M
CHRONIC TOXICITY SUMMARY

MALEIC ANHYDRIDE

(2,5-furandione; cis-butenedioic anhydride; toxilic anhydride; maleic andride)

CAS Registry Number: 108-31-6

I. Chronic Toxicity Summary

Inhalation reference exposure level	**1 µg/m^3**
Critical effect(s)	Neutrophilic infiltration of the nasal epithelium; irritation of the respiratory system
Hazard index target(s)	Respiratory system

II. Chemical Property Summary (HSDB, 1995)

Description	Colorless or white solid
Molecular formula	$C_4H_2O_3$
Molecular weight	98.06
Boiling point	202°C
Melting point	52.8°C
Vapor pressure	0.1 torr @ 25°C (AIHA, 1970)
Solubility	Soluble in water, ether, acetate, chloroform, dioxane; @ 25°C, 227 g/100 g acetone, 112 g/100 g ethyl acetate, 52.5 g/100 g chloroform, 50 g/100 g benzene, 23.4 g/100 g toluene, 19.4 g/100 g o-xylene, 0.6 g/100 g CCl_4, 0.25 g/100 g ligroin
Conversion factor	4.0 µg/m^3 per ppb at 25ºC

III. Major Uses and Sources

Maleic anhydride is used as a chemical intermediate in the synthesis of fumaric and tartaric acid, certain agricultural chemicals, resins in numerous products, dye intermediates, and pharmaceuticals (HSDB, 1995). It is also used as a co-monomer for unsaturated polyester resins, an ingredient in bonding agents used to manufacture plywood, a corrosion inhibitor, and a preservative in oils and fats.

IV. Effects of Human Exposure

There are several case reports describing asthmatic responses possibly resulting from exposure to maleic anhydride. An individual showed an acute asthmatic reaction after exposure to dust containing maleic anhydride (Lee *et al.*, 1991). Workplace concentrations of maleic anhydride were 0.83 mg/m^3 in the inspirable particulate mass and 0.17 mg/m^3 in the respirable particulate mass. Bronchial provocation testing was performed with phthalic anhydride, lactose, and maleic anhydride. Exposure of this individual to maleic anhydride (by bronchial provocation testing) at 0.83 mg/m^3 and 0.09 mg/m^3 in inspirable and respirable particulate mass, respectively, showed a response of cough, rhinitis, and tearing within two minutes. Within 30 minutes, rales developed in both lungs and peak flow rate decreased 55%.

An individual occupationally exposed to maleic anhydride developed wheezing and dyspnea upon exposure (Gannon *et al.*, 1992). After a period without exposure, two re-exposures both resulted in episodes of severe hemolytic anemia. There was no evidence of pulmonary hemorrhage. Radioallergosorbent testing showed specific IgE antibodies against human serum albumin conjugates with maleic anhydride, phthalic anhydride, and trimellitic anhydride, but not with tetrachlorphthalic anhydride. A critique of the Gannon *et al.* (1992) study by Jackson and Jones (1993) questions the relationship of maleic anhydride exposure to the onset of the anemia, since there were extended periods of exposure to maleic anhydride before symptoms appeared.

Another case report described occupational asthma due to exposure to maleic anhydride (Guerin *et al.*, 1980).

Humans exposed to maleic anhydride showed respiratory tract and eye irritation at concentrations of 0.25 to 0.38 ppm maleic anhydride (Grigor'eva, 1964). No irritation was reported at 0.22 ppm maleic anhydride.

V. Effects of Animal Exposure

Short *et al.* (1988) chronically exposed CD rats (15/sex/group), Engle hamsters (15/sex/group), and rhesus monkeys (3/sex/group) to maleic anhydride by inhalation. Four groups of each species were exposed to concentrations of 0, 1.1, 3.3, or 9.8 mg/m^3 maleic anhydride for 6 hours/day, 5 days/week, for 6 months in stainless steel and glass inhalation chambers. Solid maleic anhydride was heated to 53°C to generate vapors, which were then mixed with a stream of nitrogen. Chamber target levels were monitored by gas chromatography as total maleic (maleic anhydride plus maleic acid). No exposure-related increase in mortality occurred. Of the species examined, only rats showed significant changes in body weight during the course of the experiment, with reductions among males in the high-dose groups after exposure day 40 and a transient weight reduction from days 78-127 in the mid-dose group. Animals of all types exposed to any level of maleic anhydride showed signs of irritation of the nose and eyes, with nasal discharge, dyspnea, and sneezing reported frequently. No exposure-related eye abnormalities were reported. The severity of symptoms was reported to increase with increased dose. No dose-related effects were observed in

hematological parameters, clinical chemistry, or urinalysis. No effects on pulmonary function in monkeys were observed. Dose-related increases in the incidence of hyperplastic change in the nasal epithelium occurred in rats in all exposed groups, and in hamsters in the mid- and high-dose groups. Neutrophilic infiltration of the epithelium of the nasal tissue was observed in all species examined at all exposure levels. All changes in the nasal tissues were judged to be reversible. The only other significant histopathological observation was slight hemosiderin pigmentation in the spleens of female rats in the high-dose group.

The teratogenicity and multigeneration reproductive toxicity of maleic anhydride were also investigated (Short *et al.*, 1986). To evaluate teratogenicity, pregnant CD rats were treated orally with maleic anhydride in corn oil at concentrations of 0, 30, 90, or 140 mg/kg-day from gestational days 6-15. Animals were sacrificed on gestational day 20. No statistically significant dose-related effects were observed in maternal weight gain, implantation, fetal viability, post-implantation loss, fetal weight, or malformations. Groups of 10 male rats and 20 female rats/group (F_0 animals) were orally treated with 0, 20, 55, or 150 mg/kg-day maleic anhydride in corn oil to study multigeneration reproductive toxicity. Animals within the same dose group were bred together after 80 days of treatment to produce two F_1 generation animals (F_{1a} and F_{1b}) and animals from the F_1 generation were interbred to produce two F_2 generation animals (F_{2a} and F_{2b}). A significant increase in mortality was observed among both F_0 and F_1 generation animals in the high-dose group. Total body weight was significantly reduced in animals in the high-dose group at Week 11 of exposure for the F_0 generation males and females and at Week 30 of exposure in the F_1 generation males. No consistent pattern of dose- or treatment-related effect on fertility, litter size, or pup survival was observed. Examination of F_0 animals showed necrosis of the renal cortex in the high-dose group (60% of males and 15% of females). Absolute kidney weights were significantly increased in F_1 female in the low- and mid-dose groups, although there was no histological correlate. No changes in organ weight or histology were observed in the F_2 generation animals.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Short *et al.*, 1988
Study population	Rats (15/sex/group), hamsters (15/sex/group)
Exposure method	Discontinuous inhalation exposure (0, 1.1, 3.3, or 9.8 mg/m^3)
Critical effects	Neutrophilic infiltration of the nasal epithelium; respiratory irritation
LOAEL	1.1 mg/m^3
NOAEL	Not observed
Exposure continuity	6 hr/day, 5 days/week
Average experimental exposure	1.10 mg/m^3 for repetitive acute exposures for the LOAEL group
Human equivalent concentration	0.1 mg/m^3 for LOAEL group (gas with extrathoracic respiratory effects, RGDR = 0.096, based on hamster data)

Exposure duration	6 months
LOAEL uncertainty factor	3
Subchronic uncertainty factor	1
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	3
Cumulative uncertainty factor	30
Inhalation reference exposure level	0.001 mg/m^3 (1.0 µg/m^3, 0.0002 ppm, 0.2 ppb)

Short *et al.* (1988) examined the toxicity of maleic anhydride to rats, hamsters, and monkeys by the inhalation route of exposure. Dose- and exposure related effects, although mild and reversible, were observed at all exposure levels. Specifically, exposure to maleic anhydride vapors resulted in hyperplastic change in the nasal epithelium of rats and hamsters (obligate nose breathers). Neutrophilic infiltration of the nasal epithelium was observed in all three species at all levels of exposure. All species also showed signs of irritation at all exposure levels. The observation that acute maleic anhydride is a strong respiratory irritant to humans (ACGIH, 1992) suggests that this is a valid endpoint of toxicity to humans as well. Human exposure at levels as low as ~1 mg/m^3 appears to trigger acute asthmatic reactions in sensitive individuals (Lee *et al.*, 1991). The histological changes observed by Short occurring as a result of inhalation exposure to a known strong irritant such as maleic anhydride are considered to be the adverse effect of repetitive acute exposures, rather than a chronic response in the development of the REL. Because of the smilarities among species and the inclusion of monkeys in the study an interspecies uncertainty factor of 3, rather than 10, was used. Although there is no evidence of a toxic response similar to the development of asthma in animals, the 1.1 mg/m^3 LOAEL from the animal studies of Short *et al.* (1988) results in a REL which should protect asthmatics.

The major strengths of the REL are the availability of multiple-species, multiple-dose subchronic inhalation studies, and the observation of a mild effect LOAEL. The major uncertainties are the lack of human data and the lack of a NOAEL observation.

VII. References

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CHRONIC TOXICITY SUMMARY

METHYL ISOCYANATE

(MIC, CH_3-N=C=O)

CAS Registry Number: 624-83-9

I. Chronic Toxicity Summary

Inhalation reference exposure level	**1 μg/m^3**
Critical effects(s)	Decreased weight gain and lung pathology at cessation of exposure in rats
Hazard index target(s)	Respiratory system; reproductive system

II. Chemical Property Summary (HSDB, 1995)

Description	Colorless liquid
Molecular formula	C_2H_3NO
Molecular weight	57.06
Boiling point	39.1-40.1^{o}C
Vapor pressure	348 mm Hg @ 20°C, 600 mm Hg @ 30°C (Varma and Guest, 1993)
Solubility	10 percent in water @ 15°C
Conversion factor	2.3 μg/m^3 per ppb at 25°C

III. Major Uses and Sources (Dave, 1985; U.S. EPA, 1986; HSDB, 1995)

Methylisocyanate (MIC) is prepared industrially by reacting methylamine with phosgene, oxidizing monomethylformamide at high temperatures ($\geq$ 550°C), or heating metal methylisocyanates. Because of its high reactivity, MIC is used as an intermediate in organic synthesis, most notably in the production of carbamate based pesticides. Tobacco smoke from some brands of cigarettes also contains MIC (about 4 μg per cigarette). Workers exposed to the MIC 8-hour threshold limit value (0.02 ppm) are exposed to approximately 460 μg MIC in a workday.

IV. Effects of Human Exposure

Although occupational exposures to MIC have been documented (Varma, 1986), few known exposures to the general public have occurred. A major exposure occurred in Bhopal, India in 1984. Because of the sudden, short-term release (30-45 minutes), no measurements occurred and the air concentration was estimated as 13 ppm (Dave, 1985) to 100 ppm (Varma, 1986).

The chemical identity of the ultimate toxicant has not been unequivocally determined and may consist of more than one chemical species. Although the chemistry of MIC suggests that hydrolysis to methylamine and dimethylurea is rapid, such hydrolysis in moist air is probably slow, and the reaction with photochemically produced hydroxyl radical is also slow (chemical $T_{1/2}$ about 3 months) (U.S. EPA, 1986). Brown *et al.* (1987) have shown that the alkylisocyanates (e.g., MIC) are relatively resistant (compared to the arylisocyanates) to hydrolysis in water. Hence, despite the high water reactivity of MIC, this compound could possibly persist in the environment for many days after an initial release.

Within 5 days of the initial exposure to MIC at Bhopal, more than 2,000 deaths occurred (Dave, 1985), while 4,000 more deaths were documented during the following decade (Lepkowski, 1994). The initial symptoms among the population living near the MIC plant were irritation and difficulty in breathing (Varma, 1986). Blindness occurred in more than 10,000 exposed persons but later resolved in most cases (Andersson *et al*., 1990) The acute damage that led to death was mainly to the respiratory system, most likely pulmonary edema, bronchospasm, and electrolyte imbalance (Varma, 1986). However extrapulmonary damage, including tissue anoxia, gastrointestinal symptoms, and muscular weakness, were also observed (Dave, 1985). Within a year of the exposure, survivors continued to exhibit damage to the lung and eyes. Fibrosis of the lungs was seen in 30 percent of this group (Dave, 1985).

Reproductive toxicity was observed among women exposed to MIC in Bhopal. Varma (1987) reported 43 percent unsuccessful pregnancies among 865 women who were pregnant at the time of the MIC release. Among the live births, 14 percent of the infants died within 30 days, whereas a death rate of only 3 percent for the same interval was recorded 2 years prior to the release. Bhandari *et al.* (1990) reported increased spontaneous abortions and neonatal deaths among exposed women who were pregnant at the time of exposure compared to a control group in another city. In the latter study, still births and congenital malformations were similar in the exposed and non-exposed groups.

Non-reproductive, non-pulmonary responses were evident in a group of exposed Bhopal residents, 3-years following exposure to the MIC vapors. Loss of vision and loss of visual acuity were more prominent among exposed residents than among unexposed people, and the losses appeared to be dose-dependent (Andersson *et al.*, 1990). In this study, the surrogate for dose was extent of early deaths in a housing cluster. Similarly, cataracts were reported more often among the exposed than among the unexposed group.

The lesions associated with lung damage may be expressed as pulmonary edema for immediate effects (Varma, 1986), and lesions associated with the bronchoalveolar area for long-term effects (Dave, 1985, Varma, 1986). Vijayan *et al.* (1995) studied cellular components of bronchoalveolar lavage (BAL) and pulmonary function in Bhopal patients 1.3, 2.7, and 5.1 years after exposure to MIC. All had lived within 3-miles of the factory and all experienced acute respiratory and ophthalmic symptoms on the day of exposure. All were experiencing continued respiratory symptoms. Among the exposed people, decrements in forced vital capacity and forced expiratory volume (at 1-minute) were observed. In general, the decrements ranged from 12 - 21 percent of predicted values, whereas the control group exhibited decrements of 2 - 4 percent of the expected values. Analysis of the BAL revealed

increases in total cells (all exposed groups), increased absolute numbers of macrophages (all exposure groups), decreased percentage of lymphocytes (2.7 and 5.1 year groups), and increased numbers and percentage of neutrophils (5.1 year group). These cell types are involved, through the secretion of various factors, in inflammatory and immunologic processes in the lung (Reiser and Last, 1986). The Vijayan *et al.* (1995) study thus suggests a long term damage to lung parenchyma among people who survived the initial acute effects of MIC exposure.

In summary, humans exposed acutely by inhalation to MIC may experience long-term (as well as immediate) damage to pulmonary and extrapulmonary systems. In terms of cause of death, the lung is probably the critical target organ for long-term effects from acute exposure, although adverse effects on other organs (e.g., eye, reproductive, and gastrointestinal) also exist. The late responses to the acute exposure suggest an immunological component which could involve several systems, including lung, eye, liver, and kidney. The chemical identity of the ultimate toxicant is unknown and may be represented by more than one compound.

V. Effects of Animal Exposure

Experimental animal studies have been designed to address the experiences of the victims of the Bhopal disaster, in which the exposure has been described as acute because of the short duration (30-45 min). No studies were found that described exposure duration greater than 10 days. However, a chronic component to MIC exposure may exist as a result of slower rates of hydrolysis in air (compared to water), the presence of carbamylated hemoglobin in MIC-exposed people, and the change from edematous to inflammatory and/or fibrotic lesions with time. Further, a glutathione-dependent reversible MIC transport system has been suggested in experimental animals (see below).

MIC is absorbed through the respiratory tract and distributed to non-respiratory organs in experimental animals. In an acute (30 min) inhalation exposure to a dose of ^{14}C-MIC (labeled in the isocyanate moiety) equivalent to one-LC_{50} (23 mg/L), rats accumulated protein-bound radioactivity (including carbamylated proteins) in brain, liver, and kidney, and lung, but not in blood (Bhattacharya *et al.*, 1988). Ferguson *et al.* (1988) exposed guinea pigs by inhalation to 0.47 ppm ^{14}C-MIC (methyl group) for 6-hours. At the end of exposure, the label was found in arterial and venous blood, bile, and urine. At 2.7 days post-exposure, the label decreased to 2-7 percent. MIC was retained in the nasal-laryngeal area of the guinea pigs.

MIC, like reactive isocyanates in general, can react with biological molecules containing amino, alcohol, or sulfhydryl groups, as well as with water. While hydrolysis in an aqueous environment, such as the lung, is theoretically possible, measurements show that alkyl isocyanates are relatively resistant (compared to arylisocyanates) to such hydrolysis (Brown *et al*. 1987). The absence of a role for MIC hydrolytic products, methylamine (MA) or dimethylurea (DMU), is also suggested by the work of Jeevaratnam and Sriramachari (1994) and Sriramachari *et al*. (1994). Inhalation (30 min) or subcutaneous exposure to rats to either hydrolytic product at levels equivalent to the LC_{50} or LD_{50} did not result in death. Similarly, the acute effects of respiratory necrosis and congestion were not duplicated by methylamine

or dimethylurea. However, exposure to these hydrolytic products did lead to interstitial pneumonitis, an observation that suggests MA and/or DMU could lead to subsequent inflammatory responses if sufficient amounts are present.

A role for methylamine in reproductive / developmental toxicity was investigated by Guest and Varma (1991). In a mouse study, pregnant dams were exposed to varying doses (ip) of methylamine as well as the di- and trimethyl compounds. Reproductive toxicity was not observed for the methylamine. However, in cultured embryo experiments, decrements in crown-rump length, yolk-sac diameter, head length, and embryo survival were observed. The concentrations were high (>0.75 mM) and the interpretation of the biological activity of methylamine in terms of inhalation exposure is difficult.

MIC is a carbamylating intermediate; this is the basis for its use in the manufacture of carbamate based pesticides. In the same way, MIC should react with the appropriate functional groups of proteins, peptides, and nucleic acids. However, i*n vitro* studies with cholinesterases, show that such a reaction is not efficient (Brown *et al.*, 1987), an observation which may be explained by the presence of protonated amino groups at physiological pH (Baillie and Slatter, 1991). Brown *et al.* (1987), however, described nonspecific interactions. They reported that the reversal of cholinesterase activity, previously inhibited to 28 percent by an isocyanate, did not occur when the original MIC inhibition was below 28 percent. These considerations indicate that MIC related symptoms are multifactorial.

A transport system for MIC via reduced glutathione (GSH) has been suggested by the discovery of the MIC-adduct, S-(N-methylcarbamoyl)glutathione (SMG), in the bile and the MIC-adduct of N-acetylcysteine (mercapturic acid, AMCC) in the urine of rats exposed to MIC by non-inhalation routes (Pearson *et al.*, 1990; Slatter *et al.*, 1991). The reaction of MIC with GSH and with cysteine is reversible, and can provide a source of free MIC in the tissues (Baille and Slatter, 1991). Similar studies in experimental animals exposed to MIC by inhalation have not been reported. However, humans exposed by inhalation to N,N-dimethylformamide (H-C-(=O)-N$(CH_3)_2$) excrete AMCC in urine (Mraz and Nohova, 1992). Hence a reversible MIC-transport system in animals, including humans, is possible, and the presence of high levels of GSH in human lavage fluid (Cantin *et al.*, 1987) would permit the initiation of this mechanism.

The toxicity of the adduct SMG was tested in mouse embryo culture (Guest *et al*., 1992). Mouse embryos, at day 8 of gestation, were removed from mice and cultured in the presence (and absence) of SMG. Dose-dependent (0.25 - 2 mM) decrements were observed for yolk sac diameter, crown-rump length, somite number, and protein content. Delayed DNA synthesis in the embryos and in yolk-sacs occurred in the presence of 0.25 mM SMG. Similar to the results obtained with methylamine, the SMG concentrations were high and the exposures were not inhalation. However, the data show that a MIC metabolite, SMG, has toxic properties. In the presence of GSH (1 or 3 mM), the extent of the SMG - dependent toxicities were decreased. Such data demonstrate the reversibility of the binding between MIC and GSH.

Three inhalation studies were identified in which experimental animals were exposed to more than one dose of MIC. Among these studies, two used exposure durations for more than one day (Dodd and Fowler, 1986; Mitsumori *et al.*, 1987). Rats and mice were exposed by inhalation to 0, 1.1, and 2.8 (female) or 3.0 (male) ppm MIC for 6 hr/day for a total of 4 days, and then followed during a 91 day post-exposure interval (Mitsumori *et al.*, 1987). Among the rats, post-exposure deaths occurred by 49 days (male) and 14 days (female) at the high dose. Among the mice, only 1 male mouse died at 16 days post-exposure. Reduced weight gain was observed among the female and male rats in the high dose group, prior to death, although the absolute weights were not different from the unexposed rats one day before the end of exposure. Among the mice, a weight gain loss was observed at 3- and 6-days post exposure (male) and 1 day post exposure (female) at the high dose, but normal weight gain returned by 1 week following cessation of exposure. At 7 days post-exposure, microscopic changes were observed in the respiratory system among the high dose rats of both sexes. Between 8- and 27 days post-exposure, increased lesions in the respiratory tract and also in liver, thymus, spleen and heart, and brain were observed at the high dose. Similar lesions were not observed in rats exposed to 1.1 ppm MIC and followed to the 8-27 day post-exposure. Among survivors, the incidence of lesions decreased to control values by 91 days. Among the mice, treatment related changes in the respiratory tract were observed at high dose at 7 days post-exposure. Between 28 and 91 days, the lesions associated with the upper respiratory tract disappeared, whereas those associated the major bronchi remained, although somewhat attenuated. These data suggest the rat is more sensitive than the mouse to the effects of MIC. A LOAEL of 2.9 ppm is indicated, based on post-exposure decreased weight gain and respiratory tract changes in rats.

Dodd and Fowler (1986) exposed rats to 0, 0.15, 0.6, and 3.1 ppm MIC for two 4-day sessions at 6- hours/day and examined the animals within 1-day following exposure. The 2-cycle exposure included a 2-day recess from exposure. No deaths occurred at any MIC concentration during the exposure. Lesser weight gain occurred for rats in the 3.1 ppm groups, whereas weight among the rats in the 0.15 and 0.6 ppm MIC groups was indistinguishable from the air-exposed control animals. On exposure days 3 and 8, mean food consumption values in the high dose group were below those for the non-exposed group. At the time of termination, male rats exposed to 3.1 ppm MIC exhibited a 38 percent increase in hemoglobin concentration and a 26 percent decrease ($p<0.001$) in oxygen saturation, compared to the unexposed rats ($p<0.001$). Such changes were not observed for the female rats exposed to 3.1 ppm or for rats of either sex exposed to 0.15 or 0.6 ppm. Absolute lung weights increased ($p<0.001$) among both sexes in the 3.1 ppm exposure group, compared to the control rats. Decreases in liver, kidney and testes absolute weights were observed in this exposure group, but the authors interpreted these data as a reflection of the body weight losses. No weight changes were observed in rats exposed to 0.15 or 0.60 ppm MIC. Gross and microscopic lesions were observed in rats (female and male) exposed to 3.1 ppm, but not in rats exposed to 0, 0.15, or 0.6 ppm MIC. The microscopic lesions occurred in the respiratory tract and consisted of inflammation, epithelial necrosis, squamous metaplasia, and epithelial hyperplasia. These lesions extended into the bronchioles. These data suggest a NOAEL of 0.6 ppm MIC, based on weight gain loss, absolute lung weight and lung histopathology in rats, immediately following cessation of exposure.

Post-exposure changes in lung pathology also occurred in the 3.1 ppm surviving rats in the Dodd and Fowler (1986) study. The early lesions associated with inflammation, epithelial necrosis, squamous metaplasia, and epithelial hyperplasia extending to the bronchioles either decreased in severity or receded toward the upper respiratory tract by 85-days post-exposure. In males, the intraluminal and submucosal fibroplasia changed in appearance during this interval, due in part to the maturation of fibrous tissue. Mucous plugs were also seen in the terminal bronchioles and alveoli in some rats. The importance of this observation is the progressive character of MIC induced lung disease. Such progression may be difficult to follow at lower doses, if the times involved are beyond experimental capability.

Sethi *et al.* (1989) exposed rats by inhalation to 0, 0.21, 0.26, and 0.35 ppm MIC for 6 days at 0.5 hr/day. Statistical evaluation was not presented. No post-exposure deaths were reported, although lethality was recorded for rats exposed to 3.5 and 35 ppm for only 10 minutes. Following the 0.5 hr x 6-day exposure, the weight gain declined in proportion to the exposure dose. At the lowest dose (0.21 ppm) the weight gain was 111 gm after 91 days post-exposure, compared to a weight gain of 218 gm during the same interval among the non-exposed rats. The absolute weights of the rats at the end of the exposure were not given. According to the narrative, inflammatory lesions of bronchopulmonary tissue were present; their extent increased with dose. A dose-response increase in markers of lung infection were present and suggest that the MIC exposed rats were more prone to infectious agents than were the unexposed animals. Non-specific lesions in liver and kidneys were also observed and appeared to be dose dependent, but the authors suggest these effects could be a result of the lung infections.

Fetotoxicity was observed in two experimental animal studies (Schwetz *et al.*, 1987; Varma, 1987). Among female mice exposed to 0, 1, or 3 ppm MIC during gestation days 14 - 17 for 6 hr/day, an increased incidence of fetal deaths was observed at 1 ppm (Schwetz *et al.*, 1987). At 3 ppm, the average number of pups/litter decreased relative to the air-exposed controls. The dams were unaffected in terms of survival, body weight, or length of gestation. Non-gestational exposure (6 hr/day, 4 days) did not affect the number of pregnancies or the live litter sizes, suggesting that the fetotoxic effect may be specific to the female reproductive tract rather than a general attribute of systemic toxicity. Similarly, female mice exposed for 3 hours on gestation day 8 to 0, 2, 6, or 9 ppm MIC gave birth to pups with decreased body weights at the lowest dose, although a good dose-response was not observed (Varma, 1987). At 9 or 15 ppm MIC, the surviving dams lost 75 - 80 percent of their fetuses. Maternal mortality and decreased skeletal lengths were also observed at 9 and 15 ppm. A distinction between maternally induced fetotoxicity and a direct effect on fetal health could not be made. Because the inhalation exposure to the dams occurred for only 3 hrs on one day, a chronic LOAEL is not suggested. Exposure of male rats to one dose of 3.2 mg/L for 8-min resulted in a 21 percent fertility rate among the cohabited female rats within the first 14 days post-exposure, but the rates increased after 15 days post-exposure (Agarwal and Bose, 1992). There was no evidence of fetotoxicity among the dams impregnated by the MIC-exposed male rats. Exposure of male and female mice to 0, 1, or 3 ppm MIC did not result in altered body weights, fertility, or litter size (Schwetz *et al*, 1987). The results suggest that exposures to MIC at doses that are not toxic to adult male or female (pregestational) mice or rats do not result in adverse reproductive outcomes.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Dodd and Fowler (1986)
Study populations	F344 rats
Exposure method	Inhalation (0, 0.15. 0.58, or 3.1 ppm)
Critical effects	Decreased weight gain and lung pathology at immediate cessation of exposure
LOAEL	3.1 ppm
NOAEL	0.58 ppm
Exposure continuity	6 hours/day, 8 days/10 day experiment (2-cycles, with one 2-day recess from exposure)
Exposure duration	10 days
Average experimental exposure	0.12 ppm for the NOAEL group
Human equivalent factor	0.15 ppm for the NOAEL group (gas with pulmonary respiratory effects, RGDR = 1.23, based on BW = 152 g, MV = 0.12 L/min, SA = 225 cm^2)
LOAEL uncertainty factor	1
Subchronic factor	10
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	300
Inhalation reference exposure level	0.5 ppb (1 $\mu g/m^3$)

The Dodd and Fowler (1986) study includes the longest exposure duration of the available investigations and also uses some of the lower exposure levels (down to 0.15 ppm). The microscopic findings of the respiratory tract were statistically analyzed, although an observation of the tabulated data at the four doses (0, 0.15, 0.6, and 3.1 ppm) clearly shows a NOAEL of 0.6 ppm. Other endpoints with the same NOAEL were increased hemoglobin and increased absolute lung weights. The symptomatic ramifications of the increased hemoglobin are unknown, although similar increases were reported for humans exposed to MIC in Bhopal (Srivastava *et al.*, 1988). The lung weight gain may be a reflection of the pathological changes seen in the microscopic studies.

Decreased body weight gain was also seen in the experimental 4 day rat inhalation study of Mitsumori *et al.* (1987) (NOAEL = 1.0 ppm), except that the decrease in the latter study did not occur until 1 and 3 days (female and male, respectively) post-exposure. The apparent discrepancy could be explained, in part, on the basis of the length of exposure, which was twice as long in the Dodd and Fowler (1986) study. However, the weight gain loss in the Dodd and Fowler (1986) study was initiated within one day of the start of exposure.

The MIC chronic REL of 0.4 ppb is based on endpoints observed within 1 day of cessation of exposure. Post-exposure evaluation showed that, at a higher exposure level (3.1 ppm), progressive changes, including death, occurred. Post-exposure observations, however, were

not reported at the 0.15 and 0.6 ppm MIC levels. The attribute of delayed MIC inhalation toxicity has also been observed in other experimental animals studies (Dodd and Fowler, 1986, Mitsumori *et al.*, 1987). In the case of the human MIC exposure in Bhopal India, death did not occur during the immediate 30 - 45 minute exposure, but exhibited a lag phase. A few deaths occurred during the first few hours, the maximum occurred at 2 - 3 days, and by the end of a week about 2500 deaths were documented (Dave, 1985; Varma, 1986; Varma and Guest , 1993), although Varma (1986) suggests that the immediate number may be closer to 5,000. One report suggests that during the intervening decade as many as 6,000 deaths may be attributed to the initial exposure in Bhopal (Lepkowski, 1994). Such information suggests that the presence of an adverse effect at the NOAEL of 0.6 ppm (Dodd and Fowler, 1986) might be possible if the rats were observed during an extended post-exposure interval. Experimental evidence is needed to test this hypothesis.

Only one study was identified in which post-exposure observations were made on experimental animals exposed subchronically by inhalation to multi-doses of MIC. Mitsumori *et al.* (1987) exposed rats to 0, 1.1, and 2.9 ppm MIC for 6 hr/day for 4 days and observed the rats for 91 days. No deaths and no weight gain loss (compare Dodd and Fowler, 1986) were present until the post-exposure period at 2.9 ppm. Using a NOAEL of 1.1 ppm MIC, a chronic REL of 0.28 ppb (0.6 $\mu g/m^3$) was derived. The REL based on the Mitsumori *et al.* (1987) study is similar to the REL based on immediate effects (Dodd and Fowler, 1986), and may indicate that the time of occurrence of exposure related effects may not be as important as the MIC air concentration.

The strengths of the inhalation REL include the availability of controlled exposure inhalation studies in multiple species at multiple exposure concentrations and with adequate histopathogical analysis and the observation of a NOAEL. Major areas of uncertainty are the lack of adequate human exposure data and the lack of chronic inhalation exposure studies.

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CHRONIC TOXICITY SUMMARY

4,4′-METHYLENE DIANILINE

(MDA; 4,4'-diaminodiphenylmethane; 4,4'-diphenylmethanediamine; DAPM; dianilinemethane)

CAS Registry Number: 101-77-9

I. Chronic Toxicity Summary

Inhalation reference exposure level	**20 µg/m^3**
Critical effect(s)	Ocular toxicity to the retinas of guinea pigs
Hazard index target(s)	Eyes; alimentary system (hepatotoxicity)

II. Chemical Property Summary (HSDB, 1995)

Description	Colorless to pale yellow flakes; tan
Molecular formula	$C_{13}H_{14}N_2$
Molecular weight	198.3
Boiling point	398-399°C
Vapor pressure	1 mm Hg @ 197°C
Solubility	Soluble in alcohol, benzene, ether; 273g/100g acetone; 0.1g/100g water @ 25°C
Conversion factor	8.1 µg/m^3 per ppb at 25ºC

III. Major Uses and Sources

4,4'-Methylene dianiline (MDA) is synthesized by the reaction of aniline with formaldehyde. MDA's major uses are as a chemical intermediate in the synthesis of certain isocyanates and polyurethane polymers, as a corrosion inhibitor, in the preparation of azo dyes, as a rubber preservative, and in the curing of epoxy resins and neoprene (HSDB, 1995; ACGIH, 1992).

IV. Effects of Human Exposure

Several cases of human exposure to MDA have clearly identified the compound as a hepatotoxicant which produces a condition resembling viral hepatitis (Kopelman *et al.*, 1966; McGill and Motto, 1974; Williams *et al.*, 1974; Bastian, 1984). Bastian (1984) described cases of acute hepatic illness in four workers exposed from laying floors using an epoxy resin base which contained MDA as a curing agent. The workers were exposed via fumes and dusts in the air as well from contact with powder to the hands and had worked with epoxy resins for periods ranging from one to 12 years. The level of exposure was not quantitated. The workers initially reported to the hospital with symptoms of abdominal pain three days after the most recent

exposure and all were discharged within four days. Two workers continued to show severe symptoms five days after the onset, with abdominal pain, jaundice, a tender liver, nausea, dyspnea, and muscular pain. Plasma bilirubin, alkaline phosphatase, and aspartate aminotransferase levels were elevated. Some symptoms did not subside until two months after the onset. One worker, after another exposure, experienced nausea, abdominal pain, and muscular pain. A second worker reported further symptoms of headache, tiredness, and decreased libido.

Williams *et al.* (1974) reported symptoms in 6 of approximately 300 workers exposed to MDA by surface coating concrete walls with epoxy resins. Exposure probably occurred by inhalation, ingestion, and skin contact as a result of mixing powder containing MDA. Symptoms of clinical hepatitis in the 6 workers appeared two days to two weeks after beginning work, with five of the six having elevated bilirubin levels, and one liver biopsy showing bile stasis. All the workers recovered completely after an unspecified time.

McGill and Motto (1974) described hepatitis among 13 men who, over the course of 6 years, were occupationally exposed to MDA in the blending of epoxy resins used in the manufacture of insulating material. Among the 13 patients showing symptoms, all reported weakness, jaundice, and dark urine, 11 reported abdominal pain, nausea or vomiting, and anorexia; and over half reported fever, chills and/or headache. All the workers recovered within a 10 week period. After the first cases of hepatitis occurred, air sampling showed initial levels of MDA to be 0.1 ppm in the work area. After additional cases of hepatitis occurred, measures were taken to reduce worker exposure and air levels were reduced to as low as 0.0064 ppm. The authors concluded that percutaneous absorption was the likely major route of exposure in light of the fact that cases occurred in spite of measures taken to reduce air levels and there was evidence that significant hand contact with the compound occurred during the workday. Since the symptoms appeared within one to 18 days after “working intensively” with the compound and exposure routes were not clearly established, quantitation of exposure levels was considered difficult to establish.

The most well-known case of MDA toxicity to humans resulted from ingestion of bread made with flour contaminated with MDA during transport (Kopelman *et al.*, 1966a). Eighty-four persons showed symptoms of abdominal pain and some degree of jaundice. All patients had elevated serum alkaline phosphatase and glutamic oxaloacetic transaminase levels. Seventeen had serum bilirubin levels over 5 mg/100 ml. Liver biopsy was performed on 8 persons and evaluated in a separate study (Kopelman *et al.*, 1966b). The primary finding was an unusual lesion described during the early course of the disease as portal zone cholangitis and later as centrilobular cholestasis with necrosis. The initial study reported that all but 2 patients had complete recovery within several weeks. However, a two year follow-up study of 14 individuals showed that 10 still had symptoms of some severity 7 to 23 months after initial onset including food intolerance, gastrointestinal disturbances, fatigue, and visual disturbances (Kopelman, 1968).

Human effects other than hepatotoxicity have been described including several cases of contact dermatitis and skin sensitization (LeVine, 1983; Van Joost *et al.*, 1987; de Pablo *et al.*, 1992; Bruynzeel and van der Wegen-Keijser, 1993). A case report of a man exposed to MDA with

potassium carbonate and γ-butyrolactone by accidental ingestion has been described (Roy *et al.*, 1985). In addition to hepatitis and abnormal liver function, which persisted over 18 months, the patient developed a progressively worsening retinopathy described as a "malfunction of the retinal pigment epithelium" accompanied by diminished visual acuity. The patient improved after approximately 3 months, but after examination at 18 months had not completely recovered.

Another report described the development of acute cardiomyopathy in addition to hepatitis in a worker exposed to a large quantity of MDA dust as the result of air filtration malfunction (Brooks *et al.*, 1979). The patient showed an abnormal ECG and an elevated cardiac LDH isoenzyme profile, which returned to normal within one month of onset.

V. Effects of Animal Exposure

The carcinogenicity of MDA was investigated in F344/N rats and $B6C3F_1$ mice (50/sex/dose group) administered in the drinking water at concentrations of 0, 150, and 300 ppm MDA (dihydrochloride) for 103 weeks (Lamb *et al.*, 1986). A 14-day range finding study was also conducted with 5 animal/sex/species/dose group, with exposure levels of 0, 200, 400, 800, 1600, and 3200 ppm MDA. A 13-week subchronic study was conducted with 10 animals/sex/species/dose group, with exposure levels of 0, 25 (mice), 50, 100, 200, 400, and 800 (rats) ppm MDA. Using body weight and drinking water values from the study, low and high daily doses in the chronic study were calculated to be 9 and 16 mg/kg-day for male rats, 10 and 19 for female rats, 25 and 57 for male mice, and 19 and 43 for female mice. In the chronic study, survival was reduced among male mice treated with 300 ppm MDA. Final mean body weights were reduced in the 300 ppm dose group of female rats (-9%), male mice (-13%), and female mice (-16%). Among rats, non-cancer effects included follicular cysts and follicular-cell hyperplasia of the thyroid (significantly increased incidence in high-dose females; $p<0.05$ by Fisher's exact test). In the liver, the incidence of fatty and focal cellular change was elevated in low-dose male and female rats and also in high dose male rats. Incidence of unspecified dilatation of the liver was also elevated in high-dose male rats. Increased incidence of kidney mineralization was found in male rats treated with 300 ppm MDA. Among mice, incidence of liver degeneration was elevated in males in both treatment groups and females in the high-dose group ($p<0.01$ by Fisher's exact test). Incidence of kidney nephropathy was increased in male and female mice in both treatment groups and mineralization of the renal papilla was increased in both sexes in the high-dose group ($p<0.01$). From the 13-week study, the authors noted thyroid and bile duct effects in rats at 800 ppm MDA and in mice at 400 ppm.

Albino and pigmented guinea pigs were exposed to aerosols of methylene dianiline in polyethylene glycol 200 (PEG) in nose-only exposure chambers (Leong *et al.*, 1987). Animals (8 of each strain) were exposed to a time-weighted average aerosol concentration of 0.44 g MDA/m^3 in air for 4 hours/day, 5 days/week for 2 weeks. Eight control animals were neither exposed to aerosol nor placed in the exposure chamber. Two weeks after the exposure period, animals were evaluated for dermal sensitization and irritation by challenge with 0.05 ml of 0, 2, 20 and 200 mg MDA/ml in PEG for up to 24 hours. No evidence of dermal irritation or sensitivity was found. Subsequently, the animals were also examined for pulmonary sensitization by challenge with aerosols containing 0.01 and 0.05 ml of 200 mg MDA/ml PEG.

Lung insufflation pressures were measured as an indication of changes in lung distensibility. No evidence of pulmonary sensitization was found. After the pulmonary challenge, the animals were examined histopathologically, with emphasis on eye, lung, liver, and kidney toxicity. Ocular toxicity ranging from mild to more severe was observed in all MDA-treated animals, but in none of the control animals. Pigmented animals did not differ in sensitivity or effect compared to albino animals. Mild lesions were described as "retraction and thickening of the outer segments of the photoreceptor cells" while more severe effects included swelling "through the inner segments of the photoreceptor cells to the outer nuclear layer." Some evidence of inflammatory cell infiltration was also noted and the pigmented epithelial layer was also degenerated. The authors conclude that the effects were attributable to MDA because no retinal lesions have been associated with exposure to the PEG vehicle. Furthermore, the inhalation exposures to MDA are the likely cause rather than the dermal and lung sensitization study exposures because these subsequent studies were conducted on control as well as treated animals. Pulmonary granulomas consisting of "an aggregate of macrophages surrounded by a thin mantle of lymphocytes" were found in 7 of the 16 MDA-exposed animals and one of the 8 control animals (level of significance was not stated). Treated and control animals had a high background incidence of pulmonary lesions including slight to mild bronchitis. No liver or kidney effects were detected in treated animals.

Nine purebred beagle dogs were treated orally (by capsule) with 70 mg "crude" (4 dogs) or "purified" (5 dogs) MDA in corn oil three days per week for a period ranging from approximately 3 to 7 years (Deichmann *et al.*, 1978). No concurrent controls were included since untreated animals were regularly maintained in the laboratory. After 2 years, cystoscopic examination was performed at 15-month intervals. After 4½ years, clinical chemistry tests were performed at 4 month intervals on 3 dogs from each group. Microscopic examination of urinary bladder, liver, heart, ovaries, uterus, and lymph nodes was performed on moribund animals or at the end of the experimental period (7 years, 2 months). Liver toxicity was noted in all the treated animals. Effects were described as fatty change, cell degeneration and necrosis, and lymphoid cell infiltration. One dog from each treatment group died from the toxic effect on the liver. The kidneys of four treated animals (two from each group) showed toxic effects including granuloma, glomerular nephritis, and congestion with cloudy swelling. Two dogs treated with "purified" and one dog treated with "crude" MDA showed toxicity to the spleen described as hemosiderosis and swelling with lymphocyte infiltration.

Wistar rats (5/sex/dose) were treated orally with 0, 0.0083 and 0.083 g MDA/kg body weight in propylene glycol daily for 12 weeks (Pludro *et al.*, 1969). Doses were 1% and 10% of the experimentally determined median lethal dose. No significant changes in body weight or hematological parameters were found, although serum albumin, β-globulin, and γ-globulin were elevated in animals in the 0.083 mg/kg dose group. The livers of all the animals in the high dose group showed signs of degeneration, including atrophy of the parenchyma and stromal hyperplasia in the portal areas. Also in this dose group, all animals showed hypertrophy of the lymphatic nodules of the spleen. In the low dose group, one animal showed a liver lesion and one a lesion in the spleen.

Schoental (1968) treated rats (8/sex) with MDA in 25% aqueous ethanol by stomach tube. Rats were given 20 mg doses a total of 2-5 times over several weeks up to 7½ months (frequency not

specified). Animals showed necrosis of the liver and kidney and showed congestion and edema of the lungs.

Visual toxicity was reported in 15 cats treated perorally with 25-100 mg MDA/kg body weight in a 1% aqueous suspension (Schilling von Canstatt *et al.*, 1966). In four animals treated once with 100 mg/kg, no blindness was reported. In all the other treated animals (four with one dose of 100 mg/kg, two with one dose of 150 mg/kg, and two with three doses of 25 mg/kg and 3 doses of 50 mg/kg), blindness occurred within 8 days. Three of the eight recovered sight within 4 days. Two other treated animals were examined microscopically, one treated with 25 and then 50 mg/kg and one treated once with 200 mg/kg. The first was examined after 7 days and showed signs of granular degeneration of the rods and cones with some proliferation of the pigmented epithelium. The second was examined after 4¼ years and showed atrophy of the retinal neuroepithelium. The authors note that no visual disturbances were found in other MDA treated experimental animals including dog, rabbit, guinea pig and rat.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Leong *et al.*, 1987
Study population	Guinea pigs
Exposure method	Discontinuous inhalation exposure (nose only) of aerosols
Critical effects	Degeneration of retinal epithelium
LOAEL	440 mg/m^3 (54 ppm)
NOAEL	Not observed
Exposure continuity	4 hours/day, 5 days/week
Exposure duration	2 weeks
Average experimental exposure	52 mg/m^3 for LOAEL group (440 x 4/24 x 5/7) (6.4 ppm)
LOAEL uncertainty factor	10
Subchronic factor	10
Interspecies uncertainty factor	10
Intraspecies uncertainty factor	10
Cumulative uncetainty factor	3,000 (maximal uncertainty factor - due to lack of independence of 4 areas of uncertainty (USEPA, 1994))
Inhalation reference exposure level	0.02 mg/m^3 (20 µg/m^3; 0.002 ppm; 2 ppb)

Two specific types of toxicity have been associated with exposure to MDA: hepatotoxicity and ocular toxicity. Several studies have demonstrated hepatotoxicity in experimental animals. The best study of long term toxicity of MDA was the report by Lamb *et al.* (1986). In addition to addressing the carcinogenicity of MDA, Lamb described non-cancer health effects, which resulted from lifetime exposure of two species, rats and mice, to MDA at two concentrations in the drinking water. The 150 ppm dose level was a LOAEL for fatty change and focal cellular change to the livers of male and female rats as well as for liver degeneration in male mice. The corresponding effects were also observed in high-dose male rats and male mice. Nephropathy

was observed in mice of both sexes at the 150 and 300 ppm. There is abundant evidence from both human and animal studies that MDA is hepatotoxic. Bastian (1984), Williams *et al.* (1974), and McGill and Motto (1974) reported hepatitis in people exposed by inhalation and dermal absorption routes. Kopelman *et al.* (1966a,b) demonstrated human hepatotoxicity from exposure by the oral route. Limited data detailing exposure levels associated with adverse health effects in humans preclude the development of a chronic REL from studies in humans.

The other toxic effect of potential concern from MDA exposure is ocular toxicity. Leong *et al.* (1987) reported damage to the retinas of guinea pigs exposed for 2 weeks to MDA aerosols (0.44 g/m^3 for 4 hr/day, 5 days/week; average experimental exposure = 52 mg/m^3) by inhalation. Schilling von Canstatt *et al.* (1966) also report blindness in cats treated orally with MDA. A single case of retinopathy and visual toxicity in humans was reported in a man who accidentally ingested MDA with potassium carbonate and γ-butyrolactone. The Leong *et al.* (1986) study was selected for the development of the chronic REL because, although conducted for a relatively short period of time, the study appears to address the most sensitive endpoint of toxicity by the most appropriate route of exposure (inhalation). The studies which establish the hepatotoxicity of MDA were conducted by the oral route of exposure.

The strengths of the inhalation REL include the availability of a controlled exposure inhalation study. Major areas of uncertainty are the lack of adequate human exposure data, the lack of chronic inhalation exposure studies, the lack of reproductive and developmental toxicity studies, the lack of observation of a NOAEL, and the use of animals under additional stress due to the restraint used to obtain nose-only exposure. (Control animals were not restrained.) Liver toxicity has been included as a potential critical effect because of uncertainty regarding the relative potency of this compound in causing this effect in different species by different routes of exposure.

VII. References

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CHRONIC TOXICITY SUMMARY

METHYLENE DIPHENYL ISOCYANATE POLYMER

(diphenylmethane diisocynate)

CAS Registry Number: 101-68-8

I. Chronic Reference Exposure Level

Inhalation reference exposure level	**0.5 µg/m^3**
Critical effect(s)	Hyperplasia of the olfactory epithelium in rats
Hazard index target(s)	Respiratory system

II. Physical and Chemical Properties (HSDB, 1995)

Description	Light yellow solid
Molecular formula	$C_{15}H_{10}N_2O_2$ (monomer)
Molecular weight	Variable (monomer = 250.27)
Density	1.197 g/cm^3 @ 70°C (monomer)
Boiling point	196°C (monomer)
Melting point	37°C (monomer)
Vapor pressure	0.001 mm Hg @ 40°C (monomer)
Solubility	Soluble in acetone, benzene, kerosene, and nitrobenzene (monomer)
Conversion factor	Not applicable for polymer (for monomer 1 ppm = 10.2 mg/m^3 at 25°C)

III. Major Uses or Sources

Methylene diphenyl isocyanate (MDI) is used for bonding rubber to nylon. MDI is also used in the manufacture of lacquer coatings and in the production of polyurethane resins and spandex fibers (HSDB, 1995).

IV. Effects of Human Exposure

A 5-year occupational study of 107 workers from a polyurethane plastic manufacturing plant examined pulmonary function, respiratory symptoms, and smoking habits (Musk *et al.*, 1982, 1985). No significant changes in pulmonary function or respiratory symptoms were observed when controlled for smoking. Mean MDI concentrations measured ranged from 0.0003 to 0.0006 ppm.

Significant increased prevalence of asthma in female workers and chronic bronchitis in male and female workers was observed following occupational exposure to low levels of MDI (<0.02 ppm) (Pham *et al.*, 1988). Workers from two plants were grouped by job classification and evaluated in this study conducted in 1976; workers were grouped as unexposed (62 men, 21 women), indirectly exposed (61 men, 56 women), or directly exposed (91 men, 27 women). Further characterization of the exposure groups was not presented. Decrements in pulmonary function (measured by VC, FEV_1 and single-breath carbon monoxide diffusion tests) were observed in men in the direct and indirect exposure groups: decrements in men with a history of direct exposure to MDI were statistically significant. Workers were also grouped by duration of occupational exposure (<20 months, 20-60 months, >60 months). Workers with known (direct or indirect) occupational exposure to MDI for greater than 60 months exhibited statistically significant decrements in pulmonary function tests. The follow-up examination of this study describes data from male workers only. At the time of the 5-year follow-up, air levels had been reduced to below the maximum allowed air concentration of 0.005 ppm by a modification of the ventilation system. Statistically significant decrements in pulmonary function were observed again in workers with known direct occupational exposure to MDI. Workers who were exposed at the time of the 1976 study but had since been removed from exposure did not exhibit decrements in pulmonary function, leading the authors to conclude that the effects of low-level exposure to MDI are to some extent reversible. Flaws in study design, including lack of exposure characterization, attrition, and inclusion of asthmatics in cohorts, preclude a quantitative assessment of MDI exposure on lung function.

An epidemiologic study of foundry workers reported more respiratory symptoms and significantly lower mean FEV_1 and maximum mid-expiratory flow at 25-75% in exposed workers compared to controls (Johnson *et al.*, 1985). However, MDI-exposed workers also had unquantified exposure to silica, metal dust, phenol formaldehyde, and a pyridine derivative precluding the evaluation of respiratory effects resulting from MDI exposure.

A worker with 5 years occupational exposure and suspected MDI hypersensitivity was exposed continuously in a controlled chamber to 5 ppb for 15 minutes, then 10 ppb for 30 minutes, and 20 ppb for 15 minutes (Marczynski *et al.*, 1992). The worker had not been exposed to MDI in the workplace for 5 days prior to the test challenge. Exposure to MDI resulted in an immediate, moderate, asthmatic reaction associated with significant hypoxemia.

V. Effects of Animal Exposure

Rats were exposed to 0.2, 1.0, and 6.0 mg/m^3 aerosolized MDI polymer 6 hours per day, 5 days per week for 24 months (Reuzel *et al.*, 1990; 1994). Statistically significant increased incidences of basal cell hyperplasia, olfactory epithelial degeneration, alveolar duct epithelialization, localized alveolar bronchiolization, and adenomas were observed in male and female rats exposed to 6.0 mg/m^3 MDI. An accumulation of macrophages with yellow pigment was also noted in the lungs and mediastinal lymph nodes. Male rats exposed to this concentration also exhibited a statistically significant increase in the incidence of Bowman's gland hyperplasia. Male rats exposed to 1 mg/m^3 MDI also exhibited statistically significant increased incidences of

basal cell hyperplasia and Bowman's gland hyperplasia. An accumulation of macrophages with yellow pigment was observed in the lungs of female rats and the lungs and mediastinal lymph nodes of male rats exposed to 1 mg/m^3. No adverse effects were noted in rats exposed to 0.2 mg/m^3 MDI.

Guinea pigs were exposed to 2 ppm MDI 3 hours per day for 5 days (Aizicovici *et al.*, 1990). Qualitative immunostaining techniques indicated that MDI was localized in the respiratory tract. The spleen, lymph nodes, and thymus had very little staining. However, another study exposed guinea pigs to 4 ppb radiolabelled toluene diisocyanate (TDI) for 1-hour and found measurable radioactivity in extrathoracic tissues and body fluids (Kennedy *et al.*, 1989). Therefore, there is a possibility that MDI may be transported to sites other than the respiratory tract, such as the ovaries and testes, following inhalation exposure.

Gravid Wistar rats, Crl:(WI)BR, were exposed by whole-body inhalation to clean air (control) and to 1, 3, and 9 mg/m^3 MDI, respectively, for 6 hr per day from days 6 to 15 post conception (Buschmann et al., 1996). Rats were killed on day 20. The lung weights in the high-dose group were significantly increased compared to the sham-treated control animals. Treatment did not influence any other maternal and/or fetal parameters investigated (including maternal weight gain, number of corpora lutea, implantation sites, pre-and postimplantation loss, fetal and placental weights, gross and visceral anomalies, and degree of ossification). A slight but significant increase in litters with fetuses displaying asymmetric sternebra(e) was observed after treatment with the highest dose. Although the relevance of an increase of this minor anomaly in doses which maternal toxicity is limited and within the limits of biological variability, a substance-induced effect in the high-dose group cannot be excluded with certainty. Thus, the authors reported a NOAEL of 3 mg/m^3 for embryotoxic effects.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Reuzel *et al.*, 1990; 1994
Study population	Rats
Exposure method	Inhalation of polymeric aerosolized MDI (0.2, 1.0, and 6.0 mg/m^3)
Critical effects	Hyperplasia of the olfactory epithelium
LOAEL	1 mg/m^3
NOAEL	0.2 mg/m^3
Study continuity	6 hours per day, 5 days per week
Study duration	24 months
Average experimental exposure	0.036 mg/m^3 for NOAEL group (0.2 x 6/24 x 5/7)
Human equivalent concentration	0.016 mg/m^3 for NOAEL group (particle with extrathoracic respiratory effects, RDDR = 0.453, based on MMAD = 0.68 µm and sigma g = 2.93)
LOAEL uncertainty factor	1
Subchronic uncertainty factor	1
Interspecies uncertainty factor (UF)	3

Intraspecies uncertainty factor (UF)	10
Cumulative uncertainty factor	30
Inhalation reference concentration	0.5 $\mu g/m^3$

Strengths of the REL include the use of a well-conducted long-term inhalation study and the observation of a NOAEL. A major limitation of the REL is that it is based on data on exposures to MDI "polymer" which actually contains nearly 50% monomer. Monomers frequently are much more toxic than polymers. Effects of pure monomeric MDI may occur at concentrations somewhat lower than observed in the reported study on MDI polymer.

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CHRONIC TOXICITY SUMMARY

NITRIC ACID

(aqua fortis; hydrogen nitrate; red and white fuming nitric acid)

CAS Registry Number: 7697-37-2

I. Chronic Toxicity Summary

Inhalation reference exposure level	**40 μg/m^3**
Critical effect(s)	Pulmonary edema, emphysema, inflammation in rats
Hazard index target(s)	Respiratory system

II. Physical and Chemical Properties (HSDB, 1995)

Description	Fuming colorless or yellowish liquid with a characteristic choking odor
Molecular formula	HNO_3
Molecular weight	63.02
Boiling point	83°C
Vapor pressure	0.3513 torr @ 25°C
Solubility	Soluble in all proportions in water, soluble in ether
Conversion factor	1 ppm = 2.58 mg/m^3 at 25°C

III. Major Uses or Sources (HSDB, 1995)

Nitric acid (HNO_3) is the most common strong acid which is also a strong oxidizing agent. It is used to dissolve noble metals, and in the etching and cleaning of metals. It is also used to make nitrates and nitro compounds, especially organic compounds, many of which are commercial or military explosives. HNO_3 is also used to destroy residues of organic matter. The primary use of nitric acid is the production of ammonium nitrate fertilizer. Decomposition of HNO_3 releases nitrogen dioxide (NO_2) and nitric oxide (NO). In practice, HNO_3 is usually found in conjunction with NO_2 which appears to be more hazardous (ACGIH, 1991). HNO_3 is also present in indoor and outdoor air pollution.

IV. Effects of Human Exposure

Fairhall (1957) noted that continued exposure to nitric acid vapor and mist may result in chronic bronchitis. More severe exposures may cause chemical pneumonitis. No exposure duration or concentrations were provided.

NIOSH (1976) indicated that prolonged exposure to high concentrations of nitric acid mists or vapors may cause erosion of exposed teeth.

Ostro *et al.* (1991) studied a panel of 207 male and female asthmatics residing in the Denver area for several months in the winter. Daily concentrations of nitric acid, sulfates, fine particulates, and hydrogen ion were measured. A multiple regression time-series model was used to measure associations of pollutant exposure and self reported daily health outcomes, respiratory symptoms, and medication use by study participants. Investigators found that nitric acid and nitrates were not significantly associated with any respiratory symptom analyzed. Although personal exposure measurements were crude and symptoms were self-reported, a NOAEL up to 13.54 $\mu g/m^3$ (5.25 ppb) (range: 0.06 - 13.54, mean 1.81 $\mu g/m^3$) was found in this sensitive population.

V. Effects of Animal Exposure

Gray *et al.* (1952) exposed four groups of 10 rats for 4 hours/day, 5 days/week to red fuming nitric acid for 40-96 hours for a period of 10-24 days. The concentration was given as 9 to 14 ppm (23.2 - 36.1 mg/m^3) NO_2. In general, 14% of red fuming nitric acid is in the form of NO_2 (ACGIH, 1991) and 86% is generally in the form of HNO_3. Therefore, animals were exposed to approximately 64 - 100 ppm HNO_3 (160 - 250 mg/m^3). Animals examined shortly after exposure exhibited an inflammatory condition throughout the entire respiratory tract, with the upper respiratory tract being more severely affected. Rhinitis, tracheitis, and pneumonitis were also seen. Animals examined eight or more weeks after exposure exhibited less inflammation; however, localized areas of emphysema were seen in all lobes of the lung. Gray *et al.* (1954) later conducted a chronic exposure study on 90 rats, 30 mice, and 10 guinea pigs exposed to a nitric acid concentration of 4 ppm (10.3 mg/m^3) for 4 hours/day, 5 days/week for a duration of 6 months. Results indicated no significant pathologic changes among treated animals compared to controls.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Gray *et al.* (1952; 1954)
Study population	Mice, rats, guinea pigs
Exposure method	Discontinuous inhalation (0 and 9 to 14 ppm in Gray, 1952); 0 and 4 ppm in Gray, 1954)
Critical effects	Pulmonary changes, inflammation, emphysema
LOAEL	Mean of 11.5 ppm (9 to 14 ppm) (Gray, 1952)
NOAEL	4 ppm (Gray, 1954)
Exposure continuity	4 hours/day, 5 days/week (Gray, 1952; 1954)
Exposure duration	10 to 24 days (Gray, 1952); 6 months (Gray, 1954)
Average exposure concentration	0.48 ppm for NOAEL group
Human equivalent concentration	0.41 ppm for NOAEL group (gas with pulmonary effects, guinea pig RGDR = 0.86, based on BW = 435 g, MV = 0.20, SA(PU) = 9,000 cm^2)
LOAEL uncertainty factor	1
Subchronic uncertainty factor	1
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	30
Inhalation reference exposure level	0.014 ppm (0.036 mg/m^3; 40 $\mu g/m^3$)

The Gray *et al.* (1954) study was selected since it was the only study which demonstrates a NOAEL in animals chronically exposed to HNO_3. Although the results indicate that animals exposed to 4 ppm (10 mg/m^3) red fuming HNO_3 showed no significant pathologic changes when compared to control animals, few details are given as to the types of changes the researchers were looking for. In an earlier study, Gray *et al.* (1952) exposed four groups of 10 rats to 64 - 100 ppm (160 - 250 mg/m^3) red fuming nitric acid 4 hours/day, 5 days/week or a total of 40 - 96 hours over 10 - 24 days. Even animals in the lowest exposure group (4 hours/day for 10 days; mean concentration = 66 ppm; range = 45 - 91 ppm) exhibited inflammation throughout the entire respiratory tract, rhinitis, tracheitis, and pneumonitis. Animals examined eight or more weeks after exposure exhibited pulmonary effects such as pneumonitis and emphysema in all lobes of the lung.

An epidemiological study of confirmed asthmatics by Ostro *et al.* (1991) demonstrated a similar NOAEL to that calculated from the Gray studies. A free-standing NOAEL up to 13.54 $\mu g/m^3$ (5.25 ppb) (range: 0.06-13.54, mean 1.81 $\mu g/m^3$) was found in this sensitive subpopulation. The free-standing NOAEL does not allow for evaluation of the dose-response relationship. In comparison, the 2 studies by Gray *et al.* (1952; 1959) collectively indicate both a LOAEL and NOAEL in the same species. Although epidemiological methods in the Ostro *et al.* (1991) study are appropriate, this study was not used in the derivation of the chronic REL because exposure methods were somewhat crude and the results represent a free-standing NOAEL. There are several factors that lend strength to the Ostro *et al.* study. Although exposure duration was only six months, all participants were residents of the Denver area and had been chronically exposed

to the HNO_3 levels reported. The large number of observations in this study provided increased degrees of freedom and stability of estimates. While problems of confounding and exposure assessment exist, they were decreased since each individual served as his or her own control over time. Moreover, this study detected a relationship between airborne H^+ and fine particulates and the self reported indicators of asthma status.

The strengths of the inhalation REL include the availability of subchronic inhalation exposure data and the observation of a NOAEL. Major areas of uncertainty are the lack of adequate human exposure data and the lack of reproductive and developmental toxicity studies.

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CHRONIC TOXICITY SUMMARY

PHOSPHINE

(hydrogen phosphide; phosphorus trihydride; Celphos; Phostoxin)

CAS Registry Number: 7803-51-2

I. Chronic Toxicity Summary

Inhalation reference exposure level	**0.3 µg/m^3** (U.S. EPA RfC) This document summarizes the evaluation of non-cancer health effects by U.S. EPA for the RfC
Critical effect(s)	Decreased body weight gain in mice
Hazard index target(s)	Respiratory system; alimentary system; nervous system

II. Chemical Property Summary (HSDB, 1995, except as noted)

Description	Colorless gas
Molecular formula	PH_3
Molecular weight	34
Boiling point	-87.7°C
Vapor pressure	20 atm @ -3°C; 760 mm Hg @ -87.5°C (Weast, 1980)
Solubility	0.26 volumes in water @ 20°C; soluble in alcohol, ether (Sax and Lewis, 1989)
Conversion factor	1.39 µg/m^3 per ppb at 25ºC

III. Major Uses and Sources

Phosphine is primarily used as an agricultural fumigant against insects and is among the most acutely toxic of the fumigant gases (HSDB, 1995). In its use as a fumigant, application of aluminum, magnesium, or zinc phosphide pellets generates phosphine gas upon exposure to moisture. Because of high volatility, phosphine residue dissipates from treated material upon ventilation. Inadequate sealing of materials during the course of treatment can result in unplanned environmental exposure.

Phosphine is also used by the semiconductor industry as a chemical doping agent for electronic components (n-type semiconductors) (HSDB, 1995). Other minor sources/uses of phosphine are in chemical syntheses: specifically, in preparations of phosphonium halides, for polymerization initiation, and as condensation catalysts.

IV. Effects of Exposures to Humans

Toxicity among 22 workers intermittently exposed to phosphine levels of 0.17-2.11 ppm in air from fumigation activity ranging from 0.5 to 29 years (mean = 11.1 years) has been reported (Misra *et al.*, 1988). The most frequently reported symptoms include dyspnea (31.8%), headache (31.8%), chest tightness (27.3%), cough (18.2%), anorexia and epigastric pain (18.2%), finger paresthesia and numbness (13.6%), and giddiness, numbness and lethargy (13.6%). The subjects were interviewed within one day of fumigation activity and reported that symptoms subsided when phosphine was not in use. No change in motor or sensory nerve conduction velocity was found.

A similar spectrum of toxic effects among workers involved in grain storage at a seaboard terminal has been reported (Jones *et al.*, 1964). Among 69 men exposed to breathing zone phosphine levels of 0-35 ppm for as long as 16 hours per day, the authors report symptoms of multiple origins. These include gastrointestinal (diarrhea, nausea, epigastric pain, vomiting), cardio-respiratory (chest tightness, dyspnea, pain in chest, palpitations, retrospinal pain), and central nervous (headache, dizziness, staggering gait) systems. Symptoms were reported to appear only at the time of exposure and appeared reversible.

In another report of chronic occupational exposure, authors cited the appearance of chronic bronchitis, anemia, and digestive disorders (Eichler, 1934).

Most literature reports of human toxic health effects of phosphine, however, come from case reports of acute exposures. Some are suggestive of potential chronic toxicity endpoints because of the irreversible nature of the effect. In a case report of phosphine poisoning of 29 people exposed by inhalation on a grain freighter, pathological findings included evidence of urinary tract injury (occult blood), liver damage (bilirubinuria and increased SGPT, GGPT, and LDH), and myocardial damage (increased MB fraction of CPK, abnormal ECG) (Wilson *et al.*, 1980). A two year old child who died as a result of the exposure showed myocardial necrosis with mononuclear infiltrates, pulmonary edema with damaged epithelia, pleural effusion, and an enlarged spleen. Exposure of a 7-months pregnant, 24-year-old woman to aluminum phosphide from a nearby grain storage site was lethal (Garry *et al.*, 1993). There was evidence of severe pulmonary edema, necrosis of individual hepatic cells, and anoxic change in Purkinje cells of the cerebellum. These reported deaths of a small child and a pregnant woman exposed together with individuals who survived exposure to phosphine suggest that there may be sensitive human subpopulations. An acute phosphine poisoning by inhalation was described (Schoonbroodt *et al.*, 1992). Findings included mucosal necrosis and cardiac abnormalities (due to hypoxemia). Renal failure (1/16), proteinuria (1/16), and increased blood transaminases (2/16) have resulted from oral exposure to phosphine (Chopra *et al.*, 1986). The multi-organ involvement in toxicity suggests that phosphine is a broad spectrum toxicant.

V. Effects of Exposures to Animals

A subchronic inhalation toxicity study of phosphine was conducted in Balb-c mice (Barbosa *et al.*, 1994). Twelve animals/sex/dose group were exposed for 6 hours/day, 5 days/week for 13 weeks to 0, 0.3, 1.0 or 4.5 ppm phosphine. Non-cancer toxicity endpoints included weight gain and relative organ weights of kidneys, lungs, liver, heart, brain and spleen. In the highest dose group, itching and scratching of the eyes, feet and tail, and decreased overall activity was observed. No diarrhea, loss of equilibrium, convulsions, seizures, or other neurological disturbances were noted. A dose-dependent decrease in total body weight gain was observed at all exposure levels with a greater effect observed in females ($p<0.0001$). Statistically significant decreases in relative organ weights (kidney, heart, and brain) were observed in males only at the 0.3 ppm exposure level ($p<0.001$). On the other hand, female mice showed increased relative organ weights (lungs, heart, and spleen) predominantly at higher doses (1.0 and 4.5 ppm; $p<0.001$).

A short-term repeated dose experiment was also conducted by this group, exposing 6 mice/sex/group to 5.5 ppm phosphine for 2 weeks (6 hrs/day, 5 days/wk). No statistically significant changes in weight gain were observed at the end of this exposure period.

Another subchronic inhalation toxicity study of phosphine was conducted in which male and female Fischer 344 rats (10/sex/group) were exposed to levels of 0, 0.37, 1.0 and 3.1 ppm phosphine (Newton *et al.*, 1993). Animals were exposed for 6 hours per day, 5 days per week, for 13 weeks. A higher dose group (10 ppm) was terminated prematurely (at 3 days) because of high mortality. A satellite group exposed to 5.1 ppm for 2 weeks was sacrificed after 13 days recovery. Observations of overt toxicity and viability were made at the time of each exposure, body weight and food consumption were monitored weekly, ophthalmic examination was done the day before sacrifice, and hematological and clinical chemistry indices were measured after 4 and 13 weeks. Postmortem examination included gross necropsy, with particular attention to orifices, the cranial cavity, surfaces of the brain and spinal cord, nasal cavity and sinuses, the thoracic, abdominal and pelvic cavities and viscera, and the cervical tissue and organs. Histopathology was performed on 10% buffered formalin-fixed/hematoxylin-eosin-stained tissues (adrenal gland, aorta, sternum, brain, esophagus, eyes, heart, cecum, colon, duodenum, ileum, jejunum, kidneys, liver, right lung lobes, lymph nodes, mammary gland, larynx, nasal turbinates, nerve, ovaries, pancreas, pituitary, prostate, salivary glands, seminal vesicles, skin, spinal cord, spleen, stomach, testes with epididymes, thymus, thyroid/parathyroid glands, trachea, urinary bladder, uterus, and vagina).

Significant observations after 13 weeks of phosphine exposure include decreased hemoglobin, hematocrit, and erythrocytes in males in the 3.1 ppm dose group. Male rats in the 1 ppm dose group showed decreased weight gain. Increased incidence of small seminal vesicles was noted at 1 and 3.1 ppm, although no histological correlate was observed. Absolute and relative decreases in liver weight were observed in all exposed groups, but there was no evidence that this effect was dose-related. A significant decrease in serum glutamic pyruvic transaminase (SGPT) was observed at 3.1 ppm, although the authors note unusually high control levels. None of these effects were observed after the 4 week recovery period. Other effects of a transient nature noted during the course of exposure include decreased weight gain in female rats at 1 ppm, decreased

food consumption at 0.37 ppm in males and females, and increased blood urea nitrogen (BUN) at 3.1 ppm. Observations in the 10 ppm group sacrificed after 3 days of exposure included decreased erythrocytes, increased alkaline phosphatase, and increased kidney weight with coagulative necrosis of the tubular epithelium of the outer cortex.

Newton *et al.*(1993) also examined developmental toxicity by exposing 24 pregnant female CD^R rats per group to 0, 0.03, 0.33, 2.8, 4.9, and 7.0 ppm phosphine. The highest dose group was terminated prematurely because of high mortality; all other animals were sacrificed after 20 days for evaluation of maternal and fetal toxicity. Maternal toxicity endpoints included weight of ovaries and uteri, number of corpora lutea, pregnancy and implantation rate. Fetal toxicity was evaluated by weight, number and location of fetuses and resorptions, visceral malformations and variations, and skeletal changes after alizarin staining. No statistically significant differences from control animals were observed for any parameter at any dose, with the exception of a change in mean number of resorption sites ($p \leq 0.01$), mean resorption/implant ratio ($p \leq 0.05$), and incidence of females with resorption ($p \leq 0.05$), all at 0.03 ppm only. In the absence of this effect at higher dose levels, these observations are not considered useful in establishing a low adverse effect level.

A 35-day phosphine inhalation study was conducted exposing rats continuously to 0, 0.05, 0.2, 1.5 and 8.0 mg/m^3 phosphine (0, 0.036, 0.14, 1.1, and 5.8 ppm) in which hematological endpoints and histopathological changes of the lungs and kidneys were examined (Pazynich *et al.*, 1984). Observations include a statistically significant change in erythrocytes (increase followed by a decrease at day 35) and decreased hemoglobin at the 0.05 and 0.2 mg/m^3 dose levels, although the 1.5 mg/m^3 dose group did not show this change. Other significant changes noted in the lowest dose group include decreased peroxidase activity after 35 days exposure, decreased sulfhydryl group content in blood after 27 days, and decreased phagocytotic index after 21 days. Some histological changes were noted in the lungs, kidneys, and to a lesser extent, the liver, particularly in the higher dose groups, although the exact nature of the degenerative change is not well described. Unclear dose-response relationships and temporal aspects of the endpoints also make establishment of a low adverse effect level unreliable.

Rats were exposed to 20 ppm phosphine for 14 days (4 hours/day) (Waritz and Brown, 1975). Animals were monitored for weight gain and organs/tissues fixed in Bouin's solution and stained with trichrome were examined histopathologically. There were no reported histopathological effects, although there was slightly reduced weight gain in exposed animals.

VI. Derivation of U.S. EPA Reference Concentration (RfC)

Study	Barbosa *et al.*, 1994
Study population	Mice (12 animals/sex/group)
Exposure method	Discontinuous inhalation exposure (0, 0.3, 1, or 4.5 ppm)
Critical effects	Decrease in body weight gain
LOAEL	4.5 ppm
NOAEL	1.0 ppm
Exposure continuity	6 hr/day, 5 days/ week
Average experimental exposure	0.18 ppm for NOAEL group
Human equivalent concentration	0.18 ppm for NOAEL group (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h))
Exposure duration	13 weeks
LOAEL uncertainty factor	1
Subchronic uncertainty factor	10
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Modifying factor	3 (lack of multigenerational reproduction studies)
Cumulative uncertainty factor	1,000
Reference exposure level	0.0002 ppm (0.2 ppb; 0.0003 mg/m^3; 0.3 μg/m^3)

The lack of adequate data on levels of chronic phosphine exposure to humans precludes development of a REL from human studies. Barbosa *et al.* (1994) present an adequate animal study for the derivation of a chronic REL. The endpoint used in the determination of the REL (total body weight gain) showed a dose-related decrease with phosphine exposure in Balb-c mice. The endpoint chosen from this animal study is consistent with that found by Newton *et al.* (1993), who also noted dose-dependent decreases in body weight gain in Fischer 344 rats after a 13 week exposure regimen (at 1 ppm), and Waritz and Brown (1975), who report slightly decreased weight gain in rats exposed for 14 days (20 ppm). Although body weight changes or changes in food consumption were not addressed in human studies, the scant human data do relate phosphine exposure to a broad spectrum of toxic effects (gastrointestinal, cardio-respiratory, CNS). The decrease in weight gain found in the animal studies and reported changes in some relative organ weights (Barbosa *et al.*, 1994) suggest systemic toxicity.

The strengths of the inhalation REL include the availability of data on multiple inhalation exposure concentrations and the observation of a NOAEL. Major areas of uncertainty are the lack of adequate human exposure data, the limited nature of the study, and the lack of reproductive and developmental toxicity studies.

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CHRONIC TOXICITY SUMMARY

PHTHALIC ANHYDRIDE

(1,3-isobenzofurandione; phthalic acid anhydride)

CAS Registry Number: 85-44-9

I. Chronic Toxicity Summary

Inhalation reference exposure level	**10 μg/m^3**
Critical effect(s)	Eye and respiratory irritation, asthma, and bronchitis in occupationally exposed workers
Hazard index target(s)	Respiratory system

II. Chemical Property Summary (HSDB, 1995)

Description	White or pale yellow crystals
Molecular formula	$C_8H_4O_3$
Molecular weight	148.11
Boiling point	295°C
Vapor pressure	5.14×10^{-4} mm Hg @ 25°C; 1 mm Hg @ 96.5°C
Solubility	Soluble in 162 parts water, 125 parts carbon disulfide; soluble in hot benzene
Conversion factor	6.1 μg/m^3 per ppb at 25ºC

II. Major Uses and Sources

The primary use of phthalic anhydride (PA) is as a chemical intermediate in the production of plastics from vinyl chloride. Phthalate esters, which function as plasticizers, are derived from phthalic anhydride. Phthalic anhydride has another major use in the production of polyester resins and other minor uses in the production of alkyd resins used in paints and lacquers, certain dyes (anthraquinone, phthalein, rhodamine, phthalocyanine, fluorescein, and xanthene dyes), insect repellents, and urethane polyester polyols. It has also been used as a rubber scorch inhibitor and retarder (HSDB, 1995; National Cancer Institute (NCI), 1979).

II. Effects of Human Exposure

Symptoms in workers exposed to phthalic anhydride by inhalation in two plants (A and B) manufacturing alkyd and unsaturated polyester resins were studied (Nielsen *et al.*, 1988). Two groups of exposed workers were identified in each plant. One group worked directly loading the reactors from bags of phthalic anhydride ("heavy" exposure – 35 workers) and the other group was involved with "other work" which led to "low" exposure (25 workers). Mean employment

times for the "heavy" and "low" exposure groups were 13.3 and 11.9 years, respectively. Time-weighted average air concentrations for workers from the loading of PA was 6.1 (range: 1.8-14.9) and 6.8 mg PA/m^3 (range: 1.5-17.4) in plants A and B, respectively. Similar exposure levels in both plants led to pooling of data. The exposure duration of the "heavy" group was estimated at approximately 30 minutes two times a day, corresponding to the time of loading, and resulted in a full-day time weighted exposure estimate of 0.4 mg PA/m^3. For those engaged in "other work" exposure levels were estimated at < 0.1 mg PA/m^3 (the limit of detection). Other chemicals in use in smaller amounts included maleic anhydride, isophthalic anhydride, and trimellitic anhydride. Comparison of symptom incidence between the "heavy" and "low" exposure groups included conjunctivitis (46% vs. 20%), rhinitis (40% vs. 20%), rhinoconjunctivitis (17% vs. 12%), asthma (17% vs. 0%), and chronic bronchitis (17% vs. 4%). Serum antibodies were measured in both groups of workers and compared to 22 nonexposed workers (employed at a food processing factory). The only significantly changed level was an increase in specific IgG in the "heavy" exposure group. A correlation was also noted between specific IgG level and exposure level, although not all individuals with elevated specific IgG reported symptoms.

In a study conducted at another plant manufacturing alkyd and/or unsaturated polyester resins, serum immunoglobulins and lung function were examined in 23 workers exposed to phthalic anhydride and 18 control subjects (Nielsen *et al.*, 1991). Estimated exposure levels were 6.6 mg PA/m^3 (range: 1.5-17) (Nielsen *et al.*, 1988). Workers were examined for sensitization to PA and other allergens and possible development of small airways disease. Among the exposed workers, there was significantly increased reporting of conjunctivitis and rhinoconjunctivitis. One worker showed an asthmatic response to anhydrides. No significant differences in lung-function tests were observed between exposed and unexposed groups.

Symptoms in workers occupationally exposed to PA during the course of producing alkyd and/or polyunsaturated polyester resins were described (Wernfors *et al.*, 1986). Exposure estimates of breathing zone PA levels ranged from 3 to 13 mg/m^3 for workers engaged directly with the handling of PA. In other areas the estimated level was <0.3 mg/m^3. The study examined 48 workers who were employed at the time of the study and 70 former employees who responded to a survey of symptoms related to exposure. No unexposed control group was included in the study. Workers who were employed for at least two months reported symptoms of rhinitis (28%), chronic bronchitis (11%), and asthma (28%). Among a subset of 11 workers with asthma, 3 had positive skin tests for PA sensitivity. Bronchial provocation tests with 6 or 0.5 mg/m^3 PA for 5 or 10 minutes were positive in 2 workers.

II. Effects of Animal Exposure

Male albino rats (6/treatment group) were exposed to phthalic anhydride vapors at 0, 0.02, 0.2, and 1 mg/m^3 continuously for 45 days (Protsenko, 1970). After a two week recovery period the testes were examined for spermatozoa motility time as well as for ascorbic acid, dehydroascorbic acid, and nucleic acid content. Motility time was defined as the time it took for spermatozoa to cease motion completely under microscopic examination. Spermatozoa motility time was decreased ~50% in the 1 mg/m^3 dose group and ~25% in the 0.2 mg/m^3 dose group. Significant

decreases in ascorbic acid and dehydroascorbic acid levels were found in animals exposed to 0.2 and 1.0 mg/m^3 phthalic anhydride, and dehydroascorbic acid levels were decreased in the 0.02 mg/m^3 dose group. At 1 mg/m^3, RNA levels and combined RNA and DNA levels were significantly increased over controls. No significant changes were observed in the 0.02 mg/m^3 dose group.

Five and six female Hartley guinea pigs were exposed to 0.05-0.2 mg/m^3 and 0.6-6 mg/m^3 phthalic anhydride dust, respectively, for 3 hours/day for 5 consecutive days (Sarlo and Clark, 1992). Exposures were expressed as ranges due to difficulty in regulating dust levels in the chambers. Sampling of dust showed particles were 65-80% < 10 μm diameter and had a mean mass diameter of 5.8-9.8 μm. Eight control animals were exposed to filtered air only. Two weeks after the last exposure, animals were challenged for 30 minutes with aerosolized PA-guinea pig serum albumin conjugate. All animals in the "high" dose group showed immediate bronchoconstriction and transiently increased respiratory rate. Animals in this dose group also showed elevated IgG antibody titers. No detectable increase in antibody levels was found in the "low" dose group.

Type I hypersensitivity was examined in female Hartley guinea pigs exposed to phthalic anhydride dust (Sarlo *et al.*, 1994). Two groups of 8 animals were exposed to 0.5 or 1.0 mg/m^3, and two groups of 16 animals were exposed to 0 (filtered air only) or 5.0 mg/m^3 phthalic anhydride dust (respirable size – 5 μm) in stainless steel chambers for 3 hours/day for 5 consecutive days. Groups of 8 animals from the control and 5 mg/m^3 groups were challenged after a two week recovery period for 30 minutes with 5.0 mg/m^3 phthalic anhydride dust. Respiratory data were collected using a plethysmograph from 30 minutes before the exposure to 60 minutes after the exposure. No significant difference (defined as a change of 3 standard deviations from the same parameter in the control animals) in respiration rate or plethysmograph pressures was found between the exposed and unexposed animals. Eight animals in each of the four exposure groups were also challenged after two weeks of recovery with 2.0 mg/m^3 aerosolized PA-guinea pig serum albumin (GPSA) conjugate as described above. Respiratory rate was increased in 4/8 of the high-dose group animals and 1/8 of the low-dose animals. Plethysmograph pressures were increased in 3/8 animals in the high-dose group and one animal each in the low- and mid-dose groups. Serum IgG antibodies to PA-GPSA were elevated in all exposed animal groups and the effect showed a dose-response. Passive cutaneous anaphylaxis testing for anti-phthalic anhydride-GPSA IgG1a immunoglobulins showed positive results for 3/8, 1/8, and 5/8 animals in the 0.5, 1.0, and 5.0 mg/m^3 dose groups, respectively. Results in control animals were not described. Three of eight animals in the highest dose group had >189 hemorrhagic foci in their lungs. No control animal had more than 2 such foci. No foci were observed in albumin conjugate challenged animals. Serum IgG titer correlated with the presence of these foci.

Slavgorodskiy (1969) studied the toxicity of phthalic anhydride to animals from inhalation exposure. Sixty white male rats (group distribution not stated, but presumed to be 15 animals/treatment group) were exposed in 100 L chambers to 0, 0.18, 0.54, and 1.52 mg PA/m^3 aerosol continuously for 70 days. General condition and behavior, body weight, motor chronaxy of flexor and extensor muscles (every 10 days), cholinesterase activity (every two weeks), and hematological parameters were monitored during the course of the study. (Chronaxy is the

minimum time for which a current must flow, at a voltage twice the minimal current necessary to produce muscle stimulation, in order to cause a muscle to contract.) No changes in body weight or behavior were observed in the treated animals. In animals in the high-dose group, the chronaxy ratio of flexors and extensors differed from the controls beginning on day 31 of exposure and continued until two weeks after exposure ceased. Significantly decreased whole blood cholinesterase activity occurred in the high- and mid-dose groups, with the change occurring after 42 days of exposure. An increase in thrombocyte count occurred in the high- and mid-dose groups after 70 days of exposure, but returned to normal during the two-week recovery period.

A chronic feeding study was conducted with phthalic anhydride in rats and mice to evaluate the carcinogenicity of the compound (National Cancer Institute (NCI), 1979). F344 rats (50/sex/dose group plus 20/sex control animals) were treated with diet containing 0, 7500, or 15,000 ppm phthalic anhydride for 105 weeks (which corresponds to approximately 0, 300, and 600 mg/kg-day, assuming that food consumption is 4% body weight/day). Animals were monitored for changes in body weight and for survival, and, upon death or the end of the study, were examined histopathologically. The only group showing significantly lower body weights was male rats in the high-dose group after week 13. No significant change in mortality was observed. Adverse non-cancer effects observed in the dosed groups, but not in the control animals, included "arched back, rough hair coat, ulceration, and corneal opacity", however, incidences were described as "low". No significant histopathological effects were found to be associated with exposure to phthalic anhydride. $B6C3F_1$ mice (50/sex/dose group plus 20/sex control animals) were initially treated with diet containing 0, 25,000, or 50,000 ppm phthalic anhydride (approximately 0, 3000, and 6000 mg/kg-day, assuming that food consumption is 12% body weight/day). Because of excessive weight loss after week 32, exposure levels were reduced during the course of the study such that the time-weighted average exposure for males was 16,346 and 32,692 ppm and for females was 12,019 and 24,038 ppm phthalic anhydride. Evaluation of toxicity was conducted at 104 weeks as with the rats. Mean body weight change was reduced in male and female mice in a dose-related manner. No other significant treatment-related adverse effects were observed in the mice.

Pregnant female CD-1 mice (10/dose group) were treated intraperitoneally with phthalic anhydride in 0.5%(w/v) carboxymethyl cellulose solution on gestational days 8-10 (Fabro *et al.*, 1982). Dosing was variable, beginning within the 95% confidence limits of the LD_{01} and progressing geometrically downward until no effect was observed. Animals were sacrificed on Day 18 and examined for teratogenic effects including fetal viability and number, resorption, and gross malformations. The 95% lower confidence limit on the dose producing teratogenicity (grossly observable malformations and fetal internal malformations) in 5% and 50% of animals were 0.40 and 1.37 mmol/kg-day (59 and 203 mg/kg-day), respectively.

II. Derivation of Chronic Reference Exposure Level (REL)

Study	Neilsen *et al.* (1988; 1991)
Study population	23 Occupationally-exposed workers
Exposure method	Discontinuous occupational inhalation exposures
Critical effects	Increased incidence of conjunctivitis, rhinitis, asthma, and chronic bronchitis
LOAEL	6.5 mg/m^3
NOAEL	Not observed
Exposure continuity	8 hours/day, 5 days/week
Exposure duration	Mean of 13.3 years
Average experimental exposure	1.5 mg/m^3 for LOAEL group ($6.6\ mg/m^3 \times 8/24 \times 5/7$)
LOAEL uncertainty factor	10
Subchronic uncertainty factor	1
Interspecies uncertainty factor	1
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	100
Inhalation reference exposure level	0.01 mg/m^3 (10 $\mu g/m^3$)

Adverse effects were demonstrated to occur in humans occupationally exposed to phthalic anhydride in the workplace over long periods of time (Nielsen *et al.*, 1988). The symptoms reported primarily affected the respiratory system, with increased incidence of rhinitis, rhinoconjunctivitis, asthma, and chronic bronchitis. Conjunctivitis was also reported in exposed workers. Specific anti-PA IgG was significantly elevated compared to a non-exposed group. Increased incidences of rhinoconjunctivitis, conjunctivitis, or chronic bronchitis have also been reported in workers exposed to similar levels of PA dust (Nielsen *et al.*, 1991; Wernfors *et al.*, 1986). In these reports, adverse effects were clearly observed at the exposure level reported (6.5 mg PA/m^3; full-day time weighted exposure of 0.4 mg PA/m^3). Although symptoms were reported by Nielsen (1988) in the lower exposure level group, the level of significance is not clear since a true control group (unexposed workers) was not included in the symptomatology section of the study. The low exposure group's level of exposure was less than the detection limit for phthalic anhydride cited in the study, and this group was considered as a control group.

The strengths of the inhalation REL include the use of human exposure data from workers exposed over a period of years. Major areas of uncertainty are the uncertainty in estimating exposure and the potential variability in exposure concentration, the potential low exposures of the group considered as controls, potential confounding by exposures to other chemicals, the limited nature of the study, the lack of reproductive and developmental toxicity studies, and the lack of observation of a NOAEL. Another area of uncertainty is the apparent 10-fold greater sensitivity to bronchoconstriction from PA exposure in guinea pigs (a model for human asthmatics) in comparison to occupationally exposed workers.

The study in rats by Protsenko (1970) identified a LOAEL of 0.2 mg/m^3 for decreased sperm motility. However, this result from 1970 has not been verified or further explored in more recent toxicological or epidemiological studies. The small sample size of 6/group further weakens confidence in this result. Therefore, the study in workers by Nielson *et al.* (1988, 1991) was chosen as the basis for the REL for PA.

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CHRONIC TOXICITY SUMMARY

SELENIUM AND SELENIUM COMPOUNDS

Molecular Formula	*Synonyms*	*Molecular Weight*	*CAS Reg. No.*
Se	elemental selenium	78.96	7782-49-2
SeO_2	selenium dioxide; selenium oxide; selenious anhydride	110.96	7446-08-4
H_2SeO_3	selenious acid	128.97	7783-00-8
H_2Se	hydrogen selenide; selenium hydride; selenium anhydride	80.98	7783-07-5
$SeOCl_2$	seleninyl chloride; selenium oxychloride; selenium oxichloric	165.86	7791-23-3
Na_2SeO_3	disodium selenite	263.01	10102-18-8
Na_2SeO_4	disodium selenate	188.94	13410-01-0
SeS	selenium sulfide; sulfur selenide	111.02	7446-34-6

I. Chronic Toxicity Summary

A. Hydrogen Selenide

Inhalation reference exposure level **0.08 µg Se/m^3**
Critical effect(s) Respiratory effects; increased mortality in guinea pigs
Hazard index target(s) Respiratory system

B. Selenium and Selenium Compounds (other than Hydrogen Selenide)

Inhalation reference exposure level **20 µg/m^3**
Oral reference exposure level **0.005 mg/kg/day (USEPA RfD)**
Critical effect(s) Clinical selenosis
Hazard index target(s) Alimentary system; cardiovascular system; nervous system

II. Chemical Property Summary (HSDB, 1995; Weast, 1980; Canady and Hodes, 1994; ACGIH, 1992)

Description
Se^0 crystal: metallic gray
H_2Se: colorless gas
H_2SeO_4, Na_2SeO_3: white crystals
H_2SeO_3, Na_2SeO_4: colorless crystals
SeO_2: lustrous crystals; yellow vapor

	SeS: yellow to orange powder
Molecular formula	see above
Molecular weight	see above
Vapor pressure	Se^0: 0.001 mm Hg at 20°C; 1 mm Hg at 356°C
	H_2Se: 760 mm Hg at –41.1°C
	SeO_2: 1 mm Hg at 157°C
Boiling point	H_2Se: -41.3°C
Melting point	H_2Se: -65.73°C
	SeO_2: 340°C
	SeS: decomposes at 118-119°C
Solubility	Se^0: insoluble in water, alcohol; slightly soluble in CS_2; soluble in ether
	H_2Se: 270 ml/100 ml water at 22.5°C; sol. In CS_2, carbonyl chloride
	H_2SeO_4: sol. In water; decomposes in alcohol
	H_2SeO_3: sol. In hot water, alcohol
	Na_2SeO_3: sol. In water
	Na_2SeO_4: 84 g/100 ml water at 35°C
	SeO_2: 38.4 g/100 ml water at 14°C
	SeS: insol. In water
Conversion factor	Se^0: not applicable (particulate)
	H_2Se: 3.3 μg/m^3 per ppb at 25ºC
	SeO_2: 4.5 μg/m^3 per ppb at 20°C

III. Major Uses and Sources

Selenium occurs in four valence states: selenates (Se^{6+}), selenites (Se^{4+}), selenides (Se^{2-}), and elemental selenium (Se^0) (Goyer, 1991) which include compounds formed with oxygen, sulfur, metals, and/or halogens. Selenium compounds are used in the glass industry as decolorizing agents and in the rubber industry as vulcanizing agents. Selenium compounds are also found in toning baths used in photography and xerography, and in insecticides and photoelectric cells. Selenium sulfide is used in shampoos as an anti-dandruff agent. The most widely used selenium compound in industry is selenium dioxide (SeO_2) which catalyzes reactions of organic compounds and is produced by the oxidation of selenium with nitric acid followed by evaporation or by burning selenium in oxygen (HSDB, 1995). Hydrogen selenide is formed by the reaction of acids or water with metal selenides or the contact of nascent hydrogen with soluble selenium compounds (Gingell *et al.*, 1982). Hydrogen selenide itself does not have a reported commercial use. The largest anthropogenic sources of atmospheric selenium are from the combustion of fossil fuels and the production/refining of copper; particulates are the primary expected form of the compound (National Academy of Sciences (NAS), 1976; U.S. EPA, 1984).

Selenium is an essential trace element in humans and other species, with deficiency leading to cardiomyopathy in humans (Goyer, 1991). For dietary intake, the National Research Council has set a U.S. Recommended Daily Allowance (RDA) of 0.87 μg/kg (55-70 μg/person/day)

(Subcommittee on the Tenth Edition of the RDAs, 1989). The average daily oral intake of selenium is 125 μg/person (U.S. EPA, 1991).

IV. Effects of Human Exposures

Humans occupationally exposed to H_2Se gas at concentrations of 0.21 ppm H_2Se (0.7 mg Se/m^3) showed signs of acute toxicity (Buchan, 1947). Symptoms included irritation of the respiratory tract, severe bronchitis, bronchial pneumonia, and pulmonary edema. Exposure duration was not stated.

Acute occupational exposure to SeO_2 gas resulted in bronchospasm, irritation of the upper respiratory passages, violent coughing, and gagging with nausea and vomiting (Wilson, 1962).

The relationship between inhalation exposure to selenium and the presence of selenium in the urine was investigated in a five year study of workers at a selenium rectifying plant (Glover, 1967). Workers were exposed to fumes and dusts of elemental red selenium which, the author reported, is converted 80% to SeO_2 in the presence of air. Average air concentrations of selenium were reported to be 3.6 mg/m^3 in grinding processes, 0.04 mg/m^3 in annealing processes, and a range of averages of 0.23-0.87 mg Se/m^3 in various "special", punching, scraping, sorting, refining and testing processes. The same author previously reported symptoms among selenium exposed workers including garlic-like odor of the breath, skin rashes, indigestion, and poorly-defined "socio-psychological" effects including lassitude and irritability (Glover, 1954).

Clinical signs of toxicity were observed among a population exposed to high levels of selenium in soils and food supplies in China (Yang *et al.*, 1983). Approximately half of 248 people in this region showed symptoms including hair and nail loss, discoloration and decay of the teeth, and CNS disturbances including pain and anesthesia of the extremities. Animals in the region were also affected, with hoof damage and horn sloughing reported in water buffalo, cattle, and pigs. Populations in low-, medium-, and high-selenium areas of China were later studied to associate the symptoms with selenium intake. Estimated daily intake for adults in these areas were 70, 195, and 1438 μg Se for males and 62, 198, and 1238 μg for females, respectively (Yang *et al.*, 1989). Selenium intake was highly correlated with whole blood, breast milk, and 24-hour urine selenium levels. The authors also suggested the possibility of liver dysfunction as indicated by a delay in prothrombin time among persons with intake of 750-850 μg Se/day. More clearly recognized and characteristic clinical signs, however, were only observed in a group exposed to greater than 1261 μg Se/day and not among those exposed to less than 853 μg Se/day. Assuming a 55 kg body weight, these respective daily dose rates were 0.023 and 0.015 mg/kg-day.

A total population of 142 subjects in seleniferous areas of South Dakota and Wyoming was examined for signs of selenosis over a two-year period with monitoring of selenium levels in whole blood, serum, urine, and toenails (Longnecker *et al.*, 1991). Average intake among the population was estimated at 239 μg Se/day. No clinical signs and no changes in hematological function, clinical chemistry, or liver function were observed in the population.

V. Effects of Animal Exposures

Toxic effects from inhalation exposure to selenium dust were examined in rats, guinea pigs, and rabbits (Hall *et al.*, 1951). Twenty female rats were exposed for 8 hours to 33 ± 10 mg Se/m^3. Many animals showed signs of pulmonary effects at both one and 4 weeks after exposure, however, no control group was included in the experiment with which to compare incidence. Similarly, six female rabbits and 10 male guinea pigs exposed to the same level of selenium dust for four 4-hour periods every 48 hours (8 days total duration) showed signs of interstitial pneumonitis at one week (2 animals of each species) and lung congestion and alveolar infiltration of large macrophages.

Guinea pigs exposed to concentrations "less than 0.021 mg H_2Se/L" (22 mg Se/m^3 as hydrogen selenide) for 2, 4, or 8 hours exhibited difficulty breathing and a red-tinged discharge from the nose (Dudley and Miller, 1941). Mortality studies were conducted with guinea pigs (16/group) using the same exposure duration and selenium concentrations ranging from 1 to 43 mg Se/m^3. Fifty percent mortality was observed at 30 days among animals exposed to 12 mg Se/m^3. Mortality after 30 days was 50% among animals exposed to 1 mg Se/m^3 for 8 hours. Histopathological evaluation of guinea pigs exposed for 4 hours to 8 mg Se/m^3 showed fatty change to the liver, pneumonia, lymphoid hyperplasia, and increased reticuloendothelial tissue in the spleen. These effects did not begin to resolve until more than 17 days after the exposure.

The absorption of selenium following inhalation exposure was studied in Fischer rats (Medinsky *et al.*, 1981). Male and female rats were exposed for 10 minutes in nose-only exposure chambers to aerosols at 2.6 mg Se/m^3 of either H_2SeO_3 (Se^{4+}) or elemental (red) selenium containing radioactive ^{75}Se. The compounds were also painted on the pelt in an effort to examine the contribution of dermal absorption from selenium deposited by the aerosols during the course of inhalation exposure. In the case of elemental selenium administration, the largest fractions of the initial body burden distributed to the pelt (39%), large intestine (15%), small intestine (9.3%), liver (9.1%), and blood (7.9%), with only 5% remaining in the lung. In the case of H_2SeO_3, distribution was to the pelt (55%), liver (13%), blood (5.6%), small intestine (3.2%), and kidney (1.8%), while 1.4% of the original body burden remained in the lung. The calculated total absorption of the administered dose 4 hours after exposure was 94% for H_2SeO_3 and 57% for elemental Se. Studies in dogs also demonstrated extensive absorption of selenium metal and selenious acid aerosols to the gastrointestinal tract, blood and nasal membranes following inhalation exposure (Weissman *et al.*, 1983).

Several studies have addressed the toxicity of selenium compounds to animals when administered in either food or drinking water. Mice (50/group) treated with 0, 1, 4, or 8 ppm Na_2SeO_3 in drinking water over 50 weeks showed decreased growth rates at 8 ppm (Jacobs and Forst, 1981). The same group reported gross liver pathology in male mice treated by oral gavage for 3 days with 0.5 ml of 64 ppm Na_2SeO_3. Hamsters (8/sex/group) treated with 0.1 (unsupplemented), 1, 5, 10, or 20 ppm Na_2SeO_3 in the diet for 42 days showed histopathological changes to the liver (Beems and van Beek, 1985). Rats (6-8/group) treated in the diet with SeS_2, Na_2Se, Na_2SeO_3, or Na_2SeO_4 showed increased relative liver weights and/or decreased body weight gain at 10 ppm (for each compound) over a 5 week exposure (Dausch and Fullerton,

1993). A 13-week drinking water study of Na_2SeO_3, and Na_2SeO_4 in rats and mice showed increased mortality, decreased body weights, and histopathological changes to the kidneys in rats and decreased body weight and decreased water consumption in mice (Abdo, 1994). Decreased body weights were observed in rats treated for 6 weeks in drinking water with 2 ppm Na_2SeO_3 or Na_2SeO_4 (Palmer and Olson, 1974).

Decreased percentage of live spermatozoa, altered sperm morphology, and decreased body weight gain were observed in rats (6/group) treated for 5 weeks with 2 ppm Na_2SeO_3 in the diet (Kaur and Parshad, 1994). Rats (7-12/group) exposed to 0, 4, 8, or 16 ppm Na_2SeO_3 in drinking water for 240 days showed alterations in testicular LDH and β-glucuronidase activity at 4 ppm (Nebbia *et al.*, 1987).

Developmental toxicity endpoints were examined in hamsters (5-10/group) exposed by oral gavage on gestational day 8 to Na_2SeO_3 and Na_2SeO_4 at concentrations ranging from 0-110 μmol/kg body weight (Ferm *et al.*, 1990). Effects observed at 100 μmol Na_2SeO_3/kg included decreased fetal crown-rump length and increased percentage of abnormal litters. At 90 μmol Na_2SeO_4/kg, an increased percentage of abnormal litters was observed. Mice (10 or 14/group) treated with 0, 3, or 6 ppm Na_2SeO_3 in drinking water from 30 days pre-gestation through gestation showed altered estrus cycle length, decreased fetal growth, and a decreased number of ossified vertebrae in offspring (Nobunaga *et al.*, 1979).

VI. Derivation of Chronic Reference Exposure Levels (REL)

A. Derivation of Chronic Inhalation Reference Exposure Level (REL) for H_2Se

Study	Dudley and Miller, 1941
Study population	Guinea pigs
Exposure method	Inhalation exposure
Critical effects	Respiratory irritation; bronchopneumonia; increased mortality
LOAEL	1 mg/m^3 (Se as H_2Se)
NOAEL	Not observed
Exposure continuity	Continuous
Exposure duration	8 hours
Average experimental exposure	1 mg/m^3 for LOAEL group
Human equivalent concentration	0.23 mg/m^3 for LOAEL group (gas with tracheobronchial and pulmonary respiratory effects, RGDR = 0.23 based on BW = 435 g, MV = 0.20 L/min, SA(TB) = 200 cm^2)
Subchronic uncertainty factor	10
LOAEL uncertainty factor	10
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	3,000
Reference exposure level	0.00008 mg/m^3 (0.08 μg/m^3)

Strengths of the hydrogen selenide REL include the use of measured inhalation exposure data. Weaknesses include the very short-term exposure involved and the lack of a NOAEL. The Dudley and Miller (1941) study was selected as the basis. Athough of short duration, the toxicity of H_2Se is high enough relative to other selenium compounds that it is important to protect against possible adverse effects from exposure to this compound. There are no long-term studies on the toxicity of H_2Se.

B. Derivation of Inhalation and Oral Chronic Reference Exposure Levels for Selenium Compounds other than Hydrogen Selenide

Study	Yang et al., 1989
Study population	400 people in China
Exposure method	high environmental levels of Se
Critical effects	clinical selenosis (liver, blood, skin, CNS)
LOAEL	0.023 mg/kg-day* (1.26 mg/day / 55 kg)
NOAEL	0.015 mg/kg-day* (0.85 mg/day / 55 kg)
Exposure continuity	Continuous
Exposure duration	Lifetime
Average experimental exposure	70, 195 and 1438 μg/day for adult males; 62, 198 and 1238 μg for adult females
LOAEL uncertainty factor	1
Subchronic uncertainty factor	1
Interspecies factor	1
Intraspecies factor	3
Cumulative uncertainty factor	3
Oral reference exposure level	0.005 mg/kg/day (USEPA RfD)
Inhalation extrapolation factor	3,500 $\mu g/m^3$ per mg/kg-day
Inhalation reference exposure level	20 $\mu g/m^3$

*Factors: NOAEL (0.853 mg/day) and LOAEL (1.261 mg/day) calculated from regression analysis (log Y = 0.767log X - 2.248, where Y = blood selenium and X = selenium intake) based upon the correlation (r = 0.962) between dietary selenium intake and blood selenium level for data showing incidence of clinical selenosis in adults based on an average adult body weight of 55 kg.

The oral REL is the USEPA's oral reference dose (RfD) (U.E. EPA, 1996). The principal study used was that of Yang et al. (1989) entitled: Studies of safe maximal daily dietary Se-intake in a seleniferous area in China. II. Relation between Se-intake and the manifestation of clinical signs and certain biochemical alterations in blood and urine. Yang et al., in a follow-up to an earlier study, studied a population of approximately 400 individuals living in an area of China with unusually high environmental concentrations of selenium (Se). The subjects were evaluated for clinical and biochemical signs of Se intoxication. Three geographical areas with low, medium and high selenium levels in the soil and food supply were chosen for comparison in the studies. The earlier study was conducted in response to endemic selenium intoxication in two separate areas with sample sizes of only 6 and 3. Comparisons were then made to a selenium-adequate

area (n=8) and low- selenium area (n=13). The Yang et al. studies provide a much larger sample size and include additional analysis of tissue selenium levels. This allows a more accurate estimation of the dose-response relationship observed for selenium toxicity. Selenium levels in soil and approximately 30 typical food types commonly eaten by the exposed population showed a positive correlation with blood and tissue Se levels. The daily average Se intakes, based on lifetime exposure, 70, 195 and 1438 μg for adult males and 62, 198 and 1238 μg for adult females in the low-, medium- and high-selenium areas, respectively. Significant correlations demonstrated between Se concentrations of various tissues were used to estimate the minimal daily Se intake values that elicited various alterations in biochemical parameters indicative of possible Se-induced liver dysfunction (i.e., prolongation of clotting time and serum glutathione titer) and clinical signs of selenosis (i.e., hair or nail loss, morphological changes of the nails, etc.). In this manner, a marginal safe level of daily Se intake was estimated. Analysis of the results indicated that persistent clinical signs of selenosis were observed only in 5/349 adults, a potentially sensitive subpopulation. The blood selenium concentration in this group ranged from 1.054 to 1.854 mg/L with a mean of 1.346 mg/L. Clinical signs observed included the characteristic "garlic odor" of excess selenium excretion in the breath and urine, thickened and brittle nails, hair and nail loss, lowered hemoglobin levels, mottled teeth, skin lesions and CNS abnormalities (peripheral anesthesia, acroparesthesia and pain in the extremities). Alterations in the measured biochemical parameters occurred at dietary intake levels of 750-850 μg/day. These alterations were described as a delay in prothrombin time, i.e., increase in blood coagulation time and reduction in blood glutathione concentration. However, these indicators were poorly characterized and are not typically used as an index for clinical selenosis resulting from chronic exposure to selenium (NAS, 1989). Based upon the blood selenium levels shown to reflect clinical signs of selenium intoxication, a whole blood selenium concentration of 1.35 mg/L corresponding to 1.261 mg of daily selenium intake is indicative of the lowest correlative selenium intake causing overt signs of selenosis. The next lowest whole blood selenium concentration of 1.0 mg/L, corresponding to 0.853 mg selenium/day, produces no clinical signs of selenosis. The NOAEL for this study is 0.85 mg Se/day and the LOAEL is 1.26 mg Se/day.

An intraspecies uncertainty factor of 3 was applied to the NOAEL to account for sensitive individuals. A full factor of 10 was not deemed necessary since similar NOAELs were identified in two moderately-sized human populations exposed to selenium levels in excess of the RDA throughout a lifetime without apparent clinical signs of selenosis. No modifying factor was applied.

The USEPA stated its confidence in the RfD as: Study - Medium; Data Base - High; and RfD - High. Confidence in the chosen principal study is medium. Although this is a human epidemiological study in which a sizable population with sensitive subpopulations was studied, there are still several possible interactions that were not fully accounted for, e.g., fluoride intake and protein status. Also, except for clinical signs of selenosis there are no other reliable indicators, biochemical or clinical, of selenium toxicity. Confidence in the data base is high because many animal studies and epidemiologic studies support the principal study. An additional human study with a freestanding NOAEL (Longnecker et al., 1991) strongly corroborates the NOAEL identified in the principal study. Therefore, high confidence in the RfD is selected based upon support of the critical study and the high level of confidence in the data base.

There are insufficient data relating human inhalation exposure to selenium compounds to adverse health effects to use for the development of a chronic REL although toxicity has been reported from occupational exposure to gases of both H_2Se and SeO_2 (Buchan, 1947; Wilson, 1962). Experiments in animals have shown that H_2Se is toxic following inhalation exposure, with 8-hour exposures to concentrations as low as 1 mg H_2Se/m^3 causing "irritation sufficiently damaging to cause pneumonitis" and subsequently increasing 30-day mortality (Dudley, 1937; Dudley and Miller, 1941

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CHRONIC TOXICITY SUMMARY

SULFURIC ACID

(dithionic acid; pyrosulphuric acid)

CAS Registry Number: 7664-93-9

II. Chronic Toxicity Summary

Inhalation reference exposure level	**1 µg/m^3**
Critical effect(s)	Bronchiolar epithelial hyperplasia, and thickening of the bronchial walls in monkeys
Hazard index target(s)	Respiratory system

II. Physical and Chemical Properties (HSDB, 1995)

Description	Colorless liquid
Molecular formula	H_2SO_4
Molecular weight	98.1
Density	1.84 g/cm^3 @ 15° C
Boiling point	315-388° C
Vapor pressure	0.001 torr @ 20° C
Solubility	Soluble in water
Conversion factor	Not applicable

III. Major Uses or Sources

Sulfuric acid is a strong acid used as an intermediate in the synthesis of linear alkylbenzene sulfonation surfactants used in dyes, in petroleum refining, for the nitration of explosives, in the manufacture of nitrocellulose, in caprolactam manufacturing, and as a drying agent for chlorine and nitric acid.

IV. Effects of Human Exposures

Workers in the lead battery industry showed etching and erosion of the teeth after 4 months exposure to an average concentration of 0.23 mg/m^3 H_2SO_4 (Gamble *et al.*, 1984). Dental erosion increased in a dose-dependent manner with longer duration of exposure.

A study of 33 storage battery plant workers exposed to H_2SO_4 concentrations as high as 35 mg/m^3 showed a greater group mean decrease in FEV_1 across the time of their work shift compared to workers who were not exposed to sulfuric acid (El-Saddik *et al.*, 1972). The

salivary pH of the sulfuric acid exposed workers, a qualitative measure of acid exposure, was lower than the controls during the course of the work shift.

A large number of epidemiologic studies have been conducted showing that elevated levels of several air pollutants, including acid aerosols, sulfur and nitrogen oxides, and particulate sulfates are correlated with an increased prevalence of pulmonary disease (U.S. EPA, 1989). Elevated sulfate levels (1.6 ppb or 6.6 $\mu g/m^3$) have been associated with statistically significant decrements in FVC and FEV_1 in a cohort of Canadian children (Stern *et al.*, 1989). Further analysis of these data led Bates and Sitzo (1989) to conclude that H_2SO_4 was the most likely cause for the pulmonary changes observed. Similarly, Ostro *et al.* (1989) reported a statistical association between asthma-related symptoms reported by 209 asthmatics and sulfate and acidity levels in ambient air in Denver.

Sulfuric acid and oleum are absorbed as salts of sulfate anion (SO_4^{2-}), and are excreted as organic sulfates, neutral sulfur, or neutral sulfur compounds such as sulfur-containing amino acids. The low systemic toxicity of these metabolites is likely of secondary importance to the irritation caused by the inhaled acid.

V. Effects of Animal Exposures

An exposure of 9 monkeys per group to H_2SO_4 concentrations of 0, 0.38, 0.48, 2.43, and 4.79 mg/m^3 continuously for 78 weeks resulted in dose-dependent adverse histological changes in lung and bronchiolar epithelial and parenchymal tissue in addition to a dose-dependent decrease in blood oxygenation (Alarie *et al.*, 1973). In the animals exposed to 0.38 mg/m^3, significant bronchiolar epithelial hyperplasia was observed in 5 of 9 animals; thickening of the bronchiolar walls was observed in 3 of 9 animals. A slight focal bronchial epithelial hyperplasia was present in 4 of the 9 animals. One animal died after 4 weeks exposure to 0.38 mg/m^3. Although signs of pulmonary edema and cardiac hypertrophy were found, the cause of death was not determined.

Alarie *et al.* (1973) also exposed groups of 50 guinea pigs of each sex to 0, 0.08, or 0.1 mg/m^3 continuously for 52 weeks. The group receiving 0.1 mg/m^3 also received larger sized particulates than the 0.08 mg/m^3 group (2.78 µm vs.0.84 µm, respectively). No exposure related effects were observed in the animals exposed to 0.08 mg/m^3 whereas exposure of 0.1 mg/m^3 resulted in decreased body weights in the female guinea pigs. No other histological changes in any organs were observed at the end of the 52-week study.

Rabbits (4 per group) were exposed to 250 $\mu g/m^3$ H_2SO_4 1 hour/day, 5 days/week for 4, 8, or 12 months. They showed significantly increased bronchoconstriction upon acetylcholine challenge after 8 and 12 months exposure, compared with a control group of 4 animals that received no H_2SO_4 (Gearhart and Schlesinger, 1986, 1988). Mucociliary clearance was also impaired by H_2SO_4 exposure and did not improve 3 months after cessation of exposure. A decline in dynamic lung compliance was observed after 12 months exposure. There was no evidence of inflammatory cell infiltration in the lungs of the exposed animals.

In guinea pigs, significantly slower and irregular breathing patterns were noted when the animals had inhaled albumin followed by 30 minute exposures to H_2SO_4 at 1.91 mg/m^3 twice per week for 5 weeks (Kitabatake *et al.*, 1979). Similarly, when guinea pigs were exposed to 2.49 mg H_2SO_4/m^3 for 4 hours/day, 6 days/week for 4 weeks, *in vitro* lung histamine release was significantly enhanced following heterogeneous albumin inhalation compared to control animals unexposed to albumin (Fujisawa *et al.*, 1986; Iguchi *et al.*, 1986). In guinea pigs, sulfuric acid caused significantly greater lung function changes when adsorbed on the surface of zinc oxide particles as compared with pure sulfuric acid (Amdur and Chen, 1989). An exposure to 24 µg/m^3 sulfuric acid, layered on zinc oxide, produced significant reductions in lung function when followed by a brief exposure to 0.15 ppm ozone (Chen *et al.*, 1991).

A chronic exposure of beagle dogs to an average concentration of 889 µg/m^3 for 21 hours/day over a 620 day period resulted in increased expiratory resistance, reduced carbon monoxide diffusing capacity, reduced total and residual lung volume, and decreased lung and heart weights (Lewis *et al.*, 1973).

In apparent contrast to the above studies, rats and guinea pigs exposed to H_2SO_4 at 10 mg/m^3 for 6 hours/day, 5 days/week for 6 months exhibited no adverse histologic changes in lung tissue. Lung function measurements were not reported in this study (Cavender *et al*., 1978).

Mice inhaled sulfuric acid mist at a concentration of 1.4 mg/m^3 in combination with a carbon particle mixture (1.5 mg/m^3) for 3 hours/day, 5 days/week for up to 20 weeks. The exposure resulted in significant alterations in specific antibody titer (decreased IgG, Ig_{2a}, IgM; increased IgG_{2b}), depression of primary splenic antibody response, and decreased resistance to respiratory infection as measured by mortality and survival time compared to controls (Fenters *et al.*, 1979).

There are no reliable studies indicating that sulfuric acid is a developmental or reproductive toxicant. In the absence of massive overexposure leading to maternal acidemia, H_2SO_4 will be neutralized in the maternal circulation and is unlikely to reach the fetus.

VI. Derivation of Chronic Reference Exposure Level

Study	Alarie *et al.*, 1973
Study population	Cynomolgous monkeys (5 males and 4 females per group or vice versa)
Exposure method	Continuous inhalation exposures (0, 380, 480, 2400, or 4800 µg/m^3) for 78 weeks
Critical effects	Significantly increased bronchial epithelial hyperplasia and bronchial thickening
LOAEL	380 µg/m^3
NOAEL	Not observed
Exposure continuity	The exposure was continuous during the experiment.
Exposure duration	78 weeks
Average experimental exposure	380 µg/m^3 for the LOAEL group

Human equivalent concentration	130 μg/m^3 (RGDR assumed to equal 0.33)
LOAEL uncertainty factor	3 (slight, low incidence effects)
Subchronic uncertainty factor	1 (see text)
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	100
Reference exposure level	1 μg/m^3

The study by Alarie *et al.* (1973) identified a LOAEL for chronic exposure to sulfuric acid of 380 μg/m^3. The principal uncertainties of this study are the small sample size of the test groups and the absence of an observed NOAEL. A lower chronic LOAEL for bronchial reactivity is presented by Gearhart and Schlesinger (1986, 1988) for rabbits (250 μg/m^3). The reasons why this study was not selected as the basis of the REL were that Gearhart and Schlesinger used only a single concentration of sulfuric acid, exposed the animals only for 1 hour per day for 5 days/week, used only 4 animals per group, and measured effects over the course of up to 12 months. The predominant weakness in the rabbit study however, was the extreme discontinuity of the exposures (1 hour/day, 5 days/week) which would have necessitated a very large continuity adjustment assumption. For these reasons, in addition to obvious physiological and genetic similarity arguments, the study in monkeys by Alarie *et al.* (1973) was felt to be more appropriate as the basis for the chronic REL for sulfuric acid.

A free-standing NOAEL for histological changes in 100 guinea pigs exposed continuously for 1 year to 0.08 mg/m^3 was reported by Alarie *et al.* (1973). Guinea pigs respond to high concentrations of sulfuric acid by occasional laryngeal spasms that appear similar to a human asthmatic attack (Silbaugh *et al.*, 1981; Amdur and Chen, 1989). As a result, guinea pigs are thought to be sensitive models for the acute effects of sulfuric acid. For chronic effects of sulfuric acid on the lung, monkeys are likely a suitable model due to physiological and structural similarites to humans. Although the duration of exposure in the monkey study was 1.5 years (less than 10% of an average monkey's lifespan of 35-40 years; U.S. EPA, 1988) and therefore technically subchronic, no histological effects were observed in guinea pigs exposed chronically (i.e., 1 year out of a 5 year lifespan) to a concentration one-fifth that of the LOAEL in monkeys. Therefore, an uncertainty factor for subchronic to chronic exposures appears to be unnecessary.

For comparison, an REL based on the guinea pig NOAEL of 0.08 mg/m^3 would be 0.8 μg/m^3.

The major strength of the study is the use of health effects observations from continuous long-term exposures to a primate. The major weaknesses are the lack of adequate human health effects data and the lack of a NOAEL observation.

The occupational standard for sulfuric acid is based on a study in human subjects by Amdur *et al*. (1952). In their study, 22 healthy male subjects were exposed to 0, 0.35, to 5 mg/m^3 for 5-15 minutes. The odor, taste, and irritation threshold was 1 mg/m^3. Since the basis for this standard is an acute exposure, it is not useful in determining a chronic non-cancer REL for sulfuric acid. A review of chronic human exposures to sulfuric acid and resulting carcinogenicity outcomes can be found in IARC (1992). However, none of the studies in that review examined non-cancer endpoints.

V. References

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CHRONIC TOXICITY SUMMARY

2,4- AND 2,6-TOLUENE DIISOCYANATE

(2,4- and 2,6-TDI; 2,4- and 2,6-diisocyanato-1-methylbenzene; 2,4- and 2,6-diisocyanatoluene)

CAS Registry Number: 584-84-9 or 26471-62-5 (mixture)

I. Chronic Toxicity Summary

Inhalation reference exposure level	**0.07 µg/m^3** (U.S. EPA RfC) This document summarizes the evaluation of non-cancer effects by U.S. EPA for the RfC.
Critical effect(s)	Decreased lung function in occupationally exposed workers
Hazard index target(s)	Respiratory system

II. Chemical Property Summary (HSDB, 1995)

Description	Colorless to pale yellow liquid
Molecular formula	$C_9H_6N_2O_2$
Molecular weight	174.15
Boiling point	251°C
Vapor pressure	0.01 mm Hg @ 20°C
Solubility	Miscible with ether, acetone, benzene, carbon tetrachloride, chlorobenzene, diglycol monomethyl ether, kerosene, olive oil, alcohol; soluble in ethyl acetate
Conversion factor	7.1 µg/m^3 per ppb at 25ºC

III. Major Uses and Sources

Commercial toluene diisocyanate is comprised of approximately 80% 2,4-TDI and 20% 2,6-TDI. TDI is used in the manufacture of polyurethane foams, elastomers, and coatings (HSDB, 1995; Howard, 1989). It is also used in the manufacture of floor and wood finishes, lacquers, foam plastics, polyurethane foam coated fabrics, and insulation materials (HSDB, 1995; Howard, 1989; Duncan *et al.*, 1962). Emissions of TDI to the atmosphere can occur during production, handling, and processing of polyurethane foam (Howard, 1989).

IV. Effects of Human Exposures

Diem *et al.* (1982) conducted a prospective study beginning in 1973 of 277 male workers involved in the production of TDI. The study examined pulmonary function, with nine

examinations conducted over a five year period. A large group of workers (168) with no previously reported TDI exposure was examined 6 months prior to TDI production in the plant to provide baseline pulmonary function measurements. Personal sampling by continuous tape monitors provided exposure levels, but was not used until 2 years after the study was initiated. Sampling information resulted in a division of the workers into two groups: those exposed to levels below 68.2 ppb-months (which reflects the level of exposure of a worker for the entire 5 year duration in the low-exposure area (geometric mean = 1.1 ppb)) and those above this level. The arithmetic mean exposure level for the non-smokers was 1.9 ppb TDI in the high-exposure group and 0.9 ppb TDI in the low-exposure group (calculated by Hughes, 1993). The higher exposure group was further limited to those individuals who showed a normal FEV_1 to height ratio. Data were analyzed by the maximum likelihood weighted regression approach (Diem and Liukkonen, 1988). Both FEV_1 and forced expiratory flow (25-75%) among workers who never smoked were found to be significantly reduced in the high-exposure group (n=21) compared to the low-exposure group (n=35). Categorizing workers based on time spent at exposure levels above 20 ppb demonstrated a significant difference in FEV_1 and FEV(25-75%) and this effect was also observed among current smokers. Among low-exposure workers, a smoking effect was observed, with smokers showing a significant decline in FEV_1.

A similar longitudinal study of lung function was conducted among workers exposed to TDI during the course of polyurethane foam production (Jones *et al.*, 1992). Participants (181 males and 46 females) were required to have 3 or more spirometric examinations over the 5 year study period. Exposure of males was evaluated by personal monitors and resulted in arithmetic mean low exposure levels of 0.3, 0.4, and 0.4 ppb TDI for never-smokers, ex-smokers, and current smokers, respectively. Among workers with high-level exposure, mean TDI levels were reported to be 1.3, 1.2, and 1.2 ppb for never-, ex-, and current smokers, respectively. Stepwise multiple linear regression methods (excluding asthmatics) were used in evaluating the data (Diem and Liukkonen, 1988). No relationship between TDI exposure and change in lung function was observed, although the prevalence of chronic bronchitis was significantly associated with exposure.

A longitudinal study of 780 workers exposed to TDI in the production of polyurethane foam was also conducted (Bugler *et al.*, 1991; unpublished). Exposure levels were established using continuous-tape personal monitoring devices. The mean exposure level was 1.2 ± 1.1 (SD) ppb TDI among 521 workers and 0.3 ± 0.18 ppb TDI in the control group. Another control group who handled cold urethane products had an 8 hour time-weighted average exposure of 0.6 ppb TDI. No significant longitudinal changes in FEV_1 were found after regression analysis, although FEV_1 decline was high among the control group and exposure levels among the different groups were close, limiting the power of the study to detect changes. Approximately 3% of the 780 workers showed signs of TDI sensitization and, of these, over 80% were in the exposed group.

Meta-analysis of the three data sets (Jones *et al.*, 1992; Bugler *et al.*, 1991; Diem *et al.*, 1982) showed that the difference in significance among the findings of each of the studies could have been due to chance. The change in the probability density for the decline in FEV_1 shifted in the same direction for all data sets and the smoker/non-smoker slope difference became less meaningful with the data set combination (Hasselblad, 1993).

Another toxicological area of concern with exposure to TDI is the development of sensitization, resulting in a well-documented condition known as "isocyanate asthma" of either immediate or delayed-type onset (Moscato *et al.*, 1991). The level of exposure required to either develop or trigger a sensitization reaction is not well documented, however. Weaknesses of studies showing pulmonary effects of TDI exposure include use of area sampling vs. breathing-zone measurement of exposure, poor statement of criteria for evaluating hypersensitivity, and the presence of other compounds in the environment which may influence lung function.

V. Effects of Animal Exposures

Mice were exposed to TDI concentrations ranging from 0.007 to 1.18 ppm for 3 hours/day for 5 days consecutively (Sangha and Alarie, 1979); decreased respiratory rate was observed in groups exposed to levels higher than 0.023 ppm TDI. Groups of four mice were also exposed to 0.031 and 0.250 ppm TDI for 3 hours/day for 3 days. Lesions of the external nares and respiratory epithelium were observed in the high dose group.

Female guinea pigs were exposed to 0.12, 0.36, 0.61, 0.96, and 10.00 ppm TDI (head-only) for 3 hours/day for 5 consecutive days (short protocol) or to 0.02 ppm TDI (whole body) plus controls for 6 hours/day, 5 days/week for 70 days (long protocol). The animals showed decreased respiration rate two hours into exposure at levels above 0.12 ppm TDI and cytophilic antibody response at 0.96 ppm and above (Karol, 1983). All animals exposed to 10 ppm died. Dermal sensitivity was evident among animals in the short protocol down to 0.12 ppm TDI. No antibody response or dermal sensitivity developed in the animals exposed to 0.02 ppm TDI in the long protocol, although antibody titer was high.

Similarly, guinea pigs (8 females) were exposed head only to 1.40 ppm TDI for 3 hours/day for 4 days (no control group). In a second exposure regimen, animals (n=24) were exposed to 0.02 ppm TDI for 6 hours/day, 4 days/week for 70 days (whole body) including a control group (n=8) exposed to room air in a similar manner (Wong *et al.*, 1985). Half the animals (4/8) exposed to 1.40 ppm TDI showed pulmonary hypersensitivity (measured on days 37 and 38) and all developed TDI-specific IgE antibodies, whereas none of the animals in the 0.02 ppm TDI group showed either of these effects. Histopathological effects in the 1.40 ppm TDI group included interstitial inflammation, pleural thickening, and peripheral lymphoid hyperplasia. Interstitial inflammation was noted in 2/24 animals exposed to 0.02 ppm TDI.

SD rats and CD-1 mice were exposed to 0.05 or 0.15 ppm TDI for 6 hours/day, 5 days/week for 2 years (Loeser, 1983; nasal histopathology reported by Owen, 1984). Among female rats at both dose levels and male rats at the high dose level, histopathological effects observed included necrotic rhinitis, metaplasia, and inflammation of the respiratory epithelium. Female animals showed dose-dependent increases in incidence and severity of this effect. Similar lesions were reported in mice, although they were not well characterized.

Reproductive toxicity of TDI was evaluated in a two-generation study conducted in rats (Tyl and Neeper-Bradley, 1989). Weanling rats (28/sex/dose) were exposed to 0, 0.020, 0.079, and 0.290 ppm TDI for 6 hours/day, 5 days/week, for 10 weeks, at which time the animals were randomly

mated. Exposure of the females continued through gestation (excepting gestational day 20 through the fourth day postpartum), and exposure of the males continued only until the delivery of the F_1 generation. Weanlings in the F_1 generation were exposed in a manner similar to the parental (P_0) generation and bred after weaning to produce the F_2 generation. Body weights were significantly reduced among animals of both sexes in the highest dose group and weight gain was reduced among males in the highest dose group. Effects on the respiratory system in the P_0 generation animals included rhinitis of the epithelium in the two highest dose groups of both male and female animals. Hyperplasia of the respiratory epithelium was also increased in the high dose groups of both sexes among P_0 animals. Among males in the F_1 generation, the incidence of rhinitis was significantly increased at all exposure levels and the incidence of submucosal lymphoid infiltrates of the larynx and trachea was increased in the highest dose group. F_2 generation animals showed reduced pup weight and weight gain during the lactation period in the two highest dose groups.

Developmental toxicity of TDI was evaluated by exposing pregnant Sprague-Dawley rats (25/group) for 6 hours/day on gestational days 6-15 to 0, 0.021, 0.120, or 0.48 ppm TDI (Tyl, 1988). Reduced maternal body weight, decreased food consumption, and rales occurred among the dams in the 0.48 ppm TDI dose group. A significant fetal effect, a statistically significant increase in a specific skeletal malformation, was reported in the highest dose group.

VI. Derivation of the U.S. EPA Reference Concentration (RfC)

Study	Diem *et al.*, 1982
Study population	Human TDI production workers (168)
Exposure method	Occupational inhalation exposure
Critical effects	Decreased lung function
LOAEL	0.014 mg/m^3
NOAEL	0.006 mg/m^3
Exposure continuity	8 hrs/day (10 m^3/day occupational exposure), 5 days/week
Exposure duration	5 years
Average occupational exposure	0.002 mg/m^3 for NOAEL group (0.006 x 10/20 x 5/7)
Human equivalent concentration	0.002 mg/m^3 for NOAEL group
LOAEL uncertainty factor	1
Subchronic uncertainty factor	3
Interspecies uncertainty factor	1
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	30
Inhalation reference exposure level	0.00007 mg/m^3 (0.07 µg/m^3; 0.00001 ppm; 0.01 ppb)

The U.S. EPA selected the human occupational study examining pulmonary effects of TDI for the derivation of the RfC (U.S. EPA, 1995; Diem *et al.*, 1982). The rationale for selection of this study has been adopted for the establishment of the chronic REL. This study presented evidence

of a decline in lung function, as indicated by decrements in FEV_1, among workers involved in TDI production. In its evaluation of the study (Diem *et al.*, 1982), U.S. EPA cited factors supporting its quality including:
(1) the absence of other confounding compounds in the work environment,
(2) establishment of baseline lung function prior to exposure to TDI,
(3) "parallel internal comparison" of study groups for lung function,
(4) appropriate statistical analysis which took into account interindividual variability,
(5) breathing zone measurement of TDI (although commenced 2 years into the study), and
(6) a smoking effect on lung function.

The major strengths of the U.S. EPA RfC are the use of human exposure data from workers exposed over a period of years and the observation of a NOAEL. The major weaknesses are the uncertainty in estimating exposure, the potential variability in exposure concentration, and the limited nature of the study which focused on lung effects.

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CHRONIC TOXICITY SUMMARY

TRIETHYLAMINE

(diethylaminoethane; ethanamine; N,N-diethylethanamine)

CAS Registry Number: 121-44-8

I. Chronic Toxicity Summary

Inhalation reference exposure level	**7 µg/m^3 (** (U.S. EPA RfC) This document summarizes the evaluation of non-cancer health effects by U.S. EPA for the RfC
Critical effect(s)	Squamous metaplasia in lung tissue; necrotic inflammation in nasal tissue in rats.
Hazard index target(s)	Respiratory system; immune system; eyes

II. Physical and Chemical Properties (Nelson and Bull, 1990)

Description	Colorless liquid and gas
Molecular formula	$C_6H_{15}N$
Molecular weight	101.9
Density	0.726 g/cm^3 @ 25°C
Boiling point	89.3°C
Vapor pressure	400 mm Hg @ 31.5°C
Solubility	soluble in acetone, benzene and chloroform
Conversion factor	1 ppm = 4.14 mg/m^3 25°C

III. Major Uses or Sources

Triethylamine (TEA) is primarily used as a cross-linking catalyst in the production of polyurethane foam used in the manufacture of cores for metal castings (Albrecht and Stephenson, 1988). Triethylamine is also used as a catalyst for epoxy resins, and as a corrosion inhibitor for polymers (Nelson and Bull, 1990).

IV. Effects of Human Exposures

Acute, high level triethylamine exposures (20 mg/m^3 (4.8 ppm) for 8 hours) resulted in reversible ocular effects that included corneal swelling and halo vision in 4 out of 5 volunteer subjects (Akesson *et al.*, 1988). A medical examination of workers exposed to a time-weighted average concentration of 13 mg/m^3 (3.1 ppm) showed reversible corneal edema (Akesson *et al.*, 1986). However, small quantities of dimethylethanolamine, toluene diisocyanate and methylene diphenyl isocyanate were also present in the workplace atmosphere.

V. Effects of Animal Exposures

Lynch *et al.* (1990) exposed male and female rats to triethylamine at concentrations of 0, 25, or 247 ppm (0, 103.4, or 1022.2 mg/m^3) for 6 hours/day, 5 days/week for 28 weeks. Endpoints examined included gross and histopathological examination of all major organs, including the lungs, nasal passages, and eyes. Clinical enzyme levels (BUN, ALT, AST, CPK, and creatinine, and hematological values (hemoglobin, RBC count) were also measured. No gross or histological effects in any organ were observed in any group. Clinical and hematological parameters were unchanged with exposure.

In a short-term study by Virginia Chemicals (1987), necrotizing inflammation of the nasal cavity, metaplasia of the trachea, and thymic atrophy were observed after exposure to 1000 ppm (4140 mg/m^3) triethylamine 6 hours per day for 10 days. Two of five males and one of five females died from pulmonary edema after the seventh day. Thymic atrophy was noted in 7 out of 10 animals, and all animals exhibited necrotizing inflammation in the nasal epithelium.

Rabbits (12 per group), exposed to 48 or 100 ppm (199 or 414 mg/m^3) triethylamine for 7 hours/day, 5 days/week, for 6 weeks, showed concentration-dependent pathology in the eyes, lungs, liver, kidney, and heart (Breiger and Hodes, 1951). The lesions in the 48 ppm group were less severe than those seen in the 100 ppm group. No control animals were included in this study, nor were the specific incidences of histologic effects reported.

A chronic 3-generation reproductive study in rats (10/sex/group) was inconclusive due to excessive mortality in controls (Davison *et al.*, 1965). In this study, rats were exposed to 0, 2, or 200 ppm triethylamine. The third generation of the 200 ppm group was changed to 500 ppm since no effects were noted in the 200 ppm group. Exposure of this group to 500 ppm resulted in decreased body weight and decreased water consumption.

VI. Derivation of U.S. EPA Reference Concentration (RfC

Study	Lynch *et al.*, 1990; Virginia Chemicals, 1987; U.S. EPA, 1995
Study population	Rats (10/sex/group)
Exposure method	Discontinuous whole-body inhalation
Critical effects	Nasal passage inflammation
LOAEL	1,000 ppm (Virginia Chemicals, 1987)
NOAEL	247 ppm (Lynch *et al.*, 1990)
Exposure continuity	6 hours/day, 5 days/week
Exposure duration	10 days (Virginia Chemicals, 1987); 28 weeks (Lynch *et al.*, 1990)
Average experimental exposure	44.2 ppm for NOAEL group (247 x 6/24 x 5/7)
Human equivalent concentration	4.7 ppm for NOAEL group (based on a gas with respiratory effects, RGDR= 0.107)
LOAEL uncertainty factor	1
Subchronic uncertainty factor	10
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Modifying factor	10 (database deficiencies)
Cumulative uncertainty factor	3,000
Reference exposure level	0.002 ppm (2 ppb; 0.007 mg/m^3; 7 μg/m^3)

The major strength of the triethylamine RfC is the observation of a NOAEL in a controlled exposure experiment. The major weaknesses are the lack of adequate human health effects information, the lack of dose-response data or a LOAEL and NOAEL in a single experiment, and the lack of long-term exposure data.

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CHRONIC TOXICITY SUMMARY

VINYL ACETATE

(1-acetoxyethylene; acetic acid, vinyl ester; acetic acid, ethenyl ester; VAC; vinyl A monomer; ethenyl ethanoate)

CAS Registry Number: 108-05-4

I. Chronic Toxicity Summary

Inhalation reference exposure level	**200 µg/m^3** (U.S. EPA RfC) This document summarizes the evaluation of non-cancer health effects by U.S. EPA for the RfC.
Critical effect(s)	Nasal epithelial lesions in rats and mice
Hazard index target(s)	Respiratory system

II. Physical and Chemical Properties (HSDB, 1994)

Description	Colorless liquid
Molecular formula	$C_4H_6O_2$
Molecular weight	86.09
Density	0.932 g/cm^3 @ 20°C
Boiling point	72.7° C
Melting point	-93.2°C
Vapor pressure	115 mm Hg @ 25°C
Solubility	Slightly soluble in water, soluble in ethane, acetone, chloroform; >10% soluble in ethanol and benzene
Conversion factor	1 ppm = 3.52 mg/m^3 @ 25°C

III. Major Uses and Sources

The major use of vinyl acetate monomer is in the manufacture of polyvinyl and vinyl acetate copolymers, which are used in water-based paints, adhesives, paper coatings, and applications not requiring service at extreme temperatures (HSDB, 1994). It is also used in safety glass interlayers and in hair sprays (HSDB, 1994).

IV. Effects of Human Exposures

Deese and Joyner (1969) conducted an occupational study of 21 chemical workers with a mean length of employment of 15.2 years and exposed to a time-weighted average of 8.6 ppm (30.3 mg/m^3) VA. No adverse effects were noted following chest x-ray, electrocardiogram, blood chemistry, and urinalysis. The control group (sample size unspecified) consisted of workers in units not exposed to VA. An additional study was performed by Deese and Joyner (1969) that showed intolerable eye irritation in 3 out of 3 subjects exposed for an unspecified extended period of time to 21.6 ppm (76 mg/m^3) VA. Upper respiratory irritation was also experienced by a majority of 5 subjects. Odor was detected at 0.4 ppm (1.4 mg/m^3) in 3 out of 3 subjects.

V. Effects of Animal Exposures

A 104-week inhalation study in rats and mice (90/sex/group) was conducted using concentrations of 0, 50, 200, or 600 ppm (0, 176, 704, or 2113 mg/m^3) vinyl acetate (VA) (Owen, 1988). The Owen (1988) study was later published by Bogdanffy *et al.* (1994). Exposures were for 6 hours/day, 5 days/week. Histology was performed on all major organs. There was no mortality resulting from these exposures. A close examination of the effects of VA on the lung and nasal passages showed significant lesions in the nasal cavity, bronchi, and lungs of rats exposed to 600 ppm VA. Lesions included olfactory epithelial metaplasia/atrophy and nest-like epithelial folds in the nasal cavity, exfoliation of bronchial epithelium, fibrous intraluminal projections in the bronchi, and pigmented histiocyte accumulation in the lungs. Body weight gain of rats was significantly decreased in the 600 ppm VA group. Rats treated with 200 ppm VA showed some evidence of epithelial atrophy and metaplasia in the nasal cavity. No effects were observed in the rats from exposure to 50 ppm VA.

Mice also exhibited significant histological lesions in the respiratory tract following exposure to 200 ppm VA or greater. The lesions included atrophy of the olfactory epithelium and submucosal gland. At the 600 ppm concentration, hyperplasia of the trachea was observed, in addition to exfoliation/flattening of the bronchial epithelium and decreased body weight gain. Relative brain and kidney weights were increased in the 600 ppm group at the end of the study, and absolute liver, heart and kidney weights were also significantly elevated. No adverse effects were observed in the 50 ppm group.

A 13-week study on the effects of VA in mice was conducted by Owen (1980a). In this study, mice (10/sex/concentration) were exposed to 0, 50, 200, or 1000 ppm (0, 176, 704, or 3520 mg/m^3) VA for 6 hours/day, 5 days/week for 13 weeks. A concentration-dependent increase in the incidence of diffuse rhinitis, beginning at the 200 ppm concentration, was detected using histopathological examination. Focal pneumonitis was observed in the 1000 ppm treatment group. No adverse effects were seen in the 50 ppm treatment group. An identical study in rats was also conducted by Owen (1980b). In this study, body weight gain was significantly reduced in male and female rats exposed to 1000 ppm VA. An increase in the incidence of mild histiocytic alveolitis was observed in the 1000 ppm group.

Irvine (1980) conducted a study on the developmental toxicity of VA in rats. Groups of 24 pregnant female rats were exposed to 0, 52, 198, or 1004 ppm (0, 182, 696, or 3533 mg/m^3) VA for 6 hours/day on days 6-15 of gestation. Significant maternal toxicity, as measured by reduced weight gain from day 10 through day 15, was observed in animals exposed to 1004 ppm. Fetotoxicity, as measured by reduced crown-rump length, reduced body weight, and increased incidence of ossification defects in the sternebrae and occipital regions, was observed in the 1004 ppm group. No maternal or fetal effects were seen at the lower two VA treatments.

VI. Derivation of U.S. EPA Reference Concentration (RfC)

Study	Owen, 1988; Dreef van der Meulen, 1988; Beems, 1988; U.S. EPA, 1995
Study population	Male and female Sprague-Dawley rats and CD-1 mice (90/sex/group)
Exposure method	Discontinuous inhalation exposures (0, 50, 200, or 600 ppm) over 104 weeks
Critical effects	Histological lesions of the nasal epithelium
LOAEL	200 ppm
NOAEL	50 ppm
Exposure continuity	6 hours/day, 5 days/week
Exposure duration	104 weeks
Average experimental exposure	8.9 ppm for NOAEL group (50 x 6/24 x 5/7)
HEC	1.4 ppm for NOAEL group (RGDR = 0.16 based on a gas with respiratory effects)
LOAEL uncertainty factor	1
Subchronic uncertainty factor	1
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	30
Inhalation reference exposure level	0.05 ppm (50 ppb, 0.2 mg/m^3, 200 μg/m^3)

Acetaldehyde, a hydrolysis product of vinyl acetate, was present in the Owen (1988) study at a concentration of 49 ppm (89 mg/m^3). The duration-adjusted concentration for acetaldehyde was 16 mg/m^3, whereas the NOAEL for histological lesions in rats by Appleman *et al.* (1982) was 48.75 mg/m^3. Therefore, the concentration of acetaldehyde was not considered to account for significant irritation in the Owen (1988) study.

The strengths of the inhalation REL include the availability of controlled exposure lifetime inhalation studies in multiple species at multiple exposure concentrations and with adequate histopathogical analysis, and the observation of a NOAEL. The major area of uncertainty is the lack of adequate human exposure data.

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