the LOAEL is 2.7 mg/m^3 (15 x 6/24 x 5/7).

Incidence of laryngeal lesions (Gamer *et al*., 1996)

Aerosolized diethanolamine	Chronic inflammation of the larynx	Squamous hyperplasia	Focal squamous metaplasia of laryngeal epithelium at base of the epiglottis
0	None*	None	None
15 mg/m^3	4/20	0/20	20/20
150 mg/m^3	20/20	13/20	20/20
400 mg/m^3	20/20	17/20	20/20

* The report does not give control incidences. Assumed 0/20.

In an abstract Hartung *et al*. (1970) reported that inhalation by male rats of 6 ppm (25.8 mg/m^3) DEA vapor 8 hours/day, 5 days/week for 13 weeks resulted in depressed growth rates, increased lung and kidney weights, and even some mortality. Rats exposed continuously for 216 hours (nine days) to 25 ppm (108 mg/m^3) DEA showed increased liver and kidney weights, elevated

blood urea nitrogen (BUN), and increased serum glutamate oxaloacetate transferase (SGOT), an indicator of liver damage (Hartung et al., 1970). In studies at lower DEA levels, Eastman Kodak (1967) exposed dogs, weanling and adult rats, and guinea pigs to 0.26 ppm (1.1 mg/m^3) DEA for 90 days and found no pathology attributable to DEA. In a 45-day study with 0.5 ppm (2.2 mg/m^3) DEA they also found no pathology attributable to DEA except for a possible slight retardation in rat growth rate.

Gamer *et al*. (1993) exposed groups of 25 pregnant Wistar rats on gestation days 6-15 to a (nose-only) liquid aerosol of DEA at 10, 50 and 200 mg/m^3. Maternal toxicity, indicated by vaginal hemorrhage in 8 of the dams on gestation day 14, and fetotoxicity, evidenced by a statistically significant ($p<0.05$) increased incidence of total fetal skeletal variations, were observed at 200 mg/m^3. No teratogenic effects were seen at any level. Thus 50 mg/m^3 was a NOAEL for maternal toxicity and for embryo-fetal effects.

A 13-week drinking water study in rats (10 per sex per group) showed significant dose-dependent hematological changes following exposure to DEA at all concentrations tested: 320, 630, 1250, 2500, and 5000 ppm in males, and 160, 320, 630, 1250, and 2500 ppm in females. Hematological effects included decreased hemoglobin and mean corpuscular volume (Melnick *et al.*, 1994a). Similar hematological changes were observed following daily topical treatment. In addition to the hematological effects, female rats also showed dose-dependent spinal cord and medullary demyelination beginning at a drinking water concentration of 1250 ppm DEA. Male rats displayed demyelination beginning at 2500 ppm. Female rats gained significantly less weight than controls beginning at 63 mg/kg/day topical treatment. In a companion drinking water study (Melnick *et al.*, 1994b), mice (10 per sex per group) were exposed to concentrations of 0, 630, 1250, 2500, 5000, and 10,000 ppm DEA and displayed dose-dependent hepatotoxicity, nephrotoxicity, and cardiac toxicity. Daily topical treatment in a separate study resulted in skin lesions in mice. Significant hepatic toxicity was observed at all drinking water concentrations, and skin lesions were observed at all topical doses.

Data from female rats exposed to diethanolamine by Melnick *et al*. (1994)

Dose (ppm)	mg/kg/day DEA consumed	Survival	Mean bw change (g)	Hgb (g/dL)	Mean cell volume	Mean cell Hgb (pg)
0	0	10/10	120±6[a]	15.1±0.3	56±0.2	17.9±0.2
160	14	9/10	106±3	15.2±0.1	55±0.2**	17.8±0.1*
320	32	10/10	98±3**	13.8±0.1**	54±0.2**	17.7±0.1**
630	57	10/10	95±4**	13.0±0.1**	53±0.3**	17.2±0.1**
1250	124	10/10	85±4**	11.3±0.2**	51±0.3**	16.7±0.1**
2500	242	10/10	63±4**	10.50±.2**	49±0.2**	16.30±.1**

[a] Values are means±SEM; * $p<0.05$ or ** $p<0.01$ versus control group

Barbee and Hartung (1979a) found that repeated treatment of rats with 330 mg DEA/kg/day significantly inhibited formation of phosphatidyl choline and phosphatidyl ethanolamine in the liver as compared with control rats. In a subsequent study, Barbee and Hartung (1979b) noted changes in liver mitochondrial activity in rats (4 per group) following exposure to DEA in

drinking water for up to 5 weeks. Mitochondrial changes were observed at 42 mg/kg/day after 2 weeks.

Daily oral treatment of male rats with 0, 250, 500, or 750 mg/kg/day for 5 days, or 100 mg/kg/day for 14 days resulted in reduced activities of the liver enzymes microsomal hydroxylase and N-demethylase (Foster *et al.*, 1971).

In a developmental study Marty *et al.* (1999) administered DEA cutaneously to pregnant CD rats during gestation days 6-15 at doses of 0, 150, 500, and 1500 mg/kg/day. Dams exhibited reduced body weight at the highest dose, skin irritation and increased kidney weights at both 500 and 1500 mg/kg/day, and a slight microcytic anemia with abnormal red blood cell morphology at all 3 dose levels. The blood results are consistent with the results of topical application of DEA by Melnick *et al*. (1994b). Rat fetuses had increased incidences of six skeletal variations at 1500 mg/kg/day. Lower doses were without effect on the fetuses. Marty *et al*. (1999) also administered DEA cutaneously to pregnant New Zealand White rabbits on days 6-18 of gestation at 0, 35, 100, and 350 mg/kg/day. Dams administered the highest dose exhibited various skin lesions, reduced food consumption, and color changes in the kidneys, but no hematological changes. Body weight gain was reduced at ≥ 100 mg/kg/day. There was no evidence of maternal toxicity at 35 mg/kg/day and no evidence of developmental toxicity in rabbits at any dose. Developmental toxicity was observed only in the rat and only at doses causing significant maternal toxicity, including hematological effects. Due to a dose discrepancy, the authors adjusted the no observable effect level (NOEL) for DEA developmental toxicity to 380 mg/kg/day for rats. In rabbits, the embryonal/fetal NOEL was 350 mg/kg/day.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Gamer *et al*. (1996)
Study population	Wistar rats (male and female)
Exposure method	Inhalation 6 h/day, 5 d/wk
Critical effects	Chronic inflammation and squamous hyperplasia and metaplasia of the larynx
LOAEL	15 mg/m^3
NOAEL	Not observed
Exposure duration	90 days
Average experimental exposure	2700 $\mu g/m^3$ for LOAEL group (15 mg/m^3 x 6h/24h x 5d/7d x 1000 $\mu g/mg$)
LOAEL uncertainty factor	3 (see below)
Subchronic uncertainty factor	3
Interspecies uncertainty factor	10
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	1000
Inhalation reference exposure level	3 $\mu g/m^3$ (0.6 ppb)

No chronic inhalation studies with diethanolamine were located in the peer-reviewed literature. Thus the 90 day study by Gamer *et al*., which found a LOAEL of 15 mg/m^3 for irritation of the

rat larynx, was used to derive the REL. All 20 of the rats in the 15 mg/m^3 exposure group showed focal squamous metaplasia of the laryngeal epithelium at the base of the epiglottis, and 4 of the 20 had inflammatory cells present in the larynx. The former lesion seemed to be very limited and did not justify use of the full LOAEL uncertainty factor of 10.

For comparison, the BASF (1993) developmental study by the inhalation route found a LOAEL of 200 mg/m^3 DEA and a NOAEL of 50 mg/m^3 for fetotoxic effects. The equivalent continuous exposure at the NOAEL is 12.5 mg/m^3. Multiplying by an RGDR of 1 and dividing by an interspecies uncertainty factor (UF_A) of 3 and an intraspecies uncertainty factor (UF_H) of 10 results in a REL estimate of 40 µg/m^3.

As another comparison, the study by Melnick *et al.* (1994a) shows dose-dependent adverse hematological and CNS effects in rats exposed to DEA in drinking water. Similar systemic effects were observed following dermal exposure. The Melnick *et al.* subchronic study was of the longest duration and was the most comprehensive report of the systemic effects of DEA in the literature. However, portal-of-entry effects of DEA have not been examined and should be addressed in future studies since this compound has irritant properties. The data from female rats were used since females were more sensitive than males to the hematologic effects of DEA. The LOAEL was 160 mg/L, or 14 mg/kg-day based on water consumption rates. Dividing by a LOAEL UF of 3, a subchronic UF of 3, an interspecies UF of 10, and an intraspecies UF of 10 (cumulative UF = 1000) results in a oral REL of 0.014 mg/kg-day. Using route-to-route extrapolation and assuming that a 70 kg person inhales 20 m^3 of air per day leads to an inhalation REL estimate of 50 µg/m^3 (10 ppb) DEA.

VII. Data Strengths and Limitations for Development of the REL

The diethanolamine database is relatively weak. Major areas of uncertainty are the lack of adequate human exposure data, the absence of a NOAEL in the major study, the lack of reproductive and developmental toxicity studies, and the lack of chronic inhalation, multiple-species, health effects data.

VIII. Potential for Differential Impacts on Children's Health

Since the proposed chronic REL of 3 µg/m^3 based on laryngeal effects is much lower than the comparison REL of 40 µg/m^3 based on fetotoxic effects, the REL should adequately protect infants and children. Diethanolamine is a respiratory irritant and thus might exacerbate asthma, which has a more severe impact on children than on adults. The large uncertainty factor of 1000 should protect against that potential hazard. However, there is no direct evidence in the literature to demonstrate that DEA exacerbates asthma or to quantify a differential effect of diethanolamine on the larynx or on other organs in infants and children.

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CHRONIC TOXICITY SUMMARY

ETHYLENE DIBROMIDE

(1,2-dibromoethane; dibromoethane; alpha, beta-dibromoethane; EDB; ethylene bromide; glycol bromide)

CAS Registry Number: 106-93-4

I. Chronic Toxicity Summary

Inhalation reference exposure level	**0.8** m**g/m^3** (0.1 ppb)
Critical effect(s)	Decreased sperm count/ejaculate, decreased percentage of viable and motile sperm, increased semen pH, and increased proportion of sperm with specific morphological abnormalities in human males
Hazard index target(s)	Reproductive system

II. Chemical Property Summary (HSDB, 1995; CRC, 1994)

Description	Colorless, heavy, nonflammable liquid with a mildly sweet, chloroform-like odor.
Molecular formula	$C_2H_4Br_2$
Molecular weight	187.88 g/mol
Boiling point	131-132°C
Melting point	9.9°C
Vapor pressure	0.11 torr at 20°C
Solubility	Slightly soluble in water (3400 mg/L water at 20°C). Miscible with most organic solvents.
Conversion factor	7.68 µg/m^3 per ppb at 25°C

III. Major Uses and Sources

Ethylene dibromide (EDB) is used as a solvent for resins, gums, and waxes, and as a chemical intermediate in the synthesis of dyes and pharmaceuticals (HSDB, 1995). EDB was once widely used as a fumigant for the control of pests in the U.S. Because of concerns regarding its carcinogenicity, the agricultural uses of EDB were banned in 1983 (RECT, 1988). EDB was also commonly used as a gasoline additive to scavenge inorganic lead compounds. The transition to the use of lead-free gasoline has drastically curtailed the use of EDB in this country (REPROTOX, 1995). EDB is now used mainly in industry. EDB may be formed naturally in the ocean as a result of macro algae growth. Exposure to the general population, via inhalation,

may occur in the vicinity of industries and in industrial settings where this compound is manufactured and used. 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 1179 pounds of EDB (CARB, 2000).

IV. Effects of Human Exposures

Pharmacokinetic studies of EDB in humans could not be found in the literature. However, *in vitro* studies of EDB metabolism in human liver samples have been performed (Wiersma *et al.*, 1986). These experiments have shown that the enzyme systems known to metabolize EDB in rodent liver also metabolize EDB in the human liver. EDB was metabolized by human liver cytosolic glutathione S-transferases (GST), microsomal GST, and microsomal mixed function oxidases (MFO). MFO activity resulted in adducts irreversibly bound to protein, while GST activity was mostly responsible for adducts irreversibly bound to DNA. Rodent liver enzymes similarly activate EDB to metabolites that bind to cellular macromolecules. In human fetal liver (16-18 weeks gestation) cytosolic GST was also found to metabolize EDB with high efficiency (Kulkarni *et al.*, 1992). Since detoxification via MFO activity may be limited at this stage of development, the results suggest that the human fetus and neonate may be at greater risk from EDB toxicity than adults.

A study of mortality from cancer and respiratory diseases was conducted among 161 employees exposed to EDB in 2 production units operated from 1942 to 1969 and from the mid-1920s to 1976, respectively (Ott *et al.*, 1980). No apparent connection was found between mortality due to respiratory diseases and exposure to EDB, when compared to U.S. white male mortality figures.

Due to the structural similarity of EDB to dibromochloropropane (DBCP), a known toxic agent in human male reproductive organs, a number of epidemiological studies concerning male reproduction and spermatogenesis were conducted.

In a study of 59 employees exposed to EDB at the Ethyl Corporation plant in Magnolia, Arkansas, the sperm counts of the exposed men were divided into 2 groups depending on estimated exposure (Ter Haar, 1980). Twenty percent of the low exposure group (<0.5 ppm) had sperm counts below 40 million, whereas 42% of the high exposure group (0.5 to 5 ppm) had sperm counts below this figure. The sperm counts were intermediate between counts reported for 2 types of U.S. samples (for normal men). The observed births among the two exposure groups were found to be similar to the number of expected births. The author determined that EDB had no effect on sterility or reproduction in the workers. Weaknesses of this study include the small population of exposed workers and the lack of a concurrent unexposed control group. Taking these defects of the study into account, Dobbins (1987) concluded that the results provide evidence that EDB exposure between 0.5 and 5.0 ppm is associated with lower sperm counts.

A comparison of observed marital fertility with expected fertility (based on U.S. fertility rates) was conducted among 297 men working at 4 U.S. plants that manufacture EDB (Wong *et al.*, 1979). Fertility was 20% below expected for the four plants combined. This was largely due to

one plant (plant D), which was 49% below the expected level. After omitting the incidence of vasectomies and hysterectomies among married couples, observed fertility was still 39% below the expected figure for plant D but was now no longer statistically significant. Exposure levels of EDB at plant D were not known but were estimated to be no more than 5 ppm. Later review determined that expected (control) levels of fertility and the power of the study were too low, resulting in the inability to identify a possible adverse effect (Dobbins, 1987). The lower fertility at plant D indicates that EDB has the potential to reduce fertility, but the extent of the reduction cannot be estimated from this study. Further treatment of the data by a method that uses the proper statistical adjustments of reproductive experience in the U.S. population (used as the control) suggests borderline significance for reduced fertility among the combined workers at the four plants (Wong *et al.*, 1985). The fertility evaluation indicates that more in-depth epidemiologic or physiologic studies are needed.

Semen analysis of 83 pineapple workers at two plantations was performed by Rogers and associates (1981). EDB-exposed workers were removed from each group and placed in a separate group. The remaining two groups of workers acted as control groups. Sperm counts, motility, and morphology were similar among the three groups. However, 43.8% of exposed workers had abnormally low counts (<40 million/ml), while abnormally low sperm counts of controls were 34.2% and 17.8%. Of the four exposed workers that had fertility tests done, all tested in the infertile range. Forty percent or less tested in the infertile range among the control groups. The results suggest that workers exposed to EDB had reduced sperm counts, but exposure levels were not known.

Semen analysis among 46 men employed in the papaya fumigation industry was conducted to determine if EDB affected semen quality (Ratcliff *et al.*, 1987; Schrader *et al.*, 1987). Average duration of exposure was 5 years and the geometric mean breathing zone exposure to airborne EDB was 88 ppb (8 hr time weighted average) with peak exposures of up to 262 ppb. The comparison group consisted of 43 unexposed men from a nearby sugar refinery. Following consideration of confounding factors, statistically significant decreases in sperm count/ejaculate, the percentage of viable and motile sperm, and increases in the proportion of sperm with specific morphological abnormalities (tapered heads, absent heads, and abnormal tails) were observed among exposed men. Semen pH was significantly more alkaline than that of unexposed workers. Other measured sperm quality parameters were unchanged. This study suggests that EDB can result in reproductive impairment. However, no measurement of male fertility was conducted.

In a study that examined similar indices of semen quality, 6 week exposure of 10 forestry workers to EDB (60 ppb time weighted average, with peak exposures of up to 2165 ppb) resulted in decreased semen volume and slower sperm velocity (Schrader *et al.*, 1988). Six unexposed men were used as controls. The researchers suggest that short-term exposure to EDB results in decreased sperm velocity, while long-term exposure, as in the previous study of EDB-exposed papaya workers, results in sperm immotility and cell death.

V. Effects of Animal Exposures

EDB is readily and rapidly absorbed from the lung when breathed as a vapor, from the GI tract when taken orally, or through the skin when applied dermally (HSDB, 1995). In rats, the rate of absorption of EDB from the respiratory tract reached a plateau within 10 to 20 minutes following exposure to 75 ppm EDB for up to 2 hours (Stott and McKenna, 1984). About 58% of the EDB was absorbed. Intraperitoneal injection of [^{14}C]EDB into guinea pigs resulted in the highest concentrations in liver, kidneys, and adrenals (Plotnick and Conner, 1976). Sixty-five percent of the dose was excreted as metabolites in urine, 3% in feces, and 12% excreted unchanged in expired air. In rats, the highest concentrations of [^{14}C]EDB label were found in liver, kidney and spleen following an oral dose of 15 mg/kg body wt (Plotnick *et al.*, 1979). Studies with rats have provided evidence that 2 pathways of metabolic bioactivation exist for EDB (RECT, 1988). The oxidative pathway yields the metabolite 2-bromo-acetaldehyde, which is associated with cell macromolecule binding and liver damage. The conjugative pathway principally yields glutathione products, such as *S*-(2-bromoethyl)-glutathione, which are mainly responsible for DNA binding and mutagenesis. In rats, orally administered EDB is excreted primarily in the urine as mercapturic acid derivatives (Jones and Edwards, 1968). The biologic half-life for elimination of [^{14}C]EDB in rats is 5.1-5.6 hours (Watanabe *et al.*, 1978) and less than 48 hours in mice and guinea pigs (HSDB, 1995). Besides the small amount irreversibly bound to cell macromolecules and DNA, EDB shows little, if any, bioaccumulation in mammalian systems.

In a subchronic toxicity study of experimental animals, rats and guinea pigs were given EDB by oral administration for about 4 months (Aman *et al.*, 1946). Body weights and mortality of animals at or below an average daily dose of 40-50 mg/kg body wt-day were unaffected. However, only one control animal/species was used, the dosing regimen was not well described, and pathologic examination was apparently not performed.

Subchronic exposure of rats (20/sex/group) to 50 ppm EDB for as many as 63 seven-hour exposures in 91 days resulted in no significant change in body weights (Rowe *et al.*, 1952). Liver and kidney weights were increased in both sexes while testis weights were decreased in males. Also, lung weights in males were elevated and spleen weights in females were decreased. Histopathological examination revealed no changes. Guinea pigs (8/sex/group) subjected to as many as 57 seven-hour exposures of 50 ppm EDB in 80 days exhibited reduced body weights. Organ weights were unchanged, but microscopic examination of the livers showed slight central fatty degeneration. In kidneys, slight interstitial congestion and edema with slight parenchymatous degeneration of the tubular epithelium were observed. Four rabbits exposed to 59 seven-hour sessions at 50 ppm in 84 days showed no signs of adverse effects. Clinical signs of monkeys exposed to 50 ppm EDB (49 seven-hour exposures in 70 days) included an ill, unkempt appearance and nervousness. Slight central fatty degeneration in livers was observed, but pathology was not seen in other tissues. Exposure of the same four species to 25 ppm EDB for up to 220 days (145 to 156 seven-hour exposures) showed no signs of adverse effects.

In a 13-week inhalation study, 5 Fischer 344 albino rats/group/sex and 10 B6C3F1 mice/group/sex were exposed to 0, 3, 15, or 75 ppm EDB for 6 hr/day, 5 days/week (Reznik *et al.*, 1980). At 75 ppm, rats and mice exhibited severe necrosis and atrophy of the olfactory epithelium in the nasal cavity. Squamous metaplasia, hyperplasia and cytomegaly of the

epithelium were also seen in nasal turbinates, larynx, trachea, bronchi, and bronchioles. Minor alterations were seen in the nasal cavity of only a few male and female rats at 15 ppm. No compound-related lesions were observed in the olfactory and respiratory epithelium at 3 ppm. No lesions were seen in other tissues at any dose.

In another 13-week inhalation study, 40 male and 20 female CDF(F344) rats/group were exposed to 0, 3, 10, or 40 ppm EDB 6 hr/day, 5 days/week (Nitschke *et al.*, 1981). Male rats in the 40 ppm group exhibited decreased weight gain throughout most of the exposure period. However, reduced weight gain was never more than 6-8% below control levels. With the exception of decreased specific gravity of urine in females of the 40 ppm group, no treatment-related changes were observed in any rat group with respect to urinalysis, hematology, and clinical chemistry. At the end of 13 weeks, relative liver and kidney weights of males exposed to 40 ppm EDB were significantly elevated, while relative liver weights of females in the two highest exposure groups were significantly elevated. Absolute liver weight of females in the 40 ppm group was also significantly elevated. Histopathological examination revealed lesions primarily confined to the anterior sections of the nasal turbinates. Hyperplasia and nonkeratinizing squamous metaplasia of the respiratory epithelium were observed in nasal turbinates of rats exposed to 40 ppm EDB. Only slight epithelial hyperplasia of nasal turbinates was noted at 10 ppm. No treatment related effects were seen at 3 ppm. Livers of females in the 40 ppm group showed a slight increase in fat. After an 88 day recovery period, there was a reversion to normal of the nasal turbinates in all but one rat.

In what was originally scheduled to be a lifetime exposure study, 50 Osborne-Mendel rats/group/sex and 50 B6C3F1 mice/group/sex were administered EDB 5 days/week by gastric lavage over a substantial portion of their life-span (NCI, 1978). Twenty untreated controls/sex and 20 vehicle controls/sex of each species were included in the study. Rats received initial doses of 80 and 40 mg/kg body wt-day for the first 17 weeks. Due to high mortality, dosing of high dose rats was discontinued for 13 weeks and resumed on week 30 at 40 mg/kg body wt-day. In week 42, all intubations of low and high dose rats ceased for 1 week followed by 4 weeks of dose administration. All surviving, treated male rats were necropsied in week 49; all surviving, treated female rats were necropsied in week 61. The resulting time-weighted average dose over the test period was 38 and 41 mg/kg body wt-day for low and high dose males, respectively, and 37 and 39 mg/kg body wt-day for low and high dose females, respectively. Mice received initial doses of 120 and 60 mg/kg body wt-day. In weeks 11-13, high and low doses were increased to 200 and 100 mg/kg body wt-day, respectively. Original dose levels were resumed after week 13. At week 40, administration of EDB was decreased to 60 mg/kg body wt-day for high dose mice. EDB administration was discontinued at week 54 with necropsy occurring at week 78 for males and high dose females. Low dose female mice were observed for 37 weeks after intubation ceased. The resulting time-weighted average dose over the test period was 62 and 107 mg/kg body wt-day for low and high dose mice, respectively. In rats, clinical signs by week 5 included reddened ears and hunched back in all treatment groups. By week 10, all treated rats had reduced body weights ($\geq$10%). Both female and male rats exhibited dose-dependent mortality. Many of the deaths occurred during or shortly after intubation, suggesting an acute toxic reaction. Pathology revealed hyperkeratosis and acanthosis of the forestomach in high dose males and females and in one low dose female. A small number of rats in both treatment groups showed adrenal cortex degeneration and peliosis of the liver (hepatitis). Dosed males showed

early development of testicular atrophy. In mice, dose-related body weight reduction and mortality were observed. Clinical signs included alopecia, thin, hunched appearance, soft feces and body sores. Hyperkeratosis and acanthosis of the forestomach were seen in high dose male and female mice. One incidence each of hyperkeratosis (in a female) and acanthosis (in a male) was seen at the low dose. Splenic changes were present in high dose mice and testicular atrophy was present in high dose males.

In a long-term inhalation exposure study, F344 rats and $B6C3F_1$ mice were exposed to 0, 10, or 40 ppm EDB 6 hr/day, 5 days/week for up to 103 weeks (NTP, 1982). In male and female rats, the high dose groups had reduced body weights and increased mortality that began at about week 60. The treatment-related non-neoplastic pathology included hepatic necrosis (both sexes), epithelial hyperplasia and suppurative inflammation throughout the respiratory system (both sexes), and nephropathy (males only). Toxic nephropathy and mineralization were also seen in high dose female rats. Testicular degeneration and atrophy occurred with greater frequency in exposed rats and may be related to observed testicular tumors. Spermatic granulomas were also more frequently seen in high-dose males. Degeneration of the adrenal cortex appeared to be dose-related in females, but only one incidence each was seen in low and high dose males. Increased incidence of retinal atrophy was observed in exposed females. In mice, body weights were reduced at the high dose in both males and females. Many of the high dose animals exhibited a progressive weakness of the limbs or body during the second year. Increased mortality occurred in a dose-related manner in females and was significantly greater in low dose males. Non-neoplastic pathology included epithelial hyperplasia throughout the respiratory system and serous and suppurative inflammation of the nasal cavity in exposed mice. In all male mice, the principal cause of death was urinary bladder inflammation. However, bladder epithelial hyperplasia was only seen in exposed animals. An increased incidence of suppurative inflammation of the prostate was present but was also seen in controls. Dose-related spleen hematopoiesis was observed in females.

Another long-term inhalation study investigated the effects of 0 or 20 ppm EDB (7 hr/day, 5 days/week) on 48 Sprague-Dawley rats/sex/group for 18 months (Wong *et al.*, 1982). Significantly lower body weight gains (>10% difference from controls) occurred by the 15^{th} month in males, and by the 18^{th} month in females. Significantly reduced food consumption was not apparent. Increased mortality rates in both sexes occurred beginning in the 12^{th} month of EDB exposure. All hematological findings were within normal ranges. The only recorded non-neoplastic gross or microscopic finding was atrophy of the spleen in males, which may be related to tumor formation (hemangiosarcoma). The nasal cavity was not examined.

In a study of the effect of EDB on sperm production in bulls (Isreal-Friesian breed), 4 calves were fed 2 mg/kg body wt-day for 12 months (Amir and Volcani, 1965). The bulls were then given EDB in gelatin capsules every other day for 2-4 months longer. EDB did not appear to affect the growth, health, and libido of the bulls. However, semen density and motility were significantly lower compared to untreated control bulls of the same age. Many abnormal spermatozoa were also present in treated bulls. A NOAEL for this effect was apparently not determined. Cessation of EDB administration resulted in normal sperm within 10 days to 3 months. Further studies confirmed that EDB adversely affected sperm production without any other apparent effects on bulls (Amir and Volcani, 1967; Amir and Ben-David, 1973). However,

feeding rams 2-5 mg/kg body wt-day for 120 days did not result in any effect on sperm or on the health of the animal (Amir, 1991).

Female B6C3F1 mice (10/group) were given 31.25, 62.5, or 125 mg/kg EDB by gastric lavage 5 days/week for 12 weeks (Ratajzak *et al*., 1995). At the highest dose, EDB significantly prolonged intervals between estrus, decreased hemoglobin and hematocrit levels, and increased cholesterol, triglycerides, total protein, and albumin. The highest dose also caused an immunosuppressive effect by lowering the *in vitro* splenic lymphocyte response to T- and B-cell mitogens.

In a developmental toxicity study, 15-17 pregnant Charles River CD rats and 17-19 pregnant CD mice were exposed to 0, 20, 38, and 80 ppm EDB by inhalation 23 hr/day during days 6 to 16 of gestation (Short *et al.*, 1978). A significant increase in mortality occurred in adult rats exposed to 80 ppm EDB and in adult mice exposed to 38 and 80 ppm EDB. Mice exposed to the highest dose experienced 100% mortality. Reduced body weights and feed consumption occurred in both species at all doses tested. Fetal mortality was increased in rats at the highest dose and in mice at 38 ppm. Reduced fetal body weights occurred at 38 ppm in rats and at all exposure levels in mice. No anomalies were seen in rat fetuses. An increase in runts at 38 ppm and a dose-dependent increase in skeletal anomalies were observed among mouse fetuses. However, these anomalies were characteristic of delayed development and occurred at doses that adversely affected maternal welfare. Therefore, these effects are indicative of fetal toxicity rather than teratogenicity.

Male reproductive toxicity of EDB has been evaluated in some other experimental animals. New Zealand white rabbits, dosed subcutaneously with 0, 15, 30, or 45 mg/kg body wt-day, showed adverse effects at the highest dose (Williams *et al.*, 1991). Increased mortality, increased serum enzymes, and liver damage were observed at this dose level. With respect to sperm quality, sperm velocity, motility, and motion parameters were reduced at the highest dose. A dose related decrease in semen pH was also noted. However, male fertility and fetal structural development were unaffected.

A dominant lethal assay in mice was negative following a single intraperitoneal injection of 100 mg EDB/kg body wt (Barnett *et al.*, 1992). Germ cell tests did not indicate that EDB was a germ cell mutagen in male mice.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Ratcliff *et al.*, 1987
Study population	46 exposed men, 43 unexposed men; 89 total
Exposure method	Variable workplace breathing zone airborne exposure (88 ppb geometric mean 8-hour time weighted average (TWA) exposure with peak exposures up to 262 ppb)
Critical effects	Reproductive toxicity; decreased sperm count/ejaculate, decreased percentage of viable and motile sperm, increased semen pH, and increased proportion of sperm with specific morphological abnormalities (tapered heads, absent heads, and abnormal tails) in human males
LOAEL	88 ppb
NOAEL	Not observed
Exposure continuity	8 hr/day (10 m^3/day occupational inhalation exposure rate), 5 days/week
Exposure duration	Average, 4.9 years (with standard deviation of 3.6 years)
Average experimental exposure	31 ppb for LOAEL group (88 x 10/20 x 5/7)
Human equivalent concentration	31 ppb
LOAEL uncertainty factor	10
Subchronic uncertainty factor	3
Interspecies factor	1
Intraspecies factor	10
Cumulative uncertainty factor	300
Inhalation reference exposure level	0.1 ppb (0.0008 mg/m^3, 0.8 $\mu g/m^3$)

The primary study by Ratcliff and associates (1987) found significant changes in sperm quality indices of papaya workers exposed to EDB vapors for an average of nearly 5 years. No other health effects were apparent. A level of EDB at which no toxicity was observed (NOAEL) was not determined.

In addition to the primary study of Ratcliff *et al.* (1987), several other epidemiological studies together strongly suggest a correlation between EDB exposure and male reproductive toxicity (Ter Haar, 1980; Wong *et al.*, 1979; Wong *et al.*, 1985; Rogers *et al.*, 1981; Schrader *et al.*, 1988). This lesion appears to occur in humans at concentrations at which other toxic effects are not seen. EDB also shares some structural similarity to dibromochloropropane (DBCP), a known reproductive toxicant in human males. The evidence for male reproductive toxicity of EDB is not as strong as that for DBCP, probably because EDB is not as potent as DBCP in producing this toxic effect. However, animal studies demonstrate testicular toxicity and the number of studies indicating a connection between male reproductive toxicity and EDB exposure cannot be ignored for the development of the REL.

Chronic oral exposure of bulls to EDB results in similar toxic effects at low concentrations (equivalent to 0.9 ppm) without affecting the general health of the animal (Amir and Volcani, 1965; Amir, 1991). However, the small sample size and the lack of a dose-response effect and an observed NOAEL limits the usefulness of this study. Long-term studies of EDB toxicity in other experimental animals also lack the determination of a NOAEL (NCI, 1978; NTP, 1982). Evidence of testicular atrophy was found in other long-term studies with experimental animals, but at concentrations that also produced toxic effects in other organ systems.

For comparison with the proposed REL based on a human study, the NTP (1982) chronic inhalation study established a LOAEL (10 ppm) for liver, kidney, eyes, and the respiratory, male reproductive, and endocrine system in rats. A LOAEL was established in mice for mortality, spleen changes in females, and respiratory system toxicity. A NOAEL was not established for either species. Use of a time adjustment (6/24 hr/day, 5/7 day/week), an RGDR of 1, and a total uncertainty factor of 300 (an interspecies UF of 3, a LOAEL to NOAEL UF of 10, and an intraspecies UF of 10) resulted in an estimated REL of 6 ppb (50 $\mu g/m^3$).

VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL for ethylene dibromide include the use of human exposure data from workers exposed over a period of years, and the presence of the toxic endpoint (male reproductive system) in several experimental animal species. Major areas of uncertainty are the lack of observation of a NOAEL, the uncertainty in estimating occupational exposure, the potential variability in occupational exposure concentration, and the limited nature of the study (fertility was not actually tested). The database for chronic toxicity of EDB in experimental animals would be enhanced if the proper doses were chosen to determine a NOAEL.

VIII. Potential for Differential Impacts on Children's Health

Little fetal toxicity was observed when pregnant rats and mice were exposed to 20 ppm EDB during gestation (Short *et al.*, 1978). Thus the REL of 0.1 ppb should adequately protect infants and children. However, we do not know if adolescent boys would be more sensitive than men to this alkylating agent. Differences in metabolic capability between infants and older children and adults may result in either more or less toxicity of EDB. Both oxidative and conjugated metabolites are toxic. Infants may produce proportionately more conjugate than oxidized metabolite relative to adults.

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CHRONIC TOXICITY SUMMARY

ISOPHORONE

(1,1,3-trimethyl-3-cyclohexene-5-one; 3,5,5-trimethyl-2-cyclohexen-1-one; isoforon; isoacetophorone)

CAS Registry Number: 78-59-1

I. Chronic Toxicity Summary

Inhalation reference exposure level	**2,000** m**g/m^3** (400 ppb)
Critical effect(s)	Developmental effects (reduced crown-rump length of female rat fetuses); hepatocytomegaly and coagulative necrosis of the liver in mice
Hazard index target(s)	Development; liver

II. Chemical Property Summary (HSDB, 1995; CRC, 1994; CARB, 1997)

Description	Water-clear liquid with a peppermint-like odor
Molecular formula	$C_9H_{14}O$
Molecular weight	138.21 g/mol
Boiling point	215.2°C
Melting point	–8.1°C
Vapor pressure	0.44 torr at 25°C
Solubility	Slightly soluble in water (12,000 mg/L water at 25°C); miscible in organic solvents.
Conversion factor	5.65 µg/m^3 per ppb at 25°C

III. Major Uses and Sources

Isophorone is used extensively as a solvent in some printing inks, paints, lacquers, adhesives, vinyl resins, copolymers, coatings, finishes, and pesticides, in addition to being used as a chemical intermediate (HSDB, 1995). Since this compound has many different applications, release to the environment may originate from a wide variety of industrial sources including iron and steel manufacturers, manufacturers of photographic equipment and supplies, automobile tire plants, and printing operations. Coal-fired power plants may also emit isophorone to the air. Although it is mostly a man-made compound, isophorone has been found to occur naturally in cranberries (ATSDR, 1989). Occupational exposure may occur by inhalation or dermal contact. 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 2809 pounds of isophorone (CARB, 2000).

IV. Effects of Human Exposures

No information is available concerning long-term exposure or pharmacokinetics of isophorone in humans. In occupational monitoring studies, the time-weighted average concentration in breathing zones and workplace air of a screening plant ranged from 8.3-23 ppm and from 3.5-14.5 ppm, respectively (Samimi, 1982). Up to 25.7 ppm was detected in air of a silk screening printing plant in Pittsburgh, PA (Kominsky, 1983). The concentration in breathing zone samples from a decal manufacturing plant in Ridgefield, NJ was 0.7-14 ppm (Lee and Frederick, 1982). It was suspected that the reported eye and nose irritation of workers at the silk screening plant and at the decal manufacturing plant was the result of acute and subacute exposure to isophorone vapors.

Workers exposed to 5-8 ppm (28-45 mg/m^3) of isophorone for one month complained of fatigue and malaise (NIOSH, 1978). When concentrations were reduced to 1-4 ppm, no adverse effects were reported. Acute exposure studies in humans (up to 400 ppm for 1 to 4 minutes) resulted in eye, nose and throat irritation, nausea, headache, and dizziness or faintness (Union Carbide, 1963). Inhalation exposure for 15 minutes to 10 ppm isophorone produced only mild effects in human subjects while 25 ppm produced irritation to eyes, nose, and throat (Silverman *et al.*, 1946).

V. Effects of Animal Exposures

Few reports have been published regarding the pharmacokinetics of isophorone in experimental animals. Isophorone was widely distributed in the major organs of the rat following 4 hour inhalation exposure to 400 ppm (ATSDR, 1989). Oral gavage of 4000 mg/kg body wt to rats and a rabbit also resulted in wide distribution of the chemical. The highest blood levels of isophorone were reached by 30 min in rabbits following oral gavage and had decreased dramatically by 21 hours, indicating rapid absorption and elimination of the chemical. Preliminary results of a pharmacokinetic study indicate that rats treated orally with ^{14}C-isophorone excreted 93% of the radiolabel in the urine, expired air, and feces in 24 hours (ATSDR, 1989). The highest levels of ^{14}C-isophorone were found in the liver, kidney, preputial gland, testes, brain, and lungs. Several metabolites were identified in the urine of orally dosed rats and rabbits, including 3-carboxy-5,5-dimethyl-2-cyclohexene-1-one, 3,5,5-trimethylcyclohexanol, and some glucuronide conjugates (Dutertre-Catella *et al.*, 1978). A portion of the chemical was excreted unchanged in expired air.

In an early inhalation study, 10 Wistar rats/group and 10 guinea pigs/group, all of mixed sex, were exposed to 0, 25, 50, 100, 200, or 500 ppm isophorone 8 hr/day, 5 days/week for 6 weeks (Smyth *et al.*, 1942). Increased mortality and reduced body weights were observed at 100 ppm and up in both species. However, eye and nose irritation was noted only at the highest dose. Minor changes in blood chemistry and histopathological changes in the kidney and lungs were noted in treated animals. However, later investigations determined that the isophorone used in this study was contaminated with appreciable amounts of compounds (Rowe and Wolf, 1963).

Therefore, some of the adverse effects (i.e., the lung lesions) may have been due to the contaminants. The accuracy of the concentration data in the 1942 study is also questionable.

No treatment-related histopathological lesions were found in lungs, livers, or kidneys of male and female rats exposed intermittently (6 hr/day, 5 days/week) to 37 ppm isophorone for 4 weeks compared to controls (Hazleton Labs, 1968; summarized by ATSDR, 1989). Histological examination was limited to 30% of the control and treated rats. Body weight gain, mean absolute liver weights, and mean liver-to-body weight ratios of treated rats were significantly reduced compared to controls. Slight variations in hematological findings were noted in treated rats (increased lymphocytes and hemoglobin content; decreased neutrophils) but were not considered different from controls.

Rats (10/sex) were exposed to 500 ppm isophorone 6 hr/day, 5 days/week for up to 6 months (Dutertre-Catella, 1976; summarized by ATSDR, 1989). Irritation of eyes and nasal mucosa was observed. One female and three males in the treatment group died during the study, which was considered to be a treatment-related effect. But no exposure-related histopathological lung or liver lesions were observed compared to controls. Dutertre-Catella (1976) also exposed rats and rabbits (number per group per sex not stated) to 250 ppm isophorone 6 hr/day, 5 days/week for 18 months (Dutertre-Catella, 1976). Irritation of eyes and nasal mucosa was observed in both species, but no deaths occurred in the treatment groups. Histopathological examination of the lungs and kidneys, urinalysis, and hematological analysis revealed no exposure-related changes in either species. However, cytoplasmic microvacuolization of hepatocytes was observed in both species (ATSDR, 1989).

In a 90-day feeding study, 20 CFE albino rats/group/sex were given isophorone in their diet at concentrations of 0, 750, 1500, or 3000 ppm. Four beagle dogs/group/sex received isophorone in gelatin capsules at concentrations of 0, 35, 75, or 150 mg/kg body wt-day (AME, 1972a,b). High dose rats exhibited slightly reduced weight gain compared to controls (8-10%) for most of the study. Average weight gain among the exposure groups of beagle dogs remained essentially unchanged during the entire study. Urinalysis, hematology, and clinical chemistry indices found no treatment-related effects in the animals at either the interim or final toxicological examinations. Gross pathology and a limited histopathological examination observed no treatment-related effects in either species. Data on isophorone purity and possible loss of isophorone from rat diet due to vaporization were not presented.

In the most comprehensive isophorone toxicity study to date, 50 F344/N rats/group/sex and 50 B6C3F1 mice/group/sex were administered 0, 250 or 500 mg isophorone/kg body wt 5 days/week by oral gavage (in corn oil) for 103 weeks (Bucher *et al.*, 1986; NTP, 1986). Clinical signs of toxicity were not seen during the length of the study. However, several deaths in male and female rats at the high dose occurred early in the study. A steep decline in survival rate of high dose male rats occurred after week 90. Male and female rats and female mice in the high dose group exhibited only a slight decrease in body weight (<10%) compared to controls. A 13-week range finding study for the 2-year study did not find compound-related lesions in the kidney (or any other organs) of rats and mice exposed up to 1000 mg/kg body wt-day. However, pathological examination of rats exposed to isophorone for 2 years revealed non-neoplastic lesions in the kidney. Increased mineralization of the collecting ducts in isophorone-exposed

male (but not female) rats was observed. This lesion was characterized by basophilic aggregates of mineral most often found in the medullary collecting ducts and occurred coincidentally with lesions of chronic nephropathy. Nephropathy was observed in almost half the female controls and nearly all the male controls. Isophorone exposure appeared to increase both the severity of nephropathy in low dose male rats and the incidence of nephropathy in dosed female rats, but the effects were not pronounced. However, the isophorone potentiation of nephropathy in rats may be due to 'male rat-specific nephropathy' and may not have any relevance to human exposure (Strasser *et al.* 1988). Other adverse effects in kidneys of isophorone-treated male rats include tubular cell hyperplasia (in a dose-related manner) and epithelial hyperplasia of the renal pelvis. In mice, an increased incidence of chronic focal inflammation was observed in the kidneys of males, but was not considered treatment-related. A dose-dependent increase in fatty metamorphosis occurred in the adrenal cortex of male rats, but the biological significance of this change is unknown. All isophorone-exposed male mice had an increased incidence of hepatocytomegaly and coagulative necrosis of the liver. However, treatment-related liver lesions were not observed in female mice. Increased incidence of hyperkeratosis of the forestomach was observed in dosed male and high dose female mice, but was probably not a relevant treatment-related effect.

Published studies on possible reproductive effects of isophorone are lacking. An unpublished inhalation study conducted by a commercial laboratory (Bio/dynamics, 1984b) studied possible teratogenicity due to isophorone in rats or mice at inhaled doses up to 115 ppm. Groups of 22 female rats and 22 female mice were exposed to 0, 25, 50, or 115 ppm isophorone (6 hr/day) on gestational days 6-15. Maternal toxicity in rats included dose-dependent alopecia and cervical/anogenital staining. Low body weights (7-8%) were occasionally observed in the 115 ppm group. In mice, maternal toxicity was confined to slightly decreased weight (7-8%) on one day in the 115 ppm group. No significant differences were found in uterine implantations, fetal toxicity, and external and internal malformations among the animals. However, a slight, but significant, growth retardation in the form of decreased crown-rump length was present among the high dose fetal rats. Also, a slight, but insignificant, increase in extra ribs and/or rudimentary ribs was seen in rat and mouse fetuses at the highest dose. In a pilot study for this developmental toxicity investigation (12 females/species), exencephaly was observed in 1 rat and 1 mouse undergoing late reabsorption and in 2 live rat fetuses from dams exposed to 150 ppm isophorone on gestational days 6-15 (Bio/dynamics, 1984a). Exencephaly was not observed at any dose level in the primary study.

Dutertre-Catella (1976) did not find adverse reproductive or developmental effects in rats exposed to 500 ppm isophorone (6 hr/day, 5 days/week) for 3 months before mating and throughout gestation (females only) as well. The pups were not examined for internal malformations so the study was incomplete for determination of developmental effects.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Bio/dynamics 1984a,b
Study population	22 female mice/group, 22 female rats/group
Exposure method	Discontinuous whole body inhalation exposure during gestation (0, 25, 50, or 115 ppm)
Critical effects	Developmental effects (reduced crown-rump length of female rat fetuses); teratogenicity (exencephaly in fetal rats and mice) in range finding study at 150 ppm
LOAEL	115 ppm for reduced crown-rump length of female rat fetuses
NOAEL	50 ppm
Exposure continuity	6 hr/day during gestation
Exposure duration	Days 6-15 of gestation
Average experimental exposure	12.5 ppm (50 x 6/24)
Human equivalent concentration	12.5 ppm (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h))
LOAEL uncertainty factor	1
Subchronic uncertainty factor	1
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	30
Inhalation reference exposure level	0.4 ppm (400 ppb, 2 mg/m^3, 2,000 μg/m^3)

The inhalation study by Bio/dynamics (1984a,b) presents data that indicate exposure during gestation may be the most sensitive indicator of non-neoplastic toxicity by isophorone. Exposure of pregnant rats to 115 ppm isophorone during gestation resulted in significant growth retardation of female rat fetuses (reduced crown-rump length). Exposure to 50 ppm isophorone, the NOAEL, produced no developmental effects. The authors had removed the two shortest female fetuses prior to statistical analysis. The result was that there was no significant difference in fetal growth retardation; therefore, this adverse effect is not significant. However, this selective culling before the statistical analysis is not scientifically appropriate in this case. In addition, the authors did not perform some of the scheduled fetal examinations. Otherwise, the growth retardation might have had even greater statistical significance. The pilot study (Bio/dynamics, 1984a) observed exencephaly in a few mouse and rat fetuses at 150 ppm. Exencephaly was not considered significant by the authors because it was not present in any fetuses of the primary study (Bio/dynamics, 1984b). However, exencephaly is included as a critical effect in this summary because it is considered a serious teratogenic effect that was present at a dose (150 ppm) only slightly higher than the LOAEL of the primary study (115 ppm). Alopecia of adult female rats was observed in many of the exposed animals. However, this effect may be considered more of an acute dermal irritation than a chronic effect. In addition, cervical and anogenital staining seen in many exposed rats is not considered a chronic 'adverse' effect.

For comparison with the proposed REL of 0.4 ppm, the inhalation LOAEL of 250 ppm for mild liver effects (Dutertre-Catella, 1976) in rats and rabbits intermittently exposed to isophorone for 18 months was used to estimate a REL. Use of a time adjustment (6/24 x 5/7), an RGDR of 1, and a total UF of 100 (LOAEL to NOAEL = 3, interspecies = 3, and intraspecies = 10), also resulted in an estimated REL of 0.4 ppm. These results indicate that the REL will also protect against adverse liver effects.

While the toxicological significance of this liver effect observed by Dutertre-Catella (1976) is unknown, the NTP (1986) study observed an increased incidence of hepatocytomegaly and coagulative necrosis of the liver in treated male mice, but not in female mice and rats, orally gavaged with isophorone. Using 250 mg/kg-day as a LOAEL for mice and dividing by a total UF of 1000 (10 each for LOAEL to NOAEL, 10 for interspecies, and 10 for intraspecies) results in an oral REL of 0.25 mg/kg-day. Multiplying the oral REL by 3,500 $\mu g/m^3$ per mg/kg-day for route-to-route extrapolation results in a chronic inhalation REL estimate of 900 $\mu g/m^3$ (0.16 ppm), which is in good agreement with the REL developed from Dutertre-Catella (1976) and Biodynamics (1984a,b).

VII. Data Strengths and Limitations for Development of the REL

The strength of the database for isophorone is the consistent lack of relevant severe histopathological effects in the chronic inhalation study (Dutertre-Catella, 1976) and in the oral gavage study (NTP, 1986). Weaknesses of the database for isophorone include the lack of human exposure data, the lack of comprehensive long-term inhalation studies, and the lack of published peer-reviewed reproductive/developmental studies. The lack of human data may be due to isophorone's rather low potency for causing chronic, non-neoplastic, adverse effects. Inhalation of isophorone is a relevant route of exposure under occupational settings, but is most likely a minor route of exposure for the general population. Due to the insufficient characterization of the kidney and liver lesions in the oral gavage NTP study (Bucher *et al*, 1986; NTP, 1986) and the inhalation study (Dutertre-Catella, 1976), a comprehensive chronic study in rodent and non-rodent species would enhance the database for isophorone.

VIII. Potential for Differential Impacts on Children's Health

Since the REL is based on a developmental study, it is expected to be adequately protective of infants and children. However, there is no direct evidence in the literature to quantify a differential effect of isophorone in children relative to adults. Isophorone occurs in cranberries and thus presumably in cranberry juice, which is often mixed with other fruit juices. Children tend to consume more fruit juice. However, isophorone as a Hot Spot emission is unlikely to be a multimedia chemical, and there is no evidence to suggest that normal dietary levels of isophorone are associated with adverse health effects.

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