

CHLOROFORM

Interim 3: 03/2009

CHLOROFORM
(CAS Reg. No. 67-66-3)

PROPOSED ACUTE EXPOSURE GUIDELINE LEVELS (AEGLs)

PREFACE

Under the authority of the Federal Advisory Committee Act (FACA) P. L. 92-463 of 1972, the National Advisory Committee for Acute Exposure Guideline Levels for Hazardous Substances (NAC/AEGL Committee) has been established to identify, review and interpret relevant toxicologic and other scientific data and develop AEGLs for high priority, acutely toxic chemicals.

AEGLs represent threshold exposure limits for the general public and are applicable to emergency exposure periods ranging from 10 minutes to 8 hours. Three levels — AEGL-1, AEGL-2 and AEGL-3 — are developed for each of five exposure periods (10 and 30 minutes, 1 hour, 4 hours, and 8 hours) and are distinguished by varying degrees of severity of toxic effects. The three AEGLs are defined as follows:

AEGL-1 is the airborne concentration (expressed as parts per million or milligrams per cubic meter [ppm or mg/m³]) of a substance above which it is predicted that the general population, including susceptible individuals, could experience notable discomfort, irritation, or certain asymptomatic, non-sensory effects. However, the effects are not disabling and are transient and reversible upon cessation of exposure.

AEGL-2 is the airborne concentration (expressed as ppm or mg/m³) of a substance above which it is predicted that the general population, including susceptible individuals, could experience irreversible or other serious, long-lasting adverse health effects or an impaired ability to escape.

AEGL-3 is the airborne concentration (expressed as ppm or mg/m³) of a substance above which it is predicted that the general population, including susceptible individuals, could experience life-threatening health effects or death.

Airborne concentrations below the AEGL-1 represent exposure levels that could produce mild and progressively increasing but transient and non-disabling odor, taste, and sensory irritation or certain asymptomatic, non-sensory effects. With increasing airborne concentrations above each AEGL, there is a progressive increase in the likelihood of occurrence and the severity of effects described for each corresponding AEGL. Although the AEGL values represent threshold levels for the general public, including susceptible subpopulations, such as infants, children, the elderly, persons with asthma, and those with other illnesses, it is recognized that individuals, subject to unique or idiosyncratic responses, could experience the effects described at concentrations below the corresponding AEGL.

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SUMMARY

Chloroform is a volatile liquid with a pleasant, nonirritating odor. The chemical is miscible with organic solvents but only slightly soluble in water. Although previously used as an anesthetic and in pharmaceutical preparations, these uses are no longer allowed in the U.S. The chemical is, however, produced and imported in large quantities for use in chemical syntheses, as a solvent, and in the manufacture of some plastics.

Human data regarding acute inhalation exposure to chloroform are limited to older studies involving the exposure of human subjects to various exposure regimens (3-30 minutes and 680-7200 ppm) and resulting in effects ranging from detection of strong odor, headaches and dizziness, to vertigo. Analyses of published reports of surgical patients anesthetized with chloroform, although lacking precise exposure terms, suggested that such exposures (generally in excess of 13,000 ppm) may produce cardiac arrhythmias and transient hepatic and renal toxicity. Quantitative data regarding human fatalities following acute inhalation exposure to chloroform are not currently available.

Only limited data are available pertaining to lethality in animals following acute exposure to chloroform. Definitive quantitative data are limited to a 4- hr LC₅₀ of 9780 ppm in rats and a 7- hr LC₅₀ of 5687 ppm in mice. Remaining data indicate notable lethality following exposures ranging from 5 minutes ("saturated" concentration probably equivalent to approximately 25,000 ppm) to 12-hour exposure to 726 ppm. Data regarding the nonlethal toxicity in animals focus on biochemical (elevated serum enzyme activity indicative of hepatic damage) and histopathologic indices of hepatic toxicity in laboratory species. Data regarding reproductive/developmental toxicity in animals are equivocal. One study provided evidence of fetotoxicity in rats following gestational exposure to chloroform at 30 ppm although another study found no evidence of such toxicity following gestational exposures to 2232 ppm.

Although chloroform has been shown to be tumorigenic in rats (kidney tumors in male but not female rats) and mice (hepatocarcinomas in male and female mice) following oral exposure, there are no inhalation exposure studies demonstrating carcinogenic responses to chloroform. Currently available data on the mechanism of chloroform toxicity and tumorigenicity imply that the tumorigenic response occurs following chloroform exposures great enough to cause cell death and proliferative cellular regeneration. As such a linear low-dose extrapolation for cancer risk may not be appropriate. For this reason and because the inhalation slope factor for chloroform is based upon effects following oral administration, the AEGL values for chloroform are based upon noncarcinogenic endpoints.

Metabolism and disposition studies have affirmed that metabolism of chloroform to phosgene is mediated by P-450IIE1 and that phosgene along with depletion of reduced glutathione and formation of trichlorocarbon-radical intermediates are responsible for the toxicity of chloroform. Data from several studies indicate that the metabolism and, therefore, the rate of production of reactive metabolites is greater in rodents than in humans.

AEGL-1 values were not recommended. Based upon the available data, attempts to identify a critical effect consistent with the AEGL-1 definition were considered tenuous and uncertain. Exposures of humans to concentrations approaching those inducing narcosis or possibly causing hepatic and renal effects are not accompanied by overt signs or symptoms. Furthermore, the odor of chloroform is not unpleasant or irritating.

The AEGL-2 values for chloroform were based upon fetotoxicity and embryoletality in rats (Schwetz et al., 1974) resulting from exposure of dams to 100 ppm, 7 hours/day on gestation days 6-15. For AEGL-2 development, an assumption was made that the effects could be caused by only a single 7-hr exposure. Because available data on metabolism and kinetics indicate that rodents are more sensitive than humans to the toxic effects of chloroform, an interspecies variability uncertainty factor was not applied. An intraspecies uncertainty factor of 3 was applied to account for variability in metabolism and disposition among individuals and to protect more susceptible individuals (e.g., P-450 induction by alcohol use or exposure to other inducers of P-450 monooxygenase). No additional reduction in the AEGL-2 values was warranted because the critical effect and the assumption of a single-exposure scenario provided a conservative point of departure. For AEGL development, data were unavailable for empirically deriving a chemical-specific time scaling relationship ($C^n \times t = k$). The concentration-exposure time relationship for many irritant and systemically acting vapors and gases may be described by $C^n \times t = k$, where the exponent n ranges from 0.8 to 3.5 (ten Berge et al., 1986). In the absence of data with which to empirically derive a chemical-specific scaling exponent (n), temporal scaling was performed using $n = 3$ when extrapolating to shorter exposure durations or $n = 1$ when extrapolating to longer exposure durations.

The AEGL-3 values for chloroform were based upon a 560-minute mouse LC₅₀ of 4500 ppm. Because data were unavailable for quantitatively estimating a lethality threshold, the LC₅₀ was reduced 3-fold to 1500 ppm, an exposure level unlikely to cause lethality based upon comparisons to other available human and animal exposure data. Uncertainty factor application was limited to 3 for protection of sensitive individuals. As in AEGL-2 derivations, the intraspecies uncertainty factor of 3 was selected because it is unlikely that induction of metabolism would increase toxic effects by an order of magnitude. Available data indicate that rodents metabolize chloroform at a greater rate than do humans resulting in production of reactive, toxic intermediates at a greater rate. Results of PBPK model studies have shown that rodents, especially mice, are considerably more susceptible to the lethal effects of chloroform than are human. Therefore, the AEGL-3 values were increased three-fold by a weight-of-evidence adjustment factor of 1/3. This adjustment is further justified by surgical anesthesia data

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showing cumulative exposures of >675,000 ppm·minute and exposures to 22,500 ppm for up to 120 minutes resulted in surgical anesthesia and cardiac irregularities but not death. Time scaling was performed using an *n* of 3 to extrapolate from the 560-minute exposure duration of the point-of-departure to the shorter AEGL- time periods. To minimize uncertainties of extrapolating from the 560-minute experimental exposure period to the 10-minute AEGL-3 period, the 30-minute AEGL-3 value of 4000 ppm was adopted for the 10-minute period as well.

Assessments of carcinogenic potential following single, acute exposure to chloroform indicated that AEGL-2 values based upon noncancer toxicity endpoints were slightly greater than those based on cancer risk. However, the carcinogenic response to chloroform appears to be a function of necrosis and subsequent regenerative cellular proliferation that are not relevant to a single acute exposure.

Classification	10-min	30-min	1-hour	4-hour	8-hour	Endpoint (Reference)
AEGL-1	NR	NR	NR	NR	NR	Not recommended; data insufficient to develop AEGL-1 values; AEGL-1 effects unlikely to occur in the absence of notable toxicity.
AEGL-2	120 ppm 580 mg/m ³	80 ppm 390 mg/m ³	64 ppm 312 mg/m ³	40 ppm 195 mg/m ³	29 ppm 141 mg/m ³	Fetotoxicity/embryo-lethality in rats exposed for 7 hrs/day on gestation days 6-15 (Schwetz et al., 1974); single exposure assumed
AEGL-3	4000 ppm [19,000 mg/m ³]	4000 ppm [19,000 mg/m ³]	3200 ppm [16,000 mg/m ³]	2000 ppm [9,700 mg/m ³]	1600 ppm [7,800 mg/m ³]	Estimated lethality threshold for mice; 3-fold reduction 560-min LC ₅₀ of 4500 ppm to 1500 ppm (Gehring, 1968)

References

Gehring, P.J. 1968. Hepatotoxic potency of various chlorinated hydrocarbon vapours relative to their narcotic and lethal potencies in mice. *Toxicol. Appl. Pharmacol.* 13: 287-298.

Schwetz, B.A., Leong, B.K.J., Gehring, P.J. 1974. Embryo- and fetotoxicity of inhaled chloroform in rats. *Toxicol. Appl. Pharmacol.* 28: 442-451.

ten Berge, W.F., Zwart, A., Appelman, L.M. 1986. Concentration-time mortality response relationship of irritant and systemically acting vapours and gases. *J. Hazard. Materials.* 13: 301-309.

1. INTRODUCTION

Chloroform is a volatile liquid with a pleasant, nonirritating odor. The chemical is miscible with organic solvents but only slightly soluble in water. Although previously used as an anesthetic and in pharmaceutical preparations, these uses are no longer allowed in the U.S. Chloroform is produced and imported in large quantities (≈ 93 -350 million pounds/year) and used in chemical syntheses, for refrigeration, as a solvent, and in the manufacture of polytetrafluoroethylene plastics (DeShon, 1978; Li et al., 1993). Chloroform is also a byproduct of wood pulp chlorination for production of paper products. Physicochemical data for chloroform are shown in Table 1.

The AIHA (AIHA, 1989) reported an odor threshold of 192 ppm based upon a geometric mean of acceptable values (133-276 ppm). An odor detection of 6.1 ppm was reported by the U.S. EPA (USEPA, 1992a).

TABLE 1. Physicochemical Data for Chloroform		
Parameter	Value	Reference
Synonyms	trichloromethane, methenyl chloride, methyl trichloride	DeShon, 1978
CAS Registry No.	67-66-3	Budavari et al., 1996
Chemical formula	CHCl ₃	Budavari et al., 1996
Molecular weight	119.39	Budavari et al., 1996
Physical state	liquid	Budavari et al., 1996
Vapor pressure	159.6 mm Hg @20°C	DeShon, 1978
Density	1.484 @ 20°C	Budavari et al., 1996
Boiling/melting point	61-62°C/-63.5°C	Budavari et al., 1996
Solubility	1 ml/200 ml water @ 20°C	Budavari et al., 1996
Conversion factors in air	1 ppm = 4.87 mg/m ³ 1 mg/m ³ = 0.21 ppm	

2. HUMAN TOXICITY DATA**2.1 Acute Lethality**

Quantitative data regarding acute inhalation exposures to chloroform resulting in death were not available.

2.2 Nonlethal Toxicity

Several reports are available regarding the effects of acute inhalation exposure of humans to chloroform and serve to qualitatively characterize the health effects of chloroform inhalation.

Hutchens and Küng (1985) reported nausea, appetite loss, transitory jaundice, cardiac arrhythmias, arterial hypotension, mild intravascular hemolysis, and unconsciousness in an individual following intentional, nonsuicidal inhalation of chloroform.

Lehmann and Hasegawa (1910) conducted controlled exposure studies on human subjects. The results of this study showed that a 3-minute exposure to 920 ppm induced vertigo and dizziness and a 30-minute exposure to 680 ppm produced moderately strong odor. A 30-minute exposure to 1400 ppm produced lightheadedness, giddiness, lassitude, and headache while exposure to 3000 ppm resulted in gagging and pounding of the heart. Twenty-minute exposure at 4300 to 5100 ppm or 15 minute exposure at 7200 ppm produced light intoxication and dizziness. These data appeared to be derived from exposure of only three subjects and methods of exposure generation and measurements are unavailable. The signs and symptoms of exposure described in this report appear to be consistent with early stages of narcosis.

Lehmann and Flury (1943) reported that exposure of humans to 389 ppm for 30 minutes is tolerated without complaint but that exposure to 1030 ppm resulted in dizziness, intracranial pressure, and nausea within 7 minutes and headache that persisted for several hours.

Whitaker and Jones (1965) analyzed the clinical effects of chloroform anesthesia from 1502 surgery patients. Although the duration of anesthesia varied from <30 minutes to over two hours, the chloroform concentration never exceeded 2.25% (22,500 ppm). For most (1164 of 1502) of the cases, anesthesia was for less than 30 minutes. Clinical observations regarding the chloroform anesthesia included tachypnea, bradycardia, cardiac arrhythmias, hypotension, one case of transient jaundice, and one death (this was complicated by renal insufficiency and could not necessarily be attributed to the chloroform anesthesia). Although the maximum chloroform concentration was provided, the exposure time required to attain anesthesia was not stated. It could be assumed; however, that onset of anesthesia likely occurred within a few minutes. These observations do, however, demonstrate that a short exposure to 22,500 ppm will induce a surgical plane of anesthesia concurrent with various physiologic responses.

The clinical effects associated with chloroform-induced anesthesia were also studied by Smith et al. (1973) in an attempt to justify the resurrection of chloroform as an accepted anesthetic agent. However, the utility of data from this study for AEGL development are compromised by confounders including premedication with diazepam and pentobarbital or with hydroxyzine and pentobarbital. The inspired chloroform concentration appeared to vary between 0.85 (8,500 ppm) and 1.3% (13,000 ppm) and the average duration of anesthesia was 112.0 ± 60.38 minutes among the 58 surgical patients. Upon recovery, 46% of the patients receiving chloroform experienced nausea and vomiting. Clinical assessment of liver function and toxicity indicated transient alterations. One ventricular tachycardia occurred that necessitated pharmacologic correction. Data from a single patient indicated that chloroform at 8500 ppm would induce anesthesia.

McDonald and Vire (1992) examined the possible health hazards associated with chloroform use in endodontic procedures. Two industrial hygiene monitors sampled air in the treatment operatory and additional sampling devices were attached to the dentist and the dental assistant. The operatory area samples measured <0.57 ppm for a 5.5-hour period and the individual breathing air samples (dentist and assistant) measured <0.88 ppm over a 150-minute period. Health screening tests for the dentist and assistant revealed no signs of liver, kidney or lung damage five hours post exposure or at one year after the study.

Although specific data were not presented, Snyder and Andrews (1996) report that humans may tolerate up to 400 ppm chloroform for 30 minutes without complaint but may experience dizziness and gastrointestinal upset at 1000 ppm for seven minutes, and narcosis following exposure to 14,000 ppm (no duration specified).

2.2.1 Epidemiologic Studies

Several epidemiologic studies have been conducted regarding occupational exposure to chloroform. These studies involve worker populations exposed to the chemical for periods of time in excess of what would be considered acute exposure, and are not directly applicable to developing AEGL values. They do, however, provide some insight regarding the relationship between proposed AEGL values and the health effects that may be associated with long-term exposures.

Challen et al. (1958) evaluated workers in a pharmaceutical manufacturing process that involved exposure to chloroform vapor. Data regarding exposure terms are limited to eight "long-service operators" (3 to 10-year exposures) exposed to 77 to 237 ppm, nine employees termed "short-service operators" (10 to 24-month exposures) who were replacements for the long service operators and were exposed to 23 to 71 ppm, and a group of five controls who were not exposed to processes involving chloroform. All workers in these groups were women whose ages ranged from 34 to 60 years. Some "long-service operators" had been observed staggering about the work area. All "long-service" workers experienced alimentary effects (e.g., nausea,

flatulence, thirst), increased micturition and urinary discomfort, and behavioral effects (depression, irritability, poor concentration ability, motor deficiencies) during employment. All experienced nausea and stomach upset upon smelling chloroform after leaving their employment. Two of nine “short service operators” reported no effects from chloroform exposure, five reported dryness of the mouth and throat while at work, two had similar experiences as the “long service operators”, and several reported lassitude.

Bomski et al. (1967) reported the results of a study on workers in a Polish pharmaceutical factory with a special emphasis on examining chloroform-induced susceptibility to viral infection. Chloroform exposures were determined to be 2 to 205 ppm although frequency of sampling was not provided. The authors found that the incidence of viral hepatitis was greater in chloroform-exposed workers than in non-exposed inhabitants of the city and postulated that chloroform-induced hepatic damage may have predisposed the workers to the viral infection. Increased incidences of spleen and liver enlargement were also found in the chloroform-exposed workers.

Li et al., (1993) conducted surveys of chloroform-producing facilities in Shanghai, China. Most of the workers exposed to chloroform were associated with production of perspex (polymethylmethacrylate) and chemical synthesis. In the three facilities sampled (where no effective preventive equipment or measures were in place), chloroform concentrations ranged from 4.27 to 147.91 mg/m³ (0.88 to 31.06 ppm) with a geometric mean of 21.38 mg/m³ (4.49 ppm) for 119 samples. Chloroform concentrations were <20 mg/m³ (4.20 ppm) in 45.5% of the 119 samples. Exposure groups were classified as Exposure I (13.49 mg/m³ [2.83 ppm]; 1-15 years exposure) and Exposure 2 (29.51 mg/m³ [6.20 ppm]; 1-15 years exposure). The exposure groups and control group (no obvious chloroform or other hazardous exposures) included males and females as well as smokers and nonsmokers; all groups had an average age of approximately 36 years. The investigators concluded that long-term exposure to chloroform at 29.51 mg/m³ (6.20 ppm) resulted in functional liver damage as determined by changes in various serum enzymes (ALT [alanine aminotransferase], gamaglutamyltransferase, and adenosine deaminase), prealbumin levels, serum transferrin, and blood urea nitrogen.

2.3 Reproductive/Developmental Toxicity

Wennborg et al. (2000) conducted a study in a cohort of Swedish women who had worked in laboratory or non-laboratory jobs for one or more years during 1990-1994. The investigators obtained data from questionnaires to 763 women (66 were excluded for various reasons) that assessed reproductive history, health status, time-to-pregnancy, personal habits, specific work, and exposure to various agents and specific time at which these exposures occurred. The data from these women were compared to respective birth information from the Swedish Medical Register. Parameters examined included spontaneous abortion (SAB), birth weight, preterm delivery, small-for-gestation age (SGA), large-for-gestation age, and congenital deformities. A number of confounding variables were considered (high blood pressure, smoking, gynecological

and chronic disease, sexually transmitted infectious diseases, father's work and potential exposures during time of conception, previous abortions, etc.). Information regarding consumption of alcohol, teas, and coffee, and stress levels was not included. The analysis included 869 pregnancies but did not involve specific exposure concentrations, and did not account for exposures to other chemicals. There was no association between laboratory work and SABs. A weak association was found between women who had worked with chloroform prior to conception and SABs but there was no significant association between chloroform exposure and SGA or body weight.

2.4 Genotoxicity

No studies were located in the available literature regarding the genotoxicity of chloroform in humans.

2.5 Carcinogenicity

Although epidemiology studies have been conducted to assess the carcinogenic potential of chloroform in drinking water, no inhalation studies are available regarding the carcinogenic potential of chloroform in humans following inhalation exposure. The U.S. EPA (1992b) has developed an inhalation slope factor of $6.1 \times 10^{-3} \text{ (mg/kg/day)}^{-1}$ based upon an increased incidence of renal tumors in male rats following long-term exposure to chloroform in drinking water (Jorgenson et al., 1985). Route-to-route extrapolation was required for its derivation as inhalation exposure data were not available.

2.6 Summary

Quantitative data regarding human lethality following acute exposure to chloroform are unavailable. Although lacking quantitative exposure terms and often pertaining to oral exposures, clinical reports affirm the hepatotoxicity and renal toxicity of chloroform as well as the neurological effects. The available data on nonlethal responses indicate that acute inhalation of chloroform may result in narcosis and may be preceded by signs and symptoms characteristic of early stages of anesthesia. Early reports in which the effects of chloroform inhalation were observed in human subjects are limited by uncertainties in the measurements of exposure concentrations but do provide information regarding the human experience that does not appear to be inconsistent with other data. A summary of data relevant to acute nonlethal exposure of humans to chloroform is presented in Table 2.

TABLE 2. Nonlethal Effects of Chloroform in Humans Following Acute Inhalation Exposure				
No.of subjects	Exposure concentration (ppm)	Exposure duration (min)	Effect	Reference
3	920	3	vertigo	Lehmann and Hasegawa,1910
3	680	30	strong odor	Lehmann and Hasegawa,1910
3	1400	30	light headedness, lassitude, headache	Lehmann and Hasegawa,1910
3	3000	30	pounding heart, gagging	Lehmann and Hasegawa,1910
NA	4300-5100	20	intoxication, dizziness	Lehmann and Hasegawa,1910
NA	7200	15	intoxication, dizziness	Lehmann and Hasegawa,1910
NA	389	30	no complaints	Lehmann and Flury, 1943
NA	1030	7	dizziness, intracranial pressure, nausea, persistent headache	Lehmann and Flury, 1943
1502	22,500	<30 - >120 (most <30)	surgical plane anesthesia, cardiac irregularities	Whitaker and Jones, 1965
58	8500-13,000	113 (mean duration)	surgical plane anesthesia	Smith et al., 1973
2	<0.5	330	no effects*	McDonald and Vire, 1992
2	<0.88	150	no effects*	McDonald and Vire, 1992

* Health screening conducted at 5 hours postexposure and at one year after exposure

3. ANIMAL TOXICITY DATA

3.1 Lethal Toxicity

3.1.1 Rats

Results of preliminary range-finding experiments for a large number of chemicals were reported by Smyth et al. (1962). Concentrated chloroform vapor (presumably a saturated concentration [$\approx 25,000$ ppm] but no quantitative data provided) killed all six of the albino rats (strain not specified) exposed for five minutes. A four-hour exposure to 8000 ppm (nominal concentration; no analytical determination) killed five or six albino rats.

The results of 4-hour inhalation study in rats were briefly described in report to E. I. du Pont de Nemours and Co. (Haskell Laboratory, 1964). The study, designed to assess the toxicity of Freon TC[®] and Freon-113[®], also included experiments with chloroform (a component of Freon TC[®]). For the experiments with chloroform, four rats (gender and strain not specified) were exposed to chloroform at concentrations of 5000, 3700, or 3000 ppm for 4 hours. Four rats exposed to clean air served as controls. For the 5000, 3700, and 3000-ppm exposures, mortality was 3 of 4, 3 of 4, and 0 of 4. Deaths occurred at 2 to 3 days postexposure; the four rats in the 3000-ppm group were terminated at 14 days postexposure. No information was provided regarding the methods for measurement of chloroform concentrations (atmosphere produced by heating chloroform and injection into the chamber via a nebulizer); only nominal exposure concentrations were reported. There were no histopathology data provided for the chloroform-treated rats.

In experiments to assess the effect of chloroform inhalation on barbiturate metabolism and narcosis, Puri et al. (1971) exposed male Sprague-Dawley rats to 726 ppm chloroform for up to 48 hours (continuous exposure). Although the study focused on the effect of chloroform on barbiturate activity and metabolism, one group of rats was exposed to chloroform alone. Based upon the graphic presentation of the data, continuous 12-hour exposure resulted in at least 10 deaths. It is unclear if any deaths occurred prior to the 12-hour data point.

Lundberg et al. (1986) reported a 4-hour LC₅₀ of 47,702 mg/m³ (9780 ppm) for groups of ten female Sprague-Dawley rats exposed to a geometric series of chloroform concentrations (specific exposure concentrations for the series were not provided but stated as being equivalent to equivalent to 1/2, 1/4, 1/8, 1/16, or 1/32 of the LC₅₀ or the saturation concentration. Mortality was determined at 24 hours after exposure. The exposure concentrations were measured by infrared detection in a suitably designed apparatus.

3.1.2 Mice

The results of studies with mice exposed to chloroform were reported by Fühner (1923). Groups of mice (sex and strain not reported; 30 mice total) were exposed to various concentrations of chloroform (12 to 38 mg/L or ≈ 2458 to 7782 ppm). The mice were exposed

individually in 10-liter bottles in which chloroform was vaporized to achieve the desired concentration. Concentrations were not determined analytically. Five mice exposed to 2458 to 5120 ppm exhibited reflex loss at 48 to 215 minutes of exposure but there were no deaths. Exposure to 4710 to 5529 ppm resulted in reflex loss at 30 to 90 minutes with recovery of 12 of 18 animals and the death of six. Deaths occurred at 71 to 175 minutes of exposure. Out of seven mice exposed to 6758 to 7782 ppm, six mice exhibited reflex loss at 13 to 46 minutes of exposure and one mouse died after a 35-minute exposure (reflex loss occurred at 8 minutes). The absence of validated exposure concentrations limits the quantitative validity of these data. Four additional mice were exposed to 5585 ppm for 120 or 135 minutes. For the three mice exposed for 120 minutes, death occurred at 105, 130, and 140 minutes after the start of exposure, and the one mouse exposed for 135 minutes died 95 minutes after exposure. Under the conditions of these experiments, the findings suggest that exposure concentrations in the vicinity of 4710 ppm may represent a lethal threshold for mice following 1 to 2-hour exposure.

A 7-hour LC₅₀ of 5687 ppm for mice was reported by von Oettingen et al. (1949). These experiments used 20 adult white mice (strain and gender not specified) exposed to chloroform in a bell jar. The chloroform concentrations were calculated based upon the amount of test material volatilized over time and the volume of air passed through the chamber. The concentrations were also determined by chemical analysis. Analysis of the graphic representation of the experimental results indicated an LC₃₀ of 5529 ppm and an LC₉₀ of 6963 ppm. At the concentrations tested (4915 ppm to 7372 ppm), the mice exhibited progressive central nervous system depression followed by rapidly occurring narcosis. Death of the mice started occurring at 3 to 5 hours of exposure.

In a study by Deringer et al. (1953), the nephrotoxic and lethal effects of inhaled chloroform were examined using male and female C3H mice. In this study, three groups of six male and six female mice (2 months old) were exposed for 1, 2, or 3 hours to chloroform at concentrations of 3.38 to 5.4 mg/L (693 to 1,106 ppm). Additionally, three groups (six males and six females per group) of 8-month old mice were also exposed similarly. Twenty two male and 20 female mice served as untreated controls. Mice were observed daily for deaths or morbidity, and were examined weekly for tumors or other abnormal conditions. Necropsies were performed on all moribund or dead mice and any female mice exhibiting mammary tumors. Regardless of the exposure duration or specific concentration (693 to 1106 ppm), all of the male mice (except one) exposed to chloroform exhibited evidence of kidney damage. Within 11 das following the exposure, 15 of 18 eight-month old males and 7 of 18 two-month old males had died. The remainder of the 8-month old males survived 5 to 7 months and the remainder of the 2-month old male mice survived 14 to 18 months. Generally, deaths occurred earlier in the mice exposed for 2 or 3 hours than in those exposed for only 1 hour; specific data, however, were not provided. Histologic findings in mice that died included necrosis and calcification of the proximal and distal convoluted tubules. Necrosis appeared to be more severe with earlier deaths. Additionally, hepatic necrosis was also observed in mice exposed to the higher end of the concentration range (i.e., 942 to 1106 ppm) that died within six days. For male mice surviving

longer and in all female mice, hepatic damage was not notable. Based upon the various chloroform concentrations and exposure durations reported, the results of this study show that 3-hour exposure of male C3H male mice to chloroform at a concentration as low as 692 ppm or 1-hour exposure to a concentration as low as 921 ppm resulted in severe renal damage and death.

The influence of sex hormone status on gender-specific chloroform-induced nephrotoxicity in mice was studied by Culliford and Hewitt (1957). Although the primary objective of the study was to verify the influence of androgens on chloroform-induced nephrotoxicity, the initial results of the study provided evidence of nearly complete tubular necrosis in two strains of male mice following 2-hour inhalation exposures. Male WH (Westminster Hospital in-house, uniform heterozygous) mice exposed to 3.3 to 7.0 mg/L (676 to 1434 ppm) and male CBA mice exposed to 1.2 to 5 mg/L (246 to 1024 ppm) all exhibited complete tubular necrosis 24 hours following the exposure. Female mice of these strains did not exhibit any evidence of renal damage. The study went on to show that administration of estrogen to male mice abolished the susceptibility to the nephrotoxic response, and that the administration of testosterone to female mice increased susceptibility. The chloroform concentrations were calculated based upon the amount of chloroform added to the 6-L exposure chamber, and the assumption of complete vaporization at 80°F and uniform dispersal. No analytical measurements were made, thereby imparting some uncertainty regarding the actual chamber concentrations.

In studying the hepatotoxicity of chlorinated hydrocarbons, Gehring (1968) calculated a 4500-ppm LC₅₀ of 560 minutes (540 -585 minutes, 95% C.I.) for female Swiss-Webster mice as well a 4500-ppm EC₅₀ of 35 minutes (31.0 - 39.6 minutes, 95% C.I.) for narcosis, and a 4500-ppm EC₅₀ of 2.3 minutes (1.9 - 2.8 minutes, 95% C.I.) for elevated serum glutamic pyruvic transaminase (SGPT) activity. Groups of mice (10/group for narcosis determination and 20/group for lethality determination) were exposed to 4500 ppm chloroform and the number of responders for the endpoint of concern noted relative to exposure duration. The control group consisted of 254 mice representing a composite group of controls for all of the chlorinated hydrocarbons tested. Chloroform concentrations were attained by metering the chloroform into a heated tube for vaporization. Actual concentrations were measured by continuous flow of the atmosphere through an infrared spectrophotometric cell. If the chloroform concentration varied by more than 7% the experiment was repeated. Mortality responses to 4500-ppm chloroform ranged from approximately 5% at 400 minutes exposure duration to 80% at 700 minutes exposure duration. An exposure-response for narcosis was also determined and was shown to exhibit the same slope. These data suggest that, at an exposure of 4500 ppm, there is approximately a 16-fold difference between the time-to-narcosis (35 minutes) and the time-to-death (560 minutes) for mice exposed under the conditions of this study. Elevation of SGPT was also reported and exhibited a notably different exposure-response relationship (see Section 3.2.2).

3.1.3 Dogs

The effect of chloroform-induced anesthesia in dogs was studied by Whipple and Sperry (1909). Details regarding the exposure concentrations are limited to notation of the amount of chloroform (in ounces) used on each dog. Anesthesia duration varied from 1.5 to 2.5 hours and chloroform amounts varied from <1 to 3 ounces. Some of the dogs died although it was not possible to ascertain a definitive dose response relationship from the data.

In addition to studies with mice, von Oettingen et al. (1949) also studied the effects of chloroform (exposure to 15,000 ppm nominal; 14,376 ppm determined) on dogs (10 beagles, gender not specified) that had been surgically prepared with a tracheal cannula, and carotid and femoral artery cannulae to which pressure transducers were attached. Following recovery from the pentothal-induced surgical anesthesia (beginning of voluntary movement and "lively" reflex), the dogs were exposed continuously to the chloroform. The average survival time was 202 minutes with extremes of 60 and 285 minutes.

3.1.4 Summary of Lethal Toxicity In Animals

The lethality of inhaled chloroform in laboratory species is summarized in Table 3. With the exception of the rat 4-hour LC₅₀ value (9780 ppm) reported by Lundberg et al. (1986) and the mouse LC_{t50} (4500 ppm; 560 minutes) reported by Gehring (1968), the data tend to be of a more qualitative nature. Data from older studies lack details regarding generation and measurement of exposure atmospheres. The available data do not present a clear delineation of the lethality of acute inhalation exposure to chloroform.

Species	Exposure concentration (ppm)	Exposure duration (min)	Effect	Reference
Rat	9780	240	4-hr LC ₅₀ †	Lundberg et al., 1986
Rat	3000	240	100% mortality	Haskell Laboratory, 1964
Rat	3700	240	75% mortality*	Haskell Laboratory, 1964
Rat	5000	240	75% mortality*	Haskell Laboratory, 1964
Rat	8000	240	≈80% mortality	Smyth et al., 1962
Rat	"saturated conc."	5	100% mortality	Smyth et al., 1962
Rat	726	720	lethality (no specifics provided)	Puri et al., 1971
Mouse	5529	420	7-hr LC ₃₀	von Oettingen et al., 1949
Mouse	5687	420	7-hr LC ₅₀	von Oettingen et al., 1949
Mouse	6963	420	7-hr LC ₉₀	von Oettingen et al., 1949
Mouse	4,710-5,529	71-175	66% mortality	Fühner, 1923
Mouse	6,758-7,782	35	14% mortality	Fühner, 1923
Mouse	2,458-5,120	48-215	no deaths	Fühner, 1923
Mouse	5,585	120	75% mortality‡	Fühner, 1923
Mouse	4500	560 min	50% lethality (LC _{t50})	Gehring (1968)

† Mortality at 24 hours postexposure

* Deaths determined at 2-3 days postexposure

‡ Deaths occurred at 105-140 minutes after exposure

3.2 Nonlethal Toxicity

3.2.1 Rats

In experiments reported by Brown et al. (1974b), groups of 3-9 male Sprague-Dawley rats were used to assess the effect of P-450 induction by phenobarbital on chloroform-induced effects on reduced glutathione (GSH). Both induced and control rats were exposed for two hours to

chloroform at concentrations of 0.5% (5000 ppm) or 1.0% (10,000 ppm). Although control rats (non-induced) exhibited no decrease in GSH levels, rats with induced P-450 exhibited an approximately 70% and 83% decrease in GSH for the 0.5% and 1.0% chloroform treatment groups, respectively. The absence of GSH decrease in the control rats exposed to chloroform suggests that at these exposures GSH levels are more than sufficient for scavenging reactive intermediates of chloroform metabolism.

Brondeau et al. (1983) examined the effect of a 4-hour inhalation exposure of rats on serum enzyme activities (GLDH- glutamate dehydrogenase; GOT - glutamic oxaloacetic transaminase; GPT - glutamic pyruvic transaminase; SDH- sorbitol dehydrogenase). In this study, groups of eight male Sprague-Dawley rats were exposed by whole-body inhalation to chloroform at concentrations of 137, 292, 400, 618, 942, or 1,075 ppm. A control group consisted of eight rats exposed to clean air. Chamber atmospheres were analyzed by gas chromatography (sample loop compared to a known concentration standard) and by analysis of a solid absorbent (activated charcoal or silica gel subjected to appropriate solvent extraction and gas-liquid chromatography). Exposure to the lowest concentration failed to significantly alter the activity levels of any the tested enzymes. The 4-hour exposure to chloroform, even at the highest concentration, resulted only in <2-fold to 7-fold increase in serum enzyme activities. Statistically significant elevations in GLDH and SDH were observed in rats exposed to 292 ppm for four hours. GLDH and SDH appeared to be most affected, although none of the changes in activity levels demonstrated a definitive exposure-response relationship. Although some of the increases (especially for GLDH and SDH) were statistically significant $p < 0.05$ and 0.02), the toxicological relevance of these changes is uncertain above and beyond being biological indicators of exposure. A second phase of the study exposed rats to 301 ppm (the concentration selected by the investigators as a threshold for alteration of serum enzyme activity based upon the single 4-hour experiments) for two 6-hour or four 6-hour exposures. GLDH and SDH activities exhibited somewhat greater increases following the four 6-hour exposures than the single 4-hour or two 4-hour exposures.

Statistically significant increases in serum SDH activity were also reported by Lundberg et al. (1986) for female Sprague-Dawley rats exposed for four hours to chloroform concentrations as low as 153 ppm (1/64 of the LC_{50} for chloroform as determined by Lundberg et al.). Although useful as biomarkers of exposure, the elevation of serum enzyme activity in the absence of clinical correlates would be of limited use as an endpoint for AEGL derivation.

In experiments to study the interaction of carbon tetrachloride and chloroform in ethanol-treated rats, Ikatsu and Nakajima (1992) presented data for groups of four rats exposed in a dynamic airflow chamber to chloroform-only controls (0, 50, or 100 ppm for 8 hours). Chloroform concentrations were monitored every 15 to 30 minutes by gas chromatography. Hepatotoxicity was determined by assessing changes in serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvate transaminase (SGPT), liver malondialdehyde (MDA) and plasma MDA. Only marginal and statistically insignificant changes were detected for these indices following 8-hour exposure to 50 or 100 ppm chloroform, thereby indicating that the 8-

hour exposure of rats to 50 or 100 ppm chloroform was without appreciable effect. Histopathologic examination revealed only negligible fat deposits in the liver of rats treated with 100 ppm chloroform. These findings are consistent with those of Brondeau et al. (1983) in the previously described study. In rats pretreated with ethanol (2 g ethanol/80 ml liquid diet fed daily for six weeks), only SGOT levels were increased significantly (3-fold; $p < 0.05$) following exposure to 50 ppm chloroform. Exposure of ethanol-treated rats to 100 ppm chloroform, however, resulted in significant ($p < 0.05$) increases in SGOT (7.5-fold) and SGPT (14-fold). There was no effect on liver MDA or plasma MDA. In ethanol-treated rats, ballooned hepatocytes in midzonal areas were observed but only in the high-dose (100 ppm) chloroform group. The results indicate that 8-hour exposure of rats to 50 or 100 ppm chloroform produce only minor effects that are indicative of indices of exposure rather than outright toxicity. Ethanol pretreatment followed by 8-hour exposure to 100 ppm chloroform produced notable signs of toxicity as determined by serum enzyme and histopathologic evaluations.

The hepatotoxicity and renal toxicity of inhaled chloroform was studied in male F-344 rats (five animals per group) following a 7-day exposure to 1, 3, 10, 30, 100, or 300 ppm chloroform for 6 hrs/day (Larson et al., 1994). The effects on nasopharyngeal tissue were also examined after the 7-day exposure (Méry et al., 1994). Cage-side observations indicated no observable signs of toxicity during the exposure period although there was a significant dose-dependent decrease in body weight gain at 10 ppm and above. Mild centrilobular vacuolation was observed only in the 300 ppm group and histopathologic changes (hyperplasia) were observed in the 10-ppm and above groups at the end of the 7-day exposure period. Two-treatment-related lesions were observed in the nasal region of the chloroform-exposed rats. An increase in the size of goblet cells of the nasopharyngeal meatus was observed in rats exposed to 100 or 300 ppm. Also, region-specific changes were observed in the olfactory mucosa and bone of the ethmoid turbinates of rat exposed to chloroform at or above 10 ppm. Although not providing data appropriate for derivation of AEGL values, the results of this study may be used to evaluate the protectiveness of proposed AEGL values.

In studies to assess the impact of ethanol on the metabolism and toxicity of chloroform by various routes of administration, Wang et al. (1994) provided nonlethal effects data for male Wistar rats exposed by inhalation to chloroform alone (50, 100, or 500 ppm for six hours). Indices of hepatotoxicity (GOT, GPT, and GSH) were evaluated in groups of five rats. Rats in the 50- and 100-ppm chloroform-only groups exhibited no significant changes in any serum enzyme activities. Both GOT and GPT were significantly ($p < 0.05$) elevated following the 6-hour exposure to 500 ppm (about 1.6-fold and 1.2-fold, respectively). Such increases, although numerically significant, are not indicative of severe hepatotoxicity. Ethanol pretreatment increased these enzyme activities approximately two-fold above that of chloroform alone, and failed to alter the GSH levels.

3.2.2 Mice

In addition to providing limited qualitative data on the lethality of mice exposed to chloroform, Fühner (1923) provided similar data regarding nonlethal responses of mice exposed to chloroform. Mice exposed to 2,458 to 5,120 ppm exhibited reflex loss at 48 to 215 minutes of exposure but there were no deaths. Exposure to 4,710 to 5,529 ppm resulted in reflex loss at 30 to 90 minutes with recovery of 12 of 18 animals and the death of six. Deaths occurred at 71 to 175 minutes of exposure. For mice exposed to 6,758 to 7,782 ppm, six mice exhibited reflex loss at 13 to 46 minutes of exposure and one mouse died after a 35-minute exposure (reflex loss occurred at 8 minutes). The absence of analytical measurement of exposure concentrations limits the quantitative validity of these data.

Kylin et al. (1963) reported on the hepatotoxicity of a single inhalation exposure of mice to chloroform. A pilot study to determine the time to maximum elevation of serum ornithine carbamyl transferase (OCT) was conducted using groups of five female albino mice exposed to chloroform (3000 ppm) for four hours with a group being terminated at 1, 2, 4, 8, or 16 days after the exposure. In the main study, groups of 10 female albino mice were exposed for four hours to 100, 200, 400, or 800 ppm chloroform. Controls were exposed similarly but without chloroform in the chamber. Histopathologic exam of the liver and measurement of serum OCT were used as indices of effect at 24 and 72 hours after the single exposure. The chloroform was vaporized prior to injection into the constant-flow chamber. No information was provided regarding the measurement of test material concentration in the chamber. In the pilot study, maximum serum OCT elevations were observed at four days postexposure. In the main study, fatty infiltration of the liver was observed at one day following a single exposure to 100 ppm chloroform. At higher exposure concentrations, the extent and severity of the fatty degeneration was increased. The authors concluded that the minimal chloroform concentration to produce fatty infiltration of the liver of mice after a 4-hour inhalation exposure was <100 ppm. Histologic changes (fatty infiltration and necrosis) also appeared to be greater at 24 hours after exposure than at 72 hours after exposure.

Gehring (1968), in addition to examining indices of lethality, determined 4500-ppm EC_t values for narcosis and for significant elevation of SGPT in female Swiss-Webster mice. Groups of mice (10/group for narcosis determination and 8-10/group for SGPT determination) were exposed to 4500 ppm chloroform and the response rate noted relative to exposure duration. The control group consisted of 254 mice representing a composite group of controls for all of the chlorinated hydrocarbons tested. SGPT elevations greater than 54 Reitman-Frankel units were considered as statistically significant (control values were 24.4 ± 14.7 R-F units). Chloroform concentrations were attained by metering the chloroform into a heated tube for vaporization. Actual concentrations were measured by continuous flow of the atmosphere through an infrared spectrophotometric cell. If the chloroform concentration varied by more than 7% the experiment was repeated. The 4500-ppm EC₅₀ for narcosis was 35 minutes (31.0- 39.6 minutes, 95% C.I.) with 10% response occurring at 15 minutes and 80% response occurring at approximately 40 minutes of exposure. The 4500-ppm EC₅₀ for significant elevation of SGPT was 13.5 min (10.1 - 18.1 minutes, 95% C.I.). A 20% response was observed at about 6 minutes duration and a 90%

response as early as 20 minutes of exposure. The exposure-response relationship for SGPT elevation was notably different than that observed for narcosis and lethality. The authors noted that elevation of SGPT activity occurred much earlier than narcosis or lethality and, therefore, that chloroform was inducing liver damage prior to the onset of narcosis.

The hepatotoxicity and renal toxicity of inhaled chloroform was studied in female B6C3F₁ mice (five animals per group) following a 7-day exposure to 1, 3, 10, 30, 100, or 300 ppm chloroform for 6 hrs/day (Larson et al., 1994). The effects on nasopharyngeal tissue were also examined after the 7-day exposure (Méry et al., 1994). Centrilobular hepatocyte necrosis and severe vacuolation in centrilobular hepatocytes were observed in mice of the 100-ppm and 300-ppm groups. Mild to moderate vacuolar changes were observed in the 10-ppm and 30-ppm groups. Notable renal toxicity was observed only in the 300-ppm group. Histologic changes in the nasal region of the female mice were limited to increased cell proliferation at 10 ppm and above and a slight indication of new bone growth in the endoturbinates of one mouse in the 300-ppm group. In a later report (Larson et al., 1996), however, it was noted that the nasal lesions induced in female mice following 6 hr/day exposures to chloroform (10, 30, or 90 ppm) were transient and not sustained in mice similarly exposed for up to 13 weeks.

3.2.3 Dogs

Renal toxicity in dogs following inhalation exposure to chloroform was reported by Whipple and Sperry (1909). Details of experimental protocol and were limited and lacked definitive exposure terms. The report provided only qualitative information regarding the clinical signs (vomiting, diarrhea, lassitude) of animals subjected to the chloroform treatment. Additionally, gross pathology and histopathology evidence of hepatotoxicity and renal toxicity was reported for dogs on successive days after inhaling 1-2 oz of chloroform over 1-2 hours.

von Oettingen et al. (1949) described alterations in various physiologic functions in dogs surgically prepared for monitoring of respiration and blood pressure (see Section 3.1.3). Although the continuous exposure to 15,000 ppm ultimately resulted in the deaths of all ten dogs (6-285 minutes), it was reported that the dogs exhibited notable cardiovascular responses (decreased arterial blood pressure), decreased respiratory rate and body temperature, and depression of voluntary and involuntary reflexes within 35 minutes. Although it is uncertain if discontinuation of the exposure at or below 35 minutes would have prevented a fatal response, the data serve to provide a qualitative description of the response of this species to very high concentrations.

3.2.4 Cats

Nonlethal effects of acute exposure to chloroform in cats was reported by Lehmann and Schmidt-Kehl (1936). In this study, adult cats were exposed to chloroform concentrations of 7200 to 22,000 ppm. The chloroform concentrations were determined by chemical reaction (hydrolysis with alkali in alcohol). At 7500 ppm the cats exhibited light narcosis at 78 minutes and deep narcosis after 93 minutes. Light and deep narcosis were induced after 10 minutes and 13 minutes, respectively for exposures to 22,000 ppm. This exposure also reportedly produced mucous membrane irritation in the eyes, mouth and nose.

3.2.5 Summary of Nonlethal Toxicity In Animals

The nonlethal toxicity of chloroform in laboratory species (rats, mice, and cats) following acute inhalation exposure is summarized in Table 4. As would be expected of a known hepatotoxicant, many of the nonlethal effects reported for inhalation exposures of laboratory species focused on indices of liver damage. Acute exposures (1 to 4 hours) to chloroform concentrations of 100 to 292 ppm have resulted in some degree of hepatic injury as determined by elevated serum enzyme activities and histopathologic examination. Without histopathologic correlates, however, marginal elevations (although statistically significant) in serum enzyme activities may not be indicative of a serious toxic response. Renal toxicity has also been demonstrated in mice at exposures that are relatively low (e.g., 246-665 ppm for 2 hours or 693 ppm for 1 hour) compared to those inducing narcosis (e.g., 4500 ppm for 35 minutes). Data in cats are limited to high, narcosis-inducing exposures.

TABLE 4. Nonlethal Effects of Chloroform in Laboratory Species Following Acute Inhalation Exposure				
Species	Exposure concentration (ppm)	Exposure duration	Effect	Reference
Rat	500	6 hrs	statistically significant elevation in serum enzyme activity	Wang et al.,1994
Rat	10	6 hrs/day for 7 days	histopathologic changes in the liver	Larson et al. 1994
Rat	50	8 hrs	no increase in liver weight	Ikatsu and Nakajima, 1992
Rat	100	8 hrs	marginal, biologically insignificant increase in serum enzyme activity	Ikatsu and Nakajima, 1992
Rat	153	4 hrs	elevated serum enzyme activity	Lundberg et al., 1986
Rat	292	4 hrs	elevated serum enzyme activity	Brondeau et al. (1983)
Rat	10,000	2 hrs	no effect on hepatic GSH ^a	Brown et al., 1974b
Mouse	2,458-5,120	48 min	reflex loss	Fühner, 1923
Mouse	100	4 hrs	fatty infiltration of the liver	Kylin et al., 1963
Mouse	693	1 hr	renal toxicity	Deringer et al. (1953)
Mouse	246	2 hrs	renal tubular necrosis	Culliford and Hewitt (1957)
Mouse	665	2 hrs	renal necrosis in males	Culliford and Hewitt (1957)
Mouse	4500	35 min	50% narcosis (EC _{t50})	Gehring (1968)
Mouse	4500	13.5 min	50% significantly ^b elevated SGPT (EC _{t50})	Gehring (1968)
Cat	7500	78 min	light narcosis	Lehmann and Schmidt-Kehl, 1936
Cat	22,000	10 min	narcosis, eye, mouth and nose irritation	Lehmann and Schmidt-Kehl, 1936

a Narcosis and significant reduction in GSH was found in phenobarbital-induced rats exposed for 2 hrs to 5,000 ppm chloroform.

b Approximately 2.2-fold increase relative to unexposed controls; considered by investigators to be statistically significant.

3.3 Developmental/Reproductive Toxicity

3.3.1 Rats

The embryotoxicity and fetotoxicity of inhaled chloroform in Sprague-Dawley rats was studied by Schwetz et al. (1974). Pregnant rats were exposed to 30 ppm (22 dams), 100 ppm (23 dams), or 300 ppm (3 dams) chloroform for 7 hours/day on gestation days 6 through 15; control rats (68) were exposed to filtered air (Table 5). The exposure concentrations were subanesthetic and varied <5% from the target concentrations. The chloroform concentrations were monitored three times per day using an infrared spectrophotometer. The 300-ppm exposure produced a marked anorexia at the end of the treatment period although comparison with a pair-fed control group (8 dams) later showed that inanition was not a contributor to the observed embryotoxicity and fetotoxicity. Chloroform at 30 ppm induced some evidence of embryotoxicity and fetotoxicity while the 100- and 300-ppm exposures caused significant toxicity (Table 5).

Parameter	Control	Pair-fed control	30 ppm	100 ppm	300 ppm
% pregnancy (pregnant/bred)	88 (68/77)	100 (8/8)	71 (22/31)	82 (23/28)	15 (3/20) ^b
corpora lutea/dam	14±2	14±2	16±3 ^b	14±2	14±1
live fetuses/litter	10±4	10±4	12±2	11±2	4±7 ^b
% reabsorptions/implantations	8(63/769)	7(6/87)	8(24/291)	6(16/278)	61(20/33) ^b
fetal body weight (g)	5.69±0.36	5.19±0.29 ^b	5.51±0.20	5.59±0.24	3.42±0.02 ^b
fetal crown-rump length (mm)	43.5±1.1	42.1±1.1 ^b	42.5±0.6 ^b	43.6±0.7	36.9±0.2 ^b
total gross anomalies ^a	1/68	0/8	0/30	13/23 ^b	0/3
total skeletal anomalies ^a	46/68	3/8	20/22 ^b	17/23	2/3
total soft tissue anomalies ^a	33/68	3/8	10/22	15/23	1/3

^a litters affected/litters examined.

^b Significantly different from control; p<0.05.

The investigators concluded that exposure to 30 ppm chloroform produced minor effects on the embryo and fetus, exposure to 100 ppm was highly embryotoxic and fetotoxic, and that exposure to 300 ppm was embryocidal as well as highly embryotoxic and fetotoxic. The observed effects could not be correlated with maternal toxicity or inanition.

Newell and Dilley (1978) conducted experiments in which Sprague-Dawley rats were exposed to 942, 2232, or 4117 ppm chloroform 1 hour/day on gestation days 7-14. Controls

were exposed to clean air. There was an increase in the number of resorptions (45% relative to unexposed controls) and decrease in average fetal body weight in the high-exposure group and no notable effects in the low- or mid-exposure group. There was no evidence of teratologic effects.

A series of experiments (two preliminary and one main study) were reported by Baeder and Hoffman (1988) to assess developmental toxicity of chloroform in Wistar rats. In one preliminary study, time-mated Wistar rats (4-6/group) were exposed to chloroform for 6 hours/day at concentrations of 0, 10, 30, or 100 ppm on gestation days 7-11 and 14-16. At 10 ppm, two dams had no fetuses and a single implantation site. At 30 ppm, one dam had only one fetus and three empty implantation sites. No such effects were reported for the 100-ppm group. In the second preliminary experiment, Wistar rats exposed to 100 and 300 ppm (6 hours/day) on gestation days 7-16 exhibited decreased feed consumption and body weight loss. Fetal weights in two litters in the 100-ppm group were lightly decreased while in the 300-ppm group three dams had normally developed fetuses, one dam had totally resorbed fetuses, and one dam had only empty implantation sites. In the main study, groups of 20-23 time-mated Wistar rats were exposed to chloroform (7 hours/day, gestation days 7-16) at concentrations of 0, 30, 100, or 300 ppm. During exposure, chloroform-exposed rats exhibited decreased feed consumption and body weight gain ($p < 0.05$ for all exposure groups except body weight gain for 30-ppm group on gestation day 21) relative to unexposed controls. Litter data for the main study are summarized in Table 6. Although fetal weight is significantly decreased for the 300-ppm group and crown-rump length is significantly decreased in all chloroform-exposed groups, these effects may be a function of the maternal feed consumption/body weight effects. Incidences of external and internal malformations and skeletal abnormalities were reportedly not statistically significant.

TABLE 6. Litter data from Wistar rats exposed to chloroform on gestation days 7-16. (Baeder and Hoffman, 1988)

Parameter	Exposure concentration (ppm)			
	0	30	100	300
no. pregnant/no. sperm plugs	20/20	20/20	20/21	20/23
no. lost litters	0	2	3	8
no. live litters	20	18	17	12
resorptions/live litters (mean)	0.75	0.22	0.53	0.92
live fetuses/litter (mean)	12.4	12.8	12.8	13.4
fetal wt. (g)	3.19±0.30	3.16±0.19	3.13±0.21	3.00±0.19*
fetal crown-rump length (cm)	3.52±0.17	3.38±0.12*	3.39±0.10*	3.39±0.12*

* significantly different from control group; $p < 0$.

A follow-up study was conducted by Baeder and Hoffman (1991) in which groups of 20 time-mated Wistar rats were exposed to chloroform (0, 3, 10, or 30 ppm, 7 hrs/day) on gestation days 7-16. Feed consumption during the first week of exposure was significantly ($p<0.05$) decreased and remained so for the 30-ppm group to the end of the study. Body weight of the 3-ppm group was unaffected but an exposure-dependent decrease was detected by gestation day 17. Body weights remained lower than controls on gestation day 21 for the 10-ppm and 30-ppm groups. Analysis of litter data by the investigators revealed a significant decrease in fetal weight and crown-rump length in the 30-ppm group (Table 7). Significantly increased incidences of some ossification variations were observed, especially for the 30-ppm group (Table 8).

TABLE 7. Litter data from Wistar rats exposed to chloroform on gestation days 7-16. Baeder and Hoffman, 1991				
Parameter	Exposure concentration (ppm)			
	0	3	10	30
no. pregnant	20	20	20	20
no. lost litters	0	0	30	0
no. live litters	20	20	20	19
resorptions/live litters (mean)	0.55±0.89	0.40±0.60	0.75±1.02	0.84±1.42
live fetuses/litter (mean)	12.4±2.4	12.4±3.5	12.9±3.0	12.5±1.9
fetal wt. (g)	3.4±0.3	3.2±0.3	3.2±0.3	3.2±0.3*
fetal crown-rump length (mm)	35.8±2.0	35.5±2.1	34.4±2.6	34.0±1.9*

* significantly different from control group; $p<0.05$

Parameter	Exposure concentration (ppm)			
	0	3	10	30
no. pregnant	20	20	20	20
no. lost litters	0	0	30	0
no. live litters	20	20	20	19
resorptions/live litters (mean)	0.55±0.89	0.40±0.60	0.75±1.02	0.84±1.42
live fetuses/litter (mean)	12.4±2.4	12.4±3.5	12.9±3.0	12.5±1.9
fetal wt. (g)	3.4±0.3	3.2±0.3	3.2±0.3	3.2±0.3*
fetal crown-rump length (mm)	35.8±2.0	35.5±2.1	34.4±2.6	34.0±1.9*

* significantly different from control group; p<0.05

3.3.2 Mice

Murray et al. (1979) examined the developmental toxicity of inhaled chloroform in CF-1 mice following gestational exposure. Groups of 34-40 pregnant mice were exposed to chloroform (100 ppm) for 7 hours/day on gestation days 6-15, days 1-7, or days 8-15. Controls were exposed to filtered room air. Chloroform concentrations were monitored by infrared spectrophotometry and found to vary <3% from the target concentration. Maintenance of pregnancy was significantly (p<0.05) decreased in the dams exposed on gestation days 1-7 (44% pregnant vs 74% in controls) and 6-15 (43% pregnant vs 91% in controls) but not for those exposed on days 8-15 (decreased, but not significantly so). The significant developmental toxicity findings are shown in Table 9. The incidences of delayed ossification of skull bones and sternebrae (not for days 6-15) were significantly increased in the chloroform-treated groups compared to the respective control groups. However, these data were not presented in the report tables. There was also evidence of hepatotoxicity in the chloroform-exposed dams as demonstrated by significantly increased absolute and relative liver weights, and by elevated SGPT activity. The study authors concluded that exposure of pregnant mice to 100 ppm chloroform (7 hrs/day) on gestation days 1-7 or 6-15 decreased the ability to maintain pregnancy but was not teratogenic. Exposure on gestation days 8-15 did not affect pregnancy maintenance but resulted in an increased incidence of cleft palate.

Parameter	Days 1-7		Days 6-15		Days 8-15	
	Control	100 ppm	Control	100 ppm	Control	100 ppm
Litters examined	22	11	29	12	24	18
Resorptions/litter	2±2	4±5 ^a	2±2	1±1	2±2	2±2
Fetal body weight (g)	1.02±0.10	0.92±0.07 ^a	0.99±0.11	0.95±0.13	1.00±0.12	0.85±0.17 ^a
Fetal crown-rump length	24.7±1.0	23.6±1.2 ^a	23.7±1.3	23.2±1.1	24.1±1.1	22.9±2.2 ^a
Cleft palate fetuses (litters) affected	3(1)	0	0	0	1(1)	10(4) ^a

^a Significantly different from control (p<0.05)

Land et al. (1981) studied the morphologic changes in spermatozoa of C57B1/C3H mice. The mice were observed 28 days after exposure to chloroform (4 hrs/day for 5 days) at concentrations of 0.1 or 0.05 of the MAC (Minimal Alveolar Concentration). The chloroform was delivered via calibrated vaporizers and the concentration was monitored by gas chromatography. The mice were terminated 28 days after the last exposure and the spermatozoa (1,000 /slide) examined independently by two pathologists. Based upon data from groups of five mice, the percent of abnormal spermatozoa was 1.42±0.08, 2.76±0.31, and 3.48±0.66 for the control (clean air), 0.5 and 1.0 ppm chloroform groups respectively. Both treatment groups were significantly different (p<0.01) than the controls. The abnormalities identified included flattened spermatozoa, amorphous spermatozoa and spermatozoa with abnormal hook formation.

3.4 Genotoxicity

Numerous genotoxicity assays have been performed with chloroform (ATSDR, 1997). Generally, the results of these bioassays indicate chloroform to be a weak mutagen with low potential for interaction with DNA.

3.5 Carcinogenicity

Renal and hepatic tumors have been reported for rodents following chronic oral administration of chloroform (reviewed in ATSDR, 1997). The results of cancer bioassays appear to be substantially influenced by the method of administration (gavage vs drinking water) and by the vehicle (corn oil vs water). Currently, inhalation exposure studies addressing the tumorigenic potential of chloroform are limited to a 90-day study in F-344 rats by Templin et al. (1996a), a short-term exposure study by Templin et al. (1996b), and a report on a long-term inhalation study by Yamamoto et al. (1994).

In the study by Templin et al. (1996a), male and female F-344 rats were exposed to chloroform (0, 2, 10, 20, 30, 90, or 300 ppm), 6 hours/day, 7 days/week. Groups of rats (15-60 per group) were subjected to different exposure protocols: male rats were exposed for 4 days or 3, 6, or 13 weeks, and female rats were exposed for 3 or 13 weeks. The exposure atmospheres were monitored by infrared gas analysis. Average analytically determined concentrations were always within 4.5% of the target concentration. Results of the study revealed the liver, kidneys, and nasal passages as primary targets of toxicity. Cytolethality and regenerative cell proliferation were significant at the 300 ppm exposure. Although long-term exposure to 300 ppm would likely induce a tumorigenic response, this exposure was considered by the investigators to be highly cytotoxic (in excess of the MTD) with no relevance for extrapolating to low dose responses. Statistically significant body weight loss was observed in the male rats exposed for four days but kidney lesions were seen only in rats exposed to 30 (1 of 5 rats), 90 (3 of 5 rats), or 300 ppm (5 of 5 rats).

Templin et al. (1996b) conducted studies in BDF₁ mice to affirm the role of cytotoxicity and regenerative cell proliferation in the tumorigenic response to chloroform. Groups of male and female mice were exposed to 0, 0.3, 5, 30, or 90 ppm chloroform 6 hours/day for 4 days. Bromodeoxyuridine (BrdU) was administered by osmotic pumps implanted 3.5 days prior to necropsy and served to provide a labeling index (LI) for S-phase cells. Additional groups of mice were exposed to chloroform at 30 or 90 ppm for 5 days/week for 2 weeks. Degenerative lesions and a 7- to 10-fold increase in the LI were observed in the kidneys of male but not female mice treated exposed to 30 or 90 ppm. Liver lesions and an increased hepatocyte LI were observed in male mice exposed to 30 and 90 ppm and in female mice exposed to 90 ppm. A 40% and 80% lethality, respectively, were observed in the 30- and 90-ppm groups exposed for two weeks; severe kidney damage was evident in these animals. These findings show that in the 2-year assays, these exposures actually exceeded the MTD and were tolerated only because of the step-wise exposure protocol allowing the animals to metabolically accommodate to the high exposures. Templin et al. questioned the validity of low-dose extrapolation from tumor data of this type (e.g., non-genotoxic-cytotoxic mechanism that is secondary to organ-specific toxicity).

In a preliminary report of a 2-year cancer bioassay, Yamamoto et al. (1994) observed no increase in tumor incidences in male and female F-344 rats exposed to chloroform (10, 30, or 90 ppm), 5 days/week. No further details are available on this study.

Several issues, however, are relevant to the carcinogenic potential of chloroform. These are especially relevant regarding the estimation of carcinogenic risk following a single acute exposure. As reviewed by Conolly (1995) and Golden et al. (1997), the tumorigenic dose-response of mice and rats to chloroform appears to be nonlinear and is secondary to cytotoxicity (i.e., cell necrosis and subsequent cellular regeneration) following exposures that induce frank toxicity in tissues that are tumor sites and exposure that often exceed the maximum tolerated

dose (MTD). Additionally, both *in vivo* and *in vitro* genotoxicity data indicate the absence of a genotoxic mechanism for chloroform.

The significance of regenerative cell proliferation in chloroform-induced cancer was also examined by Butterworth et al. (1995) and Wolf and Butterworth (1997). Generally, an analysis of the available data indicates that chloroform acts through a nongenotoxic, cytotoxic mechanism. In rodent studies, toxicity is not observed when chloroform is not metabolized to reactive metabolites at a rate sufficient to cause cytolethality. As such, a linearized extrapolation from high doses that produce tumors to very low doses is considered inappropriate. Additionally, the current inhalation cancer risk is $2.3 \times 10^{-5} (\mu\text{g}/\text{m}^3)^{-1}$ (USEPA, 1992b) and is based upon a tumorigenic response (hepatocellular carcinomas) in B6C3F₁ mice administered chloroform by gavage (NCI, 1976) and, therefore, involves the uncertainties associated with route-to-route extrapolation.

Butterworth et al. (1995) and Wolf and Butterworth (1997) have compared the application of the linearized multistage model for low dose extrapolation in cancer risk assessment to an assessment based upon the use of a threshold response (i.e., cytolethality and cellular regeneration). The resulting outcomes are remarkably different. Application of the linear multistage model to tumor incidence data from a gavage study with mice (NCI, 1976) results in a virtually safe dose (VSD; relative to a 10^{-6} cancer risk) of 0.000008 ppm. However, a VSD of 0.01 ppm is obtained using a safety factor approach (10 x 10 x 10 for interspecies, intraspecies, and use of subchronic study) and inhalation data from rodents showing that exposure to 10 ppm does not produce cytolethality or a regenerative cell proliferation. The investigators justify the approach because of the apparent need for cytolethality and cellular regeneration in the tumorigenic response.

Melnick et al. (1998), however, have provided data and alternate interpretations regarding the relevance of cytolethality and proliferative cellular regeneration to the tumorigenic response observed in rodents following oral administration of chloroform in corn oil. Following gavage dosing of female B6C3F₁ mice (10/group) with chloroform (5 times/week for 3 weeks at doses of 55, 110, 238, or 477 mg/kg), biochemical indices of toxicity (ALT, SDH), labeling index (bromodeoxyuridine [BrdU]) for S-phase hepatocytes, and histopathologic examination were conducted to ascertain the relationship between regenerative hyperplasia and tumor induction. As expected, a dose-related response was observed for liver-to-body weight ratio, increase in ALT and SDH activity, severity and incidence of hepatocyte hydropic degeneration, and labeling index. The investigators compared the dose-response curves for tumor incidence (using data from previous cancer bioassays) and hepatocyte labeling index and reported that the processes are not causally related. In other words, an elevated labeling index resulting from cellular proliferation is not required for a tumorigenic response.

4. SPECIAL CONSIDERATIONS

4.1 Metabolism and Disposition

The metabolism of chloroform has been thoroughly studied (reviewed in ATSDR, 1997). Although metabolism via cytochrome P-450 IIE1 is well established, a minor anaerobic pathway also exists resulting in a dichloromethyl radical intermediate. Phosgene, formed by P-450-mediated dehydrochlorination, may react with cellular proteins or be converted to hydrochloric acid and carbon dioxide (Pohl et al., 1981). Phosgene may also react with glutathione to form diglutathionyl dithiocarbonate which is then metabolized to 2-oxothiazolidine-4-carboxylic acid (Pohl et al., 1977; Mansuy et al., 1977; Branchflower et al., 1984).

Brown et al. (1974a) studied the metabolism of orally administered [^{14}C]-chloroform (60 mg/kg) in male Sprague-Dawley rats, male CBA, CF/LP, and C57 mice, and squirrel monkeys. In all test species, $^{14}\text{CO}_2$ was a major excretory product but species-dependent variability was observed in its elimination. For all three strains of mice, $^{14}\text{CO}_2$ represented approximately 80% of the administered dose while for rats only about 60% of the dose was eliminated as $^{14}\text{CO}_2$ and for squirrel monkeys only 20% of the dose was excreted as carbon dioxide.

Fry et al. (1973) reported that 17.8-66.6% of an oral dose of radiolabeled chloroform (500 mg) was expired unchanged by eight human volunteers over an 8-hour period. Maximum excretion of chloroform occurred at 40 minutes to two hours after administration. Carbon dioxide excretion was measured in one male and one female volunteer. Over a 450-minute period, 48% (woman) and 50% (man) of the dose was expired as carbon dioxide. The study authors also reported decreased excretion of chloroform by obese subjects and suggested this was due to uptake of chloroform by greater amounts of adipose tissue. Peak blood concentrations ($\approx 1 \mu\text{g/ml}$) occurred at about 45 minutes after dosing. Elimination from the blood appeared to be biphasic: an initial rapid clearance within an hour and a slower clearance over the next six hours. As chloroform concentration in the blood increased, pulmonary excretion increased.

Corley et al. (1990) developed a physiologically based pharmacokinetic model for chloroform based upon a kinetic constant from *in vivo* studies on rats and mice, *in vitro* enzymatic studies with human tissue samples, and physiologically-based estimates for absorption, distribution, metabolism, and excretion processes. Macromolecular binding was considered as a measure of internal dose. The model was validated by comparison of predicted values with experimental data from mice, rats, and humans. Human metabolic and macromolecular binding constants for $V_{\max}\text{C}$ (15.7 mg/hr/kg) and K_m (0.448 mg/L) were derived. It was also shown that metabolic activation of chloroform to reactive intermediates such as phosgene was greatest in mice. Metabolic activation was less in rats and lowest in humans. Therefore, it was estimated that exposure to equivalent concentrations of chloroform would result in a lower delivered dose in humans than in laboratory species. Species variability

was also reported by Brown et al. (1974a), who reported that conversion of chloroform to carbon dioxide was highest in mice (80%) and lowest in squirrel monkeys (18%). In rats and mice, [¹⁴C]-urea was detected in the urine along with two unidentified metabolites and parent compound was found in the bile of the squirrel monkeys. In mice, radioactivity in the blood peaked at 1 hour after dosing and decreased gradually over the next 24 hours.

The chloroform PBPK model developed by Corely et al. (1990) was used by Delic et al. (2000) to develop models for humans and rats in an effort to compare rates of metabolism in the context of assessing the validity of uncertainty factors used in developing occupational exposure limits. The study also utilized Monte Carlo analysis to determine the extent of variability within human and animal model populations. The results demonstrated that even at the most extreme ranges within the human population, levels of toxic metabolites necessary for induction of a toxic response would not be generated at rates comparable to that in rats. Specifically, the model showed that the mean peak rate of metabolism of inhaled chloroform (at the mouse NOAEL of 10 ppm) is approximately 78-fold lower in humans and that the chloroform exposure required to achieve peak metabolism rate in humans would be 65-fold higher than that in mice. Monte Carlo analysis of population variability also indicated that chloroform metabolism rates between mice and humans varied by 25 to 50-fold. Overall, the work clearly demonstrated that humans require considerably higher exposure concentrations than do mice to induce a toxic response.

Data regarding the distribution of chloroform among brain, lung and liver tissue of humans was attained by Gettler and Blume (1931) from suicide victims or deaths during chloroform anesthesia. The brain and lungs consistently contained the highest levels of chloroform (60-480 mg/g brain tissue; 24-485 mg/g lung tissue). Liver tissue tended to contain lower levels (24-238 mg/g liver tissue) than did brain and lung tissue. Due to the nature of the subjects examined, these values reflect tissue burdens following high exposures.

The distribution of [¹⁴C]-chloroform in pregnant C57BL mice following a single 10-minute inhalation exposure (approximately 16 mmoles based upon specific activity) was studied by Danielsson et al. (1986). Assessments were conducted at 0.5, 4, and 24-hour time points. At all time points, radioactivity was greatest in the lungs, liver, and kidneys. Pulmonary radioactivity decreased with time while radioactivity in the liver and kidneys peaked at 0.5 hours followed by a successive decrease. Radioactivity in the respiratory tract was associated with epithelial tissue (nasal mucosa, trachea, and bronchi). Radioactivity was also found in the fetus and placenta at all time points, peaking at 0.5 hours and gradually decreasing over the 24-hour time frame. In addition to total radioactivity, the investigators also determined bound radioactivity in various tissues and found that the respiratory tract and centrilobular portion of the liver contained bound radioactivity possibly indicative of on-site production of reactive metabolites.

Wang et al. (1997) reported on the effects of ethanol pretreatment (2 g/rat/day for 3 weeks) on the metabolism and hepatotoxicity of chloroform in rats following administration of

chloroform by various routes (ip, po, and inhalation). The ethanol pretreatment increased cytochrome P-450 from 0.74 nmol/mg protein to 1.10 nmol/mg protein and increased the metabolism of inhaled chloroform 7-fold in rats exposed to 500 ppm for 6 hours but did not increase the metabolism of chloroform in rats exposed to 50 ppm for 6 hours. Hepatotoxicity, as determined by GPT, GOT, and GSH activity, was unaffected in the 50-ppm group and increased approximately 6-fold in the 500-ppm group.

4.2 Mechanism of Toxicity

The noncarcinogenic and carcinogenic mechanisms of chloroform have been previously reviewed (Butterworth et al., 1995; Conolly, et al., 1995; Templin et al., 1996a, b; ATSDR, 1997; Golden et al., 1997; Wolf and Butterworth, 1997). Chloroform toxicity may be generally categorized as effects on the central nervous system, hepatic and renal effects, and cardiac effects (primarily the result of myocardial sensitization to epinephrine).

The precise mechanism of chloroform on neural activity is unknown. It is generally assumed that general anesthetics act by influencing synaptic transmission (e.g., potentiating transmitter release at inhibitory synapses and/or inhibiting release at excitatory synapses). These actions may be the result of interaction with protein-lipid interfaces (Kennedy and Longnecker, 1996).

The underlying mechanism of chloroform hepatic and renal toxicity is the binding of reactive intermediates, such as phosgene (Pohl et al., 1977), to cellular macromolecules, the depletion of these macromolecules and subsequent cell death.

Brown et al. (1974b) exposed phenobarbital-treated rats for 2 hours to 0.5% (5,000 ppm) or 1.0% (10,000) chloroform and found, respectively, a 70% and 83% reduction in hepatic glutathione in rats ($p < 0.001$). At these exposure levels, however, normal (non-induced) rats exhibited no significant change in GSH levels.

The importance of GSH depletion was also demonstrated by Docks and Krishna (1976), who showed that administration of chloroform (80 mg/kg, i.p.) to phenobarbital-treated rats decreased glutathione and that this depletion resulted in massive liver necrosis. Because of the greater depletion of glutathione by chloroform than by halomethanes known to be metabolized to the trichlorocarbon radical ($\text{CCl}_3 \cdot$), Docks and Krishna postulated that chloroform-mediated decrease in glutathione was not due to the trichlorocarbon radical.

The mechanism of chloroform toxicity in isolated rat hepatocytes was studied by El-shenawy and Abdel-Rahman (1993). The results of this study supported the contentions of Docks and Krishna regarding the depletion of glutathione as a causative precursor for cytotoxicity. In this study, isolated rat hepatocytes exposed to chloroform at concentrations of 1, 10, 100, or 1000 ppm exhibited a concentration-dependent decrease in viability (statistically significant at $p < 0.05$ at all concentrations). Leakage of AST occurred with all concentrations although was significant

only after 60 and 30 minutes for the 1-ppm and 10-ppm tests, respectively. Leakage of ALT was significant at 60 minutes and 30 minutes in the 100 and 1000-ppm tests. Glutathione was significantly decreased at all time points from 15 to 120 minutes following incubation of hepatocytes with 1000 ppm chloroform. At 100 ppm and 10 ppm, glutathione depletion became significant at 30 minutes and 120 minutes, respectively.

4.3 Structure-Activity Relationships

Assessment of structure-activity relationships was not instrumental in deriving AEGL values for chloroform.

4.4 Other Relevant Information

4.4.1 Species Variability

Strain, species, and gender variability in the metabolism and toxicity of chloroform has been demonstrated. As previously noted, male mice exhibit both renal and hepatotoxicity following exposure to chloroform whereas female mice exhibit only hepatotoxicity. This has been shown to be due to hormone-specific cytochrome P-450 in the kidneys of male mice. By examining differences in the biotransformation of chloroform to phosgene, Pohl et al. (1984) clearly demonstrated strain and sex differences in chloroform-induced renal toxicity. The differences could be attributed to strain and gender-dependent differences in the rate of phosgene production by microsomal and mitochondrial fractions from the kidneys. A notable difference was observed between sensitive male DBA/2J mice and less sensitive C57BL/6J mice. Male mice exhibited nearly an order of magnitude more rapid formation of phosgene than did female mice. Additionally, based upon results of PBPK model studies using metabolism and disposition data, humans appear to be less sensitive than rodent species, and the mouse appears to be the most sensitive.

4.4.2 Concurrent Exposure Issues

Because the biotransformation of chloroform to reactive intermediates is mediated by cytochrome P-450IIE1, exposures to chemicals that induce P-450 may increase the toxic response of chloroform. From a practical standpoint, special concern would be directed to alcohol consumption.

5. DATA ANALYSIS FOR AEGL-1

5.1 Summary of Human Data Relevant to AEGL-1

Human exposure data consistent with AEGL-1 effects are limited to the Lehmann and Hasegawa (1910) study using human volunteers, and a study of dental workers (McDonald and Vire (1992). Lehmann and Hasegawa reported that 2-3 minute exposures to 920-1100 ppm

resulted in vertigo and 15-30 minute exposures to concentrations as high as 1400 ppm produced a condition of lassitude, vertigo, and headache. Some individuals exposed for 30 minutes to 620 ppm reported only the sensation of a not unpleasant odor and no neurological symptoms. Because vertigo may affect escape from a potentially hazardous condition, those exposures inducing this condition may be inappropriate for development of AEGL-1 values. The data of Lehmann and Hasegawa lack details on exposure methods and validity of exposure measurements. The McDonald and Vire (1992) report is limited to very low exposures encountered during endodontic procedures (<0.57 ppm for 5.5 hrs and <0.88 ppm for over 150 minutes). These exposures did not result in any signs or symptoms even following clinical screening at five hours and one year after exposure. No additional human data consistent with AEGL-1 level effects were available.

5.2 Summary of Animal Data Relevant to AEGL-1

Animal data consistent with AEGL-1 level effects were limited to alterations in clinical chemistry determinations (specifically serum ALT, AST, GLDH and SDH activity) and minor histopathologic findings in the liver and kidneys of rats and mice. Elevated serum enzyme activities were observed in rats exposed for four hours to 153 ppm (Lundberg et al., 1986) or 292 ppm (Brondeau et al., 1983). Six-hour exposure of rats to 500 ppm produced statistically significant elevations in serum enzyme activity (Wang et al., 1994). Eight-hour exposures of rats to 50 ppm produced no increase in liver weight while exposure to 100 ppm resulted in a slight increase in serum enzyme activity (Ikatsu and Nakajima, 1992). Although statistically significant increases in serum enzyme levels were reported in several studies, the increases were not necessarily indicative of biologically relevant hepatic damage (some of the enzyme activities were increased only 2-fold and histologic correlates were negligible) and, therefore, would not be appropriate as AEGL-1 endpoints.

5.3 Derivation of AEGL-1

Human data sets for AEGL-1 determination are limited by poorly defined exposure values with poorly described methodology. Animal data consistent with the AEGL-1, although having more definitive exposure data, are limited to clinical chemistry findings that are more indicative of biological indices of exposure than overt toxicity. Based upon currently available data, it is difficult to identify exposures producing effects consistent with AEGL-1. Exposures that do not produce overt signs of toxicity in humans are neither irritating nor with unpleasant odor. As a result, it was the consensus of the NAC/AEGL that AEGL-1 values not be recommended due to the properties of the chemical (Table 10). Specifically, it would be difficult to identify exposures that would produce notable discomfort or mild sensory irritation without approaching levels that may be near a threshold for narcosis.

TABLE 10. AEGL-1 Values for Chloroform (ppm [mg/m ³])					
AEGL Level	10-min	30-min	1-hr	4-hr	8-hr
AEGL-1	NR	NR	NR	NR	NR

NR: Not recommended due to properties of the chemical.

6. DATA ANALYSIS FOR AEGL-2

6.1 Summary of Human Data Relevant to AEGL-2

In an assessment of 1502 surgical patients anesthetized with chloroform (never exceeding 22,500 ppm) for <30 minutes to >120 minutes, Whitaker and Jones (1965) reported cardiac irregularities (bradycardia 8.1%; arrhythmias 1.3%) in some patients. Protection against narcosis even in the absence of toxic effects would appear to be at least one focus of the AEGL-2, thereby rendering the Whitaker and Jones data inappropriate for AEGL-2 derivation. Lehmann and Hasegawa (1910) reported "intoxication and dizziness" following exposure of a human subject(s) to 4300-5100 ppm for 20 minutes or 7200 ppm for 15 minutes. In this same study, three human volunteers reported pounding heart and experienced gagging during a 30-minute exposure to 3000 ppm and "light-headedness" and lassitude following 30-minute exposure to 1400 ppm. Smith et al. (1973) evaluated surgical patients anesthetized with chloroform (8,500-13,000 ppm; concentration never exceeded 2% [20,000 ppm]) for a mean duration of 112.96 minutes. Cardiac arrhythmias of various types were detected in 1-17 of the patients. With the exception of a slight elevation of LDH, serum enzyme values (SGPT, SGOT, alkaline phosphatase) were not altered by the chloroform anesthesia. Nausea and vomiting occurred in 46% of the patients.

6.2 Summary of Animal Data Relevant to AEGL-2

Several studies in rats indicate that signs of hepatotoxicity (fatty infiltration) and renal damage (tubular necrosis) may occur at cumulative exposures of 400-1330 ppm · hr that encompass exposure durations of 1-4 hours and concentrations of 100-693 ppm (Deringer et al., 1953; Culliford and Hewitt, 1957; Kylin et al., 1963). Exposure of pregnant rats during gestation (7 hrs/day on gestation days 6-15) to 30 ppm chloroform produced minor effects on the embryo and fetus and exposure to 100 ppm was significantly embryotoxic and fetotoxic (Schwetz et al., 1974). Newell and Dilley (1978), however, found that gestational exposure of rats to chloroform at concentrations as high as 2232 ppm (1 hr/day on gestation days 7-14) did not cause developmental effects although exposure to 4117 ppm increased resorptions 45% and decreased fetal body weight.

6.3 Derivation of AEGL-2

Protection against severe hepato- or renal toxicity, or narcosis initially appear to be critical effects for the development of AEGL-2 values. Human data suggest that exposures to 8500 ppm will induce anesthesia; although the duration of this exposure is unknown, it is assumed that the exposure duration would be on the order of minutes. The human data reported by Lehmann and Hasegawa (1910) suggest that exposure to 7500 ppm for 15 minutes or 4300-5100 ppm for 20 minutes were approaching narcosis-inducing effects as determined by signs and symptoms of dizziness, and “intoxication”. These data and the anesthesia data reported by Whitaker and Jones (1965) are, however, compromised by the uncertainties regarding determination of exposure concentrations and specific concentration-duration relationships. Alternately, the increased fetotoxicity and embryoletality reported by Schwetz et al. (1974) for rats exposed to 100 ppm (7 hrs/day) on gestation days 6-15 was considered a sensitive critical effect and point-of-departure for developing AEGL-2 values. The assumption was made that the reported effects (increased fetotoxicity and embryoletality) occurring following the 10-day gestational exposure could result from a single 7-hour exposure. This contention is not without precedent as has been shown by analyses of developmental toxicity data for other chemicals (van Raaij et al., 2003). An intraspecies uncertainty factor of 3 was applied to account for individual variability in metabolism and disposition of chloroform. No adjustment was made for interspecies variability because available metabolism/kinetics data and PB-PK models (Corley et al., 1990) indicate that humans are less sensitive than laboratory species to the toxic effects of chloroform. The attenuated uncertainty factors were justified by the sensitive point of departure selected for AEGL-2 development and the results of another study (Newell and, 1978) that showed gestational exposure of rats to chloroform at concentrations as high as 2232 ppm (1 hr/day on gestation days 7-14) was without effect.

Data were unavailable for calculating an exponent (n) for use in temporal extrapolation ($C^n \times t = k$). The concentration-exposure time relationship for many irritant and systemically acting vapors and gases may be described by $C^n \times t = k$, where the exponent n ranges from 0.8 to 3.5 (ten Berge et al., 1986). In the absence of chemical-specific data, an exponent (n) of 1 or 3 was used in the equation, $C^n \times t = k$. The exponent of 1 applied for extrapolation to exposure durations longer than that of the experimental exposure while the exponent of 3 was applied for time scaling to shorter exposure durations. The resulting AEGL-2 values are presented in Table 11 and Appendix A.

TABLE 11. AEGL-2 Values for Chloroform					
AEGL Level	10-min	30-min	1-hr	4-hr	8-hr
AEGL-2	120 ppm 580 mg/m ³	80 ppm 390 mg/m ³	64 ppm 312 mg/m ³	40 ppm 195 mg/m ³	29 ppm 141mg/m ³

7. DATA ANALYSIS FOR AEGL-3

7.1 Summary of Human Data Relevant to AEGL-3

Definitive lethality data for humans are unavailable. Although the weight of evidence indicates that acute exposure to high concentrations of chloroform may result in narcosis and subsequent death, the precise exposure concentrations and durations for such exposures are unavailable. The available human data generally suggest that concentrations in excess of 10,000 ppm are required for an unspecified, although short, exposure duration for surgical plane anesthesia. In the analysis of surgical patients anesthetized with chloroform, Whitaker and Jones (1965) reported that the 22,500 ppm exposure for surgical anesthesia also produced evidence of potentially serious cardiovascular effects. While these data are superficially compelling for development of AEGL values, specific exposure duration terms are lacking (i.e., it is not possible to associate a specific exposure concentration with a specific exposure duration). Additionally, the concentrations were likely variable over the duration of anesthesia. This is not unexpected; chloroform anesthesia utilizes very high initial exposures (25,000 to 30,000 ppm) of very short duration (2 to 3 minutes) for the purpose of induction but lower exposure concentrations are utilized for maintenance of surgical plane anesthesia (ATSDR, 1997; NRC, 2000). Therefore, it is unlikely that these patients were exposed to the highest concentrations (e.g., 22,500 ppm) for AEGL-specific durations. Arrhythmias were also reported by Smith et al. (1973) in some patients anesthetized for 113 minutes with chloroform concentrations (at least initially) of 8,500 to 13,000 ppm. The available data suggest that surgical plane narcosis would occur at or above 8,500 ppm following short duration exposure. Based upon the available human data, it is not feasible to extrapolate to an exposure duration that would result in death.

7.2 Summary of Animal Data Relevant to AEGL-3

Data regarding the acute lethality of animals following acute inhalation exposure to chloroform are limited to rats and mice. Four-hour exposures to concentrations of 3000 to 8000 ppm resulted in 75-100% mortality in rats (lethality determined 2-3 days post exposure) (Haskell Laboratory, 1964; Smyth et al., 1962), and a 4-hour LC₅₀ of 9780 ppm was reported by Lundberg et al. (1986). For mice, 75% mortality was observed following 120-minute exposure to 5585 ppm, a 66% mortality was reported for exposures to 4710 - 5529 ppm for durations of 71-175 minutes, and 14% mortality for 35-minute exposure to 6758 - 7782 ppm (Fühner, 1923). However, Fühner (1923) observed no deaths at exposures of 2458 - 5120 ppm for 48 - 215 minutes. If the aforementioned responses are converted to cumulative exposures, the inconsistency among the data becomes apparent. For example, no deaths were observed at 2458 - 5120 ppm for 48- 215 minute exposures (i.e., a maximum of 1,100,800 ppm · min), yet 66% mortality was observed following exposures of 71 - 175 minute duration to 4710 - 5529 ppm (a minimum of 334,410 ppm · min). A well-conducted study by Gehring (1968) reported a 4500-ppm LC_{t50} of 560 minutes (540 -585 minutes, 95% C.I.) for female Swiss-Webster mice.

7.3 Derivation of AEGL-3

The available data do not allow for the identification of a definitive lethality threshold in humans for acute exposure to chloroform. Data regarding chloroform as an anesthetic for humans suggest that very high concentrations (in excess of 8,500 ppm) are tolerated for brief durations, although quantitative concentration-time data are lacking in this respect. These limitations preclude the use of the human data in the estimation of a lethality threshold for humans.

Animal data are inconsistent regarding the lethality of acute inhalation exposure to chloroform. Data for mice is highly variable although this species appears to be the most sensitive, which is also affirmed by PBPK models. Four-hour exposure to concentrations of 3000 to 8000 ppm reportedly produced 75-100% mortality in rats (Smyth et al., 1962; Haskell Laboratory, 1964). Assuming the mouse to be the most sensitive species, the 560-minute LC₅₀ of 4500 ppm reported by Gehring (1968) appears to be a valid basis for development of the AEGL-3 values. A 3-fold reduction in this value results in a point-of departure of 1500 ppm as an estimate of the lethality threshold for mice. Consistent with the AEGL Standing Operating Procedures for development of AEGLs (NRC, 2001) an exponent of 3 was applied for time scaling ($C^n \times t = k$) because data were insufficient for empirically deriving a time-scaling exponent. Because the point-of-departure was based upon a 560-minute exposure duration, the 10-minute AEGL-3 was set equivalent to the 30-minute AEGL-3 to avoid the uncertainties inherent in a 560-minute to 10-minute extrapolation (NRC 2001). Consistent with the AEGL Standing Operating Procedures, an uncertainty factor of 3 was applied to account for responses of potentially sensitive individuals such as those exposed to inducers of cytochrome P-450 IIE1 (e. g., ethanol consumption). No interspecies uncertainty factor was applied because currently available data indicate that laboratory species metabolize chloroform more rapidly than do humans and, therefore, are more susceptible to the toxic effects of the more rapidly formed toxic intermediates. PBPK models (Corley et al., 1990) justify this contention. Further, human anesthesia data show that cumulative exposures considerably greater than those associated with the AEGL-3 values are not lethal. A more recent study using the PBPK model to compare the metabolism of chloroform in mice and humans demonstrated quantitatively the overwhelmingly greater sensitivity of mice (due primarily to a 25 to 50-fold difference in the rate of metabolism of chloroform) and the overly protective nature of typically applied uncertainty factors. These findings and the overall weight-of-evidence indicating the greater sensitivity of rodents to chloroform-induced toxicity justified further adjustment in the AEGL-3 values. This adjustment, applied as a weight-of evidence factor of 1/3, effectively increases the AEGL-3. The resulting AEGL-3 values are shown in Table 12 and Appendix A.

AEGL Level	10-min	30-min	1-hr	4-hr	8-hr
AEGL-3	4000 ppm [19,000 mg/m ³]	4000 ppm [19,000 mg/m ³]	3200 ppm [16,000 mg/m ³]	2000 ppm [9,700 mg/m ³]	1600 ppm [7,800 mg/m ³]

8. SUMMARY OF PROPOSED AEGLS

8.1 AEGL Values and Toxicity Endpoints

The proposed AEGL values for chloroform are summarized in Table 13.

Classification	10-min	30-min	1-hour	4-hour	8-hour	Endpoint (Reference)
AEGL-1	NR	NR	NR	NR	NR	Not recommended; AEGL-1 effects unlikely to occur in the absence of notable toxicity.
AEGL-2	120 ppm 580 mg/m ³	80 ppm 390 mg/m ³	64 ppm 312 mg/m ³	40 ppm 195 mg/m ³	29 ppm 141 mg/m ³	Fetotoxicity/embryo-lethality in rats exposed for 7 hrs/day on gestation days 6-15 (Schwetz et al., 1974); single exposure assumed
AEGL-3	4000 ppm [19,000 mg/m ³]	4000 ppm [19,000 mg/m ³]	3200 ppm [16,000 mg/m ³]	2000 ppm [9,700 mg/m ³]	1600 ppm [7,800 mg/m ³]	Estimated lethality threshold for mice; 3-fold reduction in 560-min LC ₅₀ of 4500 ppm to 1500 ppm (Gehring, 1968)

The AEGL-1 values were not recommended because of the inability to determine an exposure that would be consistent with the AEGL definition. The properties of chloroform are such that the odor is not unpleasant and is not irritating even at exposures that approach levels inducing narcosis.

The AEGL-2 values were developed using embryoletality/fetotoxicity in rats as the critical effect. This was considered to be a very sensitive endpoint especially with the assumption of a single-exposure response (i.e., fetotoxic effects resulting from 7-hour exposure on gestation days 6-15 were assumed possible following only one 7-hr exposure).

The AEGL-3 values were developed based upon a rat 4-hour LC₅₀ value (9780 ppm) and a 3-fold reduction of this as an estimate of the lethality threshold. A 3-fold reduction of the LC₅₀ value resulted an exposure of 3260 ppm which, when compared to available human and animal data, appeared to represent an exposure that would not likely result in life-threatening exposures.

The AEGL values were developed using an uncertainty factor of 3 for protection of sensitive individuals. Because chloroform is metabolized to toxic intermediates (i.e., phosgene) by cytochrome P-450 IIE1, induction of this enzyme by inducers such as ethanol potentially increase susceptibility to chloroform-induced toxicity although they do not appear to do so by an order of magnitude (e.g., Brown et al., [1974b] reported a 2.6-fold increase in P-450 levels following induction by phenobarbital, a more effective P-450 inducer than ethanol). Furthermore, dose rate appears to be a relevant factor in toxicity outcomes following exposure to halogenated hydrocarbons such as chloroform, a fact that may justify the application of an intraspecies uncertainty factor of less than an order of magnitude. Due to effects on P-450 and GSH levels, single exposures result in toxic outcomes that are different from those following repeated exposures. Available data and application of pharmacokinetic modeling indicate that rodents metabolize chloroform more rapidly than do humans. Therefore the application of an interspecies uncertainty factor was minimized. Furthermore, human data reveal surgical anesthesia at cumulative exposures of >675,000 ppm·minute and that exposures to 22,500 ppm for up to 120 minutes resulted in surgical anesthesia and cardiac irregularities but not death. These data suggest that the AEGL-3 values represent NOAELs for lethality.

When compared to occupational exposure data reported by Challen et al. (1958) for pharmaceutical workers, the AEGL values appear to be sufficiently protective. In this study, it was found that workers exposed to ≤71 ppm (4 hrs/day for 10-24 months) experienced mild symptoms (dryness of mouth and throat) while workers exposed to 77-232 ppm over a period of 3-10 years exhibited notable signs of exposure (staggering). It should be noted that the Challen et al. findings are the result of repeated exposures and that it was not specified if any of the workers represented a sensitive population.

8.2 Comparison with Other Standards and Guidelines

Standards and guidance values for workplace and community exposures are summarized in Table 14. The cancer notation provided for some of the criteria was not considered to be appropriate for the AEGL values.

TABLE 14. Extant Standards and Guidelines for Chloroform					
Guideline	Exposure Duration				
	10 minutes	30 minutes	1 hour	4 hours	8 hours
AEGL-1 (Nondisabling)	NR	NR	NR	NR	NR
AEGL-2 (Disabling)	120 ppm	80 ppm	64 ppm	40 ppm	29 ppm
AEGL-3 (Lethal)	4000 ppm	4000 ppm	3200 ppm	2000 ppm	1600 ppm
ERPG-1 ^a			NA		
ERPG-2			50 ppm		
ERPG-3			5000 ppm		
NRC SPEGL ^b					
NRC SMAC ^c					
NRC EEL ^d			200 ppm (30 ppm 24- hr)		
NIOSH IDLH ^e NIOSH REL ^f			500 ppm 2 ppm		
OSHA STEL/Ceil. ^g			50 ppm		
ACGIH TLV-TWA ^h					10 ppm
MAK (Germany) ⁱ					2.5 mg/m ³
MAC (the Netherlands) ^j	5 ppm (15-min)				1 ppm

^aERPG (Emergency Response Planning Guidelines, American Industrial Hygiene Association (AIHA 2002)

The ERPG-1 is the maximum airborne concentration below which it is believed nearly all individuals could be exposed for up to one hour without experiencing other than mild, transient adverse health effects or without perceiving a clearly defined objectionable odor.

The ERPG-2 is the maximum airborne concentration below which it is believed nearly all individuals could be exposed for up to one hour without experiencing or developing irreversible or other serious health effects or symptoms that could impair an individual's ability to take protection action.

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The ERPG-3 is the maximum airborne concentration below which it is believed nearly all individuals could be exposed for up to one hour without experiencing or developing life-threatening health effects.

^b**NRC SPEGL (Short-term Public Emergency Guidance Level).**

^c**NRC SMAC (Spacecraft Maximum Allowable Concentration).**

^d**NRC EEL (Emergency Exposure Guideline) (NRC, 1984).**

^e**IDLH (Immediately Dangerous to Life and Health, National Institute of Occupational Safety and Health)** (NIOSH 2003) represents the maximum concentration from which one could escape within 30 minutes without any escape-impairing symptoms, or any irreversible health effects. IDLH carries a cancer notation.

^f**NIOSH REL-TWA (National Institute of Occupational Safety and Health, Recommended Exposure Limits - Time Weighted Average)** is defined analogous to the-TLV-TWA, with cancer notation (ACGIH, 2003).

^g**OSHA PEL-TWA (Occupational Health and Safety Administration, Permissible Exposure Limits Time Weighted Average)** is defined analogous to the ACGIH-TLV-TWA, but is for exposures of no more than 10 hours/day, 40 hours/week (OSHA, 1993).

^h**ACGIH TLV-TWA (American Conference of Governmental Industrial Hygienists, Threshold Limit Value - Time Weighted Average)** is the time-weighted average concentration for a normal 8-hour workday and a 40-hour workweek, to which nearly all workers may be repeatedly exposed, day after day, without adverse effect. ACGIH, 2002.

ⁱ**MAC (Maximaal Aanvaarde Concentratie [Maximal Accepted Concentration])** (SDU Uitgevers [under the auspices of the Ministry of Social Affairs and Employment], The Hague, The Netherlands 2002) is defined analogous to the ACGIH-TLV-TWA.

^j**MAK (Maximale Argeitsplatzkonzentration [Maximum Workplace Concentration])** (DFG 2002, Deutsche Forschungsgemeinschaft [German Research Association]) is defined analogous to the ACGIH-TLV-TWA. Cancer category 4 noted.

8.3 Data Quality and Research Needs

Much of the human experience data are from older studies that lacked information regarding analytical techniques used to determine exposure concentrations. The human anesthesia data focus on initial concentration and duration of anesthesia and were not sufficient for developing AEGL values.

The most obvious data deficiency regarding development of AEGL values for chloroform is the lack of data with which to determine a lethality threshold. There is also a paucity of reliable data demonstrating definitive concentration-response relationships. The human experience data are deficient in exposure-time relationships or are unreliable and difficult to validate. The animal data are variable. Acute exposure studies providing exposure-response data for specific toxicity endpoints (e.g., hepatotoxicity, renal toxicity, narcosis threshold, lethality) in two or more species would be desirable.

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**APPENDIX A
DERIVATION OF AEGL VALUES**

DERIVATION OF AEGL-1 VALUES

AEGL-1 values were not recommended by the NAC/AEGL due to properties of the chemical. Based upon the available data it was not possible to identify a definitive effect consistent with the AEGL-1 definition. Exposures to concentrations approaching those inducing narcosis or hepatic and renal effects are not accompanied by overt signs or symptoms. Furthermore, the odor of chloroform is not unpleasant or irritating.

DERIVATION OF AEGL-2

Key study: Schwetz et al., 1974

Toxicity endpoint: absence of developmental effects in rats

Scaling: $C^n \times t = k$, where $n = 1$ or 3 . Data were unavailable for empirically determining the exponent " n ". The concentration-exposure time relationship for many irritant and systemically acting vapors and gases may be described by $C^1 \times t = k$, where the exponent n ranges from 1 to 3.5 (ten Berge et al., 1986). In the absence of chemical-specific data, temporal scaling was performed using $n = 3$ when extrapolating to shorter time points and $n = 1$ when extrapolating to longer time points using the $C^n \times t = k$ equation:
 $(100 \text{ ppm})^1 \times 7 \text{ hrs} = 700 \text{ ppm}\cdot\text{hr}$
 $(100 \text{ ppm})^3 \times 7 \text{ hrs} = 7,000 \text{ ppm}\cdot\text{hr}$

Uncertainty factors: No interspecies uncertainty factor was applied because the available metabolism/kinetics data and PB-PK models (Corley et al., 1990) indicate that humans may be less sensitive than laboratory animals to the toxic effects of chloroform. Additional adjustments were considered unnecessary because a single 7-hour exposure was utilized for AEGL-2 development rather than the full exposure period specified in the study protocol (7 hrs/day on gestation days 6-15).

An intraspecies uncertainty factor of 3 was applied to account for individual variability in metabolism and disposition of chloroform. Additional adjustment was not made because the point of departure (embryo lethality) and assumption of a single-exposure effect was considered conservative.

Total uncertainty factor application of 3 was applied.

10-min AEGL-2

$$C^3 \times 0.1667 \text{ hr} = 7,000,000 \text{ ppm}\cdot\text{hr}$$

$$C = 348 \text{ ppm}$$

$$10\text{-min AEGL-2} = 348 \text{ ppm}/3 = 116 \text{ ppm (rounded to 120 ppm)}$$

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30-min AEGL-2

$$\begin{aligned} C^3 \times 0.5 \text{ hr} &= 7,000,000 \text{ ppm}\cdot\text{hr} \\ C &= 241 \text{ ppm} \\ 30\text{-min AEGL-2} &= 241 \text{ ppm}/3 = 80.3 \text{ ppm (rounded to 80 ppm)} \end{aligned}$$

1-hr AEGL-2

$$\begin{aligned} C^3 \times 1 \text{ hr} &= 7,000,000 \text{ ppm}\cdot\text{hr} \\ C &= 191 \text{ ppm} \\ 1\text{-hr AEGL-2} &= 191 \text{ ppm}/3 = 63.7 \text{ ppm (rounded to 64 ppm)} \end{aligned}$$

4-hr AEGL-2

$$\begin{aligned} C^3 \times 4 \text{ hrs} &= 7,000,000 \text{ ppm}\cdot\text{hr} \\ C &= 121 \text{ ppm} \\ 4\text{-hr AEGL-2} &= 121 \text{ ppm}/3 = 40.3 \text{ ppm (rounded to 40 ppm)} \end{aligned}$$

8-hr AEGL-2

$$\begin{aligned} C^1 \times 8 \text{ hrs} &= 700 \text{ ppm}\cdot\text{hr} \\ C &= 87.5 \text{ ppm} \\ 8\text{-hr AEGL-2} &= 87.5 \text{ ppm}/3 = 29.2 \text{ ppm (rounded to 29 ppm)} \end{aligned}$$

DERIVATION OF AEGL-3

Key study: Based upon a 3-fold reduction in a 560-minute LC₅₀ (4500 ppm) in mice (Gehring, 1968); 4500 ppm/3 = 1500 ppm.

Toxicity endpoint: Lethality

Scaling: $C^n \times t = k$, where $n = 3$. Data were unavailable for empirically determining the exponent “ n ”. The concentration-exposure time relationship for many irritant and systemically acting vapors and gases may be described by $C^n \times t = k$, where the exponent n ranges from 1 to 3.5 (ten Berge et al., 1986). In the absence of chemical-specific data, temporal scaling was performed using $n = 3$ when for extrapolating to shorter time points using the $C^n \times t = k$ equation:

$$(1500 \text{ ppm})^3 \times 9.3 \text{ hrs} = 3.1 \times 10^{10} \text{ ppm}^3 \cdot \text{hr}$$

Due to uncertainties in extrapolating from a 560-minute exposure duration to a 10-minute duration, the 10-minute AEGL-3 was set equivalent to the 30-minute AEGL-3.

Uncertainty factors: No adjustment was made for interspecies variability (animal-to-human adjustment) regarding the lethal response to chloroform. Metabolism/kinetics data and PB-PK models (Corley et al., 1990; Delic et al., 2001) indicate that humans may be less sensitive than laboratory animals to the toxic effects of chloroform.

An intraspecies uncertainty factor of 3 was applied to account for individual variability in metabolism and disposition of chloroform (e.g., induction of P-450 enzymes and subsequent enhancement of toxicity). Comparison with available anesthesia data in humans precluded incorporation of additional uncertainty factor adjustment.

Due to the results of PBPK models (Corley et al., 1990; Delic et al., 2001) showing that mice are considerably more sensitive (25 to 50-fold difference in rate of metabolism of chloroform) to the toxic effects of inhaled chloroform than are humans, an additional adjustment factor of 1/3 has been applied resulting in overall net adjustment of 1.

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10-min AEGL-3

The 10-min value is set equivalent to the 30-minute value (4000 ppm) to minimize uncertainty associated with extrapolation from the 560-minute exposure duration for the point-of-departure.

30-min AEGL-3

$$\begin{aligned}C^3 \times 0.5 \text{ hr} &= 3.1 \times 10^{10} \text{ ppm}^3 \cdot \text{hr} \\C &= 3,979 \text{ ppm} \\30\text{-min AEGL-3} &= 3,979 \text{ ppm}/1 = 3,979 \text{ ppm (rounded to 4000 ppm)}\end{aligned}$$

1-hr AEGL-3

$$\begin{aligned}C^3 \times 1 \text{ hr} &= 3.1 \times 10^{10} \text{ ppm}^3 \cdot \text{hr} \\C &= 3158 \text{ ppm} \\1\text{-hr AEGL-3} &= 3158 \text{ ppm}/1 = 3158 \text{ ppm (rounded to 3200 ppm)}\end{aligned}$$

4-hr AEGL-3

$$\begin{aligned}C^3 \times 4 \text{ hrs} &= 3.1 \times 10^{10} \text{ ppm}^3 \cdot \text{hr} \\C &= 1989 \text{ ppm} \\4\text{-hr AEGL-3} &= 1989 \text{ ppm}/1 = 1989 \text{ ppm (rounded to 2000 ppm)}\end{aligned}$$

8-hr AEGL-3

$$\begin{aligned}C^3 \times 8 \text{ hrs} &= 3.1 \times 10^{10} \text{ ppm}^3 \cdot \text{hr} \\C &= 1579 \\8\text{-hr AEGL-3} &= 1579 \text{ ppm}/1 = 1579 \text{ ppm (rounded to 1600 ppm)}\end{aligned}$$

APPENDIX B
DERIVATION SUMMARIES FOR CHLOROFORM

**ACUTE EXPOSURE GUIDELINES FOR CHLOROFORM
(CAS NO. 67-66-3)**

AEGL-1 VALUES				
10 minutes	30 minutes	1 hour	4 hours	8 hours
Not recommended	Not recommended	Not recommended	Not recommended	Not recommended
Reference: not applicable				
Test Species/Strain/Number: not applicable				
Exposure Route/Concentrations/Durations: not applicable				
Toxicity Endpoint: not applicable				
Time Scaling: not applicable				
Concentration/Time Selection/Rationale: not applicable				
Uncertainty Factors/Rationale Total Uncertainty Factor: not applicable				
Modifying Factor: not applicable				
Animal-to-Human Dosimetric Adjustments: not applicable				
Data Adequacy: AEGL-1 values were not recommended by the NAC/AEGL due to properties of the chemical. Based upon the available data it was not possible to identify a definitive effect consistent with the AEGL-1 definition. Exposures to concentrations approaching those inducing narcosis or hepatic and renal effects are not accompanied by overt signs or symptoms. Furthermore, the odor of chloroform is not unpleasant or irritating.				

**ACUTE EXPOSURE GUIDELINES FOR CHLOROFORM
(CAS NO. 67-66-3)**

AEGL-2 VALUES					
10 minutes	30 minutes	1 hour	4 hours	8 hours	
120 ppm	80 ppm	64 ppm	40 ppm	29 ppm	
Reference: Schwetz, B.A. et al., 1974.					
Test Species/Strain/Number: Sprague Dawley rats; 68, 8, 22, 23, and 3 dams for the control, pair-fed control, low-, mid-, and high-dose groups, respectively					
Exposure Route/Concentrations/Durations: inhalation (whole body); 0, 30, 100, or 300 ppm, 7 hrs/day on gestation days 6-15.					
Toxicity Endpoint: fetotoxicity (total gross anomalies) expressed as litters affected/litters examined					
<u>Effect</u>	<u>Control</u>	<u>Pair-fed</u>	<u>30 ppm</u>	<u>100 ppm*</u>	<u>300 ppm</u>
Total gross anomalies	1/68	0/8	0/22	3/23 ^a	0/3
Total skeletal anomalies	46/68	3/8	20/22 ^a	17/23	2/3
Total soft tissue anomalies	33/68	3/8	10/22	15/23	3/3
Reduced fetal bw(g)	5.69	5.19	5.51	5.59	3.42 ^a
Fetal crown/rump length (mm)	43.5	42.1	42.5 ^a	43.6	36.9 ^a
^a $p < 0.05$					
* Determinant for AEGL-2 (100 ppm); although the effects reported in the study were the result of 7-hr exposures on gestation days 6-15, for AEGL-2, it was assumed that the effects were the result of a single 7-hr exposure.					
Time Scaling: The concentration-exposure time relationship for many irritant and systemically acting vapors and gases may be described by $C^n \times t = k$, where the exponent n ranges from 0.8 to 3.5 (ten Berge et al., 1986). In the absence of chemical-specific data, temporal scaling was performed using $n = 3$ when extrapolating to shorter time points and $n = 1$ when extrapolating to longer exposure durations.					
Concentration/Time Selection/Rationale: a 7-hr exposure to 100 ppm was selected based upon total anomalies occurring in rat fetuses from dams exposed on gestation days 6-15. The mid dose was chosen in conjunction with the assumption of a single 7-hr exposure. The fetotoxicity					

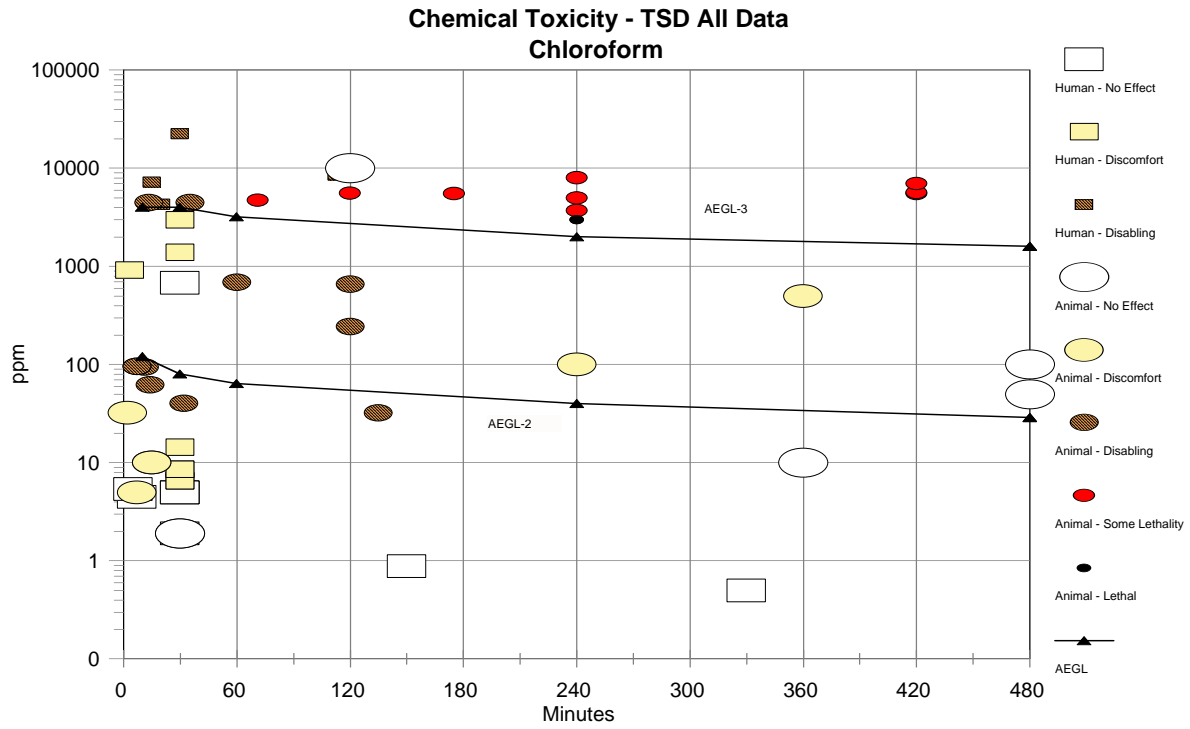
<p>endpoint is considered to represent a sensitive indicator of potential serious and irreversible effects in a susceptible population.</p>
<p>Uncertainty Factors/Rationale: Total Uncertainty Factor: 3 Interspecies: none; available metabolism/kinetics data and PB-PK models (Corley et al., 1990) indicate that humans are less sensitive than rats to the toxic effects of chloroform. Intraspecies: 3; to account for individual variability in metabolism and disposition of chloroform and protection of individuals with altered metabolism/disposition(e.g.,users of alcohol); the fetus is considered a sensitive population and, therefore, no additional reduction is warranted.</p>
<p>Modifying Factor: none</p>
<p>Animal-to-Human Dosimetric Adjustments: insufficient data</p>
<p>Data Adequacy: AEGL-2 development used a conservative approach to select the point of departure (assumption of a single 7-hr exposure). The values are considered to be protective of human health consistent with the AEGL-2 definition.</p>

**ACUTE EXPOSURE GUIDELINES FOR CHLOROFORM
(CAS NO. 67-66-3)**

AEGL-3 VALUES				
10 minutes	30 minutes	1 hour	4 hours	8 hours
4000 ppm	4000 ppm	3200 ppm	2000 ppm	1600 ppm
Reference: Gehring, 1968				
Test Species/Strain/Number: 4500-ppm LC₅₀ of 560 minutes (540 -585 minutes, 95% C.I.) for female Swiss-Webster mice (20/group)				
Exposure Route/Concentrations/Durations: inhalation/various time frames and exposures utilized				
Toxicity Endpoint: lethality threshold estimated as 3-fold reduction of the 560-minute LC₅₀ of 4500 ppm				
Time Scaling: The concentration-exposure time relationship for many irritant and systemically acting vapors and gases may be described by $C^n \times t = k$ (ten Berge et al., 1986), where the exponent n ranges from 0.8 to 3.5. In the absence of chemical-specific data, temporal scaling was performed using $n = 3$ when extrapolating to shorter time points.				
Concentration/Time Selection/Rationale: estimated lethality threshold for 4-hour exposure (3-fold reduction in the 4-hr LC₅₀ of 9780 ppm)				
Uncertainty Factors/Rationale: Total Uncertainty Factor: 1 Interspecies: No adjustment; currently available data indicate that laboratory species metabolize chloroform more rapidly than do humans and, therefore, are likely to be more susceptible to the toxic effects of the more rapidly formed toxic intermediates. PB-PK models (Corley et al., 1990) justify the adequacy of the uncertainty factor. Intraspecies: 3 to account for individual variability in the sensitivity to chloroform-induced toxicity (e.g., alcohol-potentiated hepatotoxicity) An additional adjustment (weight-of-evidence factor) of 1/3 was applied to account for the PBPK findings indicating that the mouse is notably more susceptible to chloroform toxicity due metabolism factors				
Modifying Factor: none applied				
Animal-to-Human Dosimetric Adjustments: insufficient data				

Data Adequacy: Human lethality data are lacking and lethality data in laboratory species are limited. However, when compared to human anesthesia data, the AEGL-3 values appear to be sufficient. PBPK models affirm that rodents, especially mice, are a considerably more sensitive species than are humans to the toxic effects of chloroform

**APPENDIX C
CATEGORY PLOT FOR CHLOROFORM**



**APPENDIX D
CARCINOGENICITY ASSESSMENT FOR CHLOROFORM**

CANCER ASSESSMENT OF CHLOROFORM

The currently available cancer slope factor for chloroform is $2.3 \times 10^{-5} (\mu\text{g}/\text{m}^3)^{-1}$ (USEPA, 1992b; 2005) and is based upon a tumorigenic response (hepatocellular carcinomas) in B6C3F₁ mice administered chloroform by gavage (NCI, 1976). Based upon this slope factor, the upper-bound unit risks of 10^{-4} to 10^{-7} are 4×10^{-3} to $4 \times 10^{-6} \text{ mg}/\text{m}^3$ assuming an inhalation rate of $20 \text{ m}^3/\text{day}$ for a 70 kg individual. At the 10^{-4} risk level, the virtually safe dose (d) is $4 \mu\text{g}/\text{m}^3$.

To convert a 70-year exposure to a 24-hour exposure:

$$\begin{aligned} 24\text{-hr exposure} &= d \times 25,600; \text{ where } d = 4 \mu\text{g}/\text{m}^3 \\ &= (4 \mu\text{g}/\text{m}^3) \times 25,600 \text{ days} \\ &= 102,400 \mu\text{g}/\text{m}^3 \text{ (102.4 mg}/\text{m}^3) \end{aligned}$$

To account for uncertainty regarding the variability in the stage of the cancer process at which carbon tetrachloride or its metabolites may act, a multistage factor of 6 is applied (Crump and Howe, 1984):

$$(102.4 \text{ mg}/\text{m}^3)/6 = 17.07 \text{ mg}/\text{m}^3$$

Therefore, based upon the potential carcinogenicity of carbon tetrachloride, an acceptable 24-hr exposure would be $17.07 \text{ mg}/\text{m}^3$ (3.58 ppm).

If the exposure is limited to a fraction (f) of a 24-hr period, the fractional exposure becomes $1/f \times 24$ hrs (NRC, 1984).

24-hr exposure	=	$17.07 \text{ mg}/\text{m}^3$	(3.58 ppm)
8-hr	=	$51.21 \text{ mg}/\text{m}^3$	(11 ppm)
4-hr	=	$102.42 \text{ mg}/\text{m}^3$	(22 ppm)
1-hr	=	$409.68 \text{ mg}/\text{m}^3$	(86 ppm)
0.5 hr	=	$819.36 \text{ mg}/\text{m}^3$	(172 ppm)

The AEGL-2 values based upon acute toxicity were somewhat greater than the values derived based on potential carcinogenicity. However, the data are compelling regarding the carcinogenic response to chloroform being a threshold response necessitating the need for repeated exposures that result in tissue necrosis and regeneration.

Note: A virtually safe dose of 0.01 ppm ($48.7 \mu\text{g}/\text{m}^3$) was derived by Butterworth et al. (1995) and Wolf and Butterworth (1997) based upon a 10 ppm NOAEL in mice and the contention that the tumorigenic response observed in mice is secondary to necrosis and regenerative cell proliferation (i.e., a threshold response). Cancer risk based upon this approach is 12-fold less than those derived from the 10^{-4} unit risk number.