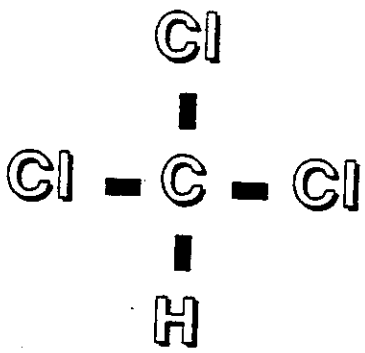
California Air Resources Board

Proposed Identification of CHLOROFORM



As a Toxic Air Contaminant

Part B
Health Assessment

State of California
Air Resources Board
Stationary Source Division

SEPTEMBER 1990

Health Effects of Chloroform

California Department of Health Services

October, 1990

Prepared By:

California Department of Health Services

Principal Author: Melanie A. Marty, Ph.D.

Document Reviewed by
Joseph P. Brown, Ph.D.
John P. Christopher, Ph.D.
George V. Alexeeff, Ph.D.

Other Contributing Reviewers:
Wa-Nang Choi, Ph.D.
Andy Salmon, Ph.D.
Calvin Wilhite, Ph.D.

TABLE OF CONTENTS

APPENDICES

LIST OF TABLES

LIST OF FIGURES

1.0 Summary

Chloroform is a colorless, volatile liquid used primarily in the production of chlorodifluoromethane, and as a solvent, cleaning agent, and fumigant. The U.S. EPA (1985b) estimates that about 9735 metric tons of chloroform per year are emitted into the ambient air in the United States. Chloroform is ubiquitous in the environment, and can be measured in the air in urban and rural areas. Some of this ambient chloroform may be from natural sources (U.S.EPA, 1985a).

About 50% of inhaled chloroform is absorbed across the human lung at low levels of exposure (13 ppb). Indirect evidence from animal and human toxicity studies indicates that chloroform is well absorbed across the gastrointestinal tract. Chloroform is absorbed across the skin. Once in the body, chloroform is distributed to all tissues, particularly to those with a high proportion of lipid. Chloroform can cross the placenta and has been measured in the fetuses of exposed mice.

Chloroform is metabolized by the cytochrome P-450 dependent mixed function oxidase enzyme system to phosgene. Phosgene may undergo spontaneous hydrolysis to form CO_2 and HCl, or it can react with cellular constituents. Phosgene is believed to be the major reactive metabolite produced during the metabolism of chloroform. Other reactive metabolites, primarily free radicals, may be produced particularly under reductive conditions.

Chloroform is excreted as CO_2 and as unchanged $CHCl_3$ in expired air. Small amounts of radiolabel have been found in urine and feces after administration of ^{14}C-chloroform. Chloroform elimination is characterized by a rapid initial phase with a half-time on the order of 20 to 30 minutes, followed by at least one slower phase with a half-time of approximately 3 hours. A third phase of elimination representing elimination from a deep compartment, most likely adipose tissue, has been suggested to have a half-time longer than 24 hours.

Unmetabolized chloroform has depressant effects on the central and peripheral nervous system. However, it appears that metabolism is essential to the hepatotoxicity and nephrotoxicity of chloroform. Induction of cytochrome P-450-dependent mixed function oxidase enzymes results in potentiation of chloroform hepatotoxicity and nephrotoxicity, while inhibitors of mixed function oxidase enzymes reduce the extent of chloroform-induced liver and kidney damage. Radiolabeled chloroform metabolites have been found bound to cellular proteins and lipids, and to a lesser extent to DNA.

Chloroform causes central nervous system depression at levels (e.g., 5000 ppm) several orders of magnitude greater than levels measured in California ambient air. Other symptoms of acute chloroform intoxication include cardiac sensitization to catecholamines, and liver and kidney damage. Subchronic and chronic toxicity occurred in animals exposed to 25 ppm for 7 hours per day, 5 days per week, for 6 months. Mild pathological changes in the liver and kidney were noted in rodents. No adverse effects were noted in animals exposed to 25 ppm chloroform in air 1, 2, or 4 hours/day, 5 days/week for 6 months.

Chloroform interferes with the maintenance of pregnancy and is fetotoxic in rodents following exposure to 30 to 100 ppm chloroform in air. Concentrations of chloroform in air producing adverse effects in experimental animals are about 5 orders of magnitude greater than ambient air concentrations of chloroform (< 0.4 ppb) in California. Department of Health Services (DHS) staff do not anticipate that noncancer health effects would result from acute or chronic exposure to ambient levels of chloroform in California air.

Results of mutagenicity tests in bacteria and fungi are largely negative. However, bacterial test systems may not be adequate for some compounds which require metabolic activation for genotoxicity. A few positive results have been reported for sister chromatid exchange in cultured human lymphocytes and mouse bone marrow cells, and in the micronucleus test in mouse bone marrow. In addition, chloroform was a mitotic poison in an invertebrate test system. Because chloroform was more frequently negative than positive in genotoxicity tests, many scientists have concluded that it is nongenotoxic. However, DHS staff believe that the positive tests in mammalian test systems indicate a possible genotoxic mechanism.

Chloroform is carcinogenic in rodents, producing liver tumors (hepatocellular carcinomas and neoplastic nodules) in both sexes of mice, kidney tubular epithelial tumors in male rats and male mice, and cholangiocarcinomas in both sexes of rats. Epidemiological evidence for human cancer from exposure to chloroform is inadequate. The International Agency for Research on Cancer (IARC) and the U.S. EPA both classify chloroform as a possible human carcinogen based on adequate evidence for carcinogenicity in animals and inadequate epidemiological data. DHS staff agree with this classification. Furthermore, DHS staff found little evidence of a carcinogenic threshold level for chloroform. Although some scientists conclude chloroform is an epigenetic carcinogen, which may imply the presence of a threshold, DHS staff, in order to protect public health, treat chloroform as a nonthreshold carcinogen.

DHS staff used data from experimental animals to extrapolate the carcinogenic risk to humans from exposure to chloroform in ambient air. Concern has been raised in the scientific literature that liver tumors produced in animals exposed to chloroform in corn oil may be vehicle-dependent. Staff used tumor sites that were not vehicle-dependent, Thus, risks from the liver tumors were excluded from the DHS range of risks and best estimate. The linearized multistage model (GLOBAL86) was applied to data from several rodent carcinogenicity bioassays where chloroform was given orally to derive a range of 95% upper confidence bounds on cancer potency and to estimate risk for a given dose. Cancer potencies derived from animal data were adjusted by dose scaling on a surface area basis to obtain theoretical estimates of human cancer potencies. These 95% upper confidence limits on slope ranged from 4.2×10^{-3} to 1.4×10^{-1} $(mg/kg\text{-}day)^{-1}$. The range of unit risks for inhalation of chloroform estimated from these analyses of animal studies assuming 50% absorption across the lung lies between 2.9×10^{-3} and $9.8 \times 10^{-2} (ppm)^{-1}$ (6.0×10^{-7} and $2.0 \times 10^{-5} (\mu g/m^3)^{-1}$). This corresponds to a potential cancer burden of 3 to 98 cancer cases per million people continuously exposed over their lifetime to 1 ppb chloroform in air. DHS contracted with the Lawrence Livermore

National Laboratories (LLNL) Environmental Health Sciences group to conduct a health risk assessment of chloroform in drinking water. The LLNL scientists used a physiologically - based pharmacokinetic model to estimate metabolized dose (Bogen et al. 1989). The metabolized dose estimates served as measures of dose in a linearized multistage model. Animal potencies were corrected by a surface area correction factor to estimate human potencies. The resulting potencies for selected data sets (those evaluated by both DHS and LLNL ranged from $4.8\ 10^{-3}$ to 1.7×10^{-1} (mg M/kg-d $^{-1}$. This is equivalent to unit risks of 4.5×10^{-6} to 1.6×10^{-4} $(ppb)^{-1}$.

DHS staff believe that a best estimate of potency lies between the high and low end of the range of estimated potencies (see Section 8.7). Almost all of the data sets were used as input for a best estimate. The potencies based on hepatocellular carcinomas in mice in the NCI study were excluded because production of these tumors appears to be vehicle-dependent. Eleven potency estimates derived from Roe et al. (1979) data on renal tumors in male mice and from Tumasonis et al. (1985) data on cholangiocarcinoma in male and female rats were geometrically averaged. This was done to take into account the large number of studies indicating positive tumor response, but to deemphasize their importance to the overall best estimate since each individual study was not as well-designed as either the Jorgenson et al. (1985) or NCI (1976) study. This value was then weighted equally with the potencies from Jorgenson et al. (1985) and NCI (1976) and arithmetically averaged. This average is presented as the best estimate of potency as a unit risk of 2.6×10^{-5} $(ppb)^{-1}$ or 2.6×10^{-2} $(mg/kg\text{-}day)^{-1}$.

Measurements of chloroform in California ambient air in 1985 - 1987 ranged up to 0.36 ppb, with a median of about 0.06 ppb and a population weighted exposure of 0.03 ppb for 20.3 million people. Using the population weighted exposure (0.03 ppb) for a lifetime and the best estimate of potency (2.6×10^{-5} $(ppb)^{-1}$), results in a potential 16 cancer cases in California.

These estimates of theoretical oncogenic potency in humans contain many sources of uncertainty. Extrapolation from animals to man involves a number of assumptions. Factors including metabolism, target site sensitivity, diet, immunological responses, and genetics, may influence the process of carcinogenicity. Differences in these factors in different species cannot at present be easily quantitated and incorporated into risk assessment. The human population is much more heterogeneous both genetically and culturally (lifestyle, diet) than experimental animals. The intraspecies variability in humans is, therefore, probably much greater than in laboratory animals. Other uncertainties arise in the assumptions underlying the model used, and in extrapolating from large experimental doses, where other toxic effects may compromise the assessment of carcinogenic potential, to much smaller environmental doses. Additional uncertainties arise from coincidental exposure to other carcinogens in the occupational or general environment. Notwithstanding these uncertainties, the lifetime risks presented above are considered to be conservative estimates and are not likely to be exceeded by the actual risk. These calculations are for the upper range of plausible excess cancer risks; the actual risk, which cannot be calculated, may be insignificant.

Based on the findings in animal studies of chloroform-induced carcinogenicity and the results of the risk assessment, DHS staff find that chloroform is an air pollutant which may cause or contribute to an increase in mortality or an increase in serious illness, or which may pose a present or potential hazard to human health. Furthermore, chloroform in drinking water (including that volatilizing into indoor air from household activities) may contribute more to the overall risk from chloroform exposure than chloroform in ambient air.

1.1 Chloroform Highlights

I. National and International Evaluation of Carcinogenicity

A. International Agency for Research on Cancer (IARC)

1. Animal Carcinogenicity Bioassays:

   Sufficient evidence exists that chloroform is carcinogenic to animals by the oral route of administration. (Inhalation route not tested).

2. Human Evidence:

   There is inadequate evidence that chloroform is a human carcinogen.

B. U.S. Environmental Protection Agency (EPA)

1. Animal Carcinogenicity Bioassays:

   There is sufficient evidence that chloroform is carcinogenic to animals by the oral route.

2. Human Evidence:

   There is inadequate evidence for carcinogenicity of chloroform in humans.

C. Conclusions: Both EPA and IARC have concluded that chloroform is carcinogenic in laboratory animals, but that evidence for human carcinogenicity is inadequate.

II. Exposure Sources

A. Air

Chloroform is a ubiquitous contaminant of urban and rural air. Ambient air levels of chloroform were measured at 22 locations in California by the Air Resources Board in 1985, 1986, and 1987. Measurements ranged from 0.02 to 3.54 ppb chloroform. The mean concentrations ranged from 0.0257 to 0.3604 ppb, with a median of 0.06 ppb and a population-weighted exposure of 0.03 ppb for 20.3 million people.

B. Water

Chloroform is formed during the chlorination of drinking water or disinfection. Levels of chloroform in chlorinated drinking water average 40 $\mu g/l$. Contamination of indoor air by chloroform volatilizing from water during household activities has been documented.

III. Quantitative Risk Assessment

A. Range of Potencies

The theoretical human risks associated with a continuous, lifetime exposure to chloroform in ambient air have been estimated from animal carcinogenicity bioassay data using the linearized multistage model. Unit risks for humans estimated from the animal data range from 2.9×10^{-6} per ppb (2.9×10^{-3} $(ppm)^{-1}$) to 9.8×10^{-5} per ppb (9.8×10^{-2} $(ppm)^{-1}$) using the linearized multistage model with administered dose. Unit risks for humans estimated from animal data based on metabolized dose ranged from 4.5×10^{-6} per ppb to 1.6×10^{-4} per ppb.

B Best Estimate of Carcinogenic Potency

The best estimate of carcinogenic potencies was derived by averaging potencies from selected data sets. The best estimate of the unit risk was 2.6×10^{-5} per ppb.

C. Extrapolations

1. Route

Animal carcinogenicity bioassays for chloroform were conducted by the oral route. No studies exist which used the inhalation route of exposure. Therefore, the assumption was made that chloroform is carcinogenic by inhalation, and that a given absorbed dose is equipotent by either route.

2. Dose

The doses in the animal carcinogenicity bioassays range from 19 to 477 mg/kg-d. A human living in an area where the average ambient air concentration of chloroform is 0.36 ppb would receive a dose from inhalation of about 0.0005 mg/kg-d. Thus, the experimental doses were about 5 to 6 orders of magnitude greater than doses resulting from inhalation of ambient air.

2.0 TOXICOKINETICS

2.1 Absorption

Raabe (1988) reported that 46 to 50% of inhaled chloroform is absorbed across the human lung at low levels of exposure (13 ± 5 ppb). About 40% of inhaled chloroform is absorbed by dogs at levels less than 600 ppb (Raabe, 1986). Little quantitative information is available for absorption across the gastrointestinal tract or skin in humans. Indirect evidence from animal and human toxicity studies implies that GI absorption is fairly rapid and complete, and that chloroform is absorbed to an appreciable extent across the skin when in a liquid phase or in aqueous solution. Experimental evidence is discussed in detail in the following sections.

2.1.1. Pulmonary Absorption

Inhalation is a primary route of human exposure to chloroform in the ambient air. Readily absorbed across the lung (Davidson et al., 1982; Fry et al., 1972; Poobalasingham and Payne, 1978), chloroform was used extensively as an inhalation anesthetic for over 100 years (Waters, 1951).

Absorption across the lung into the blood depends on the blood:air partition coefficient, diffusivity of the vapor, solubility in the various tissue compartments, and the rate of equilibration into tissues (Goodman and Gilman, 1980; Raabe, 1986, 1988). Other factors influencing absorption include concentration of chloroform in inspired air, duration of exposure, ventilation rate and cardiac output (von Oettingen et al., 1950; Davidson et al.,1982; Poobalasingham and Payne, 1978). The blood:air, water:air, and oil:air partition coefficients reported by Sato and Nakajima (1979) were 10.3, 3.5, and 401, respectively, reflecting the lipophilic nature of chloroform. Other investigators have derived similar oil:air and serum:air partition coefficients (Morgan et al. 1972).

Despite its importance and the duration of its use as an anesthetic agent, only a few experiments have been done to characterize pulmonary absorption of chloroform in experimental animals and man. Raabe (1986) measured the inhalation uptake fractions of 6 chemicals at low concentrations using adult nose-breathing beagles. Each unanesthetized trained dog was fitted with a respirator that separated and measured the volume of inhaled and exhaled air. $^{14}CHCl_3$ (0.393 to 0.594 ppm) was administered to the dogs through the respirator for 3 hours. Based on measurements of chloroform in the inhaled and exhaled air (fractional uptake), steady state was reached in the first 30 minutes of exposure. At steady state, the fractional systemic uptake of chloroform via the lungs was 39.8 ± 1.5% standard error. Raabe (1988) exposed 4 human volunteers, 2 males and 2 females, to ^{14}C-chloroform in air (7 to 25 ppb, 13 ± 5 ppb, mean ± standard deviation) via a nose-only or mouth-only breathing apparatus for 2 hours. Steady state was reached within 30 minutes, based on measurements of fractional uptake at 30-minute intervals. Steady-state uptake of chloroform, corrected for dead space, was 49.6 ± 1.6 % by mouth and 45.6 ± 1.5 % by nose. Uptake of chloroform while mouth-breathing was significantly greater than while nose-breathing ($p<0.005$, N=4, two-way analysis of variance). Uptake of chloroform, and other vapors, by humans was greater than that measured in dogs (Raabe, 1986, 1988). This study and the experiments in beagle dogs are useful for risk assessment because of the low levels of exposure which approximate

environmental conditions. Raabe's study with humans will be used as the basis for estimating absorption of inhaled chloroform at ambient levels for the risk assessment; it is assumed, then, that humans absorb 50% of inhaled chloroform at low environmental levels such as the levels measured in California ambient air (below 1 ppb) (CARB, 1987).

Lehmann and Hasegawa (1910) measured the retention of chloroform as the difference in concentration in inspired and expired air in 3 human subjects exposed to 4200 to 7200 ppm chloroform in air. Samples were taken after each of four 5 minute exposures, and analyzed for chloroform by alkaline hydrolysis and chloride titration. The retention (% inspired air concentration of chloroform retained in the body) was high initially and decreased during the 20 minutes of exposure. At 20 minutes, the retention was between 50 and 75% of the inspired concentration. However, this does not represent steady-state equilibrium absorption. Data from Smith et al. (1973) indicate that equilibrium among all body compartments is not approached until about 1 hour after induction of anesthesia with chloroform; minimal differences in arterial and venous blood concentrations occurred at 120 minutes post induction. In ten patients observed by Smith et al., the blood concentrations of chloroform during surgical anesthesia averaged 9.8 mg/dl (ranged from 7 to 16.5). The concentration of chloroform in inspired air was not constant in this study, but it was less than 1.3% (13,000 ppm). Poobalasingham and Payne (1978) measured the concentration of chloroform in the blood of 16 patients undergoing surgery. Eight spontaneously breathing patients inhaled 2 to 2.5% chloroform (20,000 to 25,000 ppm) in oxygen, and eight patients on ventilators were breathing 1% chloroform (10,000 ppm). The mean arterial concentrations of chloroform at surgical plane of anesthesia were 17.28 $\pm$ 4.1 mg/dl and 10.1 $\pm$ 3.3 mg/dl in the spontaneously breathing and ventilated patients, respectively, at equilibrium. The arterial concentration after 1 hour reached 25% and 41% of the equilibrium concentration (calculated by the investigators to be 36.7 mg/dl for 10,000 ppm exposure based on a blood:air partition coefficient of 8.4) in spontaneously breathing and ventilated patients, respectively. The uptake of chloroform into the blood in ventilated patients was more rapid in the initial stages than that occurring in spontaneously breathing patients probably due to respiratory depression induced by anesthesia (Poobalasingham and Payne, 1978). The available data are not sufficient to calculate a fractional uptake of chloroform in humans. The U.S.EPA (1985a) calculated a retention of about 67% based on data in Smith et al. (1973). However, it is not clear how this figure was derived.

2.1.2. Oral Absorption

There is appreciable absorption across the gastrointestinal tract following oral administration. Taylor et al. (1974) recovered about 89% of ^{14}C activity in exhaled air, urine and feces of mice within 48 hours of an oral dose of labeled chloroform, 60 mg/kg in olive oil. After oral administration of 60 mg ^{14}C-chloroform/kg in olive oil, Brown et al. (1974a) recovered 95 to 97% of ^{14}C activity in exhaled air, urine, feces, and the carcass of mice and 93 and 99% of administered radiolabel in exhaled air, urine and feces of rats and squirrel monkeys, respectively. In all cases, the fecal elimination was on the order of a few percent while the majority of radiolabel was exhaled, implying that chloroform is extensively absorbed (e.g., 80 to 95%) after oral administration. Mink et al. (1986) studied the absorption, distribution, and excretion of ^{14}C-labeled trihalomethanes in

mice and rats after intragastric intubation in corn oil. The total radiolabel recovered in expired air, urine, and tissues was 78% for rats given 100 mg/kg (16 uCi/kg) chloroform, and 95% in mice given 150 mg/kg (32 uCi/kg) chloroform. These data imply that at a minimum, 78 and 95% of an oral dose was absorbed by the rat and mouse, respectively.

Withey et al. (1983) found that absorption of chloroform was influenced by the vehicle in which it was administered. The blood concentration-time profile of rats given 75 mg/kg in vegetable oil differed from that obtained when chloroform was given in water. The peak concentration in blood was about 6.5-fold greater and the area under the blood concentration-time curve was 8.7 fold larger when chloroform was administered in water than in oil. The time to peak concentration differed little (5.6 min in water, 6.0 min in oil). When chloroform was given in water, an initial rise to a peak of 39 ug/ml was followed by a concave downward phase, which was followed shortly by a more rapid linear elimination phase. When chloroform was given in oil, there was a series of uptake pulses with peaks around 6, 20, and 40 minutes, followed by a fairly linear elimination phase. Withey et al. (1983) speculated that these peaks reflect uneven absorption of chloroform from water immiscible globules. They also suggested that chloroform trapped in vegetable oil is moved down the gastrointestinal tract and that the peaks represent differential absorption from different parts of the GI tract. This phenomenon was observed with other lipophilic halogenated solvents in the same study. The elimination rate constants from blood (water, 0.0218 min^{-1}; oil, 0.0258 min^{-1}) did not differ significantly.

Data reported in Fry et al. (1972) suggest that a large portion of an oral chloroform dose is absorbed across the gastrointestinal mucosa in humans and is excreted as unchanged chloroform and carbon dioxide. The blood concentration-time profile for 3 of the subjects showed a rapid rise in concentration of chloroform with peak concentrations ranging from 1 up to 5 ug/ml one hour after oral administration of 0.5 g chloroform in a gelatin capsule.

2.1.3. Dermal Absorption

The absorption of chemicals across the skin is dependent on a number of physicochemical parameters, including ionic character, hydrophilicity and lipophilicity. Tsurata (1975) studied the dermal absorption of chloroform and other solvents across intact shaved skin of urethane anesthetized mice during a 5 to 15-minute application. Chloroform, 0.5 ml, was applied to a 2.92 cm^2 area under a sealed cup designed to prevent evaporative loss. Absorption was calculated as the amount of solvent in expired air plus that retained in the whole body as determined by extraction and gas chromatographic analysis. Chloroform was absorbed across mouse skin *in vivo* at a rate of 329 nmoles/min/cm^2 of skin, for a total absorption of 1718 ug chloroform in 15 minutes. Thus, an absorption fraction of 0.2% in 15 minutes (1.7 mg absorbed/ 743 mg applied) is derived, assuming that the entire 0.5 ml chloroform was in contact with the skin. The absorption fraction could be higher if decreased dermal blood flow resulted from the urethane anesthesia, as is common. Dermal absorption increased linearly with time and was directly proportional to water solubility for 8 solvents. Tsurata (1975) also estimated the amount of chloroform that would be absorbed by a human with both hands immersed, assuming the surface area of the hands is 800 cm^2 and the absorption rate is the same as for the mouse.

The estimated absorption rate was 29.7 mg/min. Tsurata (1975) concluded that the skin is a likely route of exposure to chlorinated solvents. No other studies on dermal absorption of chloroform were found.

2.2 Distribution

Chloroform is distributed throughout the body to all tissues, particularly to those with a relatively high proportion of lipid. Equilibrium concentrations of chloroform will depend on the fat content and metabolic capacity of the tissue. The tissues of relatively greater vascularity come to equilibrium faster than those with a lower perfusion rate. McConnell et al. (1975) found highest concentrations of chloroform in body fat of post-mortem tissue samples selected randomly from the general population. The source of the chloroform was presumably environmental.

Partition coefficients for human tissue at 37°C during surgical anesthesia reported by Steward et al. (1973) reflect the lipophilicity of chloroform. The expected order of descending concentration of chloroform at equilibrium, following exposure to minimum alveolar chloroform concentration required for anesthesia, is adipose >> brain > liver > kidney = muscle > blood = lung = heart. Gettler and Blume (1931) determined the concentration of chloroform in several tissues of 9 patients who died while under chloroform anesthesia. Brain tissue contained 120-182 mg/kg wet weight, while lung and liver samples contained 92-145 and 65-88 mg/kg, respectively.

Chenoweth et al. (1962) determined the chloroform content of tissues of 2 dogs following a 2.5 hour period of chloroform anesthesia (concentration in inspired air was not determined). Highest concentrations were found in fat (2800 ppm) and the adrenals (1185 ppm, 4-fold higher than blood). The concentrations in brain, liver, and kidney were similar to those in blood.

Brown et al. (1974a) conducted whole-body autoradiography on male and female rats and squirrel monkeys, 3 hours after an oral dose of 60 mg ^{14}C-$CHCl_3$/kg (10μCi/mg). As indicated by the autoradiographs, the adipose tissue, liver, and gall bladder of the squirrel monkey contained the largest amounts of residual radioactivity, while the kidneys, lungs, stomach, heart, and testes contained relatively little radioactivity. The authors noted no apparent sex differences in distribution in rats and squirrel monkeys. However, Taylor et al. (1974) observed a sex difference in the distribution of chloroform in mice based on autoradiography 3, 5, and 7 hours after oral administration of ^{14}C-chloroform. Significantly larger amounts of radioactivity ($p<0.005$) appeared in the kidneys of the males as compared to the females of all strains. More radiolabel was found in the renal cortex than in the medulla in males, while there was little difference in the amount of label in the cortex and medulla of the kidney in the female mice. Testosterone pretreatment of female mice resulted in increased distribution of radiolabel to the kidney. Similarly, less radioactivity appeared in the kidney of castrated males relative to controls after administration of labeled chloroform. Livers of female mice contained significantly greater ($p<0.02$) amounts of ^{14}C label than those from male mice. These findings correlate well with the observation that chloroform treatment induced kidney tumors in male but not female mice (Roe et al., 1979) and in male but not female rats (NCI, 1976). In addition, female mice appear to be more sensitive to chloroform hepatocarcinogenesis than males (NCI, 1976) (see Section 7).

Mink et al. (1986) reported that the stomach (without contents), liver, kidney, and blood contained the highest amounts of radiolabel 48 hours after an oral dose of 150 mg chloroform/kg in mice and rats. Cohen and Hood (1969), using low-temperature autoradiographic techniques, found highest concentrations of radiolabel in fat (up to 12-fold > blood), with lesser amounts in the liver (1.6-fold > blood), and still smaller, roughly equivalent amounts in blood, brain, lung, muscle, and kidney, immediately following inhalation exposure of mice to $^{14}CHCl_3$ for 10 minutes. Fifteen minutes after cessation of exposure, the relative concentrations in fat and liver increased. By 120 minutes following cessation of exposure, total radioactivity in the whole body was considerably lower, and was found primarily in the liver, duodenum, and fat.

Similar results for distribution to the liver were observed in pregnant mice by Danielsson et al. (1986). In addition, Danielsson et al. observed significant radioactivity in the respiratory epithelium (nasal mucosa, trachea, bronchi) relative to surrounding tissues in autoradiograms prepared immediately after a 10 minute inhalation exposure to ^{14}C-chloroform. A large portion of this radioactivity was nonvolatile, presumably metabolites. Non-volatile radioactivity was also observed in the oral and esophageal mucosa, centrilobular region of the liver, renal cortical tubules, salivary glands, and conjunctiva of the eye. Autoradiography also revealed an accumulation of nonvolatile radioactivity, presumably metabolites, in respiratory epithelium, intestine, kidney, and urine of 4-day old mice one hour following intraperitoneal injection of ^{14}C-chloroform.

Lofberg and Tjalve (1986), using autoradiographic techniques, found radioactivity bound to the following tissues, 30 minutes and 4 hours following intravenous and intraperitoneal injection of adult rats with ^{14}C-chloroform: liver, kidney cortex, mucosa of the bronchial tree, trachea, nasal respiratory and olfactory mucosa, and mucosa of the esophagus, the larynx, the tongue, the gingiva, cheek, pharynx, soft palate, naso-pharyngeal cavity, and lower levels bound in urinary bladder and intestines. Most of the radioactivity in the heavily labeled tissues remained after washing the sections with trichloroacetic acid and organic solvents. Evidence was presented that the bound radiolabel represented covalent binding of metabolites (see following metabolism section). Cohen and Hood (1969) demonstrated that the radioactivity in adipose tissue was primarily volatile, while that in the liver was initially volatile and became mostly nonvolatile metabolites (85.6%) by 120 minutes, reflecting biotransformation of chloroform in that organ.

The distribution of radioactivity following ^{14}C-chloroform administration may differ depending on the route of exposure. In Taylor et al. (1974) more radiolabel was found in the liver than in the fat 5 hours after oral administration of chloroform, whereas in Cohen and Hood (1969) more radiolabel was present in fat than in liver at 2 hours following cessation of inhalation exposure. DHS staff suggest that this may be due to a first pass effect following oral administration of chloroform. Alternatively, these differences in tissue content may reflect the different sampling times, i.e., redistribution of radiolabel may have occurred by 5 h in Taylor et al.

Chloroform crosses the placenta and can be found in fetal blood and tissues following inhalation or oral exposure (Whipple, 1912; Schwetz et al., 1974;

Dilley et al., 1977). Danielsson et al. (1986) found volatile radioactivity in the placenta and fetuses shortly after a 10-minute exposure of pregnant mice to ^{14}C-chloroform by inhalation (concentration not specified in paper, 4 mice were exposed in an all-glass chamber to about 80 uCi equivalent to a total of 16 umol). Non-volatile radioactivity in the fetus reached a peak about 1 hour after exposure. Metabolites of chloroform accumulated with time in the fetoplacental unit. Tissue-bound metabolites of chloroform were found in fetal respiratory epithelium when pregnant mice were exposed late in gestation, indicating that this fetal tissue may have metabolic activating capability. Radioactivity accumulated in the amniotic fluid with time, reaching a peak about 4 hours after exposure. Since there was more radioactivity in amniotic fluid at later sampling times (1 and 4 hours) than immediately after exposure, the investigators assumed that metabolites had accumulated in the amniotic fluid.

2.3. Metabolism and Excretion

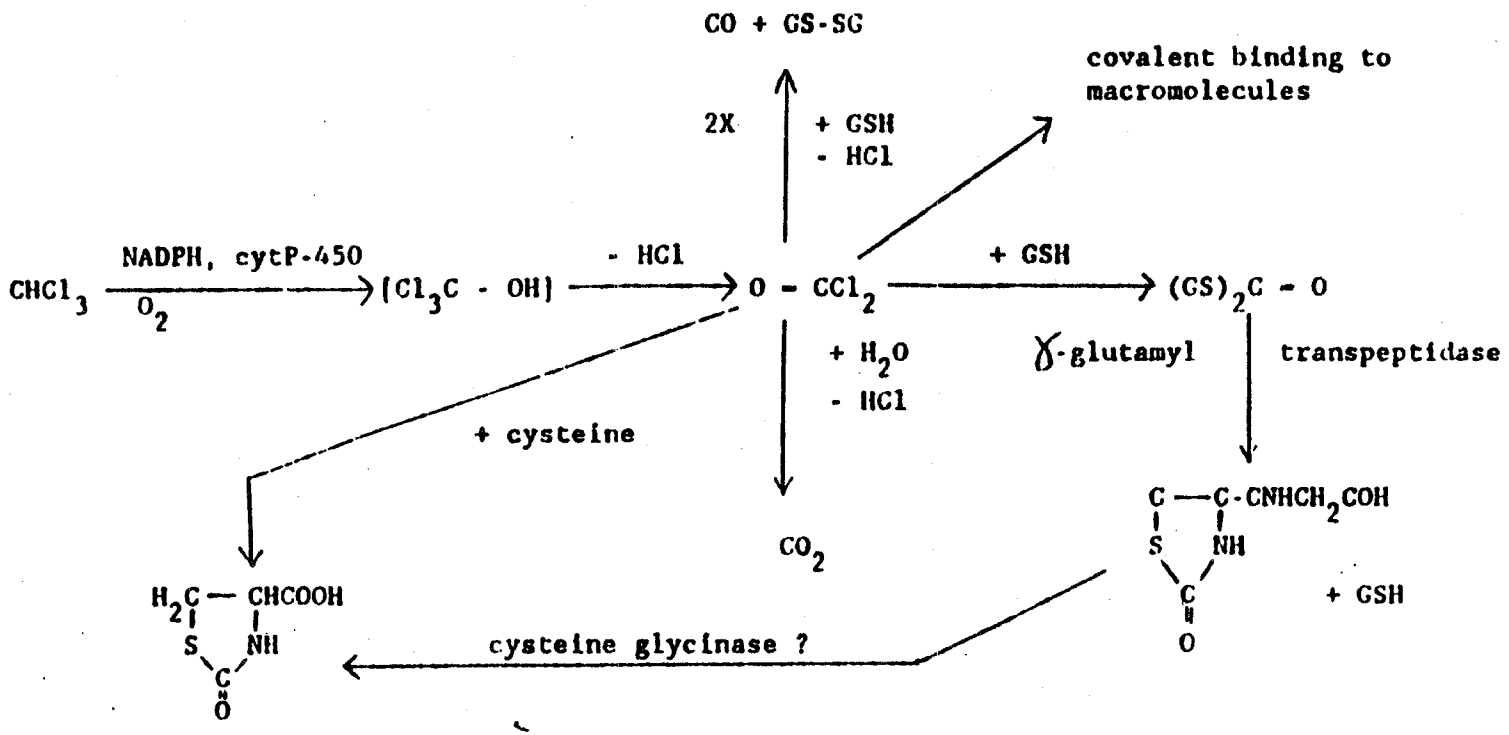
Chloroform is metabolized by the cytochrome P-450 dependent mixed function oxidase system primarily in the liver, the respiratory epithelium, and the kidney. Chloroform is metabolized to phosgene, presumably via hydroxylation to an unstable derivative, trichloromethanol, which rapidly dehydrochlorinates to phosgene (Figure 2-1). Phosgene may undergo spontaneous hydrolysis to form CO_2 and HCl, or react with cellular macromolecules (e.g., lipids and proteins). Phosgene also reacts with glutathione (GSH) to form diglutathionyl dithiocarbonate. Minor amounts of carbon monoxide are formed during chloroform metabolism. Some evidence exists for the formation of free radicals from chloroform under reductive conditions. Experimental evidence for chloroform metabolism is discussed in detail in the following section.

2.3.1. Pathways of Metabolism

Early investigators postulated that chloroform is metabolized by cleavage of chlorine atoms with the production of HCl (Lucas, 1928; Zeller, 1883; Graham, 1915). Zeller (1883) found increased urinary output of inorganic chloride in dogs given oral doses of chloroform. Lucas (1928) found excess chloride present in homogenates of liver incubated with chloroform and postulated that chloride may be produced *in vivo* in the liver by cleavage of chlorine atoms from chloroform. Van Dyke et al. (1964) found ^{36}Cl (73% inorganic, 27% unidentified) in the urine of rats following intraperitoneal (ip) injection of ^{36}Cl-$CHCl_3$.

The metabolism of chloroform to CO_2 has been described by many investigators. Following intraperitoneal (ip) injection of ^{14}C-chloroform to rats, 4 to 5 % of the injected radioactivity was recovered as labeled carbon dioxide (van Dyke et al., 1964). Much higher rates of metabolism of chloroform to CO_2 were reported by Brown et al. (1974a). Mice, rats, and squirrel monkeys excreted about 80%, 66%, and 18% of administered radioactivity as $^{14}CO_2$ (captured in an ethanolamine trap) via the lung, respectively, after oral administration of 60 mg $^{14}CHCl_3$/kg. About 20% of the radioactivity exhaled by the rat was chloroform or toluene-soluble metabolites. Small amounts of radioactivity were found in the urine and feces of all species. Urea, carbonate and bicarbonate accounted for nearly 50% of the radioactivity present in mouse urine; the remaining 50% was not identified. Taylor et al. (1974) also found that mice excreted 80% of a

Figure 2-1. Pathways of Chloroform Metabolism in Mammals



GSH = glutathione, reduced; GS = oxidized glutathione.

dose of $^{14}CHCl_3$ (60 mg/kg) as $^{14}CO_2$. Small amounts (0.4% in males; 1.8% in females) of radioactivity were exhaled as chloroform or toluene-soluble metabolites, and about 5% and 0.6% of administered radiolabel were found in urine and feces, respectively, collected over 48 hours. Raabe (1986) found that about half of the chloroform absorbed during exposure to ^{14}C-chloroform (0.393 to 0.594 ppm) was exhaled as $^{14}CO_2$, and about 18% as unchanged chloroform (or toluene-soluble metabolites) in the first 21 hours following cessation of exposure in dogs. A small fraction was excreted in the urine and feces in the 69 hours following exposure.

Fry et al. (1972) and Raabe (1988) indicated that humans can metabolize chloroform to CO_2. Two subjects were given $^{13}CHCl_3$, 0.5 g orally in olive oil, and exhaled $^{13}CO_2$ was monitored using mass spectrometry (Fry et al. 1972). Forty-eight and 51% of a single oral dose of chloroform was exhaled as CO_2 over a 7.5 hour period, with maximum excretion occurring 75 to 210 min after administration. Pulmonary excretion of chloroform and its carbon dioxide metabolite accounted for most of a single oral dose in humans. No lower chlorinated methanes were found in expired air and chloroform was not detected in the urine. Raabe (1988) exposed 4 human subjects to 13 $\pm$ 5 ppb chloroform in air for 2 hours, and found about 16% of administered radioactivity (36% of the absorbed dose) exhaled as CO_2 within a half hour of cessation of exposure.

Metabolism of $^{14}CHCl_3$ to $^{14}CO_2$ <u>in vitro</u> was reported by Rubinstein and Kanics (1964) and Paul and Rubinstein (1963). These investigations demonstrated that the formation of carbon dioxide from chloroform is an enzymatically catalyzed NADPH-dependent reaction.

Several investigations have demonstrated that chloroform is metabolized <u>in vivo</u> and <u>in vitro</u> to phosgene, $COCl_2$, a highly reactive compound which can spontaneously hydrolyze to CO_2 and HCl. Phosgene is a reactive electrophile which may react with nucleophilic groups within tissue macromolecules, and may be responsible, at least in part, for the hepatotoxicity and nephrotoxicity and possibly the carcinogenicity of chloroform. Mansuy et al. (1977) and Pohl et al. (1977) showed that phosgene is formed during NADPH and oxygen-dependent microsomal oxidation of chloroform. These investigators incubated liver microsomes from phenobarbital-pretreated rats with 2 mM chloroform in the presence or absence of 2 mM cysteine. In the presence of cysteine, a stable adduct was formed identical to the product of the reaction of cysteine with phosgene, 2-oxothiazolidine-4-carboxylate. Pohl et al. (1977) also reported that cysteine inhibited the binding of ^{14}C label from $^{14}CHCl_3$ to microsomal protein. Additionally, incubation under $^{18}O_2$ resulted in incorporation of ^{18}O into the 2-oxo position of 2-oxothiazolidine-4-carboxylate (OTZC) in the presence of cysteine. This provides evidence that chloroform is activated by oxidative dechlorination to form phosgene.

Pohl et al. (1980) found that covalent binding of ^{14}C label and the formation of $^{14}CO_2$ following <u>in vitro</u> incubation of rat liver microsomes with $^{14}CHCl_3$ was inhibited by cysteine, presumably by reaction with the metabolite phosgene.

Incubation of rat liver microsomes with ^{3}H-labeled chloroform or ^{36}Cl-labeled chloroform did not result in appreciable amounts of covalently bound radiolabel, suggesting that other reactive intermediates postulated as

possible products of chloroform metabolism, including the trichloromethyl radical ($\cdot CCl_3$), dichloromethyl carbene ($Cl_2C:$), and the dichloromethyl radical ($Cl_2HC\cdot$) are not formed in significant amounts during in vitro metabolism of chloroform (Pohl et al, 1980). Pohl et al. (1980) also showed that covalent binding, and the formation of $^{14}CO_2$ and the ^{36}Cl anion are oxygen and NADPH-dependent processes and are inhibited by carbon monoxide and SKF525A, implicating mediation by the cytochrome P-450 mixed function oxidase enzyme system.

Pohl et al. (1979) injected phenobarbital-pretreated rats with cysteine (1 g/kg, ip) followed 30 min later by chloroform (5 mmol/kg,ip, in sesame oil), or $CDCl_3$. Extraction of the liver showed that rats produced more than twice as much phosgene (trapped as OTZC) from chloroform than from deuterated chloroform. The C-D bond is more resistant to cleavage than the C-H bond, and hydroxylation of deuterated chloroform would proceed at a slower rate. This result supports the concept that a short-lived hydroxylated intermediate is formed which rapidly decomposes to phosgene. In addition, these investigators found that deuterated chloroform was 2 to 3 times less hepatotoxic than chloroform which supports the hypothesis that the hepatotoxicity of chloroform results from the production of a reactive metabolite.

Metabolism of chloroform to phosgene occurs in the kidney as well as in the liver in some species. Pretreatment with phenobarbital, a potent inducer of renal cytochrome P-450 in the rabbit, increased rabbit renal cytochrome P-450 content, and stimulated metabolism of chloroform to carbon dioxide and covalent binding of radiolabel 4 to 5-fold in renal cortical slices and microsomes (Bailie et al, 1984). Phosgene formation was indicated by the production of ^{14}C-labeled OTZC and decreased covalent binding when the kidney microsomes were incubated with $^{14}CHCl_3$ and cysteine. Pretreatment of rabbits with phenobarbital also enhanced the nephrotoxicity of chloroform.

Pohl et al. (1984) also provided evidence that cytochrome P-450 mediates the metabolism of chloroform to phosgene in the kidney. Production of phosgene was greatest in the microsomal fraction of kidney homogenates, and was inhibited by CO. Branchflower et al. (1984) examined the metabolism of chloroform in the kidney in male mice. Mouse kidney metabolized $^{14}CHCl_3$ and ^{14}C-GSCOSG to OTZC and OTZC-glycine (see Figure 2-1).

Lofberg and Tjalve (1986) studied metabolism and covalent binding in rats injected ip or intravenously (iv) with ^{14}C-chloroform, using whole-body and microautoradiography. Several tissues were identified as having marked ability to metabolize chloroform including the following: liver, kidney cortex, bronchial tree, olfactory and respiratory nasal mucosa, tracheal and esophageal mucosa, and larynx, tongue, cheek, gingivae, naso-pharyngeal duct, pharynx, and soft-palate. In vitro metabolism by tissue slices from the esophagus, trachea, and lung revealed strong labelling in the presence of oxygen and little to no covalent binding under a nitrogen or carbon monoxide atmosphere. The greatest in vitro production of $^{14}CO_2$ and tissue-bound [^{14}C] occurred in the liver, followed by nasal olfactory mucosa, nasal respiratory mucosa, kidney cortex, cheek, esophagus, tongue, larynx, trachea, and lung.

Other investigators have examined the potential for cytochrome P-450 catalyzed reductive biotransformation of chloroform. Butler (1961) reported that methylene chloride was not found in exhaled air collected for 2 hours following inhalation exposure of two dogs to chloroform vapors. Low levels of methylene chloride were found in homogenates of mouse liver incubated with chloroform for one day at 37°C. No details of the incubation conditions, purity of chloroform, chloroform concentration, and sample size were reported. Wolf et al. (1977) reported that, under anaerobic conditions, incubation of rat liver microsomes with chloroform produced minute amounts of CO.

Tomasi et al. (1985) detected the production of free radical intermediates from chloroform in isolated rat hepatocytes and in vivo in male Wistar rats using electron spin resonance techniques and the spin trapping agent phenyl-t-butyl nitrone (PBN). The authors provided evidence that the dichloromethyl radical, $\cdot CHCl_2$, is formed from chloroform. The cytochrome P-450 inhibitors SKF525A, metyrapone, and CO, and increased oxygen tension reduced free radical-PBN adduct formation in isolated hepatocytes and microsomal preparations. Free radical-PBN adducts were also found in extracts of liver homogenates 15 minutes after ip administration of 9.2 mmol $CHCl_3$/kg together with 180 mg PBN/kg in olive oil in phenobarbital-pretreated rats. Tomasi et al. (1985) concluded that reductive metabolism of chloroform to a dichloromethyl radical may contribute to the hepatotoxicity of chloroform. These investigators also speculated that chloroform-derived free radicals could be oxidized to the peroxy radical which may in turn form the alkoxy radical, $CHCl_2O\cdot$, that could interact with cellular macromolecules in a manner similar to phosgene (Tomasi et al., 1985).

Testai and Vittozzi (1986) incubated liver microsomes from uninduced rats anaerobically, and found about 2% of the covalent binding seen under aerobic conditions. However, pretreatment with phenobarbital or beta-naphthoflavone enhanced covalent binding to microsomal protein under anaerobic conditions 50 and 15-fold, respectively. Under anaerobic conditions, destruction of cyt P-450 was marked, and NADH supported P-450 loss to the same extent as NADPH. Testai et al. (1987) reported that phenobarbital pretreatment increased covalent binding of radiolabel from ^{14}C-chloroform to lipid in male B6C3F1 mouse liver microsomal preparations under both anaerobic and aerobic conditions. Addition of GSH (3mM) to the incubation medium reduced covalent binding to protein and lipid under anaerobic and aerobic incubation conditions. Anaerobic incubation of microsomes with chloroform (0.1 to 5 mM) produced a time and dose-dependent reduction in cytochrome P-450 content that was about twice that seen under aerobic conditions. These data indicate that reductive metabolism of chloroform may produce reactive, toxic metabolites.

Fodor and Prajsnar (as cited in Ahmed et al., 1977) reported elevated levels of carboxyhemoglobin in rats exposed to various haloforms, suggesting that haloforms may be metabolized to carbon monoxide. Ahmed et al. (1977) provided evidence that haloforms, including chloroform are metabolized to carbon monoxide by a cytochrome P-450 dependent mixed function oxidase in rat liver microsomes. The slow rate of formation of CO from chloroform relative to the other haloforms, is consistent with only minimal elevations in carboxyhemoglobin levels in rats exposed to chloroform (Anders et al.,

1978) and with other metabolic studies which revealed low levels of formation of CO from chloroform in the rat (Wolf et al., 1977; Stevens and Anders, 1981b).

Carbon monoxide is believed to be formed from the hydrolysis of the chloroform metabolite diglutathionyl dithiocarbonate (GSCOSG) (Anders et al., 1978). Formation of GSCOSG from chloroform was demonstrated to occur *in vivo* and *in vitro* by Pohl et al. (1981). ^{13}C- NMR revealed that the thiocarbonate carbon was derived from chloroform. GSCOSG was detected in the bile of rats 30 minutes after administration of 3.7 mmol chloroform/kg ip in sesame oil. The reaction of phosgene with GSH probably accounts for the protective effect of GSH against covalent binding of chloroform metabolites to microsomal protein and for GSH depletion observed after chloroform administration *in vivo* (Pohl et al., 1981; Brown et al., 1974).

Stevens and Anders (1981a) investigated the mechanism of haloform metabolism to CO *in vivo* in rats. Carbon monoxide was produced in rats given $CHCl_3$ or $CDCl_3$, 0.2 ml/kg ip in corn oil. Treatment with diethyl maleate (DEM), a GSH depletor, prior to chloroform administration inhibited the formation of CO *in vivo*. Stevens and Anders (1979) propose a metabolic scheme whereby carbon monoxide is formed when the phosgene reacts with cellular GSH to form a carbonyl chloride which reacts with a second molecule of GSH to form a glutathione dimer and CO and HCl. This scheme was validated *in vitro* (Stevens and Anders, 1979) and *in vivo* (Stevens and Anders, 1981b). Since DEM treatment enhances covalent binding and hepatotoxicity of chloroform (Stevens and Anders, 1981b), these investigators suggested that the glutathione-dependent production of CO may be a common detoxification pathway for haloforms (Stevens and Anders, 1981a). Carbon monoxide formation *in vivo* was also inhibited by about 50% when cysteine (1 g/kg, ip) was administered 30 minutes prior to chloroform administration (0.3 ml/kg, ip) (Stevens and Anders, 1981a). This suggests that cysteine may react *in vivo* with phosgene to detoxify this chloroform metabolite. The data presented in Stevens and Anders (1981a) supports that obtained from several studies conducted *in vitro* on chloroform metabolism. Formation of phosgene is central to the production of carbon dioxide, carbon monoxide, and OTZC from chloroform.

2.3.2. Excretion

Early investigators reported that inorganic chlorides were found in liver and urine of dogs, rabbits, and rats following administration of chloroform (Zeller, 1883; Lucas, 1928; Van Dyke et al., 1964). Mice, rats, and squirrel monkeys excreted 80%, 66%, and 18% of administered radioactivity as $^{14}CO_2$ via the lung, respectively, following oral administration of $^{14}CHCl_3$, 60 mg/kg (Brown et al., 1974a). About 20% of the exhaled radioactivity was eliminated unchanged and/or as toluene-soluble metabolites in the rat. Urinary and fecal excretion accounted for a small portion of the administered radiolabel in all three species. Taylor et al. (1974) found similar results in mice. Mice, however, exhaled less than 2% of radiolabel as unchanged chloroform and/or toluene-soluble metabolites.

Reynolds et al. (1984a, 1984b) measured exhalation of parent compound and $^{14}CO_2$ at 15-minute intervals for 8 to 12 hours following oral administration of 12 or 36 mg $^{14}CHCl_3$/kg in mineral oil to rats. These doses were not hepatotoxic by measurement of serum transaminases and histological criteria.

Pharmacokinetic analysis (using a nonlinear least squares program, NONLIN) of the data fit a two compartment model. Pharmacokinetic parameters obtained in this study (Table 2-A) indicate that about two-thirds of this oral dose was eliminated as CO_2 within 24 hours. About 5 and 12% of the parent compound were eliminated by the lung at 12 and 36 mg/kg doses, respectively, in the first 24 hours. The elimination curves of parent compound were roughly parallel and the amounts proportional to dose for rats given 12 and 36 mg/kg. However, the curves for elimination of $^{14}CO_2$ were not parallel. Rats given the higher dose of chloroform exhaled CO_2 at a rate that was about 25% greater than the rate observed in the lower dose group. The peak rate occurred later and lasted longer in the high dose animals. Apparent half-times for the phases of absorption, distribution, and elimination of parent compound, and elimination of CO_2 are presented in Table 2-A. The apparent half-time of elimination of CO_2 was about 2-fold longer at the high dose relative to the low dose.

Raabe (1986) reported that 14.9% of an inhaled dose of chloroform was excreted as $^{14}CO_2$ via the dog lung in the first 21 hours following cessation of exposure to 0.4 - 0.6 ppm. This represents about half of the amount of chloroform absorbed during exposure. In the same time period, 5.3% of the administered dose (about 20% of the absorbed dose) was exhaled as chloroform or alcohol-soluble metabolites. Excretion of radiolabel in urine and feces accounted for 2.5 and 0.5% of the administered dose (about 9% and 2% of absorbed dose), respectively, within 69 hours following exposure. The estimated clearance half-time of radiolabel derived from $^{14}CHCl_3$ from the blood was 10 hours.

Withey and Collins (1980) found that, in rats given intravenous doses of 3, 6, 9, 12, or 15 mg chloroform/kg, the elimination of chloroform followed a three compartment model at all doses. The mathematical expression for a three compartment model is as follows:

$$\ln C_t = \ln(Ae^{-\alpha t} + Be^{-\beta t} + Ce^{-\lambda t})$$

The kinetic parameters for this model are listed in Table 2-B. While the elimination at each dose appeared to fit a 3-compartment model, several of the kinetic parameters exhibited dose-dependent changes. Specifically, α, β, and λ decreased with increasing chloroform dose. B and C increased with increasing dose. In addition, the volume of distribution increased with increasing dose. The U.S. EPA (1985a) pointed out that the route of administration, intravenous in this case, influences these parameters. A bolus iv dose of volatile compound such as chloroform would be rapidly excreted via the lung, and, therefore, pulmonary elimination is occurring simultaneously with distribution and metabolism. In contrast, after oral administration, the distribution phase is more readily separated from the elimination phase. This complicates interpretation of pharmacokinetic information, and use of such information in extrapolating across routes is not realistic. Withey and Collins (1980) also looked at elimination of chloroform from major organs following iv administration of 15 mg/kg. The rates of elimination from all tissues except fat were not much different than those from blood. The investigators suggest that most of the major organs together with the blood constitute the central pharmacokinetic compartment. This is probably why most of the data presented by Withey et al. (1980) apparently fit a two compartment model as well as a three-compartment model.

Table 2-A

Pharmacokinetic Parameters for Chloroform after Oral Administration to Rats. From: Reynolds et al. (1984a, 1984b)

Parameter	Chloroform Dose 12 mg/kg	36 mg/kg
% Eliminated as $CHCl_3$[a]	5	12
Time of Peak Elimination[b] of $CHCl_3$	0-15	0-30
Peak Rate of Elimination of $CHCl_3$ (μmoles/h-kg)	6.0	15.2
% Elimination as CO_2[a]	67	68
Time of Peak Elimination[b] of CO_2	30-45	60-105
Peak Rate of Elimination of CO_2 (μmoles/h-kg)	37	46
Apparent $t_{1/2}$ (hours) of :		
$CHCl_3$ Absorption	0.08	0.13
$CHCl_3$ Distribution	0.29	0.41
$CHCl_3$ Elimination	3.8	2.3
CO_2 Elimination	2.1	5.6

a In 24 Hours
b Minutes

Table 2-B

Pharmacokinetic Parameters for Chloroform
after i.v. Administration to Rats.
Based on the Three-Compartment Model
(mean ± standard error)
From: Withey and Collins (1980)

Dose ug/kg	(N)[a]	V_d[b] ml	A ug/ml	α min^{-1}	B ug/ml	β min^{-1}	C ug/ml	γ min^{-1}	k_{12} min^{-1}	k_{21} min^{-1}	k_{13} min^{-1}	k_{31} min^{-1}
3.0	(2)	45.70 ±0.04	21.43 ±0.11	0.72 ±0.11	5.01 ±0.52	0.135 ±0.001	1.189 ±0.39	0.0287 ±0.0064	0.2346 ±0.0651	0.2575 ±0.0316	0.0921 ±0.0008	0.0421 ±0.0098
6.0	(5)	53.57 ±12.61	48.52 ±10.22	0.64 ±0.13	10.42 ±1.42	0.081 ±0.010	1.834 ±0.31	0.0158 ±0.0019	0.2681 ±0.0631	0.1862 ±0.0188	0.0730 ±0.0246	0.0233 ±0.0032
9.0	(2)	64.46 ±14.26	47.23 ±9.16	0.64 ±0.084	15.52 ±1.31	0.095 ±0.0002	2.821 ±0.92	0.0189 ±0.0009	0.2529 ±0.0489	0.2421 ±0.0064	0.0594 ±0.0034	0.0281 ±0.0042
12.0	(2)	80.62 ±20.20	57.52 ±15.23	0.32 ±0.20	15.61 ±4.28	0.060 ±0.028	1.434 ±1.13	0.0074 ±0.0056	0.1071 ±0.0774	0.1192 ±0.0676	0.0395 ±0.0253	0.0106 ±0.0084
15.0	(4)	89.13 ±12.83	55.98 ±7.39	0.35 ±0.048	20.41 ±3.07	0.070 ±0.006	3.442 ±0.64	0.0134 ±0.0005	0.1104 ±0.0256	0.1523 ±0.0188	0.0396 ±0.0104	0.0193 ±0.0018

a. Number of animals per dose
b. V_d = volume of distribution calculated as D/(A + B + C), where D was the administered amount in ug.

A recent report by Raabe (1988) provides data on the pharmacokinetics of chloroform in humans following low level inhalation exposure. Raabe (1988) reported that humans exposed to 7 to 25 ppb chloroform by inhalation exhaled a significant portion of the dose as carbon dioxide. The total amount of $^{14}CO_2$, chloroform or alcohol-soluble metabolite exhaled during and 30 minutes after exposure are presented in Table 2-C. The average net body retention of radiolabel, measured 30 minutes after exposure ceased, was 28.2 ± 1.5% of total inhaled radiolabel. About 36% of the absorbed dose (16.6% of inhaled radiolabel) was excreted as $^{14}CO_2$ via the lung, while only about 3% was excreted as unchanged chloroform or alcohol-soluble metabolite within 30 minutes of the end of exposure. The elimination half-life of radioactivity derived from $^{14}CHCl_3$ from the lung and via urine were about 3.5 and 5.2 hours, respectively. These half-life estimates were based on a simple exponential clearance process described by:

$$\ln A_1 = \ln A_2 + \lambda(t_2 - t_1)$$

where A_1 is the average uptake, A_2 is the total body retention of inhaled vapor at 2.5 hours after the beginning of exposure, t_1 is the beginning of exposure, and t_2 is 2.5 hours after exposure began. The value of A_2 was obtained by subtracting measured urinary and lung excretion from measured average uptake during exposure. The clearance half-time is calculated from the clearance rate constant, λ, where $\lambda = \ln 2 / t_{1/2}$.

Some information on pharmacokinetics following higher exposure levels can be inferred from case reports and human studies. Stewart et al. (1965) report a case of an 18 year old male who was accidentally exposed via inhalation to several solvents, among them chloroform. The patients breath was analyzed for chloroform over a 96 hour period, and the results, plotted in a semilog fashion, reveal an exponential decay with three phases. The three compartments probably correspond to vessel-rich tissues with high blood flow and high diffusion rate constants, lean body mass (muscle and skin), and adipose tissue. The elimination half-life from the adipose compartment, indicated by the graph in Stewart et al., appears to be about 24 hours. Limited information in Lehman and Hasegawa (1910) points to an elimination halflife of about 20 minutes from highly perfused tissue. In this study, a human subject was exposed to about 4400 ppm chloroform in air for 30 minutes. The amount of chloroform in the expired air was monitored during exposure and for 30 minutes after exposure. The concentration in expired air decreased from an average of 1.7 mg/l in the first 10 minutes to 0.85 mg/l in the 20 to 30 minute period following exposure. Similar estimates can be made from a graph of chloroform content of arterial and venous blood in an anesthetized patient published in Smith et al. (1973). Upon cessation of exposure to chloroform (about 10,000 ppm), the concentration fell rapidly from about 7 mg/dl to 3.5 mg/dl in 30 minutes.

The pulmonary excretion of orally-administered chloroform and its carbon dioxide metabolite was studied in healthy male and female human volunteers (18-50 yr old, 60 to 80 kg body weight) (Fry et al., 1972). The percentage of administered dose excreted via the lungs as unchanged chloroform in 8 hours ranged from 18 to 67% for eight subjects following oral administration of a gelatin capsule containing 0.5 g chloroform in olive oil (Table 2-D). Maximum pulmonary excretion of $CHCl_3$ occurred 40 to 120 min after administration. The amount of unchanged chloroform excreted appeared to be inversely proportional to body fat content. Two subjects were given $^{13}CHCl_3$, 0.5 g orally in olive oil, and exhaled $^{13}CO_2$ was monitored using

TABLE 2-C

Pharmacokinetics of ^{14}C-chloroform in Humans Following Low-Level Inhalation Exposure
From: Raabe (1988)

SUBJECT (SEX)	Conc. (ppb)	ROUTE[a]	PERCENT OF INHALED RADIOLABEL: UPTAKE AT EACH EXPOSURE INTERVAL (HOUR) 0-0.5	0.5-1	1-1.5	1.5-2.0	SSU[b]	TOTAL AT 2.5 HOURS[c] EXHALED as CO_2	EXHALED as $CHCl_3$ or ASM	EXCRETED IN URINE	NET BODY RETENTION
B.L.(M)	14	M	56.0	52.1	49.7	43.6	48.5 ± 2.5	17.8	--	0.53	--
B.R.(M)	12	M	52.0	44.9	41.8	41.0	42.6 ± 1.2	16.5	--	0.25	--
M.G.(F)	11	M	51.4	50.5	51.0	45.5	49.0 ± 1.8	15.2	--	0.22	--
P.W.(F)	25	M	56.0	55.4	59.4	52.2	55.7 ± 2.1	20.1	--	0.14	--
B.L.(M)	10	N	45.9	40.9	39.6	38.2	39.6 ± 0.8	15.6	--	0.40	--
B.R.(M)	7	N	50.4	42.3	40.6	38.8	40.6 ± 1.0	14.9	--	0.60	--
M.G.(F)	10	N	54.4	53.0	47.1	45.0	48.3 ± 2.4	15.1	--	0.14	--
P.W.(F)	13	N	55.8	52.6	47.7	45.0	48.4 ± 2.2	17.5	--	0.06	--
Average:		Nose					44.2 ± 1.5[d]	16.6 ± 0.6	1.5 ± 0.12	0.29 ± 0.07	28.2 ± 1
		Mouth					48.9 ± 1.6[d]				
Corrected for dead space		Nose					45.6 ± 1.5[d]				
		Mouth					49.6 ± 1.6[d]				

a. N = Nose breathing; M = mouth breathing

b. SSU = Steady-state uptake; Total inhaled (ug): MOUTH B.L. = 68, B.R. = 52, M.G. = 40, P.W. = 81
NOSE B.L. = 51, B.R. = 28, M.G. = 34, P.W. = 50

c. For exposure time (2 hours) plus 0.5 hour clearance.

d. Based on 12 measurements made during the last 1.5 hours of exposure.

e. ASM = alcohol-soluble metabolite.

Specific activity of ^{14}C-chloroform = 11.9 mCi/mmol, >98% pure.

Table 2-D

Pulmonary Excretion of Chloroform in Man
From: Fry et al. (1972)

Subjects			Dose of chloroform (g)	Pulmonary excretion (% of dose)	
No.	Sex	Body-wt (kg)		Found (8-hr period)	Calculated (infinite time)
1	M	61.8	0.5	66.6	67.2
2	M	64.6	"	51.0	54.8
3	M	70.9	"	50.0	52.2
4	M	80.0	"	35.9	38.4
5	M	74.6	"	17.8	18.2
6	F	62.7	"	40.4	42.0
7	F	59.0	"	34.8	35.8
8	F	58.0	"	25.6	27.6
9	M	65.0	1.0	64.7	68.3
10	M	66.0	0.25	12.4 (1)	
10	M	66.0	0.1	- (2)	

(1) After 3 hr, chloroform concentrations fell below detectable limits;
(2) Chloroform was undetected in the exhaled air.

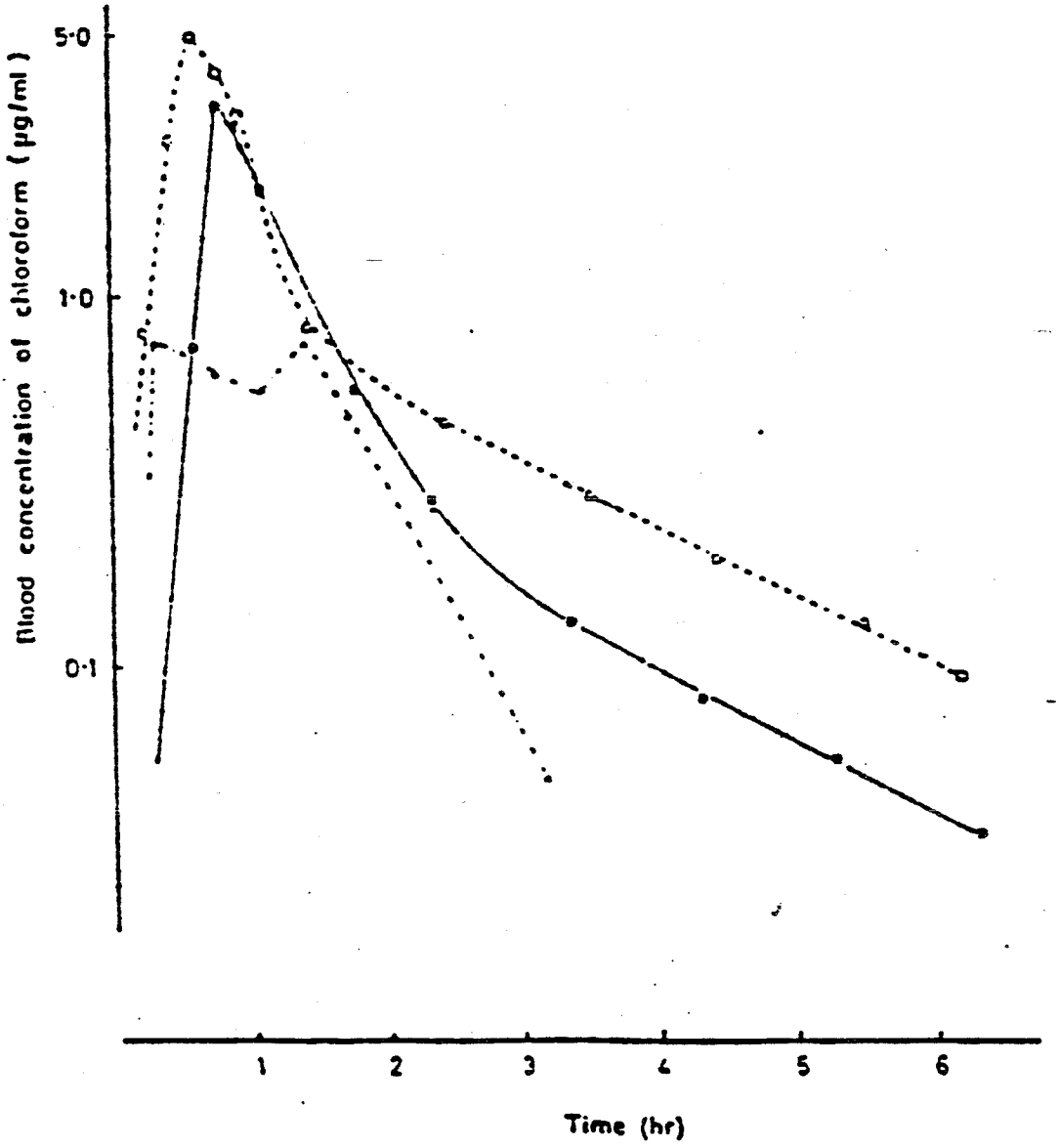
mass spectrometry. Forty-eight and 51% of the dose was exhaled as $^{13}CO_2$ over a 7.5 hour period with maximum excretion occurring 75 to 210 min after administration. In a separate experiment with the same two subjects, 67% and 40% of a chloroform dose was excreted unchanged via the lungs in an 8 hour period. No lower chlorinated methanes were found in expired air and chloroform was not found in the urine. Pulmonary excretion of chloroform and its carbon dioxide metabolite accounted substantially for a single oral dose in humans. The blood concentration - time curve for chloroform in 3 human subjects revealed a biphasic elimination profile out to 7.5 hours (Figure 2-2). The half-lives of elimination in the first rapid phase ranged from 9 to 21 minutes. The half-lives of elimination in the second slower phase ranged from 86 to 96 minutes. Chloroform was detected in the breath of some subjects for up to 24 hours after administration. Based on this information, the U.S.EPA (1985a) suggests that a third phase of elimination exists with a very long half-life, representing disappearance from adipose tissue. The variation in half-lives measured in the studies cited above reflects differences in exposure levels, in assumptions concerning the shape of the elimination curve, and in the time over which measurements were taken (Table 2-E). Low level (ppb) exposures in the Raabe (1988) study are subsaturating in terms of metabolic capacity. The higher level exposures (in Fry et al., 1972, Lehman and Hasegawa 1910, Smith et al., 1973 and Stewart et al., 1965) are probably saturating the metabolic enzymes, resulting in a backlog of unmetabolized chloroform which distributes preferentially into adipose tissue. In addition, Raabe (1988) only measured elimination during the 2 hour exposure and for 0.5 hours following cessation of exposure, while Fry et al. (1972) measured elimination for up to 8 hours thereby obtaining more information on the shape of the elimination curve.

2.3.3. Relationship Between Metabolism and Toxicity

Metabolism of chloroform by the cytochrome P-450 dependent mixed function oxidase enzyme system is central to the toxic effects observed in chloroform intoxicated animals. The potentiation and antagonism of chloroform-induced toxicity by a variety of compounds that interact with cytochrome P-450 (p-450) has been extensively investigated. The potentiation of chloroform-induced hepatotoxicity and nephrotoxicity by various alcohols and ketones is well documented (Cowlen et al., 1984; Iijima et al., 1983; Hewitt et al., 1979, 1980, 1983; Brown and Hewitt, 1984). Branchflower and Pohl (1981) investigated the potentiation of chloroform-induced hepatotoxicity by methyl-n-butyl ketone (MBK) in the rat and concluded that potentiation of chloroform-induced hepatotoxicity by MBK is the result of MBK-induced hepatic GSH depletion and increased oxidative metabolism of chloroform to phosgene. Nephrotoxicity induced by chloroform was also potentiated by MBK although P-450 and renal GSH levels were unaffected. This apparent anomaly was attributed by the investigators to possible alteration of P-450 types or localized GSH depletion not reflected in a measurement of total renal GSH levels (Branchflower and Pohl, 1981).

Jernigan and Harbison (1982), provided evidence that the potentiation of chloroform-induced hepatotoxicity by 2,5-hexanedione in mice resulted from an increase in P-450 content and amount of chloroform metabolized. The P-450 induction and potentiation of hepatotoxicity by 2,5-hexanedione was greater in the females than in the males.

Figure 2-2. Blood concentration-time curve of 3 humans following ingestion of chloroform. From: Fry et al. (1972).



Semi-log plots for the blood concentration-time relationships of chloroform in human subjects after oral administration. A gelatin capsule containing 500 mg of chloroform in 1 ml of olive oil was taken orally by each subject at zero time, and instantaneous blood levels were measured at regular times thereafter. The curve ○— — —○ represents the blood profile of subject no. 2, the broken curve △— · —△ the blood profile of subject no. 5 and the curve ■———■ the blood profile of subject no. 6.

TABLE 2-E
Estimates of Half-lives of Chloroform in Humans

Reference	[a]R	$t_{1/2}$	Reflecting:	Assumption:
Fry et al. (1972)	O	9-21 min	Rapid phase of pulmonary elimination	2 compartment model
		86-96 min	Second slower phase of pulmonary elimination	
Stewart et al. (1965)	I	24 h	Pulmonary elimination from 3rd compartment (adipose tissue)	3 compartment model
Lehman and Hasegawa (1910)	I	20-30 min	Pulmonary elimination from highly perfused tissue	Multi-compartment model
Smith et al. (1973)	I	30 min	Pulmonary elimination from highly perfused tissue	Multi-compartment model
Raabe (1988)	I	3.5 h	Pulmonary elimination	Simple exponential clearance
		5.2 h	Urinary clearance	

a. R = route of exposure, O = oral, I = inhalation

Cornish et al. (1973) reported that phenobarbital pretreatment of rats markedly potentiated the increase in SGOT in chloroform challenged animals, and increased the percentage of the liver experiencing centrilobular necrosis. Brown et al. (1974b) observed marked centrilobular necrosis and increased lethality after a 2 hour inhalation exposure to anesthetic concentrations (5000 to 10000 ppm) of chloroform in phenobarbital pretreated rats relative to controls. Gopinath and Ford (1975) reported that phenobarbitone sodium, phenylbutazone, and DDT induce hepatic P-450 activity, and potentiate the hepatotoxicity of chloroform in rats. Pretreatment with inhibitors of P-450 reduced the extent of histopathological lesions produced by administration of chloroform. Kluwe et al. (1978) reported that pretreatment of mice with various inducers of P-450 altered chloroform-induced renal damage differently than chloroform-induced hepatic damage, implying that the metabolism of chloroform in the liver and kidney may follow different pathways and may be catalyzed by different forms of P-450 in ICR mice.

Pohl et al. (1984) demonstrated a correlation between strain and sex differences in $CHCl_3$-induced nephrotoxicity and the ability of the kidney to metabolize chloroform to phosgene in mice. Kidney homogenates from male DBA/2J mice metabolized chloroform to phosgene about twice as fast as homogenates from male C57Bl/6J mice, a strain which is less sensitive to chloroform induced nephrotoxicity. Female mice are much less sensitive to the nephrotoxic effects of chloroform than male mice (Eschenbrenner and Miller, 1945a, 1945b; Klaassen and Plaa, 1967; Ilett et al., 1973). Kidney homogenates from male ICR mice produced about 9-fold greater amounts of phosgene than homogenates from female mice. Pohl et al. (1984) provided evidence that differences in the testosterone levels in plasma may be partly responsible for the observed strain and sex differential sensitivity to the nephrotoxicity of chloroform.

Branchflower et al. (1984) reported that chloroform and deuterated chloroform increased BUN, and depressed PAH and TEA accumulation by renal cortical slices. Chloroform was metabolized more rapidly, and was more effective at depressing renal GSH levels and inducing nephrotoxicity than its deuterated analogue.

Lavigne and Marchand (1974) reported that treatment of rats with phenobarbital, 3-MC, and 3,4-benzopyrene increased the hepatotoxicity of chloroform (0.5 ml/kg) increased excretion of carbon dioxide via the lung, and decreased the exhalation of unchanged chloroform. Pretreatment with SKF525A prior to chloroform administration resulted in a decrease in the amount of carbon dioxide exhaled. Kluwe and Hook (1981) showed that pretreatment of ICR male mice with piperonyl butoxide, a P-450 inhibitor, 600 mg/kg ip in corn oil 2 hours before administration of chloroform (0.25 or 0.75 ml/kg, ip in corn oil) protected the animals against hepatotoxicity and nephrotoxicity. When piperonyl butoxide was given 1 hour after the administration of chloroform, hepatotoxicity, nephrotoxicity, and lethality were potentiated, probably as a result of decreased detoxification of the toxic metabolite, phosgene. The GSH depletor diethyl maleate (0.6 mg/kg, ip in corn oil) reduced renal and hepatic GSH levels to about 19% of control levels and increased the hepatotoxicity and nephrotoxicity of chloroform. This study provides more evidence for the protective effect of GSH against chloroform-induced tissue damage.

Ahmadizadeh et al. (1984b) pretreated C57 and DBA mice with phenobarbital (PB) and noted marked increases in hepatic (but not renal) aryl hydrocarbon hydroxylase (AHH) activity and in chloroform (37 to 370 mg/kg, ip) induced hepatotoxicity (but not nephrotoxicity) in both strains, relative to nonpretreated mice. Pretreatment of mice with polybrominated biphenyls (PBB) also stimulated hepatic AHH activity and increased chloroform-induced hepatotoxicity in both strains. PBB pretreatment increased renal AHH and chloroform induced nephrotoxicity only in C57 mice and not in DBA mice.

Letteron et al. (1987) reported that methoxsalen, a potent inhibitor of P-450 mediated biotransformation, given 30 minutes prior to ip administration of chloroform, 1 ml/kg, prevented hepatotoxicity and nephrotoxicity in mice. Treatment with methoxsalen decreased P-450 content and activities in liver and kidney, and substantially decreased covalent binding in hepatic and renal microsomes. This study provides more evidence for the role of P-450 mediated biotransformation of chloroform in chloroform-induced nephrotoxicity and hepatotoxicity. McMartin et al. (1981) and Brown et al. (1974b) also showed that alterations of the P-450 content of liver and kidney resulted in alteration of chloroform-induced toxicity in those organs in rats. Masuda and Nakayama (1982, 1983) reported that supression of the bioactivation of chloroform by administration of equimolar oral doses of diethyldithiocarbamate (30 or 100 mg/kg) and carbon disulfide (10 or 30 mg/kg) decreased the hepatotoxic and nephrotoxic effects of chloroform (1 ml/kg, ip in corn oil). Lavigne et al. (1983) demonstrated that the hepatotoxicity of chloroform to male Sprague-Dawley rats varied with the time of day of administration following a circadian rhythm corresponding to the pattern of P-450 activity.

The form of P-450 involved in the metabolism of chloroform has been studied by several investigators. Enosawa and Nakazawa (1986) reported that chloroform (1.0 ml/kg, ip in corn oil) administered to phenobarbital (PB) or 3-methylcholanthrene (3-MC) treated rats resulted in a reduction of both PB and 3-MC type P-450 in the liver. Thus, based on the assumption that chloroform metabolites react with components of the P-450 enzyme complex and destroy it, both forms of P-450 are involved in the bioactivation of chloroform. This is consistent with increased hepatotoxicity observed in both 3-MC and PB treated rats relative to controls (Lavigne and Marchand, 1974). Branchflower et al. (1983) found that methyl-n-butylketone (MBK) produced qualitative changes in the composition of microsomal P-450 in rat liver and that the degree of chromatographic changes paralleled hepatotoxicity and formation of phosgene. The rate of metabolism of chloroform to phosgene was greater in microsomes from MBK treated animals than in those from PB-treated animals, when expressed as nmoles GSCOSG produced per nmole P-450.

2.3.4. Covalent Binding of Chloroform Metabolites

Chloroform metabolites bind covalently to cellular macromolecules with concomitant destruction of cellular integrity. There is considerable evidence that chloroform metabolites bind covalently to cellular protein and lipid. Covalent binding appears to be dose-related, and correlates with toxicity such that agents (e.g., GSH and cysteine) which decrease covalent binding to cellular macromolecules also protect animals against hepatotoxic and nephrotoxic effects of chloroform. There is conflicting evidence for covalent binding of chloroform metabolites to DNA or RNA. However, if

binding to nucleic acids occurs, it appears to be much less extensive than binding to proteins and lipids. Irreversible binding of chloroform metabolites to human microsomal protein and lipid has been shown, and the extent of binding was over 4-fold greater than binding to mouse and rat microsomal constituents.

Reynolds and Yee (1967) found covalently bound radiolabel in protein and lipid fractions of rat liver cells, representing 0.3 to 0.5% of the dose, 2 hours after administration of hepatotoxic doses of $^{14}CHCl_3$. Incorporation of nonvolatile ^{14}C was determined in lipid, nucleic acid, and protein fractions of liver by scintillation counting and thin layer chromatography followed by autoradiography. ^{14}C from labeled chloroform was bound primarily to phospholipids in the lipid fraction, and to methionine in hydrolyzed protein fraction. Some radiolabel was bound to nucleic acids but the specific activity was not greater than that of the homogenates, and the amount bound to nucleic acids was much smaller than that bound to protein and lipid.

Ilett et al. (1973) observed a substantial amount of covalent binding of radiolabel, determined by exhaustive extraction and counting of tissue homogenates, to liver and kidney of male C57 Black mice following ip administration of ^{14}C-chloroform (60, 297, 442, or 750 mg/kg). Binding preceeded development of demonstrable necrotic lesions. Approximately 0.5, 2, 3, and 3 nmoles ^{14}C-chloroform (metabolites) were bound per mg liver protein, 6 hours after doses of 60, 297, 442, and 750 mg/kg, respectively. These data suggest that metabolic saturation occurs somewhere between about 300 and 440 mg/kg. Covalent binding in liver occurred to a similar extent in male and female mice. However, covalent binding in kidney of female mice was an order of magnitude less than that which occurred in male kidney. In in vitro experiments, only one-third as much covalently bound radiolabel was found in renal microsomes from female mice than in those from male mice. Female mice are equally sensitive to hepatotoxic effects of chloroform, but are much less sensitive to nephrotoxic effects than males. Additionally, male mice are more sensitive to the carcinogenic effects of chloroform on kidney than females. Piperonyl butoxide, a P-450 inhibitor, reduced the severity of hepatic and renal damage and the extent of covalent binding to liver and kidney in male mice. Autoradiography and histopathological examination showed that the binding of radiolabel occurred largely in regions of hepatic centrilobular and renal tubular necrosis. Incubation of hepatic microsomes with chloroform under a nitrogen or $CO:O_2$ (8:2) atmosphere markedly reduced the extent of covalent binding, suggesting that it is a metabolite of chloroform, and not chloroform itself, which is covalently bound. However, incubation of renal microsomal preparations with chloroform under nitrogen or a CO atmosphere did not significantly affect covalent binding. Ilett et al. speculated that renal metabolism of chloroform in mice may proceed by a different pathway than that which occurs in the liver.

Smith and Hook (1984) reported that little or no metabolism of chloroform to carbon dioxide or covalent binding (determined by exhaustive extraction techniques) of chloroform-derived radiolabel occurred in renal cortical microsomes from female ICR mice. In contrast, renal cortical microsomes from male ICR mice, which are sensitive to chloroform-induced nephrotoxicity (Smith and Hook, 1984), metabolized chloroform to carbon dioxide and covalently bound metabolites. Oxygen and NADPH-dependent metabolism and

covalent binding were linear with time, and were inhibited by CO. The inability to detect metabolites of chloroform in renal microsomes from female mice is consistent with the lack of nephrotoxicity observed in vivo (Smith et al., 1983; Eschenbrenner and Miller, 1945) and in vitro (Smith and Hook, 1983), and the absence of renal tubular cell tumors in female mice (NCI, 1976; Roe et al., 1979).

Sipes et al. (1977) showed that incubation of liver microsomal preparations from PB-pretreated rats under nitrogen or in an atmosphere of $CO:O_2$ (8:2) decreased the covalent binding of chloroform-derived radiolabel to microsomal protein by about 65%. Covalently bound radiolabel was defined as that radiolabel remaining after heating and exhaustive extraction of microsomal preparations following incubation with $^{14}CHCl_3$. Addition of SKF525A (0.5 mM) or GSH (3 mM) to the incubation medium reduced covalent binding substantially, and omission of NADPH nearly abolished covalent binding. Pretreatment of rats with allylisopropylacetamide, which reduced the level of hepatic P-450 about 5-fold, resulted in a 5-fold decrease in covalent binding to liver microsomal protein in preparations from these rats relative to controls.

DiRenzo et al. (1984) observed that the extent of covalent binding (determined by exhaustive extraction) of radiolabel from $^{14}CHCl_3$ to lipid and protein of cultured primary rat hepatocytes decreased as the oxygen tension decreased. A concomitant decrease in the formation of water soluble metabolites with decreasing oxygen tension also occurred, which suggests that conjugation of chloroform metabolites decreased.

Reynolds et al. (1984b) found ^{14}C-labeled metabolite bound to liver macromolecules after oral administration of 0.1 and 0.3 mmoles ^{14}C-chloroform to male Sprague-Dawley rats. Covalently bound radiolabel was defined as that radiolabel remaining after heating precipitated lipids, protein, and nucleic acids to 80°C in 0.3 N KOH. The amount of metabolite bound was 1.1 ± 0.1 and 0.9 ± 0.1 μmoles chloroform equivalents/kg, respectively. This represents about 0.3 to 1% of the original dose. Uehleke and Werner (1975) reported that intraperitoneal administration of ^{14}C-chloroform to male NMRI mice (100 μmoles/mouse, in peanut oil; 0.2 μCi/g body weight) resulted in covalent binding (determined by exhaustive extraction and heating) of radiolabel to liver microsomal and mitochondrial protein and lipid representing 0.4% of the administered dose. This finding agrees with that of Reynolds et al (1984b). Peak binding occurred 6 hours after administration.

Under aerobic conditions, Uehleke and Werner (1975) found that radiolabel from ^{14}C-chloroform bound covalently in vitro to microsomal protein in preparations from rabbit, mouse, rat, and human liver. The amount of binding to human and rabbit liver microsomal protein was about 4-fold higher than that bound to rat and mouse liver microsomal protein.

Purified rat liver nuclear membrane preparations are capable of metabolizing chloroform in the presence of an NADPH generating system and oxygen to reactive metabolites that bind covalently to lipids and proteins (Diaz Gomez and Castro, 1980a). The extent of covalent binding was about 40% of that observed with rat liver microsomal preparations. This finding is intriguing in that reactive metabolites may be formed in close proximity to DNA and other nuclear targets. However, Diaz Gomez and Castro (1980b) found no

covalent binding of chloroform metabolites to isolated mouse or rat liver DNA or RNA by scintillation counting 6 hours following administration of 5 or 750 mg ^{14}C-chloroform/kg, either as single or multiple ip injections. In vitro binding to DNA was studied after microsomal activation of chloroform in the presence of purified mouse liver DNA. Neither DNA nor RNA were labeled to an appreciable extent above background in the in vivo or in vitro experiments. However, all fractions of nuclear proteins (acidic, histone, deoxyribonucleo-protein, and residual) were labeled.

Reitz et al. (1982) observed weak binding of radiolabel to rat and mouse liver and kidney DNA following a single oral dose of 240 mg ^{14}C-chloroform/kg (equivalent to about 10 mCi/kg body weight). DNA was isolated from liver and kidney 4 hours after exposure, enzymatically hydrolyzed, and subject to scintillation counting. About 1.5 μmole chloroform equivalents were bound per mole of DNA (detection limit = 1 μmole/mole). The levels of radioactivity were about 10 to 20 dpm over background counts in samples of DNA isolated from untreated control mice. The binding index for chloroform was about 370 times lower than that observed in previous experiments for 2-acetylaminofluorene and about 5000 times less than that for dimethylnitrosamine. No data from concurrently run positive controls were presented.

DiRenzo et al. (1982) investigated the covalent binding of a series of radiolabeled aliphatic halides in vitro to calf thymus DNA following microsomal bioactivation. ^{14}C label was found bound to DNA at a rate of 0.46 ± 0.13 nmoles/mg DNA/h after incubation of ^{14}C-chloroform with microsomes prepared from phenobarbital-pretreated rats in the presence of calf thymus DNA. Enzymatic degradation of extracted and purified DNA, followed by chromatography revealed that ^{14}C from chloroform was bound to at least 2 separated nucleosides, which were not identified in the report. The binding of chloroform metabolites to DNA provides evidence that chloroform may have initiating potential through its reactive metabolites. It is not clear why the results of DiRenzo and colleagues differ from those of Diaz Gomez and Castro (1980b) and Reitz et al. (1982). The experimental conditions were not the same in the three studies, and this may account for differences in results obtained.

Pereira and Chang (1982) and Pereira et al. (1984a) reported that radiolabel from ^{14}C-chloroform was bound to hemoglobin in vivo and in vitro. The binding of radiolabel to rat hemoglobin following oral administration exhibited a first-order relationship with dose up to 100 μmol/kg. After a single oral dose of 10 μmol/kg, binding to rat hemoglobin was maximal at 10 hours and decreased slowly with a half-life of about 2 weeks. The amount of bound chloroform ranged from 85 to 152 pmol/g hemoglobin in rats and mice. Chromatographic analysis of hemoglobin from chloroform-treated rats revealed the formation of altered amino acids in the globin portion of the molecule. The binding resulting from 10 daily doses of 0.1 or 1 μmol chloroform/kg was additive, and equaled or exceeded that amount bound after a single dose of 1 or 10 μmol/kg, respectively. Incubation of rat liver microsomes with chloroform and hemoglobin resulted in the formation of radiolabeled metabolites which bound covalently to hemoglobin (Pereira et al. 1984a). The major adduct formed was identified by gas chromatography/mass spectrometry as N-hydroxymethyl cysteine which may have formed during isolation from 2-oxothiazolidine-4-carboxylic acid (OTZC) present in the intact hemoglobin.

2.3.5. Role of Glutathione in Chloroform Metabolism and Toxicity

Glutathione (GSH) plays a major role in the metabolism of chloroform and can protect cells from injury induced by chloroform metabolites. Several studies have demonstrated that GSH decreases covalent binding of chloroform metabolites to cellular macromolecules, and that hepatic GSH levels are inversely correlated with the extent of chloroform-induced hepatotoxicity. GSH reacts with the toxic metabolite, phosgene, thereby serving to detoxify chloroform.

Brown et al. (1974b) reported that diethyl maleate (DEM) treatment reduced hepatic GSH levels of rats to 22% of controls, and increased the content of diene conjugates and liver triglycerides following chloroform challenge. In addition, phenobarbital (PB) treatment (1 mg/ml in drinking water for 10 days), prior to inhalation exposure to 0.5 or 1.0% chloroform produced a large decrease in hepatic GSH content, resulted in P-450 destruction, and increased conjugated diene and liver triglyceride levels relative to chloroform treated noninduced rats. Kluwe and Hook (1981) reported that pretreatment with DEM (0.6 mg/kg, ip) 90 minutes prior to chloroform (0.03, 0.10, or 0.30 ml/kg, ip in corn oil) reduced kidney and liver GSH content to 19% of control values, increased SGPT activity and BUN, and decreased PAH accumulation by renal cortical slices, relative to chloroform treatment alone. Docks and Krishna (1976) found that chloroform (0.1 to 1.0 ml/kg,ip in sesame oil) induced a significantly greater reduction of hepatic GSH levels, and resulted in much greater histopathology in the liver in PB-pretreated rats relative to noninduced controls. Administration of cysteine or cysteamine (150 mg/kg, ip) to PB-induced rats 30 minutes prior to and 30 minutes after chloroform challenge (0.2 ml/kg,ip) protected the liver from chloroform-induced necrosis, presumably by acting as a scavenger for reactive chloroform metabolites as GSH does.

Sipes and colleagues (1977) found that addition of GSH (3mM) to rat liver microsomal preparations in the presence of chloroform substantially reduced covalent binding of chloroform metabolites to microsomal protein.

Cresteil et al. (1979) reported that addition of cysteine (2 mM) and histidine (2 mM) to rat and human microsomal preparations in the presence of NADPH inhibited the irreversible binding of radiolabel from ^{14}C-chloroform, presumably by reacting with the electrophilic metabolites.

Stevens and Anders (1981b) investigated the effects of cysteine, DEM, and phenobarbital treatments on the hepatotoxicity of chloroform and deuterated chloroform, and covalent binding of radiolabel from ^{14}C-chloroform. Administration of DEM (0.6 ml/kg, ip) or pretreatment with phenobarbital (50 mg/kg, ip once daily for 4 days) resulted in a 2 to 3-fold increase in covalent binding of chloroform metabolites to hepatic microsomal and cytosolic proteins, relative to treatment with chloroform alone. Cysteine (1 g/kg, ip) decreased covalent binding to hepatic proteins *in vivo* in PB-pretreated chloroform challenged rats. DEM potentiated and cysteine antagonized chloroform induced hepatotoxicity, measured as increased SGPT levels. DEM treatment reduces the amount of CO in the blood of chloroform treated rats (Stevens and Anders, 1981a) by decreasing hepatic GSH levels and production of GSH conjugates of phosgene (the precursors of CO). The increased phosgene load results in increased covalent binding and hepatotoxicity.

2.3.6. Lipid Peroxidation Induced by Chloroform Metabolites

A few investigators have examined possible lipid peroxidation induced by metabolites of halogenated methanes by measuring alkane production *in vivo* in chloroform-treated rats (Sagai and Tappel, 1979; Ekstrom et al., 1986). Sagai and Tappel (1979) measured pentane in expired air 15, 30, 60, 90, and 120 minutes after ip administration of 0.90 ml chloroform/kg in mineral oil. This dose of chloroform caused a 2.7-fold increase in the pentane content of expired air relative to mineral oil treated controls. However, measurement of conjugated dienes in tissue 30 min after chloroform administration provided no evidence for diene conjugation in tissues at doses up to 2.7 ml/kg. Carbon tetrachloride and bromotrichloromethane were much more potent initiators of lipid peroxidation than chloroform. Ekstrom et al. (1986) measured lipid peroxidation as expired ethane and malondialdehyde excretion in urine in rats exposed to chloroform. Starvation or DEM treatment reduced hepatic GSH levels, and increased ethane exhalation relative to control animals following oral doses of 0.7 ml chloroform/kg. In nonstarved animals not treated with DEM, hepatic GSH levels were normal and this same dose of chloroform did not result in an increase in the ethane content of expired air. Chloroform also produced an increase in malondialdehyde excretion via the urine in rats. This increase was potentiated and the time-to-effect shortened by starving the rats 48 hours prior to chloroform administration. The investigators concluded that GSH depletion was essential to chloroform-induced lipid peroxidation *in vivo*.

3.0 Acute Toxicity of Chloroform

Acute chloroform intoxication involves effects on the central nervous system, cardiovascular system, liver, and kidney. No-observed-adverse-effect levels (NOAEL) reported for hepatotoxicity and nephrotoxicity by parenteral routes of exposure are on the order of 7 to 17 mg/kg in rodents. Minor liver damage was noted in mice after a 4 hour inhalation exposure to 100 ppm. The lowest-observed-adverse-effect level (LOAEL) for acute CNS intoxication in rats by oral administration is about 300 mg/kg. Acute LC50 values for mice following inhalation exposures range from 1200 to 7000 ppm. In contrast, mean levels of chloroform measured in ambient air in California in the years 1985-87 ranged from 0.026 to 0.36 ppb (CARB, 1987), with a highest measured concentration of 3.54 ppb.

3.1. Animal Studies

Several investigators have determined lethal doses of chloroform in experimental animals by the inhalation and oral routes. Values reported in the literature are summarized in Table 3-A. Acute oral LD50 values for rodents are on the order of 1000 mg/kg. Intraperitoneal LD50 values were reported by Klaassen and Plaa (1967, 1969) as 1.2 and 1.3 ml/kg (1800-1900 mg/kg) for mice and rats, respectively. Acute (6-7 hour) inhalation LC50 values range from 1260 to 7161 ppm (5-35 mg/l) in mice (von Oettingen et al., 1950; Gradiski et al. 1978).

In dogs, depression and narcosis followed inhalation of 15,000 ppm chloroform (von Oettingen et al., 1950). Death resulted from respiratory arrest in combination with a direct effect on the heart resulting in cardiac arrest (von Oettingen et al. 1949, 1950). Concentrations of chloroform in brain and blood were about 35 mg% at death (von Oettingen et al. 1949). Ataxia, sedation, and anesthesia were observed in mice given 500 mg/kg (Bowman et al. 1978), and in rats given doses >546 mg/kg (Chu et al. 1980) by gavage. An acute oral LOAEL in rats of 300 mg/kg for gross symptoms of CNS intoxication is estimated from the data of Kimura et al. (1971). Fink and Hatscke (1973) observed that chloroform, like other anesthetics, depresses cerebral oxygen consumption in experimental animals.

Balster and Borzelleca (1982) reported an ED50 for ataxia of 484 mg chloroform/kg (95% confidence interval, 243-965). Mice acquired a taste aversion to saccharin, demonstrated by a preference test paradigm, after a single dose of 30 mg chloroform/kg, but not 10 mg/kg.

Cardiovascular effects observed during chloroform anesthesia include changes in arterial and venous blood pressure and induction of cardiac arrythmias (von Oettingen et al. 1949). Studies in rodents have demonstrated that chloroform sensitizes the heart to the action of catecholamines (Dutta at al. 1968). Taylor et al. (1976) provide evidence for depressed myocardial contractility and negative inotropic effects following short-term exposure to 5% chloroform (50,000 ppm) in rabbits.

The acute hepatotoxic effects of chloroform are well-documented. Centrilobular liver necrosis was observed in dogs after inhalation exposure to anesthetic levels of chloroform (Davis and Whipple, 1919; Whipple, 1912; Graham, 1915) and intraperitoneal injections of approximately lethal doses (Klaassen and Plaa, 1967). Gross pathologic changes in the livers of rats

Table 3-A

LD50 Values for Chloroform

Species/Strain	Sex	Age	R/V[a]	N[b]	D[c]	Value (95% CL)[d]	Method[e]	Reference
mice	NS[f]	NS	ip/c	1	5	1.2 ml/kg	Brownlee	Klaassen and Plaa, 1967
rats, Sprague-Dawley	M	Adult	ip/c	5-10	4-5	1.3 ml/kg (1.1-1.4)	L-W	Klaassen and Plaa, 1969
mice	NS	NS	O/NS	130[g]	NS	1750 mg/kg (1550-2000)	L-W	Miklashevskii et al, 1966
rat, Sprague-Dawley	M	newborn	O/U	6-12	NS	0.3 ml/kg (0.2-0.5)	L-W	Kimura et al, 1971
		young adult	O/U	6-12	NS	0.9 ml/kg (0.8-1.1)	L-W	
		old adult	O/U	6	NS	0.8 ml/kg (0.7-0.9)	L-W	
rat	M	adult	O/U	4	6	2.0 ml/kg (1.0-3.8)	Weil	Torkelson et al, 1976
mice, ICR Swiss	M	adult	O/E	10	7	1120 mg/kg (789-1590)	L-W	Bowman et al, 1978
	F	adult	O/E	10	7	1400 mg/kg (1120-1680)	L-W	
rat, Sprague-Dawley	M	adult	O/C	10	5	908 mg/kg (750-1082)	probit	Chu et al, 1980
	F	adult	O/C	10	5	1117 mg/kg (843-1514)		
mice	NS	NS	I	20	NS	5761 ppm (28.2 mg/l)		von Oettingen et al, 1950
mice	F	adult	I	20	NS	1260 ppm (1229-1292)	Bliss	Gradiski et al, 1978

Footnotes:
a. R = Route, V = Vehicle, ip = intraperitoneal, O = oral, I = inhalation, C = corn oil, NS not specified, U = undiluted, E = emulphor: ethanol: water, 8 : 2 : 1
b. N = # animals per dose group
c. D = # doses
d. CL = confidence limits
e. L-W is Litchfield - Wilcoxon
f. NS = not specified
g. 130 was total number of mice used

Purity of the chloroform used was specified as analytical grade by Kimura and as 99.3% pure by Torkelson. Otherwise no comment was made on the purity of the chloroform.

and mice given lethal doses include congestion, enlargement, fatty infiltration, and centrilobular necrosis (Chu et al. 1980; Bowman et al. 1978). Intragastric intubation of mice with chloroform produced mimimal midzonal fatty changes of the liver at 35 mg/kg, massive fatty infiltration of the total liver lobule at 140 mg/kg and severe centrilobular necrosis at 350 mg/kg (Jones et al. 1958). Subcutaneous injections of chloroform in peanut oil at doses ranging from 38-535 mg/kg produced hepatic damage manifested as prolonged pentobarbital sleep time and pathologic lesions in mice 24 hours after administration (Plaa et al. 1958). A dose of 8 mg/kg did not result in hepatotoxicity. White female mice exposed to 100, 200, 400, or 800 ppm chloroform for 4 hours in an inhalation chamber exhibited dose-dependent liver lesions, ranging from fatty infiltration to frank necrosis. Serum ornithine carbamoyl transferase (SOCT) activity was greater than control levels 24 hours after exposure of mice to 200 ppm or higher concentrations of chloroform. Brondeau et al. (1983) found no change relative to control levels of serum glutamic oxaloacetic transaminase (SGOT), glutamic pyruvic transaminase (SGPT), glutamic dehydrogenase (SGLDH), or sorbitol dehydrogenase (SSDH) in rats exposed for 4 hours to 137 ppm. Increased levels of SGLDH and SSDH were measured 24 hours after a 4 hour exposure to 292 ppm chloroform. SGOT was elevated after exposure to 942 or 1075 ppm chloroform, and SGPT was not elevated by exposures up to 1075 ppm chloroform for 4 hours. It is interesting to note that measurements of SGPT and SGOT are less sensitive indicators of hepatic damage than either SLDH or SSDH but are used much more frequently than the other two enzymes. It is apparent from the studies by Plaa et al. (1958) and Kylin et al. (1963) that chloroform is more hepatotoxic than many other halogenated hydrocarbon solvents.

Many chemicals act to increase the hepatotoxicity of chloroform. Included among these are compounds which increase the rate of metabolism of chloroform (Brown et al. 1974b; Hewitt et al. 1980, 1986; Danni et al. 1981). This phenomenon was discussed in the section on metabolism. Species differences exist in the potentiation of chloroform toxicity by cytochrome P-450 monooxygenase inducers (Ebel et al. 1987).

Chloroform is also nephrotoxic in experimental animals. Kidneys of mice and rats given lethal doses of chloroform by gavage were congested, pale, and enlarged at autopsy (Chu et al. 1980;Bowman et al. 1978). Eschenbrenner and Miller (1945a, 1945b), and Hewitt (1956) reported that extensive necrosis of the convoluted tubules of the kidney occurred in male but not in female mice following oral or inhalation exposure (246 ppm for 2 h) to chloroform. Female mice did not have kidney damage even after inhalation exposure to anesthetic concentrations (not specified, but presumably about 5000 ppm) of chloroform (Hewitt, 1956). Ilett et al. (1973) demonstrated that chloroform metabolites were bound covalently to protein of liver and kidney in male animals suffering chloroform-induced hepatotoxicity and nephrotoxicity. In female mice which did not experience nephrotoxic effects, covalent binding to kidney protein was minimal. Nephrotoxic effects of chloroform have also been observed in dogs. The ED50 for reduction of phenolsulfonphthalein excretion in dogs following a single intraperitoneal dose of chloroform was 640 mg/kg (Klaassen and Plaa, 1967). At doses near this ED50, slight changes such as mild dilatation of the collecting ducts were seen in some kidneys. At nearly lethal doses, slight calcification of the tubules was observed. The mouse may be much more sensitive to the nephrotoxic action of chloroform than other experimental animals (Klaassen and Plaa, 1967). There

are also strain differences in susceptibility within the species. Hill (1977) reported an oral LD50 of 119 mg/kg in male DBA/2J mice while the LD50 for C57BL/6J mice was 490 mg/kg.

A decrease in the ability to concentrate organic anions was observed in renal cortical slices from rats pretreated with 0.25 to 1.65 ml chloroform/kg (370-2450 mg/kg) ip, sc, or po (Watrous and Plaa, 1972). Chloroform was more potent in the mouse than in the rat, inducing a greater decrease in organic ion accumulation at a given intraperitoneally administered dose. The minimum subcutaneous dose of chloroform necessary to produce a decrease in organic ion accumulation by mouse renal cortical slices was 7.4 mg/kg. A maximal response was seen at a dose of 37 mg/kg.

Moore et al. (1982) showed that the vehicle may have an effect on the hepatotoxicity and nephrotoxicity of chloroform. No toxic effects were observed in mice following oral administration of 17.3 mg chloroform/kg in corn oil or 18.2 mg/kg in toothpaste. Chloroform given in corn oil at 65.6 mg/kg resulted in increased kidney weight and ^{3}H-thymidine uptake in the kidneys. When administered in toothpaste a dose of 59.2 mg/kg did not induce either liver or kidney damage, as estimated by organ weight, plasma urea, SGPT, and SGOT levels, ^{3}H-thymidine uptake or histological abnormalities. The highest dose of chloroform in either vehicle (273 mg/kg in corn oil, 199 mg/kg in toothpaste), produced elevated plasma urea and ^{3}H-thymidine uptake, and kidney necrosis. Liver damage was also evident as increased SGPT, elevated ^{3}H-thymidine uptake by the liver, and centrilobular enlargement relative to untreated mice.

Torkelson et al. (1976) applied chloroform under an impermeable plastic cuff to the bellies of rabbits at doses of 1, 2, or 4 g/kg for 24 hours. Two weeks after exposure, necropsy revealed degenerative changes in the kidney tubules which increased in intensity with dosage level. The livers were not grossly affected.

Other organ systems may have acute toxic reponses to chloroform. Yannai (1983) observed adrenal hypertrophy in rats one day after oral administration of a single dose of 1500 mg chloroform/kg. Plasma corticosterone levels were also elevated by chloroform treatment. The specificity of this response is questionable in light of the large number of stressors which may activate the hypothalamic-pituitary-adrenocortical axis (Selye, 1951).

Chloroform applied to rabbit skin at levels of 1, 2, or 4 g/kg for 24 hours produced hyperemia, erythema, edema, exfoliation, extensive necrosis of the skin and weight loss after one to four treatments (Torkelson et al. 1976; Duprat et al. 1976). Torkelson et al. found that chloroform caused irritation of the conjunctiva when instilled in the rabbit eye. Corneal injury was noted upon staining with fluorescein. Duprat et al. (1976) found that, of 11 compounds studied, chloroform was the most potent eye irritant, producing purulent conjunctivitis, and intense epitheliostromal necrosis.

3.2. Acute Toxicity in Humans

Information on the acute toxic effects of chloroform in humans derives from its use as an anesthetic, and from a few case reports of accidental or intentional exposure. The acute toxic effects in humans are similar to those observed in animals, and usually involve central nervous system depression at high concentrations (5000 ppm), and liver and kidney damage. Chloroform was first used as an anesthetic agent in 1847 by James Simpson at the University of Edinburgh for relief of labor pain (Payne, 1981; Waters, 1951; Winslow and Gerstner, 1978). It has fallen out of favor because of the hepatotoxicity, nephrotoxicity, and cardiovascular derangement observed during and after surgery when chloroform was used as the anesthetic agent. Concentrations used for the induction of anesthesia were 1 to 3% (10,000-30,000 ppm) followed by lower concentrations (5000 ppm) for maintenance of anesthesia. The minimum alveolar concentration (MAC) for absence of response to surgical incision in 50% of the population is about 0.5% v/v (5000 ppm) (Steward et al. 1973). Blood concentration of chloroform in humans during surgical anesthesia ranges from about 9 to 17 mg/dl (Payne 1981; Steward et al. 1973).

The cardiovascular effects of chloroform during surgical anesthesia are well-documented. Steward et al. (1973) and others (Waters, 1951) reported that 50% of patients under chloroform anesthesia experienced arrythmias and 12% experienced hypotension. Respiratory rate and depth increase during induction of anesthesia with chloroform and in light anesthesia. Bronchial smooth muscle is relaxed and bronchial secretions increase in chloroform anesthetised patients (Waters, 1951).

Gastrointestinal effects observed following chloroform anesthesia include decreased intestinal tone and motility, and nausea and vomiting during recovery (Goodman and Gilman, 1980). Chloroform anesthesia results in a decrease of urine flow, probably due to release of antidiuretic hormone and renal vasoconstriction (Goodman and Gilman, 1980).

Metabolic effects caused by chloroform anesthesia include glycogenolysis, elevated blood glucose, and metabolic acidosis (Goodman and Gilman, 1980; Smith et al. 1973). After anesthesia, an increase in polymorphonuclear leukocytes occurs, clotting time decreases and the synthesis of prothrombin by the liver is impaired (Goodman and Gilman, 1980).

Chloroform-induced hepatotoxicity following chloroform anesthesia in humans is characterized by progressive weakness, prolonged vomiting, jaundice and hemmorhage occurring 1 to 3 days after exposure (Goodman and Gilman, 1980; Wood-Smith and Stewart, 1964). Autopsy of victims revealed degeneration and centrilobular necrosis of the liver (Winslow and Gerstner, 1978; Goodman and Gilman, 1980).

Human poisonings from accidental or intentional ingestion of chloroform have also occurred (Piersol et al. 1933; Schroeder, 1965; Storms, 1973). The lethal oral dose in humans is estimated to be about 30 ml in an adult (approximately 640 mg/kg for a 70 kg person), although doses of 5 to 7 ml have caused serious poisoning and death, and one individual survived a dose of 180 ml (Schroeder, 1965; Winslow and Gerstner, 1978). Signs and symptoms of poisoning following ingestion are similar to that following inhalation except that ingestion of chloroform results in severe irritation of the

mucosa lining the mouth, esophagus, and stomach. In addition, kidney damage, manifested as oliguria, anuria, elevated BUN, and histopathologic damage in the convoluted tubules, was observed in some cases after ingestion of large amounts (113 to 170 ml) of chloroform (Piersol et al. 1933; Schroeder, 1965). Acute chloroform intoxication following intravenous injection (Timms and Moser, 1975) resulted in a chemical pneumonitis characterized by a hazy infiltrate in the chest roentgenogram, low measured vital capacity, deranged arterial blood gases, and the presence of tachypnea and rales. Evidence of hemolysis observed in this patient included anemia, elevated serum bilirubin levels, and free hemoglobin in the urine.

Chloroform causes a burning sensation, erythema, hyperemia, and vesication when applied directly to human skin. When splashed into the eye, chloroform causes burning, pain, and redness of the conjunctiva (Winslow and Gerstner, 1978).

TABLE 3-B
Acute NOAELS and LOAELS[a] for Chloroform in Experimental Animals

SPECIES	ROUTE[b]	NOAEL	LOAEL	ENDPOINT	REFERENCE
rat	oral (u)	--	300 mg/kg	CNS intoxication	Kimura et al., 1971
mice	oral (ol)	--	35 mg/kg	hepatotoxicity	Jones et al., 1958
mice	sc (pn)	8 mg/kg	--	hepatotoxicity	Plaa et al., 1958
rat	inhalation	137 ppm (4h)	--	hepatotoxicity	Brondeau, et al., 1983
mice	sc (co)	--	7.4 mg/kg	nephrotoxicity	Watrous and Plaa, 1972
rat					
mice	oral (co)	17.3 mg/kg	--	nephrotoxicity and hepatotoxicity	Moore et al., 1982

a NOAEL = no-observed-adverse-effect level; LOAEL = lowest-observed-adverse-effect level.

b Vehicle is in parenthesis: u = undiluted; ol = olive oil; pn = peanut oil; co = corn oil; sc = subcutaneous injection

4.0 Subchronic and Chronic Toxicity

4.1 Introduction

Subchronic and chronic toxicity of chloroform in experimental animals generally involves the liver and kidney. Pathological changes including frank necrosis have been observed in both organs following chronic chloroform exposure by inhalation or ingestion. Other reported effects include pathological changes in the lung and blood dyscrasias. A subchronic oral no-adverse-effect level (NOAEL) of 31 mg/kg-d for neurotoxicity in mice was reported by Balster and Borzelleca (1982). A subchronic (28-day) oral NOAEL of 50 ppm chloroform in drinking water (approximately 1.3 mg/rat-day) was reported in rats by Chu et al. (1982a). However, Chu et al. (1982b) found that exposure to 5 to 500 ppm chloroform in the drinking water for 90 days resulted in histopathology of the liver in rats. The significance of this result is difficult to assess in terms of a NOAEL or LOAEL as the controls had a similar number of lesions, and the authors state that the severity of lesions increased with dose. Torkelson et al. (1976) reported an inhalation NOAEL in rats of 25 ppm 4 h/d, 5 d/w, for 6 months. Longer daily exposures (7 h/d) to 25 ppm, 5 d/w, for 6 months resulted in pathological changes in liver and kidney of rats, rabbits, dogs, and guinea pigs. A chronic oral NOAEL of 0.4 mg/kg-d, administered for at least 5 months, was reported by Miklashevskii et al. (1966) for rats and guinea pigs. In this study, guinea pigs were more sensitive than rats to higher doses of chloroform (see Table 4-A). Heywood et al. (1979) found minor serum enzyme elevations in dogs given 15 mg chloroform/kg-d for 7.5 years.

In humans, exposure to 2 to 205 ppm chloroform in an occupational setting produced hepatomegaly, splenomegaly, and increased susceptibility to viral hepatitis (Bomski et al, 1967).

In contrast to the experimental and occupational exposure levels cited above, means of measured levels in ambient California air ranged from 0.026 to 0.36 ppb chloroform, with maximum measured concentrations up to 3.54 ppb (CARB, 1987). DHS staff do not expect noncancer health effects to result from exposure to ambient levels of chloroform in California air.

4.2. Animal Studies

4.2.1. Oral Exposure

The subchronic and chronic toxicity of chloroform has been studied in rodents and dogs (Table 4-A). Eschenbrenner and Miller (1945a) administered chloroform to mice in a corn oil gavage at doses ranging from 15 to 250 mg/kg at intervals of 4 days for a total of 30 doses. Twenty-four hours after the last dose, extensive liver necrosis was observed in both males and females with the 3 larger doses, whereas no necrosis was noted at the two lower doses. Thirty days after the last dose, the livers of mice that had received necrotizing doses of chloroform were moderately cirrhotic. A striking sex difference was noted in the incidence of renal necrosis observed 24 hours after dosing. Renal necrosis was present in all chloroform-treated male mice but completely absent in the females.
Roe et al. (1979), Palmer et al. (1979), and Heywood et al. (1979) conducted a safety evaluation of toothpaste containing chloroform in mice, rats, and beagle dogs. Chloroform was administered to mice by gavage in a toothpaste

Summary of Subchronic and Chronic Toxicity Studies in Animals

Species	Sex[1]	N[2]	Route[3]	Dose[4] (mg/kg)	Observations and Comments	Reference
mice (Strain A)	M/F	5	O (OL)	30 doses at 4d intervals a. olive oil b. 15 c. 30 e. 120 d. 70 f. 250	Renal necrosis in males in b-f; hepatic necrosis in both sexes and mild cirrhosis in d-f.	Eschenbrenner and Miller, 1945a
mice (ICI)	M/F	104C 52T	O (TP)	6d/w for 80w a. 0 b. 17 c. 60	Intercurrent respiratory disease and moderate-severe fatty degeneration of liver in all groups, more prevalent in c. Increased incidence of kidney tumors in c.	Roe et al., 1979, Expt. I
mice (ICI)	M	260C 52T	O (TP)	6d/w for 80w a. 0 b. 60	Retardation of weight gain in b; increased incidence of kidney tumors in b; respiratory, kidney and liver disease in a and b.	Roe et al., 1979, Expt. II
mice (ICI, C57BL, CBA, CF/1)	M	52-100C 52T	O (TP)	6d/w for 80w a. 0 b. 60	Respiratory and urinary tract infections in all groups; moderate to severe renal pathology in b (CBA and CF/1 mice); increased kidney tumor incidence in b.	Roe et al., 1979, Expt. III
ICI only	M	52C 52T	O (AO)	6d/w for 80w a. 0 b. 60	As above only renal disease worse when chloroform was given in AO than TP; increased kidney tumor incidence in b.	

Table 4-A continued

Species	Sex[1]	N[2]	Route[3]	Dose[4] (mg/kg)	Observations and Comments	Reference
rat (Sprague-Dawley)	M/F	50	O (TP)	6d/w for 80w a. 0 b. 60	Reduced plasma cholinesterase in group b females; severe respiratory and renal disease in control and treated animals; retardation of weight gain in both sexes in b.	Palmer et al, 1979
dogs (Beagle)	M/F	16C 8T	O (TP)	6d/w for 7.5 yrs. a. 0 b. 15 c. 30	Elevated SGPT in b and c; fatty cysts in b and c.	Heywood et al., 1979
Guinea pigs	M	6	O	Daily for 5 months a. 0 b. 0.4 C. 30	No effect in a or b. Deranged blood chemistry, impairment of conditioned reflexes, histopathologic changes in liver, myocardium, and stomach wall, death by 2-3 months in c.	Miklashevskii et al., 1966
rats	M	6	O	Daily for 5 months a. 0 b. 0.4 c. 125	No effect in a or b. Impairment of conditioned reflexes, inhibited phagocytic activity of leukocytes, histopathologic changes in liver, myocardium, and stomach wall in c.	
mice (CD-1)	M	16C 10T	O (CO)	7d/w for 14d a. 0 b. 37 c. 74 d. 148	Dose-dependent decrease in PAH accumulation by renal cortical slices, increase in BUN and SGPT, and histopathologic changes in liver and kidney.	Condie et al., 1983

Table 4-A continued

Species	Sex[1]	N[2]	Route[3]	Dose[4] (mg/kg)	Observations and Comments	Reference
mice (ICI)	M/F	6-8	O (DW)	Daily for 90 d a. 0 b. 3.1 c. 31.1 Daily for 30 d d. 100 Daily for 60 d e. 100 f. 400	No effects on several behavioral measures in a, b, or c. Impairment of operant behavior in e and f, with development of partial tolerance.	Balster and Borzelleca, 1982
mice (CD-1)	M/F	7-12	O (EM)	Daily for 14 or 90 d a. 0 b. 50 c. 125 d. 250	14 day study: Depressed body weight in group d males; Increased liver weight in b, c, and d females and d males; Increased SGPT in d males and females, increased SGOT in d females. Dose-dependent decreased number of AFC per million spleen cells in both sexes. Depressed blood glucose in c and d females. 90 day study: Increased liver weight in b, c, and d females and d males; Increased blood glucose in d females and males; Decreased microsomal protein in d males and females and decreased cyt P450 activity and prolonged hexobarbital sleeptime in females; Depressed humoral immunity in d females. Histopathologic changes in liver and kidney in both sexes in b, c, and d.	Munson et al., 1982

Species	Sex	N	Route	Dose (mg/kg)	Observations and Comments	Reference
rat (Sprague-Dawley)	M	10	O (DW)	Daily for 28 d a. 0 b. 5 ppm c. 50 ppm d. 500 ppm	No alteration in serum biochemical profiles and hepatic microsomal enzyme activities. No pathological changes observed. Decreased blood neutrophil content in d.	Chu et al. 1982a
rat (Sprague-Dawley)	M/F	20	O (DW)	Daily for 90 d a. 0 b. 5 ppm c. 50 ppm d. 500 ppm e 2500 ppm	Depressed weight gain decrease in water and food consumption in e, both sexes; Depressed lymphocyte count in d males; Histopathologic changes in liver and thyroid in a-e (more severe in d and e). Severity rating significant for thyroid in e.	Chu et al., 1982b
rat rabbit guinea pig	M/F	12rt 2-3rb 8-12gp	I	7h/d, 5d/w for 6 mo a. air b. 25 ppm c. 50 ppm d. 85 ppm	Pathological changes in liver and kidney in both sexes of rt and rb in d, in both sexes of rt in c, and in male rt, both sexes of gp and rb in b; Pneumonitis in female rb in b, and in male rt, female gp, and male rb in d.	Torkelson et al., 1976
dog	M/F	1	I	7h/d, 5d/w for 6 mo a. air b. 25 ppm	No effects noted in male dog; Pathological changes in kidney of treated female.	Torkelson et al., 1976

Species	Sex[1]	N[2]	Route[3]	Dose[4] (mg/kg)	Observations and Comments	Reference
rat	M	10	I	1,2 or 4h/d for 6 mo a. air b. 25 ppm	No adverse effects.	Torkelson et al., 1976

1. M=Males, F=Females
2. N=number of animals/dose/sex, C=control group, T=treated groups, rt=rat, rb=rabbit, gp=guinea pig;
3. Route of administration: O=o ral, ip=intraperitoneal, I=inhalation, sc=subcutaneous;
   Vehicle in parentheses: OL=olive oil, CO=corn oil, TP=toothpaste, AO=arachis oil, DW=drinking water, EM=10% Emulphor in water;
4. Dose is in mg/kg unless otherwise noted.

Other abbreviations used: d=days; w=weeks; AFC=antibody-forming cells; GSH=glutathione

base or in arachis oil, at doses of 17 or 60 mg/kg/day, 6 days/week, for 80 weeks (Roe et al. 1979). Treatment with 60 mg chloroform /kg was associated with significantly higher incidence of moderate to severe kidney lesions in the CBA and AF/1 males relative to controls. No evidence of treatment-related liver damage was noted in this study. Daily doses of 150 mg/kg/d for 13 weeks resulted in death in 8/10 males and marked retardation of weight gain in the females. There were no deaths in mice given 60 mg/kg/d by gavage for 13 weeks, but moderate retardation of weight gain was observed in both sexes. Palmer et al. (1979) reported increased liver weight, fatty degeneration and necrosis of the liver, gonadal atrophy, and increased cellular proliferation of the bone marrow in rats given chloroform in a toothpaste base at doses of 150 or 410 mg/kg-d by gavage daily for 13 weeks. Doses of 60 mg chloroform/kg/day given 6 d/w for 80 weeks by gavage in a toothpaste base reduced plasma cholinesterase in female rats but not males. No other toxic effects attributable to chloroform were noted. This study, however, was complicated by severe non-neoplastic respiratory and renal disease in the control and chloroform-treated rats.

Chloroform was given to 1 or 2 dogs of each sex in doses ranging from 30 to 120 mg/kg/day for 12 to 18 weeks (Heywood et al. 1979). Vomiting, jaundice, and general malaise were noted occasionally, and marked body weight loss occurred in dogs given doses of chloroform $\geq$ 60 mg/kg/day. Hepatocyte enlargement and vacuolization, fatty deposition in the liver, increased liver weight, and increased SGPT, SGOT, plasma bilirubin, and serum alkaline phosphatase were observed in dogs given doses of 45 mg chloroform/kg/day or greater. Heywood et al. (1979) gave beagles gelatin capsules containing chloroform (0, 15, or 30 mg/kg/day) in a toothpaste base 6 days/week for 7.5 years. Cessation of dosing was followed by a 20-24 week recovery period. The only statistically significant treatment-related adverse effect was elevated SGPT levels. During the post-treatment recovery phase, SGPT levels tended to revert toward normal. Other biochemical measurements and macroscopic and histological examination did not reveal any other adverse effects from treatment with chloroform in toothpaste at 15 or 30 mg/kg/day.

Miklashevskii et al. (1966) gave groups of 6 male guinea pigs or rats 0, 0.4, 35 (guinea pigs), or 125 (rats) mg chloroform /kg-day, p.o., for several months (regimen not described). Only 2 guinea pigs in the high dose group survived longer than 3 months. Changes observed in the high dose group of guinea pigs include increased blood globulin levels, decreased albumin levels, depressed blood catalase activity, and a decline in the phagocytic activity of leukocytes in treated animals. Histological lesions observed in high dose guinea pigs and rats include fatty infiltration, necrosis, and cirrhosis of the liver parenchyma, lipoid degeneration and proliferation of interstitial cells in the myocardium, and acute edema of the submucosal and muscular layers of the stomach. Behavioral toxicity was also observed in conditioned reflex testing paradigms. The long term administration of 0.4 mg chloroform/kg-d was not associated with any toxic effects in rats in this study, and the only effect on guinea pigs was an increase in the vitamin C content of the adrenals. The investigators stated that 0.4 mg chloroform/kg-d can be regarded as a threshold dose for prolonged oral administration.

Condie et al. (1983) administered technical grade chloroform to mice by gavage daily for 2 weeks at doses up to 148 mg/kg/day. There was a slight but significant body weight loss in the high dose group. Active uptake of PAH by renal cortical slices isolated from treated animals was inhibited in a dose-dependent fashion. BUN and SGPT levels were significantly elevated in the high dose group. Dose-dependent microscopic changes in the kidney included renal intratubular mineralization, epithelial hyperplasia and cytomegaly. Chloroform treatment was associated with liver lesions, particularly in the high dose group, which included centrilobular cytoplasmic pallor, focal inflammation and mitotic figures (indicative of marked cellular proliferation).

Balster and Borzelleca (1982) evaluated behavioral effects of chloroform administered to mice in drinking water. No effects of chloroform (3.1 or 31.1 mg/kg/day) treatment for 90 days were observed in the cling test, screen test, or hole-board test. Treatment with 100 mg chloroform/kg/day for 30 days had no effect on passive-avoidance learning. Effects on operant behavior were observed during a 60 day regimen of 100 or 400 mg chloroform/kg/day. Greatest effects were observed early in the dosing regimen in both dose groups, with partial tolerance developing during the course of treatment. Balster and Borzelleca (1982) concluded that there was no evidence for progressive neurotoxicity of chloroform at doses of 3.1 and 31.1 mg/kg/day.

Munson et al. (1982) gave chloroform by gavage to mice at doses ranging from 50 to 250 mg chloroform/kg-day for 14 or 90 days. Increased liver weight was noted in female mice in all dose groups relative to controls, and in male mice at the two highest doses after 14 days. Increased SGPT and SGOT were measured in the high dose mice. A dose-dependent decrease in the number of antibody-forming cells (AFC) per million spleen cells occurred in both sexes. Similar changes were noted in the 90-day study, although the dose-response relationship for decreased AFC was unclear. Histopathological examination revealed chronic inflammatory cells in the intertubular regions of the kidney. Hydropic degeneration of hepatocytes and focal collections of lymphocytes were noted in the liver. Aniline hydroxylase activity and microsomal protein were depressed in treated females at the two higher doses and in males at the highest dose. Glutathione levels in the liver were elevated at all treatment levels in females but not in males. Since suppression of the humoral immune system occurred in a dose-dependent fashion in the 14-day study, the investigators suggested that immunotoxicity may be an indicator of halomethane toxicity.

Chu et al. (1982a) gave rats chloroform in the drinking water at levels up to 500 ppm (w/v) for 28 days. Based on the amount of water ingested daily, the approximate doses of chloroform ranged up to 11 mg/rat/day. Growth rate and food consumption were not affected by chloroform. No treatment-related symptoms, histopathology, or biochemical changes were noted. However, mice given 500 ppm chloroform had a decreased number of neutrophils in the blood. Chu et al. (1982b) gave rats drinking water containing 0 to 2500 ppm chloroform for 90 days. Ten rats from each group were killed immediately following cessation of exposure while the remainder were retained untreated for 90 more days prior to sacrifice. Fluid intake was depressed in a dose-dependent manner. Decreased food consumption and lower weight gain were noted at the highest dose in males and females. After the 90 day recovery period, food consumption was still depressed but weight gain approached

normal. Lymphocyte counts were depressed in male rats in the 500 ppm group relative to controls. Dose-dependent changes in the liver of treated rats included fatty infiltration, a mild increase in cytoplasmic volume in the midzonal and pericentral regions, and vesiculation and hyperplasia of the biliary epithelium. After the 90 day recovery period, the histological changes were not significantly different from controls. However, it appears that this is due to an increase in liver lesions in the vehicle controls (1% emulphor) measured at the end of the recovery period, rather than improvement in the chloroform-treated groups. Histopathological changes in the thyroid gland were observed in high dose males, and consisted of a reduction in follicular size and colloid density, increased epithelial height, and occasional focal collapse of follicles.

4.2.2. Inhalation Exposure

Torkelson et al. (1976) exposed rats, rabbits, and guinea pigs 7 hours/day, 5 days/week, for 6 months to 25, 50, or 85 ppm chloroform vapor (99.3% chloroform, 0.4% ethyl alcohol, 0.3% unknown). In addition, dogs were exposed to 25 ppm chloroform 7 hours/day, 5 days/week for 6 months. Dose- and species-dependent pathological changes in the liver included mild to marked centrilobular granular degeneration, foamy vacuolization, focal areas of necrosis, and fibrosis in both sexes of all species tested. Guinea pigs were least sensitive and male rats most sensitive to chloroform hepatotoxicity. Dose-dependent cloudy swelling of renal tubular epithelium, and interstitial and tubular nephritis were observed in all species. No effects were noted in the male dog, but pathological changes occurred in the kidney of the female dog exposed to 25 ppm. Pneumonitis was observed in the high exposure groups of male rats, female guinea pigs, and male rabbits, and in the low dose group of female rabbits. Mortality was highest in the high dose male rats, and attributable to pneumonia. No effects of chloroform on hematological parameters, clinical chemistry, SGPT, SAP, or BUN were observed at any exposure level in rats and rabbits (other species not examined). Some rats exposed to 25 ppm 7 h/d were allowed to recover for 6 weeks following the 6 month exposure. These rats were histologically normal, reflecting the reversibility of the slight changes produced at this concentration. No adverse effects were noted in rats exposed for 1, 2, or 4 h/d to 25 ppm for 6 months.

4.3 Health Effects in Humans

De Salva et al. (1975) conducted 2 long-term studies in humans which evaluated the safety of chloroform-containing toothpaste and mouthwash under normal conditions of use. The subjects were patients at state hospitals for mentally retarded and physically handicapped people. A wide variety of drugs were administered to the patients throughout the study including hypnotics, haematinics, anticonvulsants, antibiotics, antihistamines, antiarrythmics, hormones, anticholinergics, tranquilizers, and stimulants. In study I, 118 subjects brushed their teeth twice daily for 5 years with 1 gram of toothpaste containing 3.4% or 0% chloroform. Serum glutamic-pyruvic transaminase (SGPT), glutamic oxaloacetic transaminase (SGOT), alkaline phosphatase (SAP), and blood urea nitrogen (BUN) were measured before exposure and periodically during exposure. No differences between control and exposed groups were noted. In study II, 57 men and women brushed their teeth with a toothpaste containing 3.4% chloroform, and rinsed with a mouthwash containing 0.425% chloroform twice daily for 1 year. A group of

54 men and women used toothpaste and mouthwash that did not contain chloroform. There were no differences in measured parameters between treated and control groups before the study and at 6, 9, and 12 months into the study. The authors concluded that chloroform in toothpaste and mouthwash is not hazardous. However, the dose to individuals in this study could not be ascertained. Furthermore, use of the toothpaste and mouthwash were carefully monitored, unlike the experience of the general population where children may be expected to swallow such products. Since many drugs can affect serum transaminase levels and BUN, use of drugs by the test population limits the usefulness of the study. It is difficult, therefore, to extrapolate the results to the general population.

Evidence of chronic intoxication by chloroform in the occupational setting was reported by Challen et al. (1958), Phoon (1983), and Bomski et al. (1967). Workers exposed to chloroform vapors during the manufacture of throat lozenges complained of fatigue, dull-wittedness, depression, gastrointestinal distress, and frequent and burning micturition (Challen et al. 1958). Ventilation of the workroom air was poor and exposure was estimated to be 77-237 ppm with episodes of >1100 ppm. Workers in this plant were observed by management to stagger about at times and behave in a giddy manner. No evidence of liver dysfunction was found based on tests of thymol turbidity, serum bilirubin, and urine urobilinogen levels in exposed individuals and controls. However, more sensitive tests may have detected liver damage. Bomski et al. (1967) reported 17 cases of hepatomegaly in a group of 68 workers exposed to chloroform in concentrations ranging from 2 to 205 ppm for 1 to 4 years. Three of the 17 individuals with hepatomegaly were judged to have toxic hepatitis on the basis of elevated serum enzymes. Ten cases of splenomegaly were found in the 68 workers. Workers exposed to chloroform were ten times more likely to contract viral hepatitis than the general population of the city in which the plant was located. The authors attributed this to toxic liver injury as a predisposing factor in the chloroform-exposed workers. However, the incidence of viral hepatitis in the workers is in itself a confounding factor in the study. Other problems with the study include a lack of description of the control cohort, and no specification of the area tested for chloroform levels in air (e.g., general workroom air, breathing zone). Phoon (1983) described outbreaks of toxic hepatitis in workers involved in the manufacture of electronics equipment in Singapore. In one plant, 13 workers from the same work.area had been diagnosed as having viral hepatitis. Upon examimation of the work area, a sweet odor and an open bottle of degreasing agent (99.5% chloroform, 0.5% ethanol) were noted. The level of chloroform in the air was above the measurable range of the detector, 400 ppm. Blood samples from 9 workers, 5 of whom were jaundiced, contained between 0.10 and 0.29 mg chloroform/100 ml. In another factory described in the report, 18 workers were diagnosed with hepatitis. All the hepatitis cases came from an area of the plant where chloroform was used from open dishes or bottles. Analyses of workroom air found between 14.4 and 50.4 ppm chloroform. Due to a lack of fever and lack of hepatitis B surface antigen in the patients, the authors attributed the jaundice to chloroform exposure rather than viral hepatitis.

Wallace (1950) described hepatitis and nephrosis in a 47 year old man who had been consuming on the average 12 ounces of the cough formula, Cheracol, daily for ten years, with occasional excursions to 20 ounces per day. Thus, the man ingested 0.8 to 1.3 g of codeine and 1.6 to 2.6 g of chloroform (about 23-37 mg/kg-d) in 3% ethanol each day. Hepatomegaly was

noted upon physical examination. The urine contained many hyaline and granular casts and a trace of albumin. Bromosulphthalein excretion was impaired. Discontinuation of the Cheracol resulted in a reversal of toxic effects and the patient was normal 6 months later.

5.0. Reproductive and Developmental Toxicity

5.1. Introduction

Chloroform is embryotoxic and fetotoxic in experimental animals when exposure occurs by the oral or inhalation routes. No evidence of teratogenicity was found in studies using the oral route of exposure. However, acaudia and imperforate anus were observed following inhalation exposure of pregnant rats to 100 ppm (488 mg/m^3) 7 h/d from days 6 through 15 of gestation by Schwetz et al. (1974). A significant increase in the incidence of cleft palate in fetuses of mice exposed to 100 ppm 7 h/d from day 8-15 of gestation was reported by Murray et al. (1979). Fetotoxicity was observed following exposure of pregnant rats to levels as low as 30 ppm, 7 h/d from days 6-15 of gestation (Schwetz et al. 1974).

Chloroform interferes with the maintenance of pregnancy in rodents, and is associated with an increase in the number of morphologically abnormal sperm in mice, and, therefore, can be considered a reproductive toxicant. Decreased incidence of pregnancy in CF-1 mice exposed to 100 ppm chloroform by inhalation for 7 h/d from days 1 through 7 or 6 through 15 of gestation, relative to controls, was reported in Murray et al. (1979). In another study, the LOAEL for interference with the maintenance of pregnancy in rats was 300 ppm in air, 7 h/d from days 6-15 of gestation (Schwetz et al., 1974). No statistically significant effects on maintenance of pregnancy were observed at 2 lower doses (30 and 100 ppm). Thus, 100 ppm was a NOAEL for reproductive effects in rats, while mice were adversely affected at this dose.

5.2. Animal Studies

Experimental studies are discussed below and summarized in Table 5-A.

Burkhalter and Balster (1979) evaluated the effects of chloroform on neurobehavioral development in albino mice using a battery of tests which clearly show ontogenetic development of behaviors in the mouse. Male and female mice were given vehicle (Emulphor:saline) or 31.1 mg chloroform/kg-day by gavage for 21 days prior to mating and during the mating period. Treatment of the dams was continued throughout gestation and lactation. The pups were also given daily oral doses starting at postnatal day 7. Pups were selected randomly for testing each day starting at postnatal day 1. Chloroform treatment was not associated consistently with retardation of neurobehavioral development. However, on days 5-8, the chloroform group scored lower than the vehicle control group in the forelimb placement response, but the difference between treated and controls was only significant on 2 of these days. The investigators concluded that this treatment regimen did not result in adverse effects on behavioral development.

Thompson et al. (1974) found no evidence of external, skeletal, or soft tissue abnormalities in rats given oral doses of chloroform ranging from 20 to 126 mg/kg-day on days 6-15 of gestation, and in rabbits given doses of 20 to 50 mg/kg-day on days 6-18 of gestation. Fetotoxicity, manifested as reduced birth weight, was evident at the highest dose in both rats and rabbits. Maternal toxicity in the high dose groups involved anorexia and depressed weight gain in both species, alopecia, rough appearance and mild

TABLE 5-A
Summary of Reproductive and Developmental Toxicity Studies of Chloroform

Species (Strain)	Sex	N	1 Route	2 Dose (mg/kg)	3 Observations	4 References
mice (albino)	M/F adults pups	 10 80	O (EM)	Daily throughout mating (M,F), and through pregnancy and lactation (F). Pups treated daily starting at postnatal day 7. a. 0 b. 31	Slight depression of body weight gain in b pups from postnatal day 7-21; No treatment-related effects in pups on righting reflex, forepaw grasp, rooting reflex, cliff drop aversion, auditory startle response, bar-holding ability, eye opening, motor performance, and passive avoidance learning. Lower score in b pups for forelimb placement, significantly lower on days 5 and 7; not significant but lower on days 6 and 8.	Burkhalter and Balster (1979)
rat (Sprague-Dawley)	F	25	O (CO)	Daily in 2 divided doses, days 6-15 of gestation. a. 0 b. 20 c. 50 d. 126	Body weight gain suppressed and mild fatty changes in liver in c and d dams; alopecia and rough coat in d dams; body weight of fetuses lower in d; no major external, skeletal, or visceral abnormalities in any group. No effect on number of implantations, corpora lutea, resorptions, or fetal viability.	Thompson et al. (1974)
rabbit (Dutch-Belted)	F	15	O (CO)	Daily on days 6-18 of gestation a. 0 b. 20 c. 35 d. 50	Some anorexia and diarrhea in all groups; Depressed body weight gain and hepato-toxicity in d dams; body weight of fetuses lower in b and d; increased incidence of incompletely ossified skull bones in fetuses of b and c. No effects on number of fetuses, implantations, corpora lutea, resorptions, or fetal viability.	Thompson et al. (1974)

TABLE 5-A Continued

Species (Strain)	Sex	N	Route	Dose (mg/kg)	Observations	References
rats (Sprague-Dawley)	F	15	O (CO)	Daily on days 6-15 of gestation a. 0 b. 100 c. 200 d. 400	Depressed maternal weight gain in b, c, and d; increased maternal liver weight in b, c, and d, and kidney weight in d; dose-dependent decrease in hemoglobin and hematocrit in b-d, elevated blood levels of inorganic phosphate in c and d; depressed sorbitol dehydrogenase in b-d dams. Depressed fetal body weight, presence of sternebral aberrations and interparietal deviations in d; no evidence of dose related visceral anomalies or other terata.	Ruddick et al. (1983)
rats (Sprague-Dawley)	F	20	I	7 h/d, days 6-15 of gestation a. air b. starved C c. 30 ppm d. 100 ppm e. 300 ppm	Dose-dependent decrease in maternal weight gain and food consumption and increase in liver weight in c-e; Decrease in live fetuses/litter and increased resorptions in e. Depressed fetal crown-rump length and body weight in b and e; altered sex ratio of pups in e; increased incidences of delayed ossification and missing or wavy ribs in c and d; increased incidence of acaudia or short tail and imperforate anus in d. increased incidence of subcutaneous edema in d.	Schwetz et al. (1974)

TABLE 5-A Continued

Species (Strain)	Sex	N	Route	Dose (mg/kg)	Observations	References
mice (CF-1)	F	34-40	I	Daily on days 1-7, 6-15, or 8-15 of gestation a. air b. 100 ppm, 1-7 c. 100 ppm 6-15 d. 100 ppm, 8-15	Maternal body weight gain significantly reduced in b and d, slightly reduced in c; decreased ability to maintain pregnancy in b and c; increased number of resorptions per litter in b; increased relative liver weight and SGPT levels in c and d. Fetal body weight and crown-rump length decreased in b and d; increased incidence of delayed ossification of skull bones in b,c, and d, and and of sternebrae in b and d; increased incidence of cleft palate in d.	Murray et al. (1979)
mice (C57B1/C3H)F1	M	9T 15C	I	4 h/d for 5d a. air b. 400 ppm c. 800 ppm	Dose-related increase in number of morphologically abnormal spermatozoa in b and c 28 days following exposure.	Land et al. (1981)
mice (CBA x BALB/c)F1	M	5	ip (CO)	Daily for 5d a. 0 b. 0.025 c. 0.05 d. 0.075 e. 0.10 f. 0.25	Death in f; no evidence of abnormal spermatozoa in a-f.	Topham (1981)

1. N = number of animals/sex/dose; C = control group, T = treated group
2. Route: O = oral, I = inhalation, ip = intraperitoneal
   Vehicle in parenthesis: EM = Emulphor: saline; CO = corn oil
3. Dose expressed in mg/kg unless noted otherwise.
4. Effects reported in treated groups are relative to vehicle treated controls (group a).

fatty changes in livers of rats, and overt hepatotoxicity in rabbits. The investigators concluded that there was no evidence for teratogenicity of chloroform in this study.

Ruddick et al. (1983) reported no teratogenic effects in rats following oral administration of chloroform at doses up to 400 mg/kg-d from day 6 to 15 of gestation. Dose-dependent maternal toxicity was found in the chloroform-treated rats (Table 5-A). Fetotoxicity was observed in pups of high dose dams. There were no histopathologic changes observed in the dams or fetuses. The negative finding in this study is in agreement with others on the teratogenicity of chloroform by the oral route.

Schwetz et al. (1974) found that inhalation exposure of pregnant rats to 300 ppm chloroform 7 h/d on days 6 - 15 of gestation was associated with a high incidence of fetal resorption ($p<0.05$, Fisher Exact Test). Only 3 of 20 females which were bred and then exposed to 300 ppm chloroform were pregnant at the end of exposure (significantly less than controls, $p<0.05$, Fisher Exact test). In 7 of the 17 nonpregnant rats, there was vascular evidence of implantation in the mesometrium. Schwetz et al. speculate that chloroform may have adverse effects at an early stage in gestation and may interfere with the process of implantation. A decrease in fetal body weight and crown-rump length (analysis of variance and Dunnett's test, $p<0.05$) was also observed in fetuses of dams exposed to 300 ppm relative to controls. There were no statistically significant changes in fertility indices at lower (30 or 100 ppm) exposure concentrations. Fetal crown-rump length was significantly less, and the incidences of delayed ossification of skull bones and wavy ribs were greater in the 30 ppm group than in controls. In the 100 ppm exposure group, the incidence of acaudia or short tail and imperforate anus (considered by Schwetz et al. as true terata), as well as the incidences of missing ribs and delayed ossification of sternebrae (evidence of retarded fetal development), and subcutaneous edema were significantly greater than controls ($p<0.05$, Fisher Exact Probability test). Anomalies of the skull and sternum, and subcutaneous edema were also observed in the 300 ppm fetuses but statistical analysis was preempted by the small number of litters (two) born to dams in this exposure group. Chloroform caused a dose-dependent decrease in maternal food consumption and body weight gain ($p<0.05$, analysis of variance and Dunnett's test). Maternal relative liver weight at termination of study was increased in the 100 ppm and 300 ppm exposure groups compared to controls ($p<0.05$, Dunnett's test); however, SGPT levels were not different in control or treated animals. With the exception of depressed fetal body weight and crown-rump length, the untoward effects seen in embryos and fetuses of dams exposed to 100 and 300 ppm chloroform were not due to anorexia in the dams, since the same degree of starvation in an unexposed control group did not produce skeletal anomalies or true terata. Similarly, restriction of food intake in these controls did not interfere with the maintenance of pregnancy and did not affect fertility. Schwetz et al. concluded that chloroform-induced adverse developmental effects observed in this study were not correlated with maternal toxicity. These results contrast with the results of the oral teratogenicity studies in which chloroform did not cause abnormalities in the fetus even at doses high enough to cause significant maternal toxicity in rats and rabbits.

Murray et al. (1979) evaluated the teratogenicity of chloroform in groups of CF-1 pregnant mice exposed to 0 or 100 ppm chloroform vapor 7 h/d from days 1 through 7, 6 through 15, or 8 through 15 of gestation. Food consumption and body weight gain were depressed in chloroform-treated dams ($p<0.05$, one-way analysis of variance). The ability of females to maintain pregnancy was significantly decreased by exposure to chloroform on days 1-7 or days 6-15, but not on days 8-15 of gestation ($p<0.05$, Fisher Exact test). Mice were more sensitive to this effect than the rats in the Schwetz et al. (1974) study. Increased maternal liver weight and SGPT levels were observed in animals exposed to 100 ppm chloroform on gestation days 6-15 or 8-15. Fetal body weight or crown-rump length were decreased by exposure to chloroform on day 1-7 or day 8-15, but not in the day 6-15 group ($p<0.05$, one-way analysis of variance). Delayed ossification of skull and sternebrae occurred more frequently in the chloroform-exposed groups than in the controls. The incidence of cleft palate was significantly higher than controls in the group of mice exposed to chloroform from day 8-15, but not in the other chloroform-exposed groups ($p<0.05$, modified Wilcoxon test). It is not clear why cleft palate occurred in the group exposed on gestation days 8 through 15, but not in the group exposed on days 6 through 15. This malformation was seen predominantly in fetuses with low body weight. Murray and colleagues suggest that the cleft palate may have resulted from an indirect rather than a direct effect of chloroform. Under conditions of maternal toxicity, it is unclear whether the chemical has direct teratogenic effects, or whether the observed effects result from imbalanced maternal homeostasis. The U.S. EPA (1986) states that current information is inadequate to assume that developmental effects at maternally toxic doses result only from maternal toxicity. When developmental effects are seen only at maternally toxic doses, it may simply mean that the fetus and adult are equally sensitive. Maternal toxicity may be reversible while effects on the offspring may be permanent. Although cleft palate occurred in association with severely growth retarded fetuses, it must be viewed as indicative of the teratogenic potential of chloroform.

Dilley et al. (1977) found no fetal malformations after exposing pregnant rats to 4100 ppm or 2 lower concentrations daily (h/d not reported) from days 7 through 14 of gestation. Chloroform caused increased fetal mortality and decreased fetal weight gain relative to controls. Borzelleca et al. (1980) reported a dose-dependent effect on survival and weight gain of pups and on viability and lactation indices following exposure of pregnant ICR mice to 0.1 to 5.0 mg chloroform/ml in drinking water (duration of exposure or other details not reported). There were no apparent dose-related dominant lethal or teratogenic effects. There was not enough detail in these two abstracts for critical review.

Land et al. (1981) exposed mice to reagent grade chloroform, 400 or 800 ppm 4 h/day for 5 consecutive days in an inhalation chamber. Chloroform exposure increased the number of abnormal sperm relative to air-exposed controls measured 28 days after exposure (Table 5-A). Abnormalities observed were the result of exposure to chloroform in the first stage of sperm development.

Topham (in de Serres and Ashby, 1981) found no evidence of sperm abnormalities five weeks after mice were given daily ip injections of chloroform in doses ranging from 0.025 to 0.25 mg/kg/d for 5 consecutive days. Some or all of the animals at the high dose died. Differences in results observed in this study and that of Land et al. (1981) may be related to dose, time of sampling after treatment, or other methodological considerations.

6.0. Genotoxicity

Results of mutagenicity testing in bacteria, fungi, and mammalian systems are largely negative. However, a few positive results are reported in the literature (see following sections and Tables 6-A through 6-D). In addition, there is evidence that chloroform is a mitotic poison.

6.1. Prokaryotic Test Systems

Of the studies discussed below, only one study reported positive results in bacteria (San Agustin and Lim-Sylianco, 1978). This study was difficult to evaluate due to a lack of procedural detail, inadequate reporting of data, and a lack of statistical analysis. Using several different bacterial mutagenicity assay systems, only one of 20 labs participating in the International Collaborative Program on Evaluation of Short-Term Tests for Carcinogens (de Serres and Ashby, 1981) reported a positive result for chloroform which could not be repeated. One other lab involved in the collaborative program reported that they could not classify chloroform as positive or negative. De Serres and Ashby (1981) concluded that chloroform is not a mutagen for Salmonella typhimurium or Escherichia coli.

Several problems can arise in testing a volatile, metabolically activated chemical, such as chloroform. Volatilization of chloroform from the test medium must be adequately controlled. Activation of chloroform to phosgene (and other reactive metabolites) by added exogenous metabolic activation systems must be sufficient to test for mutagenicity of metabolites. Finally, many of the studies did not use volatile positive controls.

6.1.1. Studies Reported in de Serres and Ashby (1981)

Chloroform was negative in most of the assays reported in the volume edited by de Serres and Ashby (1981). Ten groups of investigators indicated that chloroform was not mutagenic in the Ames assay using S. typhimurium tester strains TA1535, TA1537, TA1538, TA98, and TA100 or E. coli strains WP2(p) and WP2uvrA(p), with or without a preincubation period, and either in the presence or absence of rat liver microsomes from Arochlor 1254 pretreated rodents. Although not all investigators reported the concentration range examined, several indicated that chloroform was tested at concentrations ranging from 10 to 10,000 μg/plate.

Garner et al. (1981) reported a questionably positive result in the Ames assay in strain TA1535 when chloroform, 20 μg/plate, was tested in the absence of rat liver S9 mix. However, the investigators stated that repeated testing of chloroform gave inconsistent results, and a positive dose-response curve was not obtained. Mixed results were reported for chloroform mutagenicity in a fluctuation test with S. typhimurium strains TA98 and TA100 by Hubbard et al. (1981). However, they considered the positive results questionable and did not conclude that chloroform was mutagenic. Ichinotsubo et al. (1981) reported that chloroform was positive in the rec- assay in 2 of 5 E. coli rec- strains. A reproducible dose-response curve was obtained and the size of the zones of inhibition were at least 3 mm at one of the concentrations tested. Chloroform was weakly positive in a growth inhibition assay in DNA polymerase-deficient E. coli in the presence of S9 mix, and negative without S9 (Rosenkranz et al., 1981).

TABLE 6-A

Summary of Bacterial Mutagenicity Assays of Chloroform

Reference	Test[1]	Activation[2]	Bacterial Tester Strain		Result[3]	Comments
Uehleke et al., (1977)	Ames	± micr.	S.typhimurium	TA 1535 TA 1538	-	Only 5mM $CHCl_3$ used; survival was 90% at this dose. Bacterial suspensions were incubated under nitrogen for 60 minutes with liver microsomes from phenobarbital-treated rats and mice. Bacteria were then plated on histidine deficient medium to determine reverse mutation to histidine prototrophy.
Simmon et al., (1977)	Ames	± S9	S.typhimurium	TA 1535 TA 1537 TA 1538 TA 98 TA 100	-	The plate incorporation assay tested up to 5 mg/plate or a dose which produced a toxic response. For vapor test system, plates were incubated with chloroform vapor 7-10 h at 37°C in a sealed dessicator, covered and incubated 40 h for determination of reverse mutation to histidine prototrophy. Chloroform concentration was not reported.
Van Abbe et al., (1982)	Ames	± S9	S.typhimurium	TA 1535 TA 1537 TA 1538 TA 98 TA 100	-	Used standard plate test with concentration of 10, 100, 1,000, and 10,000 ug/plate in triplicate. S9 fraction was made from Aroclor 1254 pretreated rat and mouse liver and kidney. In vapor test systems, plates were exposed to vapor (flow rate - 32 ml per hour) for 2, 4, 6, or 8 h, sealed, and incubated 72 hours. Chloroform was negative at all concentrations tested both in the absence and presence of liver or kidney S9 fraction.

TABLE 6A, Continued.

Reference	Test[1]	Activation[2]	Bacterial Tester Strain		Result[3]	Comments
Gocke et al., (1981)	Ames	± S9	S.typhimurium	TA 1535 TA 100 TA 1538 TA 98 TA 1537	-	Five concentrations were tested up to 3,600 ug/plate, in the standard plate incorporation assay. S9 fraction was made from Aroclor treated rat liver. Plates were incubated in a dessicator to control for volatility of chloroform. Chloroform was negative in both systems with or without S9.
Kirkland et al., (1981)	Ames	± S9	E.coli	WP2 WP2uvrA-p	-	Chloroform was tested at 0.1, 1, 10, 100, 1,000, or 10,000 ug/plate. S9 fraction was made from Aroclor treated rat liver. For plate incorporation, chloroform was added to molten agar. For preincubation assay, bacterial suspensions were shaken for 20 minutes with chloroform and then added to top agar.
San Agustin and Lim-Sylianco (1978)	Ames	–	S.typhimurium	TA 1535 TA 1537 TA 98	-	Tested concentrations of 4,500 ug/plate[4] in TA 1535, 750 ug in TA 1537 and 300 ug in TA 98, dissolved in DMSO, in 25 plates/strain.
ibid.	rec assay	–	B.subtilis	H 17 (rec +) H 45 (rec -)	+ 	Concentrations of chloroform tested were not reported. Zone of inhibition was 4.0 ± 0.4 mm in rec + and 14.7 ± 0.9 in rec - strain (N = 5).
ibid.	host-mediated assay	in vivo	S.typhimurium	TA 1535 TA 1537	- +	Host was albino mouse. No procedural details reported except that they followed the method of Legator and Malling (1971).[5] Ratio of mutation frequency in treated to that in

TABLE 6A, Continued.

Reference	Test[1]	Activation[2]	Bacterial Tester Strain	Result[3]	Comments
					controls in TA 1535 was 0.12 (female mice), 0.61 (male mice). Ratio in TA 1537 was 2.30 (female mice) and 36.75 (male mice). The latter value was considered indicative of a positive response.
ibid.	spot test of mouse extracts	in vivo	S.typhimurium TA 1537	+	Urine of 10 adult male mice which had received a dose of 700 mg chloroform/kg (route not reported) was pooled and extracted with ether (presumably diethyl ether but it is not stated). Extracts were plated. No. of revertants/plate: 10 ± 2 controls, 302 ± 66 for extract I, 101 ± 20 for extract II. The positive control, 9-aminoacridine produced colonies that were "too numerous to count." Zone of inhibition for control, extract I, extract II, and positive control were 0, 29, 32, and 15 mm, respectively.
Ichinotsubo et al., in de Serres and Ashby (1981)	rec assay	± S9	E. coli rec -	+	Chloroform was tested up to 500 ug/ml. S9 was made from rat liver. Test chemicals were added to microwells and plates incubated 24 h at 37°C. Chloroform was positive without metabolic activation in 2 of the 5 strains tested (positive in E. coli strains 2921 and 5519, but not in 9239, 8471,7623, or 7689). No details were reported; eg., level of chloroform producing positive

TABLE 6A, Continued.

Reference	Test[1]	Activation[2]	Bacterial Tester Strain		Result[3]	Comments
Rosenkranz et al., in de Serres and Ashby	DNA Polymerase Deficient Growth Inhibition	± S9	E. coli	P3478 (polA1-) W3110 (polA1+)	+?[6]	response, etc. Bacteria were incubated in suspension with 250 ug/ml chloroform for 2 h at 37°C, plated on HA + T medium, and incubated for 48 h at 37°C. S9 fraction was made from Aroclor 1254 treated rat liver. Chloramphenicol (20 ug/incubation) and ethyl methanesulfonate (1 ul/incubation) were positive controls. Chloroform was weakly positive in the presence of S9 mix; survival ratio of polA1- to polA+ was 0.91. Ratio for ethyl methanesulfonate was 0.2. Chloroform was negative in the absence of S9.

1. Mutagenicity test system.

2. Metabolic activation. ± = assays were conducted with and without activation systems. Micr. = microsomes. S9 = 9000 x g postmitochondrial supernatant fraction from liver.

3. - = negative; + = positive

4. Assumes 0.1 ml of chloroform in DMSO were added per plate. This was not directly stated in the paper. However, they state that the procedure of Ames and Yanofsky (1971) was used.

5. Legator, M.S. and H.V. Malling. "Chemical Mutagens," vol. 2, Plenum Press, pp. 569-588. 1971.

6. Equivocal.

The survival index, expressed as the ratio of the survival of the polA- strain to the polA+ strain, in the presence of S9 was 0.91. In contrast, ethyl methanesulfonate showed a survival ratio of 0.2. Other carcinogens such as benzo[a]pyrene (survival ratio = 1.0) tested negative, and 2-acetylaminofluorene was only weakly positive (survival ratio = 0.89).

Chloroform was negative in several other types of bacterial mutagenicity assays reported in de Serres and Ashby (1981) including microtiter fluctuation tests with E. coli WP2uvrA and S. typhimurium TA98, TA1535, and TA1537, a reverse mutation test with E. coli WP2, a forward mutation assay to 8-azaguanine resistance in S. typhimurium strain TM677, and a lambda induction assay using E. coli strains 58-161 envA and C600. Chloroform was negative in a preferential killing assay using E. coli strain WP2, and the repair deficient strains WP67 uvrA polA, and CM871 uvrA recA lexA (Green, 1981). Green suggested that the metabolic activation of chloroform, and other mutagens which tested negative, by rat liver S9 was not sufficient in this assay. Additionally, since the preferential killing assay requires the production of lethal mutations in a large proportion of the bacterial population for a positive result, it is not a very sensitive test for genotoxicity. In all of the reports in de Serres and Ashby (1981) no detail is given on measures that were taken to prevent the evaporation of chloroform during incubation. No volatile positive controls were included. In addition, not all carcinogens tested positively in every assay. Finally, little detail is given in these reports, particularly when the results were negative which was often the case when chloroform was tested. This makes interstudy comparisons difficult.

6.1.2. Other Studies

Uehleke et al. (1977) found no evidence for mutagenicity of chloroform in the Ames test following anaerobic incubation of S. typhimurium tester strains with rat or mouse liver microsomes and an NADPH generating system. Appreciable binding of radiolabeled chloroform metabolites to liver microsomal protein and lipid was noted. Higher concentrations of chloroform should have been tested; survival at this concentration was greater than 90%. In addition, aerobic incubations were not conducted.

Simmon et al. (1977) tested chloroform by the Ames test both in suspension and in a dessicator with and without a metabolic activating system. In the suspension test system, several Salmonella tester strains were incubated with test chemical prior to determination of viability and mutation to histidine prototrophy. In the dessicator assay system, plates of bacterial tester strains were placed uncovered in a sealed dessicator containing chloroform vapor for 7 to 10 hours at 37°C. The plates were then removed, sealed, and incubated for 40 hours before counting the mutant colonies. Chloroform was not mutagenic to S. typhimurium in either the suspension or dessicator assay systems. Bromoform, bromodichloromethane, and dibromochloromethane were mutagenic in the dessicator assay in the absence of S9 mix.

Daniel et al. (1980) reported negative findings for chloroform mutagenicity in the Ames test in the presence of liver or kidney S9 from Arochlor 1254 treated CFY mice and CFLP rats, strains which have shown a carcinogenic response to chloroform. A range of known mutagens gave a positive response in their system. Concentrations of chloroform tested were not reported in

the abstract. Van Abbe et al. (1982) also reported negative results in similar experiments using Salmonella in the standard Ames test and in a vapor phase system, with or without S9 fraction. Concentrations from 10 to 10,000 μg pharmaceutical grade chloroform/plate were used in the plate incorporation assay. At the highest concentration, toxicity was evident as an incomplete bacterial lawn. There was no significant increase in numbers of revertants formed at any dose tested for any of the indicator strains. In the vapor phase system, exposure to chloroform for 6-8 hr was highly toxic to strains TA1535 and TA1538. No significant increase in revertants was observed at any exposure in the two strains. In both systems the positive controls gave the expected results. However, the positive controls in the vapor test system (ethylmethanesulphonate, 2.5%, and 2-acetylaminofluorene, 2.5 μg/plate) were not vapors and were, therefore, inappropriate.

Gocke et al. (1981) reported that chloroform was negative in the standard Ames assay. Although the protocol was not described in detail, the authors stated that at least 5 doses of each test compound were tested in all 5 Salmonella strains, usually up to 3600 μg/plate, both with and without a metabolic activating system prepared from Arochlor-pretreated rats. Test plates of chloroform, dichloromethane, and 1,1,1-trichloroethane were placed in sealed dessicators for 8 hours to control for volatility. It is not clear how efficacious this control measure was; however, both dichloromethane and 1,1,1-trichloroethane increased the frequency of revertants with and without the metabolic activating system.

Kirkland et al. (1981) found that analytical grade chloroform was not mutagenic in two strains of Escherichia coli, WP2p and WP2uvrA-p, using plate incorporation and liquid preincubation systems both with and without rat liver S-9 fractions. Bacteria were placed onto medium in the presence or absence of tryptophan to determine viability and reversion to tryptophan prototrophy, respectively. Measures taken to prevent volatilization of chloroform in the plate incorporation assay were limited to packing triplicate plates in a gas tight container for incubation. This may have been inadequate particularly since the chloroform was mixed with agar at 45°C in the plate incorporation assay. The positive controls were N-methyl-N'-nitro-N-nitrosoguanidine and 2-aminoanthracene, both nonvolatile chemicals.

San Agustin and Lim-Sylianco (1978) reported that chloroform was negative in the Ames assay in S. tryphimurium without metabolic activation, but positive in the rec assay in Bacillus subtilis. The zone of growth inhibition was over 3 times larger in the rec- strain (DNA repair deficient) of B. subtilis than in the rec+ strain. The concentration of chloroform tested was not reported. In a host-mediated assay using Salmonella strain TA1537 (frame-shift mutations), chloroform was positive when the host was a male mouse but not when the host was female. Chloroform was negative in the host-mediated assay with TA1535 (base-pair mutations). It is not clear why the mutation frequencies of TA1535 in the chloroform-treated animal were less than the control (Table 6-A). This may be symptomatic of a problem with the assay. Doses of chloroform, route of exposure, duration of exposure, time of sacrifice, and number of bacteria injected and recovered were not reported. San Agustin and Lim-Sylianco also reported that ether (diethyl, presumably; not stated) extracts of urine from chloroform-treated male mice were mutagenic in strain TA1537 using the spot test. Evaluating the results of

this paper is compromised by a lack of procedural detail, inadequate reporting of data, and a lack of statistical analysis.

6.2. Eukaryotic Test Systems

Results of genotoxicity assays of chloroform in eukaryotic systems have been largely negative. A few notable positive results were found in yeast, in mammalian test systems and in an invertebrate test system for mitotic poisons. Experimental evidence is discussed below and summarized in Tables 6-B through 6-D.

6.2.1. Tests in Fungi

Chloroform was not mutagenic in a number of genotoxicity assays using Saccharomyces cerevisiae as the test organism. As reported in de Serres and Ashby (1981), chloroform was negative in the following tests: the haploid yeast reversion assay with strain XV185-14C; a mitotic crossing over and differential growth inhibition assay with strains T1, T2, T4 and T5; mitotic gene conversion assays with strains D4, D7, and JD1; induction of mitotic aneuploidy in strain D6; and inhibition of cell growth in the repair deficient strain rad.

A positive result in a forward mutation assay in the yeast Schizosaccharomyces pombe was reported by Loprieno in de Serres and Ashby (1981) (Table 6-B). In this assay, forward mutations induced in 5 different loci were distinguished by changes in colony color. Following incubation with or without chloroform, cells were screened for white complete (indicative of forward mutation) or sectored mutant colonies. The wild type colonies are red. Chloroform did not produce an increase in the frequency of mutation, as measured by the frequency of white colonies. However, the presence of sectored mutant colonies among those grown from treated cells indicated possible treatment-related mutation; but, more experimental support is needed.

Callen et al. (1980) found that chloroform was genotoxic in Saccharomyces cerevisiae strain D7. Chloroform-exposed cells were plated on various selective media for the determination of gene conversion at the trp5 locus, mitotic recombination at the ade2 locus, or gene reversion at the ilv1 locus. Cells incubated with chloroform (purity not reported) for 1 hour showed dose-dependent decreased survival and increased frequencies of gene conversion, mitotic recombinants, and revertants. The authors considered a doubling in the number of genetically altered colonies per unit survivors as a positive genetic effect. Chloroform produced significant (by this criterion) effects at 41 and 54 mM, concentrations associated with 69 and 6% survival, respectively (Table 6-B). When the incubations proceeded for 4 hours, the genotoxic activity was weaker; the investigators attributed this to masking of genotoxic effects by the cytotoxicity of chloroform. Chloroform was inactive and less toxic when tested with yeast which had a lower level of cyt P-450 enzymes than strain D7. Callen et al. suggested that the halogenated hydrocarbons tested were metabolically activated to mutagenic compounds and that the metabolites produced the genotoxic effects observed. This yeast system may be more sensitive to halogenated hydrocarbons than the Ames assay because the metabolites are produced in close proximity to the DNA, rather than by externally added microsomes. Highly reactive metabolites may not be able to reach target bacterial DNA in

TABLE 6-B. SUMMARY OF POSITIVE RESULTS FOR GENOTOXICITY OF CHLOROFORM IN FUNGI

Reference	Test	Fungal Tester Strains	Results[1]	Comments
Loprieno, in de Serres and Ashby, 1981.	Forward mutation	Schizosaccharomyces pombe, double mutant strain P_1, genotype SPade6 60/rad 10-198/h^-	+?	Concentrations of 0, 0.04, 0.06, and 0.08 mM chloroform were tested. Growing cells were incubated with chloroform for 16 h at 32°C with or without rat liver S9, plated, and incubated for 5 days at 32°C, then for 1-2 days at 4°C. N = 10 plates/dose. Background colonies are red; white colonies are indicative of forward mutation; sectored colonies are indicative of possible treatment-related mutation. Chloroform did not induce statistically significant increased rate of forward mutation. However, the presence of sectored mutant colonies with and without S9 was considered indicative of a mutagenic response.
Callen et al., 1980.	Gene conversion, mitotic recombination, gene reversion	Saccharomyces cerevisiae, strain D7	+	Cells from log-phase cultures were incubated at 37°C for 1 or 4 h with 0, 21, 41, or 54 mM chloroform. Washed cells were incubated at 30°C on supplemented minimal medium for 2 or 5 days for determination of trp 5 convertants and ilv 1 revertants, respectively. Plates were stored at 4°C for 7 days to score mitotic recombinants. Convertants per 100,000 survivors at trp 5 locus: 1.7, 2.1, 4.6 33.1 for 0, 21, 41, and 54 mM chloroform treatment, respectively. Revertants per million survivors at ilv 1 locus:4.3, 3.5, 8.2, 60.0 for 0, 21, 41, and 54 mM chloroform treatment, respectively. Mitotic recombinants per 10,000 survivors at ade2 locus: 1.6, 1.7, 4.1, 44.8 for 0, 21, 41, and 54 mM chloroform treatment, respectively.

1. +? = equivocal response

Highly reactive metabolites may not be able to reach target bacterial DNA in the Ames procedure (Callen et al., 1980). In addition, multiple endpoints in the yeast system provide greater sensitivity than the single endpoint in the Ames test.

Chloroform was nonmutagenic in Aspergillus nidulans (Carere et al., 1985). In this assay, the haploid strain 35 was used for detection of gene mutations (methionine suppression), while the diploid P1 was used for the study of somatic segregation. Cells were exposed to chloroform in the vapor phase. Other volatile compounds, including carbon tetrachloride and benzene, were positive in this system. Details of the procedures were not reported in the abstract.

6.2.2. Mammalian Test Systems

The majority of reports on mutagenicity of chloroform in mammalian test systems is negative. However, a few positive findings are reported. It would be prudent to conclude that the evidence of mutagenicity in mammalian systems is equivocal and that more studies are needed.

6.2.2.1. Forward Mutation - 8-azaguanine Resistance

Chloroform was not mutagenic in a test for forward mutation to 8-azaguanine resistance in cultured Chinese hamster lung fibroblast cells (Sturrock, 1977) (Table 6-C). Chloroform-exposed cells were cultured in the absence or presence of 8-azaguanine to determine viability and mutation frequencies to 8-azaguanine resistance (hypoxanthine-guanine phosphoribosyltransferase deficiency), respectively. There were no significant differences in the mutation frequencies between treated and control cells.

6.2.2.2. Sister Chromatid Exchange

White et al. (1979) reported that exposure to chloroform vapors (7100 ppm) did not increase sister chromatid exchange in cultured Chinese hamster ovary cells in the presence of Arochlor-induced rat liver S9 fraction, relative to controls (Table 6-C). Of the other anesthetics tested in this study, vinyl-containing compounds, including divinyl ether, fluroxene, and ethyl vinyl ether increased the frequency of sister chromatid exchanges significantly above the controls. The exposure duration of 1 h is rather short, and only one concentration of chloroform was tested. In addition, the amount of chloroform that actually went into solution and contacted the cells was not quantified.

Perry and Thompson (in de Serres and Ashby, 1981) also reported negative results for chloroform-induction of SCE in Chinese hamster ovary cells. The investigators considered a compound to be positive if an increase of 50% in frequency of SCE over control values and a positive dose-response relationship were observed. The number of cells scored seems rather low in comparison to other investigations (Table 6-C). In addition, longer exposure durations should have been tested.

Chloroform at concentrations up to 400 μg/ml (3.3 mM) did not increase the frequency of sister chromatid exchange or chromosome breakage in cultured human lymphocytes (Kirkland et al. 1981) (Table 6-C). The positive control, benzo-a-pyrene, gave the expected response, but was not run concurrently.

TABLE 6-C: GENOTOXICITY ASSAYS OF CHLOROFORM IN MAMMALIAN TEST SYSTEMS

Reference	Test	Test Organism/Cell	Results[1]	Comments
Sturrock (1977)	8-azaguanine resistance	Chinese hamster lung fibroblasts	-	Monolayer cells were exposed to 10,000, 15,000 20,000, or 25,000 ppm chloroform vapor for one hour, sealed with the chloroform atmosphere, and incubated 23 h at 37°C. Cells were plated to determine percent survival. Resistance to 8-azaguanine (8-AzG) (20 ug/ml, final concentration) was determined after incubation with 8-AzG starting 48h after exposure commenced through day 14. Frequency of mutation to 8-AzG resistance per 10^6 survivors: control-13; average of all treated groups minus control = 1.89 ± 3.4. No metabolic activation used.
White et al. (1979)	SCE[2]	Chinese hamster ovary (CHO) cells	-	Exponentially growing CHO cells were exposed to 7100 ppm chloroform for 1 h (78% remaining after 1 h) in presence of Arochlor-treated rat liver S9, at 37°C. Washed cells were incubated with 10 uM BrdUr[3] for 24 h; colcemid was added for final 2 h. Cells were stained by fluorescence-plus-Giemsa. 100 cells were scored for SCE. Rate of SCE: 0.544 ± 0.018 SCE per chromosome for treated groups, 0.536 ± 0.018 for controls. Difference not significant by Student's range test.
Perry and Thompson (in de Serres and Ashby, 1981)	SCE	CHO cells	-	Cells were incubated with chloroform, 0.01, 0.1, 1.0, or 10 ug/ml for 1 h with S9 from Arochlor-treated rat liver. Washed cultures were incubated 23 h, with 10 uM BrdUr, treated with 0.2 uM colcemid for 2 h, harvested and stained for SCE scoring. Twenty cells/dose were scored. SCE per chromosome: control 10.3; 0.01 group, 11.1 ± 0.65; 0.10 group, 9.4 ± 0.39; 1.0 group, 10.8 0.49; 10 group, 11.8 ± 0.75. No differences among treatment groups were noted.
Kirkland et al. (1981)	Chromosome breakage	Cultured human lymphocytes	-	Cultured human lympocytes were incubated 2 h at 37°C with 50, 100, 200, or 400 ug chloroform/ml in the presence of

TABLE 6-C, Continued

Reference	Test	Test Organism/Cell	Results	Comments
				rat liver S9. Washed cells were incubated 22 h at 37°C. Colcemid was added 1 h before harvest. 100 metaphases were scored for chromosome breaks. No difference in number of chromosome breaks was noted between treated and control metaphases. Positive control (benzo-a-pyrene) not run concurrently.
ibid	SCE	Cultured human lymphocytes	-	Lymphocytes were incubated 2 h at 37°C with 25, 50, 75, 100, 200, or 400 ug chloroform/ml and 50 uM BrdUr. Washed cells were incubated with BrdUr for 22 h. 50 cells/dose were scored for SCE. No difference in SCE frequency between treated and control cells was noted. Positive control (benzo-a-pyrene) not run concurrently.
Morimoto and Koizuma (1983)	Cell cycle delay	Cultured human lymphocyte	+	Whole blood from a healthy, adult man was incubated at 37°C with chloroform (0.02, 0.08, 0.4, 2, 10, or 50 mM), 3% PHA^3, and BrdUr (20 uM). Three h prior to fixation, colcemid was added. Cells were stained with fluorescence-plus-Giemsa method. The number of cells dividing for the first, second, or third time was recorded (200 metaphase cells/dose). About 65, 50, and 5% of cells were in the third division in the 0, 10, and 50 mM groups. Corresponding changes in the number of cells in the first division were observed.
ibid	SCE	Cultured human lymphocytes	+	Procedure as above. Chromosome preparations were scored for SCE in 35 second-division cells/dose. The lowest concentration associated with increased SCE was 10 mM chloroform ($p < 0.05$, Student's t test). Number of SCE/cell was about 8, 8.5, 11, and 15 for 0, 2, 10 and 50 mM chloroform treated lymphocytes, respectively.

TABLE 6-C, Continued

Reference	Test	Test Organism/Cell	Results	Comments
ibid	SCE	Mouse bone marrow cells, in vivo exposure	+	Male ICR/SJ mice were given daily oral doses of 0, 25, 50 100, or 200 mg chloroform/kg in corn oil for 4 days. Following the last dose, mice were constantly infused with BrdUr, 30 mg/kg/h for 24 h, and given colcemid (1 mg/kg) ip 2 h before sacrifice. 25 second-division bone marrow cells were scored for SCE/animal. Increased SCE frequency was observed in mice given 50 mg/kg or greater. Doses of 200 mg/kg produced an increase of 3 SCE/cell above controls ($p < 0.05$, Student's t test).
Perocco and Prodi (1981)	SDS and UDS[4]	Cultured human lymphocytes	-	Cultured lymphocytes were incubated with 0, 2.5, 5.0 or 10 ul chloroform/ml (0, 30, 60 or 120 mM) in the presence of rat liver S9 in tritiated thymidine (^{3}HTdR) at 37°C in sextuplicate for 4 h. ^{3}HTdR incorporation in the absence and presence of 10 mM hydroxyurea (HU) was used as a measure of SDS and UDS, respectively. Incorporation of ^{3}HTdR was not affected by chloroform treatment in absence of HU (control dpm ± se = 2661 ± 57; 60 mM chloroform = 25 ± 61; no statistical analysis reported) or in the presence of HU (without S9, control = 715 ± 24, 30 mM = 795 ± 30, 60 mM = 659 ± 38, 120 mM = 661 ± 39; with S9, control = 612 ± 26, 30 mM = 568 ± 13, 60 mM = 595 ± 26, 120 mM = 578 ± 22, no statistical analysis reported).
Perocco et al. (1983)	SDS and UDS	Cultured human lymphocytes	-	Methods as in Perocco and Prodi (1981). Viability of lymphocytes determined by trypan blue staining. ^{3}HTdR incorporation by treated cells and viability were not different than control values.

TABLE 6-C, Continued

Reference	Test	Test Organism/Cell	Results	Comments
Reitz et al. (1982)	SDS and UDS	B6C3F1 mouse liver, <u>in vivo</u> exposure	-	Mice were given vehicle or 240 mg chloroform/kg orally, followed by hydroxyurea. After 4 hours, ^{3}H-thymidine was injected ip. DNA from liver was isolated and tritiated-thymidine incorporation was measured by scintillation counting. Hydroxyurea-resistant DNA repair was not different in treated and control animals.
Martin and McDermid (in de Serres and Ashby (1981)	UDS	HeLa cells	+?	Cultured HeLa S3 cells were incubated with 10 mM HU 1 h, then ^{3}HTdR (5 uCi/ml) and chloroform (0.1, 1.0, 10, or 100 ug/ml in DMSO) were added and incubation proceeded in triplicate with rat liver S9 and in duplicate without S9 for 2.5 h at 37°C. DNA extracts were analyzed for ^{3}HTdR uptake by scintillation counting. At least one dose in the presence of S9 (but not without S9) stimulated UDS. No dose-response curve was observed. Martin and McDermid state that the Student's t test indicated statistical significance; however, they believe the result is a false positive.
Mirsalis et al. (1982)	UDS	Primary rat hepatocytes, <u>in vivo</u> exposure	-	Male Fischer 344 rats were given 0, 40, or 400 mg chloroform/kg by corn oil gavage. 2 or 12 h after treatment, hepatocytes were isolated and monolayer cultures incubated for 4 h at 37°C with ^{3}HTdR. Incorporation of ^{3}HTdR, measured by autoradiography, was not different in hepatocytes from control and treated rats. Corn oil treated control results were -2.2 ± 0.5 and -5.1 ± 0.5 net gains/nucleus for 2 and 12 h isolates, respectively. Chloroform treatment resulted in -4.1 and -4.4 for 40 and 400 mg/kg, respectively.

Table 6-C, Continued

Reference	Test	Test Organism/Cell	Results	Comments
San Agustin and Lim-Sylianco (1978)	Micronucleus	Mouse bone marrow, <u>in vivo</u> exposure	+	Chloroform (100, 200, 400, 600, 700, 800 or 900 mg/kg) wa given to albino mice (route not reported), and the micronucleus test performed according to the method of Schmid (1973). This implies that chloroform is given at 0 and 24 h and bone marrow slides prepared at 30h. Number of mice/dose not reported. Nine or 27 slides (700 mg/kg group) were stained and examined for micronucleated PCE. Marrow from control animals contained 4 micronucleated PCE per 1000 PCE. Doses < 600 mg/kg did not increase frequency of micronucleated PCE. Treatment with 600, 700, 800, or 900 mg/kg resulted in incidences of 9 ± 2, 17 ± 4, 9 ± 2, and 10 ± 2 micronucleated PCE/1000 PCE, respectively, which the authors considered significant. However, no statistical analysis was presented. Vitamin E (125 IU/kg) given 1 h prior to or after 700 mg chloroform/kg reduced the frequency of micronucleated PCE/1000 from 17 to 8 ± 3, and 4 ± 2, respectively. The authors considered the reduction significant when Vitamin E was given 1 h after chloroform.
Gocke et al. (1981)	Micronucleus	Mouse bone marrow, <u>in vivo</u> exposure	-	Chloroform (0, 238, 476, or 952 mg/kg) was given to male and female NMRI mice, 2/sex/dose, ip in corn oil at 0 and 24 h. Bone marrow slides were prepared at 30 h. No differences in incidence of micronucleated PCE/1000 cells were noted (control = 1.2, 238 mg/kg group = 2.2, 476 mg/kg group = 2.6, 952 mg/kg group = 2.2).
Tsuchimoto and Matter (in de Serres and Ashby, 1981)	Micronucleus	Mouse bone marrow, <u>in vivo</u> exposure	-	B6C3F1/BR mice received 15, 30, or 60 ul chloroform per kg, ip in DMSO (doses equal to 1/8, 1/4, and 1/2 the LD50, respectively) at 0 and 24 h. Femoral bone marrow slides were prepared at 30 h and scored for micronucleated PCE. Chloroform was negative (data not shown).

Table 6-C, Continued

Reference	Test	Test Organism/Cell	Results	Comments
Salamone et al. (in de Seres and Ashby, 1981)	Micronucleus	Mouse bone marrow, <u>in vivo</u> exposure	+? -	B6C3F1 mice were give chloroform at a dose equal to 80% of an LD50 for this strain at 0 and 24 h. Bone marrow was prepared from 5 mice at 48, 72, and 96 h, and scored for micronucleated PCE. No increases in micronucleated PCE were observed in chloroform treated animals. A second test involved a single ip injection of chloroform (80% of LD50) followed by bone marrow sampling at 30, 48, 60, and 72 h. Two mice sampled at 60 h contained significantly higher numbers of micronucleated PCE than controls. This result could not be repeated in subsequent tests.

Footnotes:
1. - = negative; + = positive; +? = equivocal
2. SCE = sister chromatid exchange
3. PHA = phytohemagglutinin
   BrdUr = bromodeoxyuridine
4. SDS = Scheduled DNA synthesis; UDS = Unscheduled DNA Synthesis

No measures were taken to prevent loss of chloroform by volatilization from the incubation mixtures. Neither dose range-finding nor toxicity of chloroform to the cells were discussed. Use of higher concentrations of chloroform and longer exposure durations may have produced a different result.

Morimoto and Koizumi (1983) found that chloroform was capable of producing cell cycle delays in cultured human lymphocytes, and increasing the rate of sister chromatid exchange in cultured human lymphocytes and in mouse bone marrow cells in vivo (Table 6-C). Chromosome preparations from chloroform-exposed lymphocytes were scored for SCE, and the number of cells which had undergone 1, 2, or 3 or more divisions was recorded. Human lymphocytes incubated with chloroform at levels $\geq$ 10 mM exhibited fewer cells in the 3rd division and more cells in the 1st division, indicating a cell cycle delay. This effect is non-specific and common to many cytotoxic agents. The lowest concentration of chloroform that caused a significant ($p<0.05$, Student's t test) increase in SCE relative to controls was 10 mM. In contrast, the highest concentration used in the Kirkland et al. (1981) study was about 3.3 mM. Bromoform was about 100 times more potent than chloroform in the induction of SCE. Although an exogenous metabolic activating system was not added to the incubates, Morimoto and Koizumi note that lymphocytes cultured in the presence of phytohemagglutinin have significant mixed function oxidase activity, and metabolism of chloroform may have contributed to increased SCE frequency in treated cells. For the in vivo studies, mice were treated orally with chloroform at doses ranging from 25 to 200 mg/kg, and bone marrow cells from the femur were prepared for scoring of SCE (Table 6-C). SCE frequency exhibited a positive dose-response, and was significantly greater than control (Student's t test, $p<0.05$) at 50 mg chloroform/kg or higher doses. The four trihalomethanes tested did not differ markedly in their ability to induce SCE in vivo.

6.2.2.3. DNA Repair

Perocco and Prodi (1981) and Perocco et al. (1983) reported that chloroform did not stimulate unscheduled DNA synthesis (UDS) in cultured human lymphocytes (Table 6-C). Although other volatile compounds, such as dibromoethane and dichloroethane, were positive in this system, measures taken to prevent volatilization of chloroform from the incubation wells were not described. In Perocco et al. (1983), chloroform was used as the negative control in a study evaluating the ability of 17 chemicals to produce genotoxic effects measured as DNA repair in cultured human lymphocytes. Chloroform (99.8% pure) at final concentrations up to 10 mM did not decrease the viability of the lymphocytes relative to controls, nor was the [^{3}H]-thymidine incorporation different from that of controls. Other volatile compounds reduced cell viability. Only chloromethylmethyl ether in the presence of S9 mix stimulated UDS relative to controls. Measures to prevent volatilization from the cultures were not described.

Martin and McDermid (in de Serres and Ashby, 1981) reported that chloroform may have been positive in an assay for unscheduled DNA synthesis in HeLa cells measured as tritiated thymidine incorporation. The investigators noted that chloroform produced a statistically significant positive result, but considered the result questionable particularly since a positive dose-response was not observed. The raw data were not presented in the paper and independent evaluation is not possible.

Mirsalis et al. (1982) reported that chloroform did not increase UDS in primary rat hepatocyte cultures obtained from male rats treated with 0, 40, or 400 mg chloroform/kg by corn oil gavage. Results in chloroform-treated rats were not different from corn oil controls. Several known genotoxic agents and hepatocarcinogens were positive in this assay including 2-acetylaminofluorene, and dimethylnitrosamine. However, benzo[a]pyrene and 7,12-dimethylbenz[a]anthracene were negative. The authors noted that these two compounds are not hepatocarcinogens.

6.2.2.4. Micronucleus Test

San Agustin and Lim-Sylianco (1978) reported positive results for chloroform in the mouse bone marrow micronucleus test. Details of the procedure were not reported; the authors used the method of Schmid (1973). Chloroform was given to mice at doses up to 900 mg/kg (route not specified). Doses of 600 mg chloroform/kg or greater resulted in greater numbers of micronucleated polychromatic erythrocytes (PCE) than controls. No statistical analysis and lack of detail make this paper difficult to evaluate. The sex or number of mice used per dose were not reported. The investigators suggested that chloroform had to be metabolized to produce this effect, based on the shape of the dose-response curve. However, insufficient data are presented to support this suggestion. While the authors reported that vitamin E given 1 hour before or after administration of chloroform (700 mg/kg) reduced the number of micronucleated PCE, a statistical analysis of the data was not presented.

Gocke et al. (1981) reported that chloroform was negative in the micronucleus test. The data (Table 6-C) were analyzed for statistical significance by the Kastenbaum-Bowman tables (Kastenbaum and Bowman, 1970), and found to be insignificant. Tsuchimoto and Matter (in de Serres and Ashby, 1981) reported that chloroform did not increase the frequency of micronucleated PCE (MPCE) in femoral bone marrow of mice. A test substance was judged positive when there were 2 or more mice/group with MPCE frequencies above 0.4%, when one or more treated groups had mean MPCE above 0.3%, and when the results were statistically significant by Kastenbaum and Bowman tables. Chloroform was negative by these criteria. Salamone et al. (in de Serres and Ashby, 1981) reported a questionable positive result for chloroform in the micronucleus assay (Table 6-C). Chloroform was positive in one of three tests. The authors state that chloroform was not tested further, and a final confirmation of the negative findings would be necessary to classify chloroform as negative in this assay.

Because of the lack of detail in the San Agustin and Lim-Sylianco paper, it is not possible to critically compare these results with the other micronucleus assays. Differences in the methodology employed in the micronucleus test can be responsible for differences in results (Heddle et al., 1983). More studies using updated methods for this test system (Heddle et al., 1983) are needed to support or refute the findings. However, it is prudent to assume that chloroform may have weak clastogenicity in light of the findings published in San Agustin and Lim-Sylianco (1978) and Morimoto and Koizumi (1983).

6.2.3. Invertebrate Test Systems

Chloroform was negative in the Basc assay in Drosophila (Gocke et al. 1981). Adult flies were fed chloroform in 5% saccharose at concentrations close to the LD50 (Table 6-D). Subsequent broods were examined for sex-linked recessive lethal mutations. Of 15 chemicals which were positive in the Ames assay, 5 were mutagenic in the Basc assay. Chloroform did not induce recessive lethal mutations on the X chromosome.

Vogel et al. (in de Serres and Ashby, 1981) tested chloroform for mutagenicity based on production of sex-linked recessive lethal mutations in Drosophila melanogaster. The Basc technique for the detection of recessive lethals was used to indicate mutagenic activity. Suspected recessive lethals were examined in the F3 generation after crossing treated males with untreated females. No increase in recessive lethals was observed in broods from chloroform-treated males relative to controls. Five of ten carcinogens and one of seven putative noncarcinogens were positive in this assay.

Liang et al. (1983) described a system for evaluating volatile mitotic poisons which uses intact grasshopper (Melanoplus sanguinipes) eggs. Living embryos (7-9 days development) were exposed to chloroform vapor in sealed jars for 16 hours. The mitotic index (MI) and anaphase to metaphase ratios (A/M) were determined in squash preparations made from the embryos. Results from embryos exposed to the lowest dose of chloroform (about 30,000 ppm) were not different from controls (Table 6-D). At higher exposure levels, chloroform produced complete colchicine-like mitotic block (i.e., interference with spindle formation resulting in mitotic arrest) and the average MI was up to 11 times greater than that in the control embryos ($p<0.005$, Chi-square analysis). This indicates that chloroform did not prevent interphase cells from entering mitosis. The A/M ratios in embryos from the 2 middle dose groups were zero, indicating that chloroform-exposure arrested the cells at metaphase. The exposure levels in the higher dose jars were not quantified, and the chloroform concentration in the grasshopper embryos was not determined. Of the four chemicals which tested positive, including benzene, toluene, and halothane, chloroform was the most potent mitotic poison followed closely by benzene, based on the volume added to the jars. Amyl acetate, ethanol, water, and 10% camphor were negative in this assay. Inhibition of mitosis by chloroform observed in this system may be relevant to the production of cell cycle delays in cultured human lymphocytes observed by Morimoto and Koizumi (1983). All four solvents testing positive in this assay have consistently yielded negative results in the Ames assay and other assays for mutagenicity. Mitotic poisons may lead to mutations and cancer by inducing chromosome imbalance in daughter cells.

TABLE 6-D. GENOTOXICITY TESTS OF CHLOROFORM IN INVERTEBRATE TEST SYSTEMS

References	Test	Test Organism/Cell	Results[1]	Comments
Gocke et al. (1981)	Basc assay	Drosophila melanogaster Berlin K (wild-type) and Basc strains	-	Adult flies were fed chloroform (25 mM = LD50) in saccharose and bred. 1200 X chromosomes were examined for sex-linked recessive lethal mutations in 3 successive broods. The number of X-linked recessive lethals (RL) per chromosome were not different in broods derived from chloroform-treated or control flies. Percent of chromosomes with RL's in brood 1, 2 and 3 from controls were 0.27, 0.14, and 0.39, respectively; and from treated were 0.29 and 0.35, respectively.
Vogel et al. (in de Serres and Ashby, 1981)	Basc assay	D. melanogaster, Berlin K (wild-type)	-	Adult male flies were given 0.1 or 0.2% chloroform in 5% sucrose for 3 days and crossed with untreated females. The F_3 generation was examined for RL mutations on the X-chromosome (2200 were examined/dose). Chloroform treatment did not increase the number of X-linked RL mutations relative to controls. Percent RL in broods 1-3 were 0.37 $\pm$ 0.17 for controls and 0.09 $\pm$ 0.06 for 0.1% chloroform and 0.05 for 0.2% chloroform (no standard deviation presented for the latter value). 5/10 carcinogens tested positive.
Liang et al. (1983)	Mitotic inhibition	Melanoplus sanguinipes eggs	+	Chloroform (0.01, 0.05, 0.1, or 0.2 ml) was added to 95 ml capacity jars in which grasshopper eggs were suspended Embryos were exposed to chloroform vapors for 16 h. Squash preparations were examined for mitotic index (MI) (percentage of mitoses, both anaphases and metaphases, in 3000 cells), and anaphase to metaphase ratio (A/M). MI for controls was 1.0 and A/M was 0.65 (average, N=12) MI for 0.01, 0.05, 0.10 treatment groups were 1.2, 11.1, and 6.4, respectively. The 0.05 and 0.1 group MI were statistically significant, $p < 0.005$, Chi-square analysis. The A/M ratios for these two groups were both zero.

1. - = negative results; + = positive results

7.0 Carcinogenicity

7.1. Introduction

Chloroform is a carcinogen in rats and mice, producing kidney and liver tumors following ingestion (NCI, 1976; Jorgenson et al. 1985; Roe et al. 1979). The mechanism of action of chloroform carcinogenesis is unknown. Mixed results have been obtained in studies designed to assess initiation and/or promotion by chloroform. While several epidemiological studies have examined a possible association between chlorinated drinking water and human cancer, there are no epidemiological studies which examine an association between chloroform exposure and cancer in humans. Evidence for chloroform carcinogenicity in experimental animals is discussed in the following sections.

7.2. NCI Study, 1976

The National Cancer Institute conducted carcinogenesis bioassays of chloroform in both sexes of Osborne-Mendel rats and B6C3F1 mice (NCI, 1976). Mice and rats were given either corn oil or chloroform (USP grade, 98.0% chloroform, 2.0% ethanol) by gavage in corn oil, 5 d/w for 78 weeks. Details of the protocol are provided in Table 7-A. Following a 6 week preliminary toxicity test, survival, weight gain, clinical measurements and observations at necropsy were used to set dose levels for the chronic bioassay. Details of the short-term toxicity study were not provided in the NCI report. Doses for the female rats and both sexes of mice were changed during the course of the chronic study (Table 7-A). Time-weighted average doses for female rats were 100 and 200 mg/kg, and for male and female mice were 138 and 277 mg/kg, and 238 and 477 mg/kg, respectively. In addition to the matched vehicle-treated control group, two other control groups were used: a colony control group of rats and mice of the same strain and source as the test animals, and a positive control group which received carbon tetrachloride by gavage in corn oil.

Animals were housed in humidity and temperature controlled rooms, under a 12:12 light:dark cycle. Chloroform-treated and control animals were group-housed in a room with test animals treated with other volatile chemicals, some of which were also carcinogenic.

Animals were observed twice daily. Body weights and food consumption were recorded weekly for the first 10 weeks and monthly thereafter. Moribund animals were killed and immediately necropsied. At study termination, samples of the following tissues were examined microscopically: brain, pituitary, adrenal, thyroid, parathyroid, trachea, esophagus, thymus, salivary gland, lymph node, heart, nasal passages, lung, spleen, liver, kidney, stomach, small intestine, large intestine, pancreas, urinary bladder, prostate or uterus, testis with epididymus, seminal vesicles, ovary, skin with mammary gland, muscle, bone, nerve, bone marrow, and tissue masses.

Survival probabilities were estimated by the Kaplan and Meier procedure. Tumor incidence was evaluated for significance by the Armitage test for linear trend. The Fisher Exact test was used to compare control data to each dose level, when the departure from linear trend was significant.

TABLE 7-A

Experimental Design of the NCI (1976) Carcinogenesis Bioassay of Chloroform

Species/Strain	Sex	Dose Group	Dose[1] (mg/kg-d)	Duration (days)	TWA[2] Dose	N	Comments
rat, Osborne-Mendel	F	low	125 90	154 392	100	50	The doses in female rats had to be lowered presumably because of toxicity, although this is not stated outright. Rats were started on treatment at 52 days of age and survivors sacrificed at 111 weeks.
	F	high	250 180	154 392	200	50	
	M	low	90	546	90	50	
	M	high	180	546	180	50	
	F	CO[3]	0	546	0	20	Controls were run concurrently.
	F	CC[4]	0	546	0	98	
	M	CO	0	546	0	20	
	M	CC	0	546	0	99	
mouse, B6C3F1	F	low	200 250	126 420	238	50	The doses given to male and female mice were increased because it was believed that the high doses were below the maximum tolerated dose. No reasons for this assumption were provided in the report. Mice were started on treatment at 35 days of age and survivors sacrificed at 92-93 weeks.
	F	high	400 450	126 420	477	50	
	M	low	100 150	126 420	138	50	
	M	high	200 300	126 420	277	50	

TABLE 7-A, Continued.

Species/Strain	Sex	Dose Group	Dose[1] (mg/kg-d)	Duration (days)	TWA[2] Dose	N	Comments
	F	CO	0	546	0	20	Controls were run concurrently.
	F	CC	0	546	0	80	
	M	CO	0	546	0	20	
	M	CC	0	546	0	77	
rat, Osborne-Mendel	M	PC[5], low	47, CT[6]	546	47	50	The protocol for the positive controls w essentially the same as for chloroform. Animals were given single daily doses of carbon tetrachloride by corn oil gavage, 5 times/week for 78 weeks.
	M	PC, high	94, CT	546	94	50	
	F	PC, low	80, CT	546	80	50	
	F	PC, high	160, CT	546	160	50	
mouse, B6C3F1	M	PC, low	1250, CT	546	1250	50	
	M	PC, high	2500, CT	546	2500	50	
	F	PC, low	1250, CT	546	1250	50	
	F	PC, high	2500, CT	546	2500	50	

1. Dose of chloroform or carbon tetrachloride (positive controls only) in mg per kg body weight; administered by gavage in corn oil 5 x per week.
2. TWA dose - time weighted average dose calculated as (dose x treatment period)/number of days receiving each dose.
3. CO - corn oil controls, gavaged 5 x per week with corn oil.
4. CC - colony controls.
5. PC - positive controls treated with carbon tetrachloride.
6. CT - carbon tetrachloride.

7.2.1. Results in Rats

For both sexes of rats, food consumption, body weight gain and survival rates were depressed by chloroform treatment. Grossly observable abnormalities in treated rats included a hunched appearance, urine stains on the abdomen, redness of the eyelids, weight loss, and wheezing. Rough hair coat, localized alopecia, and sores appeared in the second year of the study, first in the chloroform treated animals, and later in the matched and colony controls. The survival rate for chloroform-treated rats was less than controls (Figure 7-1). Survival appeared to be dose-related in the male rats early in the experiment, and in females after 70 weeks.

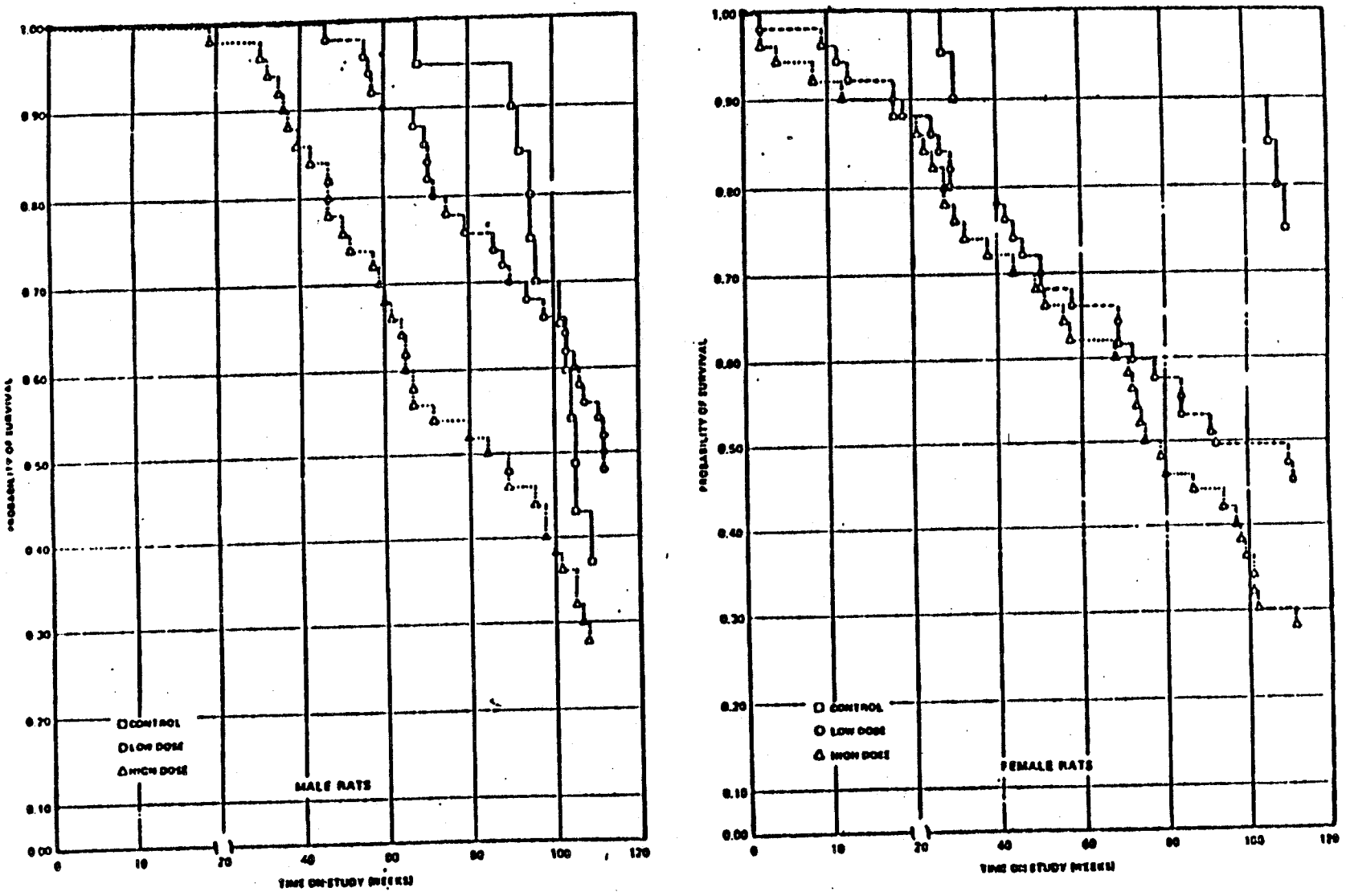
A statistically significant increase ($p<0.05$) in epithelial tumors of renal tubular origin was noted in the treated males (Table 7-B). Thirteen tumors of renal tubular origin were found in 12 high dose male rats. These included 10 carcinomas, two of which had metastasized, and 3 adenomas. In the low dose males, 2 carcinomas and 2 adenomas of tubular origin were observed in 4 out of 50 animals. The microscopic appearance of the tumors ranged from circumscribed well-differentiated tubular cell adenomas to highly pleomorphic, poorly differentiated carcinomas which had invaded surrounding tissues and metastasized. Among the 48 high dose female rats, one tubular epithelial carcinoma and one squamous cell carcinoma arising from renal pelvic transitional epithelium were observed. No epithelial tumors of the renal pelvis or tubules were noted in matched or colony controls. The NCI reported that while malignant mixed tumors and hamartomas are seen in low incidence in Osborne-Mendel rats, purely epithelial tumors of the renal tubules or renal pelvic transitional epithelium rarely occur spontaneously in Osborne-Mendel rats. No data on the historical incidence of these tumor types were provided.

The incidence of thyroid tumors in female rats (Table 7-B) was statistically higher than controls in both treated groups ($p=0.05$, Fisher Exact test). The departure from linear trend was also significant. In contrast, the incidence of thyroid tumors in male rats was higher in controls than in the treated groups. The NCI concluded that thyroid tumors were not biologically significant based on inconsistencies in the results by sex, and the variability of observations on spontaneous incidence of thyroid tumors (data not provided) in Osborne-Mendel rats in their laboratory. The incidence of hepatocellular carcinoma or neoplastic nodules was not increased in the chloroform-treated rats.

Although inflammatory pulmonary lesions occurred in all test groups, the lesions were more severe and occurred more frequently in the chloroform-treated rats. These lesions included peribronchial and perivascular lymphoid aggregates and accumulation of alveolar macrophages in interstitium and alveoli. Necrosis of the liver occurred in 3/50 low dose males, 4/50 high dose males, 3/49 low dose females, and 11/48 high dose females. The report did not present the incidence of hepatic necrosis in control animals.

7.2.2. Results in Mice

Little difference was observed in food consumption and body weight gain between control and treated mice. Survival was significantly lower in the high dose female mice but not in other treatment groups relative to controls (Figure 7-2). Both pulmonary inflammation, observed in 8/41 high dose



Survival Curves for Rats (Chloroform)

Figure 7-1. Survival curves for rats in the NCI study. From: NCI (1976).

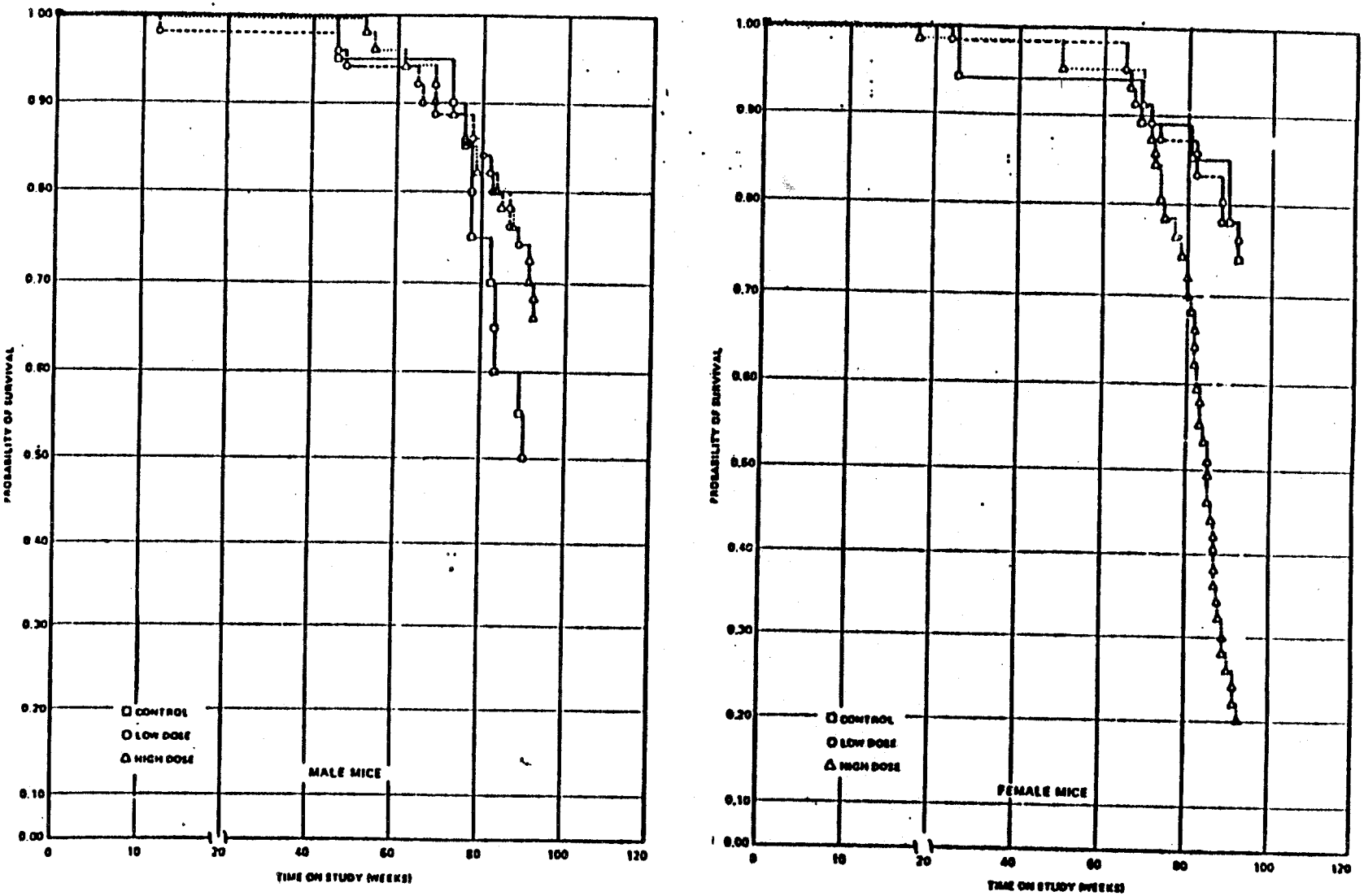
TABLE 7-B

Incidence of liver, kidney, thyroid, and total tumors in Osborne-Mendel rats from the NCI Carcinogenesis Bioassay of Chloroform.

	MALE				FEMALE			
	Controls				Controls			
TREATMENT[1]	COLONY	MATCHED	LOW	HIGH	COLONY	MATCHED	LOW	HIGH
Total Tumor-bearing Animals/Animals[2]	--	9/19	24/50	20/50	--	12/20	24/49	24/48
	--	47%	48%	40%	--	60%	49%	50%
P Value[3]	--	.2347#	--	--	--	.2733#	--	--
Time to Tumor (weeks)[4]	--	95	70	42	--	108	73	49
Hepatocellular Carcinoma/Animals[2]	1/99	0/19	0/50	1/50	0/98	0/20	0/49	0/48
	1%	0%	0%	2%	0%	0%	0%	0%
P Value[3]	.3366	.1497	--	--	1.000	1.000	--	--
Time to Tumor (weeks)[4]	97	--	--	111	--	--	--	--
Kidney Epithelial Tumors/ Animals[2]	0/99	0/19	4/50	12/50	0/98	0/20	0/49	2/48
	0%	0%	8%	24%	0%	0%	0%	4%
P Value[3]	.0000*	.0016*	--	--	.0592[6]	.1662[6]	--	--
Time to Tumor (weeks)[4]	--	--	102	80	--	--	--	102
Thyroid Tumors/ Animals[2]	8/99	4/19	3/49	4/48	1/98	1/19	8/49	10/46
	8%	21%	6%	8%	1%	5%	16%	22%
P Value[3]	.4874#	.1123#	--	--	.0000*	.0574	--	--
Time to Tumor (weeks)[4]	103	103	111	111	110	110	73	49
Survival at Terminal Sacrifice (111 weeks)	26%	37%	48%	28%	51%	75%	45%	29%

1 - Oral dose of chloroform in corn oil administered by gavage five times per week.
2 - Based on number of animals whose tissues were examined.
3 - One-tail P value from Armitage test for linear trend in proportions, unless otherwise stated.
4 - Time to detection of first tumor (at death).
5 - Data departure from linear trend (for departure statistic; $P < .05$). Fisher Exact Test is used comparing controls to a dose level. Bonferroni correction for simultaneous comparison of controls is included.
6 - P value computed using exact test (Cox, Analysis of Binary Data) as the number of tumors is too small for Armitage method.
* - Statistically significant ($P < .05$).
\# - P value given in direction of negative trend.

From: Report on Carcinogenesis Bioassay of Chloroform, National Cancer Institute, Bethesda, Maryland. March, 1976, p. 15.



Survival Curves for Mice (Chloroform)

Figure 7-2. Survival curves for mice in the NCI study. From: NCI (1976).

females, and cardiac thrombosis, observed in 9/41 high dose females, may have contributed to early mortality. The latter lesion was not observed in the low dose or control females.

The gross appearance and behavior of the control and treated mice were initially comparable, up to the first 10 months. Abdominal distension was noted in the high dose females at 42 weeks and at 78 weeks in the high dose males. Necropsy of the animals confirmed the presence of liver lesions subsequently diagnosed as hepatocellular carcinomas.

The incidence of hepatocellular carcinomas (Table 7-C) was significantly elevated in all treatment groups ($p<0.001$; Fisher Exact test with Bonferroni correction). There was a significant departure from linear trend in the data for hepatocellular carcinoma in females. Microscopically, the hepatocellular carcinomas ranged from tumors composed of well-differentiated hepatocytes in uniform arrangement to poorly differentiated anaplastic cells. Metastasis to the lung occurred in 2 low dose males and 2 high dose females. Metastasis to the kidney occurred in one high dose male. The NCI reported that in their experience the spontaneous incidence of hepatocellular carcinomas in B6C3F1 mice is about 5-10% in males and 1% in females. The observed incidence in colony and matched controls agreed with the expected incidence.

Positive controls treated with carbon tetrachloride showed poor survival and high incidences of hepatocellular carcinomas. The time-to-tumor observed with carbon tetrachloride treatment was extremely short (16 and 19 weeks in low and high dose females, respectively; 48 and 26 weeks in low and high dose males, respectively) relative to chloroform (54 and 80 weeks for low and high dose males; 66 and 67 weeks for low and high dose females). The morphology of tumors produced by carbon tetrachloride treatment was similar to that of tumors produced by chloroform treatment.

Nonneoplastic hepatic lesions were observed in chloroform-treated mice, but were not apparently dose related.

7.2.3. Analysis of Tumor Incidence by Reuber (1979)

Reuber (1979) examined the histological sections from the NCI bioassay and reported his opinion of tumor incidence in a separate publication. Reuber concluded that there was an increased incidence of liver tumors in chloroform treated rats (Table 7-C). The incidence of cholangiofibromas and cholangiocarcinomas in high dose females was significantly greater than in controls ($p=0.00599$, Fisher Exact test). Additionally, the incidence of hyperplastic nodules and hepatocellular carcinomas in high dose females was significantly greater than controls ($p<0.05$, Fisher Exact test) primarily due to an increase in hyperplastic nodules in treated female rats. The incidence of hyperplastic nodules and hepatocellular carcinomas in high dose male rats was significantly greater than the incidence in controls ($p = 0.045$, test for positive trend). This was also due to an increase in the incidence of hyperplastic nodules rather than a significant increase in carcinomas (Table 7-C). In addition, Reuber stated that the incidence of thyroid adenomas and carcinomas was much greater in the treated female rats than reported in the NCI, and that the difference in incidence between

TABLE 7-C

Incidence of liver, kidney, thyroid, and total tumors in B6C3F1 mice from the NCI Carcinogenesis Bioassay of Chloroform.

	MALE				FEMALE			
	Controls				Controls			
TREATMENT[1]	COLONY	MATCHED	LOW	HIGH	COLONY	MATCHED	LOW	HIGH
Total Tumor-bearing Animals/Animals[2]	--	4/18	26/50	44/45	--	2/20	37/45	39/41
	--	22%	52%	98%	--	10%	82%	95%
P Value[3]	--	.0000*	--	--	--	.0000*	--	--
Time to Tumor (weeks)[4]	--	72	66	54	--	27	66	67
Hepatocellular Carcinoma/Animals[2]	5/77	1/18	18/50	44/45	1/80	0/20	36/45	39/41
	6%	6%	36%	98%	1%	0%	80%	95%
P Value[3]	.0000*	.0000*	--	--	--	--	.0000*[5]	.0000*[5]
Time to Tumor (weeks)[4]	72	72	80	54	90	--	66	67
Kidney Epithelial Tumors/Animals[2]	1/77	1/18	1/50	2/45	0/80	0/20	0/45	0/40
	1%	6%	2%	4%	0%	0%	0%	0%
P Value[3]	.1414	.4873	--	--	1.000	1.000	--	--
Time to Tumor (weeks)[4]	92	92	92	92	--	--	--	--
Thyroid Tumors/Animals[2]	0/77	0/17	0/48	0/43	0/80	0/20	0/41	0/36
	0%	0%	0%	0%	0%	0%	0%	0%
P Value[3]	1.000	1.000	--	--	1.000	1.000	--	--
Time to Tumor (weeks)[4]	--	--	--	--	--	--	--	--
Survival at Terminal Sacrifice (92 weeks)	48%	50%	65%	65%	81%	75%	75%	20%

1 - Oral dose of chloroform in corn oil administered by gavage five times per week.
2 - Based on number of animals whose tissues were examined.
3 - One-tail P value from Armitage test for linear trend in proportions, unless otherwise stated.
4 - Time to detection of first tumor (at death).
5 - Data departure from linear trend (for departure statistic; $P < .05$). Fisher Exact Test is used comparing controls to a dose level. Bonferroni correction for simultaneous comparison of controls is included.
* - Statistically significant ($P < .05$).

From: Report on Carcinogenesis Bioassay of Chloroform. National Cancer Institute, Bethesda, Maryland. March, 1976, p. 31.

treated and controls was significant ($p<0.05$, Fisher exact test). Reuber's analysis of thyroid tumor incidence in male rats agreed with that of the NCI pathologists, but he described tumors in the treated rats as larger and more invasive than those in the control rats.

Reuber (1979) contended that the incidence of hepatocellular carcinoma in male and female mice is slightly higher than that reported by the NCI (see Tables 7-C and 7-D). Reuber (1979) concluded that the incidences of lymphomas in chloroform-treated male and female mice were increased relative to controls in the NCI study (Table 7-C). Lymphomas were not found in untreated and vehicle-treated mice. The incidences of lymphoma in low and high dose males and low dose females were all significantly higher than that in the controls ($p < 0.05$; Fisher Exact test.) However, a clear positive dose response is not evident, as the high dose mice had fewer lymphomas than the low dose mice.

7.2.4. Conclusions

The NCI concluded that chloroform treatment was associated with increased incidences of hepatocellular carcinomas in male and female mice and renal epithelial tumors in male rats. The methodology used in this bioassay was different in several respects from that currently recommended. The duration of exposure for the subchronic tests was only 42 days rather than 90 days. The number of matched controls currently recommended is at least 50, as compared to 20 used in this bioassay. The duration of exposure in the chronic bioassay was 18 months rather than 2 years. The dosing regimen changed during the study. And finally, mice treated with several different volatile chemicals were housed in the same room. NCI concluded that these factors did not negate the results of their assay. There was no evidence in control groups that low level exposure to other chemicals in the room air had an effect on carcinogenesis. However, the possibility of interaction of these chemicals and chloroform in the production of the carcinogenic response cannot be completely ruled out. The dose of chloroform given to these animals which was associated with tumors was probably much greater than any presumed exposure to other volatile chemicals in the air. Levels of these other chemicals in room air were not evaluated.

In addition, Reuber (1979), based on his examination of the histological sections from the NCI study, concluded that chloroform treatment was also associated with cancer of the liver in rats and an increased incidence of malignant lymphomas in mice. However, pathologists at the NCI did not agree with his findings.

7.3. Jorgenson et al. (1985) Study

The carcinogenicity of chloroform given in drinking water was evaluated in male Osborne-Mendel rats and female B6C3F1 mice (Jorgenson et al. 1985) (Table 7-E). The chloroform used (technical grade redistilled before use), was analyzed for contaminants by gas chromatography/mass spectrometry and found to contain 100 ppb diethylcarbonate, and trace amounts of 1,1-dichloroethane, dichloroethylene, carbon tetrachloride, and an unidentified

TABLE 7-D

Incidence of tumors in NCI study according to reanalysis by Reuber (1979)

Species/Sex		Tumor Type	Tumor Incidence[a]		
			Control	Low Dose	High Dose
rat	F	cholangiofibromas and cholangiocarcinomas	0/20	3/39	11/39
rat	F	hyperplastic nodules and hepatocellular carcinomas	3/40[b]	9/39	14/39
rat	F	hepatocellular carcinomas	0/40[b]	2/39	2/39
rat	F	thyroid adenomas and carcinomas	1/20	11/39	12/39
rat	F	thyroid carcinomas	1/20	3/39	5/39
rat	M	hyperplastic nodules and hepatocellular carcinomas	1/39	5/50	10/49
rat	M	hepatocellular carcinomas	0/39[b]	0/50	2/49
mouse	M	lymphomas	0/34[b]	14/46	10/44
mouse	F	lymphomas	0/39[b]	9/45	4/40
mouse	M	hepatocellular carcinomas and hyperplastic nodules	5/34[b]	31/46	44/44
mouse	M	hepatocellular carcinomas	2/34[b]	20/46	44/44
mouse	F	hepatocellular carcinomas and hyperplastic nodules	0/39[b]	41/45	40/40
mouse	F	hepatocellular carcinomas	0/39	40/45	40/40

a. Tumor Incidence = number of animals with tumor/number of animals examined for that tumor. It is not clear why, in some cases, Reuber's denominators are not the same as in the NCI report.
b. Pooled vehicle and untreated controls.

C_5H_{10} hydrocarbon. Chloroform concentrations in water remained at least 93% of target concentration during the 4 days between new preparations. Time-weighted average doses of chloroform (Table 7-E), calculated based on water consumption rates and body weight, ranged up to 160 and 263 mg/kg-d for rats and mice, respectively. Chloroform in room air was consistently below 6 ppb and none was detected in feed. Two control groups were used, an untreated control, and a control group of animals with restricted access to water such that the water consumption was equivalent to that observed in the high dose groups. Animals were observed daily. Body weights were recorded weekly for the first 14 and 18 weeks of the study for mice and rats, respectively, and monthly thereafter. A complete necropsy was performed at termination of the study or when animals were found moribund.

Water consumption by rats decreased with increasing chloroform content. Survival was inversely correlated to chloroform dose in the male rats. At week 104, the survival rates were 12% for the control, and 25, 29, 60, and 66% for chloroform-treated groups (lowest to highest dose), respectively. Untreated control rats on restricted water intake had a survival rate of 54% at 104 weeks. Jorgenson et al. speculated that leaner body composition may be related to survival in the male rats. The chloroform-treated female mice also consumed less water than controls. However, the investigators noted that the difference appeared to be primarily due to a subgroup of animals which refused to drink water. After this subgroup died, water consumption returned toward control values, although it was still significantly less.

Tumor incidence data (Table 7-F) were evaluated for significant differences by the Peto trend test or the Cochran-Armitage test. A dose-related significant increase in renal tubular cell adenomas and adenocarcinomas was observed in the male rats ($p<0.0001$, Peto trend test). Increased incidences of neurofibromas, leukemias, lymphomas, and overall circulatory system tumors were observed. However, these tumors were not clearly dose-related (see Table 7-F), and may represent progression from one tumor type to another because of the longer survival of treated rats. The incidence of nephropathy was high (> 90%) in all groups including controls.

Jorgenson et al. found no treatment related increases in tumor incidence in the female mice. The lack of liver tumors in female B6C3F1 mice is in sharp contrast to the results of the NCI study, wherein the incidence was 80% for the low dose group and 95% for the high dose group, compared to 1% and 0% in the colony and matched controls, respectively. The low dose in the NCI study, 238 mg/kg, is comparable to the high dose in the Jorgenson study. A major difference between the NCI study and the Jorgenson study is the mode of administration. Withey et al. (1983) showed that administration of chloroform to rats in a corn oil vehicle slowed the gastrointestinal absorption of chloroform relative to the rate observed after administration in water. The peak blood concentration of chloroform and the area under the blood-concentration time curve were about 7-fold and 8.7-fold greater when chloroform was administered as a bolus dose in water than when administered in oil. In the Jorgenson study, the rats received small doses each time they drank water, whereas the NCI study used bolus administration in corn oil. However, the corn oil vehicle effect (Withey et al. 1983) may have diminished the differences in absorption kinetics expected with the 2 different methods of administration. Thus, differences in peak concentrations in blood in the NCI and Jorgensen studies may not have been

TABLE 7-E

Experimental Design of Jorgenson et al. (1985)

Species/Strain	Sex	DOSE mg/l	DOSE TWA[a] mg/kg-d	N[b]	Duration[c] (weeks)	Comments
rat, Osborne-Mendel	M	0	0	330	104	Rats were started on test at 7 weeks.
		0 (RW)[d]	0	50	104	
		200	19	330	104	
		400	38	150	104	
		900	81	50	104	
		1890	160	50	104	
mouse, B6C3F1	F	0	0	430	104	Mice were started on test at 8.5 weeks of age.
		0 (RW)	0	50	104	
		200	34	430	104	
		400	65	150	104	
		900	130	50	104	
		1800	263	50	104	

a. TWA = time-weighted average dose based on water intake and body weight.
b. N = number of animals/dose group.
c. Duration of exposure.
d. RW = restricted water intake.

TABLE 7F

Kidney tumors and other tumors in male Osborne-Mendel rats treated with chloroform in drinking water. From Jorgenson et al. (1985).

			Chloroform Treatment Groups (mg/l)			
Tumor Type	Control	Matched Control	200	400	900	1800
All kidney tumors[a]	5/301	1/50	6/313	7/148	3/48	7/50[b,c]
Tubular cell adenoma	4/301	1/50	2/313	3/148	2/48	5/50[b,c]
Tubular cell adenoma and adenocarcinoma	4/301	1/50	4/313	4/148	3/48	7/50[b,c]
Neurofibroma	2/303	1/50	2/316	1/148	0/48	3/50
Lymphomas and leukemias	5/303	1/50	19/316[b]	5/148	2/48	3/50[b]

a. Includes nephroblastomas occurring in the 200 and 400 mg/l groups.
b. Individual treatment group statistically different from control group, $p<0.01$ (test not specified).
c. Overall p value calculated by Peto trend test, $p<0.0001$.

sufficient to account for the differences in results. Bull et al. (1986) showed that chloroform administered to male and female B6C3F1 mice in corn oil caused a greater degree of hepatotoxicity than the same dose given in an aqueous solution of Emulphor (2%). Other investigators have shown that corn oil may increase the yield of tumors when administered after aflatoxin B_1 (Newberne et al. 1979), and that nitrosamines and acetylaminofluorene induced tumors earlier or with a higher incidence in rats fed a high fat diet relative to controls (Rogers and Newberne, 1975). Some physiologic or metabolic changes produced by corn oil consumption might interact with chemical carcinogens altering the production of liver tumors. The incidence of renal tumors produced by chloroform in the Jorgensen study was in good agreement with the NCI tumor incidence data. The differences in method of administration had no apparent effect on renal carcinogenesis in rats.

7.4. Huntingdon Research Center Studies

A series of experiments was conducted by the Huntingdon Research Center to determine the effects of chronic ingestion of chloroform in a toothpaste base in mice, rats, and beagle dogs. In the first set of experiments (Roe et al. 1979), doses of 17 and 60 mg chloroform/kg were administered by gavage in toothpaste to male and female ICI mice, 6 d/w for 80 weeks followed by a 16 week observation period (Experiment I). Controls (N=104) were treated with 1 ml toothpaste/kg-d. Aside from increased nonneoplastic liver lesions (moderate fatty degeneration), the only significant difference in pathology reported was an increase in the incidence of kidney tumors in high dose male mice (Table 7-G). Of the 8 kidney tumors in the high dose group of ICI males, three were classified as hypernephromas (tubular adenocarcinoma, personal communication from Dr. F.J.C. Roe to Dr. John Christopher, California Dept. of Health Services), and the remainder were adenomas. Reuber (1979) reported that the incidence of renal tumors in high-dose male ICI mice, 8/37 (the original paper reported 8/38, but was in error, see Table 7-G), was significantly greater than control mice ($p = 0.00012$, Fisher Exact test). In experiment I of Roe et al. (1979), many of the animals' tissues were not examined histologically and, therefore, the true incidence of tumors is unknown. None of the female ICI mice examined developed renal tumors (Roe et al. 1979). The incidence of liver tumors in treated ICI mice was not different than in the controls.

Roe et al. (1979) also investigated other components of the toothpaste base for carcinogenicity using male ICI mice (Experiment II). Groups of male ICI mice (N = 260 controls, 52/dose) were given one of the following: toothpaste base (without peppermint oil, eucalyptol, or chloroform), 8 or 32 mg eucalyptol/kg, 4 or 16 mg peppermint oil/kg, or 60 mg chloroform/kg in toothpaste base by gavage 6 d/w for 80 weeks. Roe and colleagues stated that no lesion in this part of the study could be correlated with treatment. However, a Chi-square test on the kidney tumor data (Table 7-G) indicates that chloroform treatment was associated with an increase in kidney tumor incidence relative to controls ($p<<0.001$). Two of the kidney tumors in the chloroform-treated mice and one in a eucalyptol-treated mouse were regarded as malignant and classified as hypernephromas (tubular adenocarcinoma, see above). All other kidney tumors were classified as benign adenomas (tubular adenomas).

TABLE 7G

Incidence of kidney tumors in ICI mice given 0, 17, or 60 mg chloroform/kg-d in toothpaste base (no peppermint oil or eucalyptol).
From: Roe et al. (1979)

EXPERIMENT I

Dose[a] (mg/kg-d)	Males	Females
0 (vehicle only)	0/72	0/59
17 chl	0/37	0/35
60 chl	8/37[b]	0/38

EXPERIMENT II

Dose[a] (mg/kg-d)	Males	Females
0 (no vehicle)	1/45	0/49
0 (vehicle only)	6/237	--
60 chl	9/49[c]	--
8 euc	1/52	--
32 euc	0/46	--
4 pep	1/50	--
16 pep	0/49	--

---

a. chl = chloroform; euc = eucalyptol; pep = peppermint oil

b. The original paper reported 8/38 for kidney tumors in this group. The correct figure is 8/37 (personal communication from Dr. A. K. Palmer to Dr. J. Christopher, California Department of Health Services). Significantly greater than controls, $p<0.001$, Fisher Exact Test.

c. Significantly greater than vehicle treated controls, Chi-square test, $p \ll 0.001$.

In a third mouse experiment (Experiment III), Roe et al. (1979) compared the effects of toothpaste containing 3.5% chloroform on male mice of 4 different strains (C57BL, CBA, CF/1, and ICI). Roe and colleagues estimated that a 55 kg woman would receive about 0.15 mg chloroform/kg-d by brushing the teeth twice daily with a toothpaste containing 3.5% chloroform. Groups of mice (N = 52/dose) received either 1 mg/kg-d of toothpaste base which contained both eucalyptol and peppermint oil, 1 ml arachis oil/kg-d, or 60 mg chloroform/kg-d (400 times "human" dose from toothpaste) in toothpaste or arachis oil by gavage 6 d/w for 80 weeks, followed by a 16-24 week observational period.

Treatment with chloroform was not associated with any increase in liver or lung neoplasms relative to vehicle-treated controls in any of the 4 strains tested. Treatment with chloroform in toothpaste was associated with significantly higher incidences of moderate to severe kidney pathology in CBA and CF/1 mice relative to the controls ($p<0.0001$, Chi-square test). In addition, chloroform administered in arachis oil significantly increased the incidence of moderate to severe kidney disease ($p<0.05$, Chi-square test) and kidney tumors in ICI male mice compared with arachis oil vehicle controls. Kidney tumors were malignant in 9 treated mice (Table 7-H). No kidney tumors were found in the 83 untreated controls. When chloroform was given to male ICI mice in a toothpaste base, 5/47 animals developed kidney tumors. In three of these mice, the tumors were malignant. Both groups of vehicle (toothpaste base or arachis oil) controls each had one mouse with a benign kidney tumor. Roe et al. did not present a statistical analysis of the kidney tumor data. Analysis by the Chi-square test shows that the incidence of kidney tumors in the ICI male mice given chloroform in arachis oil is significantly higher than vehicle treated controls ($p<0.005$). Reuber (1979) reported that the incidence of kidney tumors in male ICI mice treated with chloroform in arachis oil was significant relative to the combined (vehicle plus untreated) controls ($p = 0.00156$) by the Fisher Exact test.

Survival was poor in the ICI and CF/1 mice, and relatively few CF/1 mice survived beyond the 80th week when renal neoplasia would most likely be seen (time-to-tumor = 88 weeks in ICI mice).

Palmer et al. (1979) gave groups of 50 Sprague-Dawley rats (both sexes) 0 or 60 mg chloroform/kg-d, 6 d/w by gavage in a toothpaste base for 80 weeks, followed by a 15 week observation period. There were no differences in the incidences of tumors of any site examined, including brain, lung, liver, kidney, and mammary gland, between treated and control animals.

Heywood et al. (1979) investigated the carcinogenicity of chloroform in a toothpaste base in beagle dogs. Groups of male and female dogs received toothpaste base with 0, 15 or 30 mg chloroform/kg-d, 6 d/w for 7.5 years (N = 8-16 per sex), followed by a 20-24 week recovery period. Untreated controls consisted of 8 dogs/sex. Treatment with chloroform at the high dose was associated with significant elevations in SGPT levels starting 6 weeks into the treatment phase, which rose steadily during the first 5 years and plateaued or declined slightly thereafter. Treatment with the lower dose of chloroform also produced slight but significant increases in SGPT levels after 130 weeks of treatment. No treatment related tumors were observed.

TABLE 7-II

Incidence of kidney tumors in male mice given 0 or 60 mg chloroform/kg-d in toothpaste[a] or arachis oil (Experiment III, Roe et al, 1979)

Strain	Dose[b]	Number of mice with tumors: Benign	Malignant	Total	% Survivors at Terminal Kill	(Week)
C57B1	0	0/46	0/46	0/46	52	104
C57B1	60	0/51	0/51	0.51	69	104
CBA	0	0/51	0/51	0.51	69	104
CBA	60	0/51	0/51	0/51	79	104
CF/1	0	0/45	2/45	2/45	21	93
CF/1	60	0/48	1/48	1/48	12	93
ICI	0	1/49	0/49	1/49	27	97-99
ICI	60	2/47	3/47	5/47	31	97-99
ICI	untreated	0/83	0/83	0/83	19	97-99
ICI	0 (arachis)	1/50	0/50	1/50	21	97-99
ICI	60 (arachis)	3/48	9/48	12/48	17	97-99

a. Toothpaste base contained 39.35% glycerol; 48.53% calcium carbonate; 0.25% peppermint oil; 0.50% eucalyptol; 0.45% carragheen gum; 1.1% sodium lauryl sulphate; 0.03% sodium saccharin; 1.10% white mineral oil; 5.12% water.

b. In toothpaste, unless noted otherwise; arachis = arachis oil vehicle.

7.5. Tumasonis et al. (1985) Study

Chloroform treatment of rats via drinking water was associated with hepatic neoplastic nodules and hepatic adenofibrosis (Tumasonis et al. 1985). Chloroform was administered to male and female Wistar rats (derived from a closed colony maintained by the New York Department of Health) in the drinking water initially at a concentration of 24 mM; at 72 weeks, the concentration was halved because of an observed increase in water consumption. The resulting daily doses were about 220 mg/kg/d and 160 mg/kg/d for the female and male rats, respectively. Chloroform was the positive control in this study which was designed to examine the effects of chronic consumption of dibromochloromethane, another trihalomethane commonly found in drinking water. Control groups received city water. Treatment was continued for the lifetime (>90 to 180 weeks) of the rats which were killed when moribund or when a large tumor was noted. Sections of liver and of tissues with grossly observable lesions were examined histologically. Treated animals weighed less than controls at all ages. Survival curves presented in the paper indicated a slightly lower survival rate in treated rats than in controls between about 80 and 120 weeks. However, more treated rats than controls lived longer than 160 weeks.

The incidence of hepatic neoplastic nodules was significantly elevated in treated females compared to controls ($p<0.03$, Fisher Exact test) (Table 7-I). In males, the incidence of hepatic neoplastic nodules did not differ in control and chloroform-treated groups. Increased incidences of hepatic adenofibrosis were observed in chloroform-treated males and females relative to controls. Tumasonis et al. used the term adenofibrosis for lesions which resemble cholangiocarcinoma, based on the classification of hepatic tumors proposed by the National Academy of Sciences (NAS, 1980 as cited in Tumasonis et al. 1985). As noted in the report, adenofibrosis was used because the NAS report expressed doubt that bile duct cells in the rat can give rise to adenomatous tumors, i.e., cholangiocarcinomas. However, several hepatocarcinogens can produce proliferative lesions of bile ducts (Terao and Nakano, 1974). Schauer and Kunze (1976), in the International Agency for Research on Cancer publication on tumors of the rat (IARC, 1976), described cholangiocarcinomas in the rat, and Tumasonis and colleagues state that lesions observed in their study were similar to those described in the IARC publication as chloangiocarcinomas. The reanalysis of the NCI data by Reuber (1979) indicated that he believed the female rats in the NCI study had chaloangiofibromas and cholangiocarcinomas. This is consistent with the observations made by Tumasonis et al. (1985). The time-to-tumor is not discussed by Tumasonis; however, survival curves are presented for animals with and without adenofibrosis or neoplastic nodules. From these curves, it appears that hepatic adenofibrosis was found in animals which died around week 80, at the earliest, and that this lesion was found in animals dying out to week 180.

The incidence of lymphosarcoma was lower in chloroform-treated males than in controls. Lower incidences of pituitary and mammary tumors were observed in chloroform-treated females relative to controls ($p<0.05$, Fisher exact test). In contrast to the NCI and the Jorgenson et al. studies, renal tumors were not associated with chloroform treatment. However, Tumasonis et al. indicated that kidneys were only examined when grossly observable lesions were evident. Hence, kidney tumors may have been missed by this protocol.

TABLE 7-I

Liver tumors found in Wistar rats treated with chloroform in drinking water.
From: Tumasonis et al., 1985.

	Males		Females	
Tumors	Control	Treated	Control	Treated
Hepatic neoplastic nodules	5/22	5/28	0/18	10/40[a]
Hepatic adenofibrosis[b]	0/22	17/28[a]	0/18	34/40[a]
Lymphosarcoma	14/22	6/28[c]	2/18	4/40
Pituitary Tumor	0/22	2/28	6/18	1/40[c]
Mammary Tumor	0/22	0/28	8/18	0/40[c]

a. Significantly greater than control, $p<0.001$, Fisher exact test.

b. Tumasonis et al. use this term, rather than cholangiocarcinoma, based on a classification proposed by the Institute of Laboratory Animal Resources of the National Academy of Sciences.

c. Significantly less than control, $p<0.05$, Fisher exact test.

Tumasonis et al. concluded that chloroform is a hepatocarcinogen in these closed-colony Wistar strain rats.

7.6. Other Studies

Eschenbrenner and Miller (1945a) gave 30 doses of chloroform in olive oil by gavage at 4 day intervals to strain A mice (5/sex/dose) at doses of 0, 150, 300, 600, 1200, and 2400 mg/kg. One month after the last dose, the animals were examined for the presence of hepatomas. A single dose of chloroform, administered 24 h prior to sacrifice, produced extensive necrosis of the liver in male and female mice at the 3 highest doses, and renal necrosis in male (but not female) mice. Cirrhosis of the liver was present in the chronically treated mice at the 3 highest dose levels, but was absent in the 2 lower dose groups. No hepatomas were observed in males or females in the low dose groups. However, these groups were too small, and the duration of exposure too limited to conclude that chloroform does not cause hepatomas at the lower doses employed. All the males in the higher dose groups died during the course of treatment. It was, therefore, not possible to evaluate the carcinogenicity of chloroform in the male mice. All surviving females (3/5 at 600 mg/kg and 4/5 at 1200 mg/kg) had hepatomas, which the investigators described as similar to those produced by carbon tetrachloride.

Chloroform was negative in the pulmonary adenoma assay in strain A mice (Theiss et al. 1977). Doses of 80, and 200 mg/kg were injected intraperitoneally thrice weekly. One group received 2 doses of 400 mg/kg. Twenty-four weeks after the first injection, mice were sacrificed and the lungs were examined for tumors. The frequency of lung tumors in treated mice was not greater than controls.

7.7. Tumor Promoter Activity

Results of experiments designed to test for tumor promoting activity of chloroform have been mixed. Evidence from experiments is discussed in the following sections.

7.7.1. Tumor Promotion Bioassays

Klaunig et al. (1986) used a two-stage initiation/promotion treatment protocol to evaluate the tumor promoting activity of chloroform in diethylnitrosamine (DENA) treated B6C3F1 mice. Groups of 35 male mice received drinking water containing 0 or 10 mg DENA/l for 4 weeks. After the 4 week initiation period, mice were given drinking water containing either deionized water or chloroform at levels of 600 or 1800 mg/l. These levels were believed to be the maximum tolerated dose (MTD) and 1/3 MTD, based on mortality in a 4 week study. Ten mice per group were sacrificed after 24 weeks of promotion; the remainder were sacrificed at 52 weeks. At necropsy, all tissues were examined for gross lesions and samples of liver, lung, and kidney were prepared for quantitating tumors.

Mice receiving chloroform gained less weight, had lower water consumption rates, and decreased rates of survival relative to controls ($p<0.05$, statistical test not reported). Focal areas of necrosis in liver and kidney were observed in chloroform-treated mice. Liver and lung tumor data from

chloroform treated mice that died before termination of the study were included in the final tallies. Klaunig et al. (1986) reported that chloroform treatment had no affect on the incidence of lung tumors in initiated or noninitiated mice. No statistical analysis of this result is presented. However, Chi-square analysis of lung tumor data (Table 7-J) reveals that lung tumor incidence in initiated mice treated with 1800 mg chloroform/l was less than that in initiated controls ($p<0.005$). In addition, the number of tumors per mouse was significantly lower in chloroform treated initiated animals than in initiated controls ($p<0.001$, Student's t test, 1800 mg/l group compared to control).

Treatment of noninitiated mice with chloroform did not affect the incidence of liver tumors with respect to controls. However, chloroform treatment of DENA-initiated mice at both the low and high doses produced a significant decrease in the mean number of liver tumors per mouse relative to the DENA initiated group given deionized water during the promotional period. No statistical analysis of this data was presented by Klaunig. Analysis by Student's t test shows statistical significance at $p<0.02$. The lack of an association between chloroform treatment and liver tumors in the uninitiated mice is in contrast to the findings reported by the NCI (1976), but is in agreement with the Jorgenson study, which also used drinking water as the route of administration. Doses used in mice in the NCI and Klaunig studies approximated 1.8 and 1.5 mg chloroform/g body weight per week, respectively (Klaunig et al. 1986). However, the duration of exposure was less in the Klaunig study than in both the NCI and Jorgenson studies. Direct comparison is, therefore, difficult.

Habs et al. (1983) reported that subcutaneous injection of 300 mg chloroform/mouse had no effect on the occurrence of tumors in C57B16 mice 9 weeks following a single whole-body dose of 170 or 330 rad of fast neutrons. Whole body irradiation increased the incidence of malignant tumors, especially in the liver.

Pereira et al. (1985) injected groups of 15 day old CD-1 Swiss mice of both sexes (N = 25 to 45) with either 0, 5, or 20 mg ethylnitrosourea (ENU)/kg. After weaning, the mice received 1800 ppm chloroform or 500 ppm sodium phenobarbital in the drinking water, until 51 weeks of age. At 52 weeks the animals were examined for grossly observable lesions and sections of kidney, liver, adrenals, lung, esophagus, forestomach, and all gross lesions were prepared for histological examination. ENU treatment caused a dose-related increase in the incidence of altered foci, adenomas, and carcinomas in the liver (Table 7-K). Treatment with chloroform in the drinking water decreased the incidence of ENU-initiated liver tumors (adenomas and carcinomas) significantly in male mice ($p<0.05$, Student's t test). In contrast, phenobarbital treatment caused a significant ($p<0.05$, Student's t test) increase in the incidence of liver tumors, especially carcinomas, in ENU treated animals. There were no liver tumors in the ENU-treated female mice. ENU caused a dose-related increase in the incidence of lung tumors in male and female mice, which was not altered by subsequent chloroform or phenobarbital treatment. Chloroform treatment itself, without prior initiation by ENU, did not increase the incidence of liver or lung tumors relative to drinking water controls.

TABLE 7-J

Liver and lung tumor incidence at 52 weeks in an initiation-promotion bioassay with B6C3F1 male mice.
From: Klaunig et al., 1986

TREATMENT		LIVER TUMORS		LUNG TUMORS	
Initiation[a]	Promotion[b]	Incidence	Tumors/Mouse	Incidence	Tumors/Mouse
—	—	5/25	0.08 ± 0.28	2/25	0.10 ± 0.30
+	—	25/25	29.3 ± 15.4	18/25	1.40 ± 1.40
—	600 mg/l	3/25	0.1 ± 0.3	0/25	0
+	600 mg/l	25/25	19.4 ± 14.1	13/25	0.80 ± 0.90
—	1800 mg/l	4/25	0.3 ± 0.9	0/25	0.04 ± 0.20
+	1800 mg/l	20/25	18.4 ± 14.7	6/25	0.30 ± 0.60

a. Mice were initiated by drinking water which contained 10 mg diethylnitrosamine/l for 4 weeks.

b. Following initiation, mice were given water containing 600 or 1800 mg chloroform/l for 52 weeks.

TABLE 7-K

Effect of chloroform treatment on the incidence of liver tumors at 52 weeks in ethylnitrosourea-initiated male mice. Adapted from: Pereira et al., 1985

Treatment	Tumor Incidence (# animals with tumor/# animals examined) Adenoma and Carcinoma	Adenoma	Carcinoma
ENU[a]			
0	2/37	2/37	2/37
5	8/39	8/39	2/39
20	22/30	22/30	10/30
ENU + CHL[b]			
0	0/23[d]	0/23[d]	0/23
5	1/25[d]	1/25[d]	0/25
20	12/29[d]	12/29[d]	5/29
ENU + PB[c]			
0	11/30[e]	6/30	0/30
5	17/36	14/36	10/36[e]
20	24/25	22/25	17/25[e]

a. Mice were initiated with ethlynitrosourea, 5 or 20 mg/kg, administered intraperitoneally at 15 days of age.
b. CHL = 1800 ppm chloroform in the drinking water from weaning through 51 weeks.
c. PB = 500 ppm phenobarbitol in the drinking water from weaning through 51 weeks.
d. Significantly less than ENU treatment alone, Fisher exact test, $p<0.05$.
e. Significantly greater than ENU treatment alone, Fisher exact test, $p<0.05$.

Hirose et al. (1981) reported that the induction of tubular hyperplasia and renal cell tumors were markedly enhanced in rats by combined treatment with a single dose of 20 mg dimethylnitrosamine/kg (DMN), unilateral nephrectomy, formic acid 2-[4-(5-nitro-2-furyl)-2-thiazolyl]hydrazide (FNT) in the diet, and 400 mg chloroform/kg given as a single oral dose either before or after DMN. Epithelial regeneration following chloroform-induced tubular necrosis seemed to be as effective a promoter as proliferation produced by nephrectomy. However, the investigators did not examine the effects of chloroform and unilateral nephrectomy on the incidence of FNT-initiated tumors separately. It is, therefore, difficult to elucidate the role of chloroform in the observed increase in tumor incidence.

Pereira et al. (1982) examined DNA binding, and the initiation and promotion activities of bromoform and chloroform using female B6C3F1 mice or male Sprague-Dawley rats. In rat liver and kidney, a peak of radioactivity derived from ^{14}C-chloroform was found associated with the pyrimidine bases or phosphates of DNA. Purification of labeled DNA revealed that dinucleotide bridges may have formed. The amount of label bound was about 0.05 to 0.15% of the binding index obtained with dimethylnitrosamine. No such association was found using mouse liver. Chloroform given as a single oral dose of 179 mg/kg was not active as an initiater in a two-step initiation-promotion assay using phenobarbital as the promoter, and gamma-glutamyl transpeptidase (GGTase) positive foci in the liver as an indicator of carcinogenesis. The incidence of GGTase positive foci in livers of DENA-initiated rats treated with chloroform in a promoting regimen (179 mg/kg, twice weekly for 8 weeks) was significantly higher relative to vehicle treated animals, but was not statistically higher than in groups given DENA alone or chloroform alone. Pereira et al. concluded that chloroform has little or no initiating activity, and that tumor promoting activity needs further study. Despite the work of Pereira and colleagues, DHS staff believe that chloroform may also act as a genotoxic agent.

Capel et al. (1979a) reported that growth of Ehrlich ascites tumor cells in the peritoneal cavity of mice, measured as DNA content of peritoneal cavity washes, was increased in mice treated with 15 mg chloroform/kg-d in the drinking water for 2 weeks either before and/or after innoculation with tumor cells ($p<0.001$, test not reported). The invasion of spleen, mesenteric lymph nodes, and lung by B16 melanoma cells injected sc into the tail of C57B1 mice was more extensive in chloroform treated mice. Statistical analysis of this result was not presented. Capel et al. also reported that the lungs of mice treated with 15 mg chloroform/kg-d had a greater number of metastases than controls following inoculation of the animals with Lewis lung tumor cells ($p<0.05$, test not reported). Ingestion of a smaller dose of chloroform (0.15 mg/kg-d) did not affect the growth of any of these murine tumors. Capel et al. concluded that chloroform ingestion can enhance the growth and spread of experimental tumors.

7.7.2. Biochemical Evidence of Tumor Promoter Activity

Deml and Oesterle (1987) reported that chloroform treatment following diethylnitrosamine initiation increased the number of preneoplastic foci in the liver, defined as areas of increased GGTase activity and glycogen storage, and decreased ATPase activity. Administration of 100, 200, and 400 mg/kg twice a week for 11 weeks produced a 2, 2.5, and 5 fold increase in number of ATPase deficient islands. Total area of these islands was also

increased. Areas of increased GGTase activity and glycogen storage were also increased by doses of $\geq$ 100 and $\geq$ 200 mg chloroform /kg, respectively. This study provides some evidence that chloroform may have promoting effects.

Capel et al. (1979b) theorized that chloroform may exert some of its carcinogenic activity by influencing the bioactivation and detoxification of other carcinogens. Ingestion of chloroform-containing drinking water, at doses equivalent to 0.15 or 15 mg chloroform/kg-d, for 14 days did not affect the liver weight or protein content of microsomes in male rats (N=3). Chloroform treatment was associated with decreased cytochrome P-450 content in the liver, no changes in aryl hydrocarbon hydroxylase or glutathione transferase activity, and depressed uridine diphosphoglucuronic acid (UDPGA) transferase activity relative to controls ($p<0.05$, test not reported). There were no differences observed in DNA binding of ^{3}H-benzo[a]pyrene between control and chloroform treated rats. Other investigators have demonstrated that chloroform treatment can destroy cyt P-450 in the liver (Enosawa and Nakazawa, 1986). The relationship between changes in cyt P-450 and tumor promotion are ill-defined.

A number of tumor promoters, including the phorbol esters are activators of calcium dependent, phospholipid dependent protein kinase (protein kinase C) (Roghani et al. 1987). Roghani et al. (1987) found that chloroform (2% v/v) could activate protein kinase C in vitro using washed rabbit platelets. Carbon tetrachloride and methylene chloride were also able to activate this enzyme. The investigators suggested, based on assays for protein kinase C in a reconstituted membrane system, that chloroform increases the number of binding sites for the protein substrate, and that a likely mechanism is alteration of the chemical structure of the lipid surrounding the enzyme. Ulmer and Braun (1987) reported that chloroform (2% v/v) enhanced the phosphorylation of myelin basic proteins 17 fold over control conditions, probably due to the stimulation of protein kinase C activity. The relationship between activation of protein kinase C and the process of tumor promotion is undefined. Furthermore, the relevance of these in vitro assays using 2% chloroform to what occurs in vivo at much lower concentrations is unclear.

Ornithine decarboxylase (ODC) induction may be a biochemical marker for tumor promoters (Savage et al. 1982; Pereiera et al. 1984b). This enzyme is the rate-limiting enzyme in polyamine synthesis, and is induced during tumor promotion in skin and liver and during the hyperplastic response of liver to drugs, hormones, and partial hepatectomy (Savage et al. 1982). The increase in ODC activity is rapid and is followed by an increase in polyamine synthesis, which, in turn, is followed by increased DNA, RNA, and protein biosynthesis (Pereira et al. 1984b). Injection of rats with chloroform (ip) at a dose of 750 mg/kg resulted in a 44 fold increase in liver ODC activity compared to saline treatment (Savage et al. 1982). An apparent threshold for chloroform induction of ODC activity was indicated below 100 mg/kg. Chloroform also induced hepatic nuclear RNA polymerase I, another enzyme associated with hepatic hyperplastic response. However, in the kidney, which had 300 fold higher levels of ODC activity than the liver, chloroform treatment resulted in a 35% reduction in ODC activity. Pereira et al. (1984b) reported that i.p. administration of chloroform to male mice and rats resulted in a dose-dependent increase in hepatic and renal ODC activity in both species, which was maximal at 375 mg/kg in the mouse and 750 mg/kg

in the rat. However, ODC induction did not parallel DNA synthesis. Based on the decrease in DNA synthesis following chloroform administration, Pereira et al. (1984b) stated that the induction of hepatic and renal ODC activity does not appear to be associated with a regenerative response. Much work remains to elucidate the association, if any, between ODC induction and the mechanism of tumor promotion, and to relate biochemical changes induced by chloroform to the carcinogenicity of chloroform.

7.8. Cell Transformation Assays

7.8.1. BHK Cells

Daniel and Dehnel (in de Serres and Ashby, 1981) reported that chloroform was positive in a cell transformation test with baby hamster kidney cells incubated in suspension with or without rat liver S-9 mix and chloroform. Five concentrations were used up to the LC50 derived from toxicity tests on the cells (Table 7-L). Transformed colonies (those of more than 500 um diameter) were counted and the transformation frequency calculated (number transformed colonies/million survivors). A transformation frequency of 5 times the negative control was taken to be a positive response. Chloroform was positive in the presence and in the absence of S-9 mix. The S-9 mix increased the toxicity of chloroform to this cell line, and also increased the spontaneous transformation rate, i.e., that occurring in the absence of chloroform. Sixteen of 23 known animal carcinogens and 11 of 14 putative noncarcinogens were positive in this assay. Daniel and Dehnel noted several problems with the assay, including inconsistent responses with the positive controls 2-acetylaminofluorene and N-methyl-N'-nitro-N-nitrosoguanidine and other known carcinogens, as well as with the negative control dimethylformamide used as a vehicle for some of the test substances. Daniel and Dehnel noted that the low specificity of this test system considerably reduces its value.

Styles (in de Serres and Ashby, 1981) reported that chloroform was negative in an assay for cell transformation using BHK cells. The criterion for significance was a fivefold greater transformation frequency in treated cells compared to controls. Twenty-two of 25 carcinogens and 5 of 17 noncarcinogens were positive in this assay. Chloroform did not induce an increase in transformation rate at doses up to and greater than the LC50 for this cell line (Table 7-L). These results contrast with those of Daniel and Dehnel. Methodological differences including temperature, source of serum in the medium, type of agar and viscosity, and a variety of other procedural details may account for the different results obtained in the two investigations (Styles, in de Serres and Ashby 1981).

7.8.2. Enhancement of Viral Transformation

Hatch et al. (1983) described a system designed to test the ability of volatile or gaseous chemicals to enhance the transformation of Syrian hamster embryo (SHE) cells. Exposure to chloroform vapors for 3 hours significantly enhanced the viral transformation frequency of cultured SHE cells in a dose-dependent manner (Table 7-L). Enhancement, expressed as the ratio of the transformation frequency of chloroform-treated survivors to

TABLE 7-L

Cell Transformation Assays of Chloroform

Reference	Test	Test Organism/Cell	Results[1]	Comments
Daniel and Dehnel (in de Serres and Ashby, 1981)	Cell Trans-formation	Baby hamster kidney (BHK 21 C13/HRC 1) cells	+	Duplicate cell cultures were incubated in suspension, with or without liver S9 fraction from Arochlor treated rats, in the presence of chloroform (5 concentrations up to the LC50, 6,380 ug/ml) for 4 h at 37°C. Washed cells were cultured for 21 days at 37°C and the number of transformed colonies (those of more than 500 um diameter) counted. Transformation frequency of 5 times the control value was taken as a positive response. The spontaneous TF = 1. Chloroform treatment at the LC50 in the absence of S9 resulted in a TF = 15, and a maximum observed TF = 40. The positive control N-methyl-N'-nitro-N-nitrosoguanidine produced a TF = 19. In the presence of S9, chloroform at the LC50 produced TF = 72, a Tmax observed = 800. The spontaneous TF and positive control (2-acetylaminofluorene) TF in presence of S9 were 14 and 140, respectively. See text for discussion of problem with assay.
Styles (in de Serres and Ashby, 1981)	Cell Trans-formation	BHK cells	-	Cells were incubated for 4 h at 37°C with 0, 0.025, 0.25, 2.5, 25, or 250 ug chloroform per ml in the presence of rat liver S9. No increase in TF in cells cultured for 21 days was observed at doses up to the LC50 in this cell line (250 ug/ml). A compound was judged to be positive if the TF was five fold greater than spontaneous TF.

Table 7-L continued

Reference	Test	Test Organism/Cell	Results[1]	Comments
Hatch et al. (1983)	Viral Trans-formation	Syrian Hamster Embryo Cells	+	Chloroform (0.12 to 2.0 ml) was added to petri dishes in sealed chambers containing monolayer cultures of SHE cells, and volatilized. Cells were exposed to the vapor for 3 hours, before addition of Simian adenovirus SA7. Virus was adsorbed for 3 hours, and cells then transferred for determination of viability and enhancement of transformation. Enhancement ratios (ER) were determined by dividing the transformation frequency (SA7 foci x 1/surviving fraction) of treated cells by that of controls. ER were 1.0, 0.7, 1.7, and 4.6 for 0, 0.12, 0.25, and 0.50 ml chloroform, respectively. The latter 2 ER's were significant at $p < .01$ and $p < .05$, respectively.

1. - = negative; + = positive

that of controls, was 1.7 and 4.6 for the 2 mid-dose groups. The vapors from the 2 highest exposures were 100% lethal. The survival fraction (the ratio of colonies derived from chemically treated virus-inoculated cells to that from control virus-inoculated cells) at the higher dose was 0.2, while at the lower dose, the survival fraction was 0.49. Hatch et al. (1983) stated that this bioassay correlated well with the results of *in vivo* bioassays for carcinogenicity.

7.9 Epidemiological Studies

There is no information currently available in the open literature which examines the potential relationship between exposure to chloroform in an occupational setting and human cancer. However, several studies are available which examine the relationship between drinking water and human cancer, some of which emphasize exposure to trihalomethanes, especially chloroform. Present epidemiological evidence suggests an association between chlorinated drinking water consumption and human cancer, particularly bladder and gastrointestinal cancers. However, these relationships cannot be directly correlated to chloroform exposure because many other carcinogens are found in drinking water including other chlorinated halomethanes, brominated halomethanes, industrial pollutants, and nonvolatile halogenated compounds. Epidemiological studies were not useful for the risk assessment. Some of the larger studies are discussed at length in Appendix A.

8.0. Quantitative Estimate of Cancer Risk

8.1. Introduction

Chloroform is carcinogenic to rats and mice (NCI, 1976; Roe et al. 1979; Jorgenson et al. 1985). The International Agency for Research on Cancer (IARC) has classified chloroform as a possible human carcinogen (Group 2B). Similarly, the U.S. EPA has placed chloroform in Group B2 in their classification scheme, based on sufficient evidence of carcinogenicity in animals, but inadequate epidemiologic evidence. The carcinogenicity of chloroform was reviewed in Section 7. The estimation of cancer risk to humans from exposure to chloroform based on animal studies is discussed in this section.

8.2. Possible Mechanisms of Chloroform Carcinogenesis

The mechanism by which chloroform induces tumors in experimental animals has not been fully elucidated. It is commonly accepted that carcinogens may induce cancer by damaging DNA. This damage may result from direct interaction of a chemical or agent (e.g., virus, radiation) with the DNA producing a mutation, which, if left unrepaired, is later expressed upon cellular replication. Chloroform is metabolized in mammalian species by the cytochrome P-450 dependent monooxygenases to phosgene. Phosgene may undergo spontaneous hydrolysis to form CO_2 and HCl, or react with cellular macromolecules. Several studies have demonstrated that radiolabeled chloroform metabolites covalently bind to cellular proteins and lipids, and that the extent of binding is related to nephro- and hepatotoxicity (Reynolds and Yee, 1967; Ilett et al. 1973; Smith and Hook, 1984; Uehleke and Werner, 1975). DiRenzo et al. (1982) reported that radiolabeled chloroform metabolites could bind to calf thymus DNA *in vitro*. Reitz et al. (1982) also reported that radiolabel was bound to DNA following exposure to ^{14}C-chloroform, but the binding index was weak (see section 2.3.4. on covalent binding). However, chloroform has been largely negative in bacterial and fungal mutagenicity assays. A few important exceptions include one positive result in a host-mediated assay (San Agustin and Lim-Sylianco, 1978), questionable positives in the *Bacillus subtilis* rec assay (Kada and Ichinotsuba, both in de Serres and Ashby, 1981), and positive results in *Saccharomyces cerevisiae* strain D7 (Callen et al. 1980). Similarly, chloroform has been tested in a number of mammalian systems and the results were largely negative. However, a few positive results were reported in tests for sister chromatid exchange in cultured human lymphocytes and mouse bone marrow cells *in vitro* (Morimoto and Koizumi, 1983), and in the mouse bone marrow micronucleus test (San Agustin and Lim-Sylianco, 1978). In addition, chloroform was a mitotic poison in an invertebrate test system (Liang et al. 1983).

Despite clearly positive results in a few genotoxicity assays, and because chloroform was more frequently negative than positive in genotoxicity tests, chloroform is considered a nongenotoxic or epigenetic carcinogen by some scientists (Reitz et al., 1982; Reitz, 1987). Postulated mechanisms of action for nongenotoxic carcinogens include, but are not limited to, stimulation of cell division leading to expression of background DNA damage, and stimulation of growth of neoplastic cells into tumors. The argument has been made that only doses which cause necrosis in the liver are associated

with increased incidences of tumors, and, therefore, chloroform is probably an epigenetic carcinogen (Reitz et al., 1980). However, doses which caused kidney tumors in male rats did not greatly stimulate cellular regeneration (Reitz et al. 1982). The metabolism of chloroform in liver and kidney, target organs for carcinogenicity, leads to highly reactive phosgene, a plausible alkylating agent that binds to cellular protein and lipid. This observation coupled with the weak genotoxic activity of chloroform provides some evidence for classifying chloroform as a genotoxic agent. However, current evidence and understanding of the carcinogenic process is insufficient to classify chloroform as either a genotoxic or epigenetic carcinogen, and it is possible that both types of effects are involved.

8.3. Choice of Risk Model

Since there is no adequate information regarding the carcinogenicity of chloroform to humans from epidemiological studies, data from animal bioassays must be extrapolated to estimate cancer risk for humans. This requires extrapolation across routes (from oral to inhalation), and across species, and extrapolation from high experimental doses to low environmental exposure experienced by humans. The latter two of these extrapolations are problematic. There are several models available for high dose to low dose extrapolation within species. No model is universally accepted as the best, since, given current understanding of the carcinogenic process, there is little scientific basis for choosing one model over another. Evidence from mutagenicity studies with ionizing radiation and some chemicals indicates that the quantal type of biological response observed in mutagenesis and possibly in carcinogenesis may exhibit linear non-threshold dose-response characteristics (Upton, 1988). This model may be more relevant to carcinogens which are initiators than to those that may be working by an epigenetic mechanism. However, there is not enough evidence to classify chloroform as either an epigenetic or genotoxic carcinogen, and chloroform may induce both types of effects. Cancer is a multistage process, and chloroform or its metabolites may affect one or more stages in the carcinogenic process. Assuming that chloroform is acting at least partially through a genotoxic mechanism to produce cancer, a non-threshold linear model was chosen for this risk estimate.

The linearized multistage model of Howe and colleagues (1986), GLOBAL86, was used to estimate the cancer potency of chloroform. This model uses bioassay data to compute maximum likelihood estimates and upper 95% confidence limits of risk associated with a particular dose. The 95% upper bound is regarded as the upper limit of the true risk. The true risk is not likely to be higher than the upper limit, may be lower than the upper limit or could be zero. The linearized multistage model yields upper bound estimates of risk which are generally regarded as protective of public health.

The linearized multistage model is based on several assumptions about the process of carcinogenesis. It is assumed that cancer is an irreversible process which originates in a single cell, and involves a number of biological events or stages. The rate of occurrence of each stage varies linearly with dose. In addition, it is assumed that the incidences of background and chemically-induced cancer are additive.

The mathematical expression of the linearized multistage model is:

$$P(d) = 1 - \exp -(q_0 + q_1 d + q_2 d^2 + \ldots + q_k d^k) \qquad \text{[Eq. 1]}$$

where P(d) is the lifetime probability of tumor developing at dose d, k is the number of stages, and $q_i \geq 0$, $i = 0, 1, 2, \ldots, k$, are coefficients calculated to give the best fit to the experimental data (maximum likelihood estimates) subject to the constraint that all qi are ≥ 0.

In order to determine the extra risk over background rate at dose d, or

$$P_e(d) = \frac{P(d) - P(0)}{1 - P(0)} \qquad \text{[Eq. 2]}$$

the equation takes the form:

$$P_e(d) = 1 - \exp -(q_1 d + q_2 d^2 + \ldots + q_k d^k) \qquad \text{[Eq. 3]}$$

At low doses, the extra risk function is approximated by:

$$P_e(d) = q_1 d \qquad \text{[Eq. 4]}$$

The model calculates point estimates and 95% upper confidence limits of the coefficients q_i, i=0,1,2,...,k, and the extra risk at a given dose d, by maximizing the likelihood function of the data. The 95% upper confidence limit of the coefficient q_1, q_1^*, is an estimate of the cancer potency of the carcinogen in question, and is expressed in units of inverse dose. The q_1^* is used to obtain an upper bound on the risk associated with a given dose and the lower bound on dose producing a given risk. The model computes directly the largest value of risk (upper bounds) consistent with the data and the smallest dose corresponding to a fixed risk which is consistent with the data. The selection of the number of stages, k, used in the multistage is done within the program according to an algorithm described in Howe et al. (1986). The asymptotic Chi-square distribution of the log-likelihood ratio is the basis for judging goodness-of-fit of the model to data in the observed range. In addition, GLOBAL86 incorporates a second technique for assessing goodness of fit based on a Monte Carlo simulation (Howe et al. 1986). Both techniques were used in the calculations of q_1^*.

8.4. Bioassay Data Used in the Risk Estimation

Data from animal carcinogenesis bioassays used in the risk estimation were chosen based primarily on statistical significance of the results. Liver tumor data in male and female B6C3F1 mice, and renal tubular cell tumors in male Osborne-Mendel rats from the NCI (1976) study were chosen because statistically significant increases in these tumor types were observed in chloroform treated animals relative to controls. In addition, renal tubular cell tumor data in male Osborne-Mendel rats from the Jorgenson et al. (1985) study and in male ICI mice in the Roe et al. (1979) study were used for risk estimation based on a statistically significant increase in kidney tumors in chloroform treated animals relative to controls. In addition, liver cholangiocarcinoma ("adenofibrosis", see Section 7.5) data in female rats from Tumasonis et al. (1985), and from Reuber's reanalysis of the NCI (1976)

slides (Reuber, 1979) were also analyzed with the linearized multistage model. Data input is summarized in Table 8-A. Administered doses were transformed to lifetime doses by adjusting for the number of days exposed per week and the ratio of the length of exposure to the length of the experiment (exposure plus observation period) as indicated in Table 8-A, footnote a.

8.5. Results of Linearized Multistage Model Analysis

The maximum likelihood estimates (q_1) and 95% upper confidence limits (q_1^*) of the cancer potency slopes are presented in Table 8-B. Calculated q_1^* values ranged from 8.1×10^{-4} to 1.9×10^{-2} $(mg/kg\text{-}d)^{-1}$. These represent cancer potency estimates for rats and mice and must be converted to theoretical equivalent potency values for humans. This conversion can be based on assuming either equivalency of dose per body weight or per unit surface area. In the former case, it is assumed that a dose, expressed as amount of chemical per unit body weight, is equally effective across species. In the latter case, it is assumed that the best method of dose scaling is on a surface area basis, such that an equally efficacious dose is expressed as amount per unit of body surface area. There is very little information supporting one scaling assumption over the other. However, evidence for scaling on a surface area basis has been reported for alkylating agents (Freiereich et al. 1966). In addition, the U.S. EPA in their Health Assessment Document for Chloroform (1985a) concluded that dose scaling on a surface area basis may be warranted for chloroform, based on metabolic rates reported in Brown et al. (1974a), Taylor et al. (1974), and Fry et al. (1972) in rodents, primates and humans. The amount of chloroform metabolized by rats, mice, monkeys, and humans was estimated by EPA staff for a common dose of 60 mg/kg given orally, using data from the above studies. When plotted as the logarithm of the amount metabolized versus the logarithm of the species body weight in accordance with the allometric relationship $Y = aW^n$ or, $\ln Y = \ln a + n \ln W$, it appears that the amount metabolized is closely related to surface area (Figure 8-1, U.S. EPA, 1985a). The slope of the line is 0.65, providing some evidence that chloroform metabolism is proportional to surface area ($W^{2/3}$). While the EPA's analysis does provide some support for dose scaling on a surface area basis, it must be noted that data on metabolism in humans is limited to amount of unchanged ^{13}C-chloroform and $^{13}CO_2$ excreted via the lungs (Fry et al. 1972). These data are based on a small number of human volunteers (N = 1 at the high dose), and is highly variable (amount excreted unchanged ranged from 18 to 67% after a single dose of 0.5 g chloroform). In addition, the assumption inherent in surface area scaling based on metabolism is that metabolites of chloroform are solely responsible for the carcinogenic effect. Despite these limitations, a surface area dose scaling was used in DHS' risk assessment. Therefore, the animal cancer potency estimates are corrected for surface area differences according to Anderson et al. (1983). Animal cancer potency is multiplied by the cube root of the ratio of a reference human body weight, in this case 70 kg, and a reference

TABLE 8-A
Tumor Incidence Data Used to Estimate Cancer Potency

STUDY	STRAIN/SPECIES	SEX	LIFETIME DOSE[a]	TUMOR TYPE	TUMOR INCIDENCE 1	2	3	4	5
NCI (1976)	B6C3F1 Mouse	M	1. Control 2. 83 (138) 3. 167 (277)	hepatocellular carcinoma	1/18	18/50	44/45	--	--
NCI (1976)	B6C3F1 Mouse	F	1. Control 2. 143 (238) 3. 287 (477)	hepatocellular carcinoma	0/20	36/45	39/41	--	--
NCI (1976)[b]	Osborne-Mendel Rat	M	1. Control 2. 45 (90) 3. 90 (180)	renal tubular adenoma or adenocarcinoma	0/19	4/38	12/27	--	--
Jorgenson[c] et al. (1985)	Osborne-Mendel Rat	M	1. Control 2. 18 3. 38 4. 79 5. 155	renal tubular adenoma or adenocarcinoma	4/301	4/313	4/148	3/48	7/50
Roe et al. (1979) Expt. I	ICI Mouse	M	1. Control 2. 12 (17) 3. 43 (60)	renal tubular adenoma or adenocarcinoma[d]	0/72	0/37	8/37	--	--
Roe et al. (1979) Expt. II	ICI Mouse	M	1. Control 2. 40 (60)	renal tubular adenoma or adenocarcinoma[d]	6/237	9/49	--	--	--
Roe et al. (1979) Expt. III (t)[e]	ICI Mouse	M	1. Control 2. 42 (60)	renal tubular adenoma or adenocarcinoma[d]	1/49	5/47	--	--	--
Roe et al. (1979) Expt. III (ao)[e]	ICI Mouse	M	1. Control 2. 42 (60)	renal tubular adenoma or adenocarcinoma[d]	1/50	12/48	--	--	--

TABLE 8-A, Continued

STUDY	STRAIN/SPECIES	SEX	LIFETIME DOSE[a]	TUMOR TYPE	1	2	3	4	5
Tumasonis et al. (1985)	Wistar Rat	F	1. Control 2. 220	cholangiocarcinoma[f]	0/18	34/40	--	--	--
Tumasonis et al. (1985)	Wister Rat	M	1. Control 2. 160	cholangiocarcinoma[f]	0/22	17/28	--	--	--
Reuber (1979)[g] using NCI (1976)	Osborne-Mendel Rat	F	1. Control 2. 50 (100) 3. 100 (200)	cholangiocarcinoma and cholangiofibroma	0/20	3/39	11/39	--	--

a. Lifetime dose, expressed as mg/kg-d, is calculated as in Anderson et al. (1983), adjusting from administered dose (in parentheses) to lifetime daily exposure as follows:

$$\text{Lifetime dose} = D \times d/7 \times l_e/L_e$$

where D = administered dose (in mg/kg-d).
d = number of days dosed per week.
l_e = length of exposure (weeks).
L_e = length of experiment (weeks) (dosing period plus observation period).

In Jorgensen et al. and Tumasonis et al., the dose given is the applied dose; no correction to lifetime dose was needed.

b. The tumor incidence used to estimate cancer potency is corrected to exclude from the denominator animals which died before the occurrence of the first tumor of this type.

c. The doses presented here are slightly different than those reported by Jorgenson et al. (1985). The authors collected data on body weight and water consumption every week for the first 18 weeks, and every 4th week thereafter. In calculating the mean dose in mg/kg-d, the investigators weighted each data point equally. Recalculation by Dr. John Christopher, CDHS, resulted in the doses presented here.

d. The original paper reported these tumors as benign (adenomas) or malignant (adenocarcinomas). However, in a conversation with Dr. John Christopher of the California Department of Health Services, Dr. F.J.C. Roe has confirmed that these tumors were adenomas and adenocarcinomas.

Table 8-A, Continued

e. t = toothpaste base was the vehicle; ao = arachis oil was the vehicle.

f. These tumors were identified as hepatic adenofibrosis by Tumasonis et al. (1985), based on a report by the National Academy of Sciences. However, the paper qualifies this by stating that the lesions are similar to those described by others as cholangiocarcinomas. See text for discussion.

g. Rueber (1979) examined tissue slides from the NCI (1976) bioassay of chloroform and reported on increased incidence of cholangiocarcinoma and cholangiofibroma in female rats.

TABLE 8-B

Upper 95% Confidence Limits for Cancer Potency Estimate from Linearized Multistage Model (GLOBAL 86)

DATA BASE	MLE[a] q_1	ANIMAL q_1*[b] $(mg/kg\text{-}d)^{-1}$	HUMAN q_1*[c] $(mg/kg\text{-}d)^{-1}$	UNIT[d] RISK $(ppb)^{-1}$
NCI (1976), male mouse, hep.[e] carc.	0	3.4×10^{-3}	4.3×10^{-2}	3.0×10^{-5}
NCI (1976), female mouse, hep. carc.	1.1×10^{-2}	1.9×10^{-2}	2.6×10^{-1}	1.8×10^{-4}
NCI (1976), male rat, ren. tub. aden. or adenocarc.	6.6×10^{-4}	4.6×10^{-3}	2.6×10^{-2}	1.8×10^{-5}
Jorgenson et al. (1985), male rat, ren. tub. aden. or adenocarc.	1.1×10^{-4}	8.1×10^{-4}	4.2×10^{-3}	2.9×10^{-6}
Roe et al. (1979), Expt. I, male mouse, ren. tub. aden. or adenocarc.	0	3.9×10^{-3}	4.3×10^{-2}	3.0×10^{-5}
Roe et al. (1979), Expt. II, male mouse, ren. tub. aden. or adenocarc.	2.2×10^{-3}	7.8×10^{-3}	8.7×10^{-2}	5.9×10^{-5}
Roe et al. (1979), Expt. III(t), male mouse, ren. tub. aden. or adenocarc.	1.1×10^{-3}	4.7×10^{-3}	5.3×10^{-2}	3.7×10^{-5}
Roe et al. (1979), Expt. III (ao), male mouse, ren. tub. aden. or adenocarc.	3.2×10^{-3}	1.2×10^{-2}	1.4×10^{-1}	9.8×10^{-5}
Tumasonis et al. (1985), female rat, cholangiocarc.[f]	4.3×10^{-3}	1.2×10^{-2}	8.7×10^{-2}	5.9×10^{-5}
Tumasonis et al. (1985), male rat, cholangiocarc.[f]	2.9×10^{-3}	8.6×10^{-3}	5.3×10^{-2}	3.4×10^{-5}
Reuber (1979), using NCI (1976), female rat, cholangiocarc. and cholangiofib.	6.3×10^{-4}	3.6×10^{-3}	1.8×10^{-2}	1.3×10^{-5}

a. MLE = maximum likelihood estimate of the coefficient, q_1.

b. Animal q_1* is the 95% upper confidence limit of the coefficient q_1 adjusted for less-than-lifetime exposure, where applicable, by the factor $(L/L_e)^3$, where L_e = length of experiment, and L = normal lifespan of animal, equal to 104 weeks or L_e, whichever is greater.

TABLE 8-B, continued

c. Human q_1^* is the equivalent human cancer potency calculated based on a surface area extrapolation of dose such that:

$$\text{Human } q_1^* = \text{Animal } q_1^* \ (70 \text{ kg}/BWa)^{1/3}$$

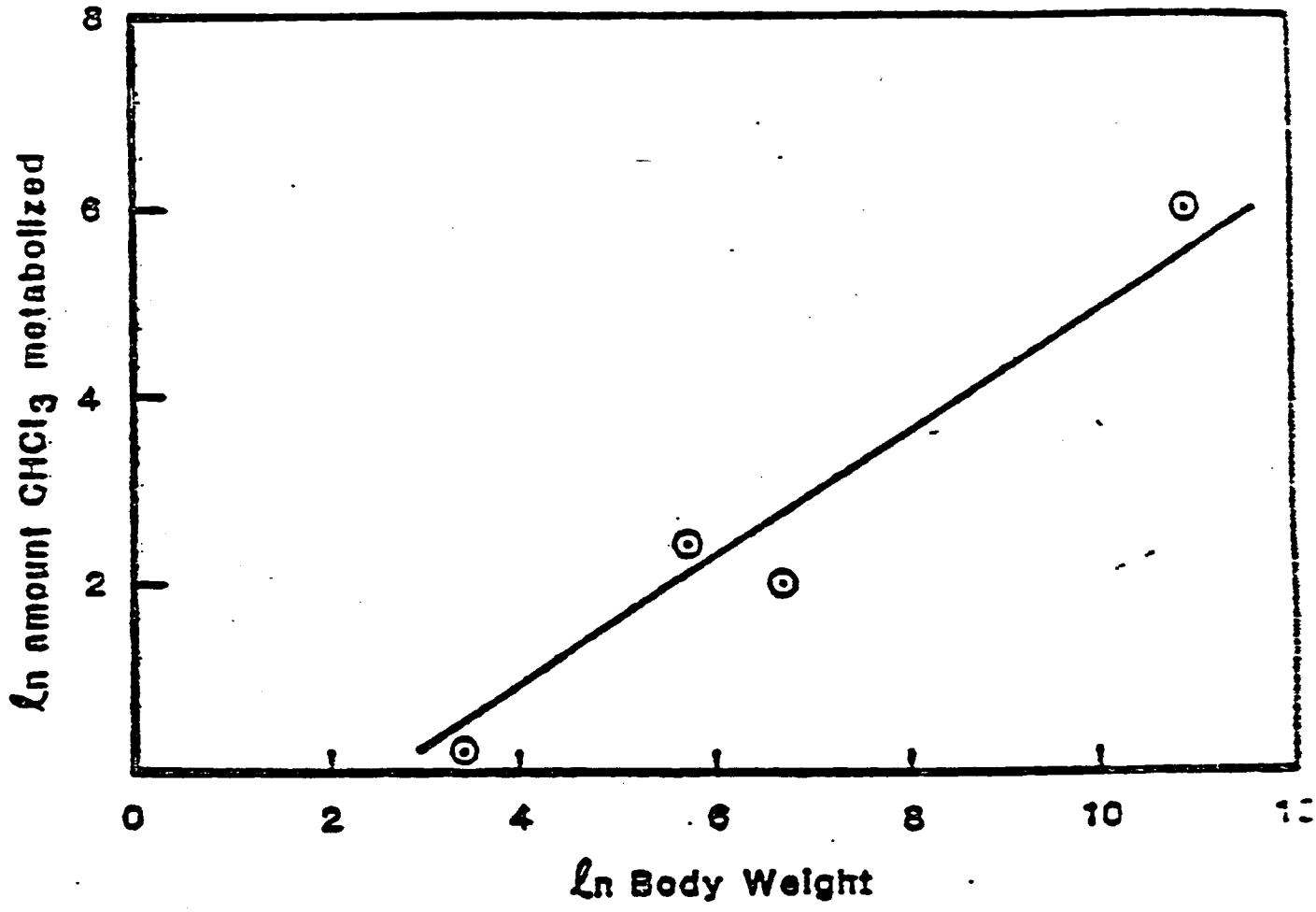
where BWa = body weight of the animal (kg) at midstudy.

$$\text{Risk of cancer} = (\text{Human } q_1^*) \ (\text{dose}).$$

d. Unit risk = Risk associated with continuous lifetime exposure to a chloroform concentration of 1 ppb in air. See text for discussion. Calculation based on 70 kg reference human breathing 20 m^3/day and absorbing 50% of inhaled chloroform at ambient levels (ppb range).

e. Other abbreviations: hep. carc. = hepatocellular carcinoma
ren. tub. aden. = renal tubular adenoma
adenocarc. = adenocarcinoma
cholangiocarc. = cholangiocarcinoma
cholangiofib. = cholangiofibroma

f. These tumors were identified as hepatic adenofibrosis by Tumasonis et al. (1985), based on a report by the National Academy of Sciences. However, the paper qualifies this by stating that the lesions are similar to those described by others as chloangiocarcinomas. See text for discussion.



Allometric relationship ($Y = aW^n$) between species body weight (in order: mouse, rat, squirrel monkey, and man) and the amount metabolized of a common oral dose of chloroform as calculated from the data of Fry et al. (1972) and Brown et al. (1974). The species body weights assumed were: mouse, 30 g; rat, 300 g; squirrel monkey, 850 g; and man, 70 kg. The slope of the regression line is 0.65, indicating that metabolism of chloroform in these species is proportional to their surface area.

Figure 8-1. Relationship between body weight and metabolism of chloroform by different species. From: U.S. EPA (1985a).

animal body weight (taken from the study as body weight mid-study). For example, adjusting the animal potency derived from female mouse liver tumor data in NCI (1976) to a human potency estimate would proceed as follows:

$$\text{Human } q_1^* = \text{Animal } q_1^* \ (70 \text{ kg}/0.028 \text{ kg})^{1/3}$$

$$= (1.9 \times 10^{-2}) \ (13.57) = 2.6 \times 10^{-1}$$

These "human" cancer potencies (Table 8-B), adjusted by applying a surface area correction factor, range from 4.2×10^{-3} to 2.6×10^{-1} $(\text{mg/kg-day})^{-1}$. Scaling factors ranged from 5.19 to 13.57.

Potency slopes were derived from many sets of data, some of which are more appropriate for use than others. In particular, the NCI (1976) and Jorgenson et al. (1985) studies were the most thorough studies in terms of the number of doses tested, sample size, histological examination of the animals, and other procedural and statistical methods presented. As such, DHS places more confidence in the potency slopes from these studies than in the other studies. The potency slopes derived from Roe et al. (1979) and Tumasonis et al. (1985) fall within the range of those from the NCI and Jorgenson studies.

8.6. Other Estimates of Cancer Potency Slope for Chloroform

DHS contracted with the Lawrence Livermore National Laboratories Environmental Health group to assess the risk associated with chloroform in drinking water (Bogen et al., 1989). LLNL scientists used a physiologically based pharmacokinetic (PBPK) model to estimate metabolized dose for chloroform to use in the analysis of cancer potency with the linearized multistage model. LLNL employed the Ramsey-Andersen PBPK model (Ramsey and Andersen, 1984), which consists of a series of differential equations defining the rates of change of the amount of absorbed chloroform in four physiological compartments. In the application of the model, the liver was considered to metabolize chloroform through a saturable enzyme system following Michaelis-Menten kinetics. The model predictions of metabolism rates are compared with published empirical data for rats, mice, and humans. Values for V_{max} used in the model are adjusted so that the model predicts chloroform metabolism observed in selected studies. The PBPK model then is used to estimate the metabolized doses from administered doses in rodent carcinogenicity bioassays to use in estimating cancer potency. This approach is consistent with the evidence that chloroform metabolites are responsible for toxicity and probably for the carcinogenicity of chloroform. A linearized multistage model was applied to carcinogenicity bioassay data from NCI (1976), Jorgenson et al. (1985), Roe et al. (1979), and Tumasonis et al. (1985). The potency estimates made from these studies (excluding Reuber's (1979) reanalysis of NCI data) ranged from 4.8×10^{-3} to 5.0×10^{-1} $(\text{mg M/kg-d})^{-1}$. These correspond to unit risks of 4.5×10^{-6} to 4.7×10^{-4} $(\text{ppb})^{-1}$. The lower estimate was derived from data on renal adenomas and carcinomas in male Osborne-Mendel rats reported in Jorgenson et al. (1985). The upper end of the range was derived from the data on hepatocellular carcinoma in female B6C3F1 mice reported in NCI (1976). The potency estimates derived using a PBPK model to estimate metabolized dose are higher than those calculated by DHS staff based on administered dose. These

potency estimates are incorporated into DHS staff's best estimate of cancer potency for chloroform described is section 8.7.

The U.S. EPA Carcinogen Assessment Group (CAG) used data from the NCI (1976), Roe et al. (1979), and from Jorgenson et al. (1985) to estimate cancer potency with several models (U.S. EPA, 1985a). Emphasis was placed on the linearized multistage model (EPA used GLOBAL83), which was presented as a reasonably conservative model useful for estimating cancer risk to humans from the available animal data. The major difference between DHS' estimate of cancer potency and that of the U.S.EPA CAG is the assessment of a lifetime average daily exposure. The EPA decreased the administered dose by 6% for mice and 20% for rats on the premise that mice and rats metabolized 94% and 80% of the administered dose, respectively, and that the remainder was excreted unchanged and therefore did not affect carcinogenesis. No adjustment was used for the amount metabolized by humans other than use of a surface area dose scaling factor.

The EPA's estimates of cancer risk for a dose of 1 mg/kg/day (equivalent to the q_1^* in units of $(mg/kg\text{-}day)^{-1}$) calculated from animal data using the linearized multistage model are shown in Table 8-C, together with estimates based on other models. The q_1^* values derived by the EPA are quite similar to those derived by DHS using Global 86 (Table 8-B), despite the different assumptions regarding dose. The risk for a dose of 1 mg/kg/d derived by the EPA from both the Weibull and one-hit models are within one order of magnitude of those derived by the linearized multistage model for all the data sets. The U.S. EPA used a geometric mean of the 95% upper bounds on q_1^* calculated with the linearized multistage model from liver tumor data in male and female mice in the NCI study as an estimate of the cancer potency slope for chloroform. The cancer potency slope presented in U.S. EPA (1985a) is 8.1×10^{-2} $(mg/kg\text{-}day)^{-1}$. In addition, the U.S. EPA published a revised risk estimation for chloroform in the Integrated Risk Information System (U.S. EPA, 1987). In this publication, the cancer potency slope previously derived (U.S.EPA, 1985a) was retained for use with inhalation exposures. Another cancer potency slope was suggested for use with oral exposures. The oral exposure number, 6.1×10^{-3} $(mg/kg\text{-}day)^{-1}$, was derived using the linearized multistage model with data on kidney tumors in male rats following exposure to chloroform in drinking water from Jorgenson et al. (1985). It is not clear why this number differs slightly from the number reported for the same data set in U.S. EPA (1985a) (Table 8-C).

8.7. Estimate of Risk Associated with Exposure to Chloroform in Ambient Air

There are no studies on the carcinogenicity of chloroform by the inhalation route. Therefore, estimation of the cancer risk from exposure to chloroform in the ambient air requires extrapolation from the oral route. In so doing, it is assumed that chloroform is also carcinogenic by the inhalation route, and that the risk posed by an absorbed inhaled dose of chloroform is equivalent to that posed by the same dose absorbed after oral administration. These assumptions are not necessarily health-protective and introduce further uncertainty in the risk estimation process.

TABLE 8-C

95% Upper Confidence[a] Limits of Lifetime Cancer Risk for Humans from a Dose of 1 mg/kg/day Calculated from Animal Data by the U.S. Environmental Protection Agency.
From: U.S. EPA (1985a)

DATA BASE	MULTISTAGE	PROBIT	WEIBULL	ONE-HIT
Liver tumors in female mice (NCI, 1976)	1.8×10^{-1}[b]	2.1×10^{-1}	4.8×10^{-1}	1.8×10^{-1}[b]
Liver tumors in male mice (NCI, 1976)	3.3×10^{-2}	6.7×10^{-11}	3.2×10^{-3}	1.6×10^{-1}
Kidney tumors in male rats (NCI, 1976)	2.4×10^{-2}	3.9×10^{-4}	3.0×10^{-3}	2.5×10^{-2}
Kidney tumors in male mice (Roe et al., 1979)	1.0×10^{-1}	NA	NA	1.0×10^{-1}
Kidney tumors in male rats (Jorgenson et al., 1985)	4.4×10^{-3}	9.0×10^{-5}	4.8×10^{-4}	5.4×10^{-3}

a - Upper-bound estimates are calculated by the one-sided 95% confidence limit.
b - At 1 mg/kg/day, the dose-response curve diverges from a straight line. For lower doses the dose-response slope is 2.0×10^{-1} per mg/kg/day.
NA - Not applicable. Models are not applicable because there is only one dosed group.

Staff relied on tumor sites that did not appear to be vehicle-dependent. Thus, the liver tumors were not included in the range of risks or the best estimate of risk.

8.7.1. Best Estimate of Unit Risk

DHS staff believe that a best estimate of potency lies somewhere between the lowest and highest estimates of potency presented in this document. In deriving a best estimate, DHS staff evaluated the quality of the data sets used to derive potency estimates with the linearized multistage model.

The study by Jorgenson et al. (1985) was well-conducted and well controlled. Large numbers of animals were used in the control and lower dose groups to increase the probability of detecting an effect. The study used 5 doses including controls. In addition, water-restricted controls were used because drinking water consumption decreased proportionally to the dose of chloroform. Survival, which was inversely related to dose, did not adversely impact the assessment of carcinogenicity. The mode of administration, drinking water, may be more relevant to human exposure and is not likely to be a major factor in addressing potency as is the case with corn oil administration.

The NCI (1976) study has some limitations with respect to assessment of the carcinogenic potency of chloroform. In particular, the use of corn oil as a vehicle limits the usefulness of this study. As described in Section 7.3, published reports indicate that corn oil exacerbates hepatotoxicity of chloroform. Corn oil or fatty diets may increase tumor yield in rodents fed other carcinogens. The drinking water study of Jorgenson et al. (1985) provided no evidence for induction of hepatocellular carcinomas in female B6C3F1 mice at doses comparable to those in NCI (1976). Thus, the production of liver tumors may have been vehicle-dependent. Other problems with the applicability of the NCI (1976) study to assessment of chloroform cancer potency include a relatively small control group (20 animals/sex), poor survival in the treated rats, and change of dosing regimen during the study. DHS staff believe that the potencies derived using data sets on renal tumors from NCI (1976) should be included in the range of risks and best estimate. However, the hepatocellular carcinoma data in B6C3F1 mice should not be included in the range of risks or in the best estimate of potency or in the reported range of potencies because these tumors may be vehicle-dependent. The renal tumor data in male rats from NCI is included in the range and best estimate of risk. Renal tumors were produced regardless of the vehicle (eg, drinking water or corn oil gavage).

Other data sets which support the estimate of potency were included in the best estimate. The studies of Roe et al. (1979) and Tumasonis et al. (1985) have some limitations in usefulness. Most of the data is obtained after using only a control and one dose group. Roe et al. (1979) used toothpaste base or arachis oil as the vehicle. Nonetheless, potencies derived from these studies fell in the range of risks presented. In addition, data from Tumasonis et al. (1985) includes some female animals while the Jorgenson et al. (1985) study does not. A geometric mean was taken of the potencies generated from Roe et al. (1979) and Tumasonis et al. (1985) using the linearized multistage model based on the administered dose (CDHS, column D, Table 8-D) and the metabolized dose (Bogen et al., 1989 Table 8-D. column B for inhalation potency or column C for ingestion potency). Consequently,

Table 8-D Potencies used in the best estimate of unit risk and oral potency slope.

Data Set[a]	q_1^*(M)(mg M/kg-d)$^{-1}$[b]	q_1^*Ar(mg/kg-d)$^{-1}$[c]	q_1^*(Al(mg/kg-d)$^{-1}$[d]	DHS q_1^*
NCI (1976) MR, RA or RC	0.035	0.0232	0.0345	0.021
Jorgenson (1985) MR, RA or RC	0.0048	0.0032	0.0047	0.0043
Roe (1979)I MM, RA or RH	0.072	0.048	0.071	0.043
Roe II	0.11	0.075	0.108	0.087
Roe III (ao)	0.17	0.113	0.167	0.14
Roe III (tp)	--	--	--	0.053
Tumasonis (1985)[e] MR, HAF	0.012	0.008	0.0118	0.053
FR, HAF	0.019	0.0126	0.0187	0.087

[a]Data Sets from indicated references; MR = male rat, MM = male mouse, FR = female rat, RA = renal adenoma, RC = renal carcinoma, RH = renal hypernephroma, HAF = hepatic adenofibrosis, ao = arachis oil vehicle, tp = toothpaste vehicle, Roe I, II, III as described in Table 8-A.

[b]These potencies are those of Bogen et al. (1989) expressed as (mg metabolite/kg-d)$^{-1}$

[c]These potencies are equivalent to 0.662 q_1^*(M) and represent the potency of an administered dose of chloroform by inhalation. The correction factor is derived by Bogen et al. (1989).

[d]These potencies are equivalent to 0.986 q_1^*(M) and represent the potency of an orally administered dose of chloroform. The correction factor is derived by Bogen et al. (1989).

[e]DHS called the hepatic adenofibrosis tumors cholangiocarcinomas based on qualifying statements in the published article and pers communication with one of the authors (Brian Bush, NY State Health Department).

information from these eleven potency estimates were incorporated into the best estimate, but only following geometric averaging of the values to mininize the influence of the extremes. This composite value represented one of five values utilized in calculating the best estimates. This number was then weighted equally with potencies derived by CDHS (Table 8-D, column D) and by Bogen et al. (Table 8-D, column B for inhalation potency or column C for ingestion potency) from data on renal tumors in male rats from Jorgenson et al. (1985) and NCI (1976).

In order to include the potency slopes generated by Bogen et al. (1989) in the derivation of the best estimate for a unit risk, the upper 95% confidence limit for potency based on metabolized dose must be corrected to the potency based on administered inhaled dose. This is done with a correction factor of 0.662, as derived by Bogen et al. (1989) (see column B, Table 8-D). The correction factor incorporates pharmacokinetic information regarding the percent of inhaled chloroform absorbed at low levels of exposure as well as the percent of inspired volume reaching the alveoli. Unit risks were then calculated based on the assumptions that 70 kg humans breathe 20 m^3/day and that 1 ppb chloroform is equivalent to 4.88 $\mu g/m^3$.

In summary, the best estimate of unit risk is the arithmetic average of unit risks generated by CDHS and Bogen et al. (1989) for renal tumors in Jorgenson et al. (1985) and NCI (1976) and of the geometric mean for supporting data sets (from Roe et al., 1979 and Tumasonis et al., 1985) indicated in Table 8-D. This unit risk, 2.6×10^{-5} per ppb (equivalent to an inhalation potency slope of $2.6 \times 10^{-2}(mg/kg\text{-}day)^{-1}$) (Table 8-E), represents the best estimate using a nonthreshold linear model and most of the data on the carcinogenicity of chloroform. It includes analysis by PBPK modeling of metabolized dose, as well as analysis of potency based on applied dose. The best estimate of an oral potency slope is also presented (Table 8-E). This oral potency slope is different than the inhalation potency in that the upper bound for potency based on metabolized dose in Bogen et al. (1989) is corrected by the factor 0.986 (derived by Bogen et al.) to derive an oral potency based on administered dose. This correction factor applies pharmacokinetic information on absorption and metabolism of chloroform at low doses.

The risk associated with a given quantity of chloroform in air can be calculated by assuming a 70 kg person breathes 20 m^3 of air/day (NRC, 1986), and that a certain fraction of inhaled chloroform is absorbed across the lung. Raabe (1988) showed that about 50% of an inhaled dose of chloroform is absorbed across the human lung at low levels of exposure under steady-state conditions in mouth-breathing individuals, with slightly lesser amounts (46%) absorbed in nose-breathing individuals. This study is discussed in section 1.a. For the purposes of this risk assessment, it is assumed that 50% of inhaled chloroform at low environmental levels of exposure is absorbed across the human lung. A dose by inhalation for a given concentration of chloroform in air is calculated as follows:

Table 8-E Best Estimate of Unit Risk and Potency Slope for Chloroform

Unit Risk	2.6×10^{-5}	per ppb
Oral Potency Slope	3.1×10^{-2}	per mg/kg-d
Inhalation Potency Slope	2.6×10^{-2}	per mg/kg-d

$$d_I = \frac{(X \text{ ppb})\ (4.88\ \mu g/m^3/\text{ppb})\ (10^{-3} mg/\mu g)\ (20\ m^3/\text{day})\ (0.5)}{70\ \text{kg}} \qquad \text{[Eq. 6]}$$

$$= (X)\ (0.5)\ (1.39 \times 10^{-3})\ \text{mg/kg-d} = (X)\ 7.0 \times 10^{-4}\ \text{mg/kg-d}$$

Thus, the dose to a 70 kg reference human for a concentration X = 1 ppb in air is 7.0×10^{-4} mg/kg-d. The lifetime risk associated with this concentration in air, calculated as (human q_1^*) (dose) (Eq. 4), ranges from 2.9×10^{-6} to 9.8×10^{-5} based on applied dose, and up to 1.6×10^{-4} based on PBPK modeling in Bogen et al. (1989). This range represents the plausible upper bounds of risk associated with a concentration of 1 ppb in air, based on the extrapolation of experimental animal data to humans. The best estimate of risk of cancer from lifetime inhalation of 1 ppb in air is 2.6×10^{-5}, and includes estimates of cancer potency based on both administered and metabolized dose. The actual risk cannot be calculated but is not likely to be higher than the upper bounds and may be insignificant.

Chloroform is emitted into ambient air from a number of sources. The three largest sources are pulp and paper production, drinking water treatment, and production of pharmaceuticals (U.S. EPA, 1985b). Taken together, these three sources emit about 6900 metric tons per year of chloroform into ambient air in the United States. Concentrations of chloroform in ambient air were measured at various locations throughout California by the California Air Resources Board (CARB, 1987). Mean concentrations of chloroform measured in the years 1985 through 1987 ranged from 0.0257 ppb (0.125 $\mu g/m^3$) to 0.3604 ppb (1.759 $\mu g/m^3$). The population-weighted exposure for the state of California is 0.03 ppb for 20.3 million people. The risk of cancer by inhalation exposure to chloroform can be calculated using the unit risks as follows:

$$\text{Risk} = (\text{Risk/ppb})\ (\text{ppb}) \qquad \text{[Eq. 7]}$$

The risk, calculated according to Eq. 7 using the lowest and highest estimate of unit risk ranges from:

$$\text{Risk} = (2.9 \times 10^{-6}/\text{ppb})(0.03\ \text{ppb}) \approx 8.7 \times 10^{-8},$$

to $$\text{Risk} = (1.6 \times 10^{-4}/\text{ppb})(0.03\ \text{ppb}) \approx 4.8 \times 10^{-6}$$

for the population-weighted exposure. The top of the range of upper bounds on risk can be calculated using the highest average concentration of chloroform in air encountered in California and the highest unit risk obtained from the linearized multistage model (from female mouse liver tumor data in NCI, 1976, adjusted to a human potency) as in Eq.7. The highest estimate of risk from chloroform inhalation to people living in the area with the highest measured average chloroform concentration would be:

$$\text{Risk} = (1.6 \times 10^{-4}/\text{ppb})(0.3604\ \text{ppb}) \approx 5.8 \times 10^{-4}$$

Thus, if one million persons were exposed continuously for a lifetime to 0.36 ppb chloroform in air, there could be as many as 170 excess cancer cases. Using the population-weighted exposure for chloroform in ambient air in California (0.03 ppb), and the best estimate of unit risk (2.6×10^{-5} per

ppb), in the above equation the estimate of excess cancer cases drops to 0.8 per million persons exposed.

The California Department of Health Services (CDHS) has calculated potencies for a number of other chemicals classified as "toxic air contaminants". The unit risk estimates for these chemicals, reported as a range and expressed in units of $(\mu g/m^3)^{-1}$, are presented in Table 8-F. The range of chloroform potencies, i.e. 6.0×10^{-7} to $2.0 \times 10^{-5}/(\mu g/m^3)$, would fall in the lower half of potencies for those chemicals previously evaluated by DHS, based on mass (not corrected for molecular weight).

Exposure to chloroform occurs by other routes as well as through inhalation of ambient air. Most importantly, chloroform exposure occurs by ingestion of chlorinated drinking water. Most Californians live in urban areas where the water is chlorinated. Based on a hypothetical drinking water chloroform concentration of 40 $\mu g/l$ (the average of measurements made at 6 sites in California in the NORS survey, Symons et al. 1975), and a reference water consumption of 2 l/day, the 70 kg human would consume 1.14 μg chloroform/kg/d from the drinking water.

The risk to this hypothetical individual from drinking water can be calculated as in Eq. 4, using the total dose to the individual, and a human cancer potency estimate adjusted from the q_1^* calculated from animal data. For example, using the best estimate of human potency for ingestion exposure, the individual lifetime risk from ingestion of chloroform in drinking water would be:

$$[1.14\ \mu g/kg\text{-}d]\ [10^{-3}\ mg/\mu g]\ [3.1 \times 10^{-2}/mg/kg\text{-}d] = 3.5 \times 10^{-5}$$

If a million persons were thus exposed for a lifetime, the excess cancer cases may approach 35 from drinking water contaminated with chloroform.

These estimates of risk do not consider exposure to chloroform in indoor air, which may be substantial in homes as a result of volatilization from water during household activities (showering, clothes and dish washing, cooking) (Wallace, 1988). As stated previously, it is unlikely that the actual risk is higher. It may be much lower and may be insignificant.

These calculations assume 100% absorption across the gastrointestinal tract after ingestion in the drinking water, and 50% absorption across the lung. It is also assumed that an absorbed inhaled dose is equally effective in producing cancer as an absorbed oral dose. The latter assumption is not necessarily conservative, and both assumptions add further uncertainty to the risk calculation. Most of the average chloroform levels measured in ambient air are about an order of magnitude or so lower than the highest average value used in this example (CARB, 1987). The population-weighted average is 0.03 ppb. Therefore, the cancer risk to the average Californian from exposure to chloroform through water is probably greater than that from exposure to chloroform in ambient air, exclusive of potential occupational exposure. However, there are important public health benefits to chlorinating drinking water (e.g., reduction of water-borne diseases). There is no health benefit derived from exposure to chloroform in ambient air.

TABLE 8-F

Unit Risk Numbers* and Reported Range for Identified Toxic Air Contaminants

Toxic Air Contaminant	Reported Range of Unit Risks $(\mu g/m^3)^{-1}$
1. Benzene	$(0.75 \text{ to } 5.3) \times 10^{-5}$
2. Ethylene dibromide	$(1.3 \text{ to } 7.1) \times 10^{-5}$
3. Ethylene dichloride	$(1.3 \text{ to } 2.2) \times 10^{-5}$
4. Chromium (+6)	$(1.2 \text{ to } 14.6) \times 10^{-2}$
5. Dioxin (TCDD)	24 to 38
6. Asbestos	$(38 \text{ to } 190) \times 10^{-6}$ [a]
7. Cadmium (+2)	$(0.2 \text{ to } 18) \times 10^{-2}$
8. Carbon tetrachloride	$(1 \text{ to } 15) \times 10^{-5}$
9. Ethylene oxide	$(0.6 \text{ to } 8.8) \times 10^{-5}$

* 95% Upper Confidence Limits

[a] Units are expressed as $(100 \text{ fibres}/m^3)^{-1}$

The estimate of risk presented here is only an approximation of the true risk, which cannot be calculated. Differences between experimental animals and humans may influence actual risk. Such factors as target site susceptibility, diet, immunological responses, toxicokinetic variables, and genetics play a role in the carcinogenic process. The human population is much more heterogenous both genetically and culturally (diet, lifestyle) than experimental animal populations. The intraspecies variability in humans may be greater than in experimental animals because of such heterogeneity. The risk extrapolation process cannot account for these differences between humans and experimental animals. In addition, the assumption is made that the risk is linear with dose for chloroform. This assumption may or may not be valid. Nonetheless, risk estimates do provide a rough indication of the relative potency of carcinogens.

9.0 Chloroform References

Adriani J (1970) The Pharmacology of Anesthetic Drugs. Charles C. Thomas. Springfield, IL.

Ahmadizadeh M, Echt R, Kuo C-H, Hook JB. (1984a) Sex and strain difference in mouse kidney: Bowman's capsule morphology and susceptibility to chloroform. Toxicol Lett 20:161-172.

Ahmadizadeh M, Kuo C-H, Echt R, Hook JB. (1984b) Effect of polybrominated biphenyls, beta-naphthoflavone and phenobarbital on arylhydrocarbon hydroxylase activities and chloroform-induced nephrotoxicity and hepatotoxicity in male C57BL/6J and DBA/2J mice. Toxicology 31:343-352.

Ahmed AE, Kubic VL, Anders MW. (1977) Metabolism of haloforms to carbon monoxide. I. In vitro studies. Drug Metab Dispos 5:198-204.

Alavanja M, Goldstein I, Susser M. (1978) A case control study of gastrointestinal and urinary tract cancer mortality and drinking water chlorination. Water Chlorination: Environmental Impact and Health Effects, V.2. Jolley RL, Gorchev H, Hamilton DH Jr., eds. pp 395-409. Ann Arbor Science Publishers. Ann Arbor, MI.

Anders MW, Stevens JL, Sprague RW, Shaath Z, Ahmed AE. (1978) Metabolism of haloforms to carbon monoxide. II. In vivo studies. Drug Metab Dispos 6:556-560.

Anderson EL, Carcinogen Assessment Group of the U.S. EPA. (1983) Quantitative approaches in use to assess cancer risk. Risk Analysis 3:277-295.

Aniya Y, Anders MW. (1985) Chloroform-induced alteration of glutathione S-transferase activity. Biochem Pharmacol 34:249-255.

Armitage P, Doll R. (1954) The age distribution of cancer and multi-stage theory of carcinogenesis. Br J Cancer 8:1-12.

Bailie, MB, Smith JH, Newton JF, Hook JB. (1984) Mechanism of chloroform nephrotoxicity. IV. Phenobarbital potentiation of in vitro chloroform metabolism and toxicity in rabbit kidneys. Toxicol Appl Pharmacol 74: 285-292.

Balster RL, Borzelleca JF. (1982) Behavioral toxicity of trihalomethane contaminants of drinking water in mice. Environ Health Perspect 46:127- 136.

Belfiore F, Zimmerman HJ. (1970) Cytotoxicity of chlorinated hydrocarbons in vitro. Observations of chloroform-induced hemolysis (34728). Proc Soc Exp Biol Med 134:61-66.

Bogen KT, Hall LC, McKone TE. (1989). Draft. Health Risk Assessment of Chloroform in Drinking Water. Report Number UCRL - 21170. Environmental Sciences Division. Lawrence Livermore National Laboratory, Livermore, CA.

Bomski H, Sobolewska A, Strakowski A. (1967) Toxische schadigung der leber durch chloroform bei chemiebetriebswerkern. Int Archiv fur Gewerbepathologie und Gewerbehygiene 24:127-134.

Bowman FJ, Borzelleca JF, Munson AE. (1978) The toxicity of some halomethanes in mice. Toxicol Appl Pharmacol 44:213-215.

Branchflower RV, Pohl LR. (1981) Investigation of the mechanism of the potentiation of chloroform-induced hepatotoxicity and nephrotoxicity by methyl n-butyl ketone. Toxicol Appl Pharmacol 61:407-413.

Branchflower RV, Schulick RD, George JW, Pohl LR. (1983) Comparison of the effects of methyl-n-butyl ketone and phenobarbital on rat liver cytochromes P-450 and the metabolism of chloroform to phosgene. Toxicol Appl Pharmacol 7:414-421.

Branchflower RV, Nunn DS, Highet RJ, Smith JH, Hook JB, Pohl LR. (1984) Nephrotoxicity of chloroform: Metabolism to phosgene by the mouse kidney. Toxicol Appl Pharmacol 72:159-168.

Brault D, Morliere P, Rougee M, Bizet C. (1978) Action du tetrachlorure de carbone et du chloroforme sur les hemes, en relation avec le role du cytochrome P-450 dans le metabolisme et l'hepatotoxicite des composes polyhalogenes. Biochimie 60:52.

Brenniman GR, Vasilomanolakis-Lagos J, Amsel J, Namekata T, Wolff AH. (1980) Case-control study of cancer deaths in Illinois communities served by chlorinated or nonchlorinated water. Water Chlorination: Environmental Impact and Health Effects, V.3. Jolley RL, Brungs WA, Cumming RB, eds. Ann Arbor Science. Ann Arbor, MI.

Brondeau MT, Bonnet P, Guenier JP, DeCeaurriz J. (1983) Short-term inhalation test for evaluating industrial hepatotoxicants in rats. Toxicol Lett 19:139-146.

Brown DM, Langley PF, Smith D, Taylor DC. (1974a) Metabolism of chloroform. I. The metabolism of ^{14}C-chloroform by different species. Drug Chem Toxicol 4:151-163.

Brown BR Jr, Sipes IG, Sagalyn AM. (1974b) Mechanisms of acute hepatic toxicity: Chloroform, halothane, and glutathione. Anesthesiology 41: 554-561.

Brown ES, Hewitt WR. (1984) Dose-response relationships in ketone- induced potentiation of chloroform hepato-and nephrotoxicity. Toxicol Appl Pharmacol 76:437-453.

Bull RJ, Brown JM, Meierhenry EA, Jorgenson TA, Robinson M, Stober JA. (1986) Enhancement of the hepatotoxicity of chloroform in B6C3F1 mice by corn oil: Implications for chloroform carcinogenesis. Environ Health Perspect 69:49-58.

Burkhalter JE, Balster RL. (1979) Behavioral teratology evaluation of trichloromethane in mice. Neurobehav Toxicol 1:199-205.

Butler TC. (1961) Reduction of carbon tetrachloride in vivo and reduction of carbon tetrachloride and chloroform in vitro by tissues and tissue constituents. J Pharmacol 134:311-319.

California Air Resources Board (1987) Memorandum from James F. Boyd to Kenneth Kizer on Evaluation of Chloroform. December 28, 1987.

Callen DF, Wolf CR, Philpot RM. (1980) Cytochrome P-450 mediated genetic activity and cytotoxicity of seven halogenated aliphatic hydrocarbons in Saccharomyces cerevisiae. Mutat Res 77:55-63.

Cantor KP, Hoover R, Mason TJ, McCabe LJ. (1978) Associations of cancer mortality with halomethanes in drinking water. JNCI 61:979-985.

Cantor KP, Hoover R, Hartge P, Mason TJ, Silverman DT, Altman R, Austin DF, Child MA, Key CR, Marrett LD, Myers MH, Narayana AS, Levin LI, Sullivan JW, Swanson GM, Thomas DB, West DW. (1987) Bladder cancer, drinking water source, and tap water consumption: A case-control study. J Natl Cancer Inst 79:1269-1279.

Capel ID, Dorrell HM, Jenner M, Pinnock MH, Williams DC. (1979a) The effect of chloroform ingestion on the growth of some murine tumours. Eur J Cancer 15:1485-1490.

Capel ID, Dorrell HM, Jenner M, Williams DC. (1979b) Effect of chloroform ingestion on some carcinogen-metabolising enzyme systems of rats. Bull Environ Contam Toxicol 23:112-116.

Capel ID, Jenner M, Dorrell HM, Williams DC. (1980) The effect of chloroform inhalation on hepatic glucuronidation and de-glucuronidation mechanisms. Drug Chem Toxicol 3:73-81.

Carere A, Bellincampi D, Conti G, Conti L, Crebelli R, Gualandi G, Morpurgo G. (1985) Genotoxic activity of selected chemical carcinogens in Aspergillus nidulans. Mutat Res 147:18.

Carlo GL, Mettlin CJ. (1980) Cancer incidence and trihalomethane concentrations in a public drinking water system. Am J Public Health 70: 523-525.

Castro JA, Diaz Gomez MI. (1972) Studies on the irreversible binding of ^{14}C-CCl_4 to microsomal lipids in rats under varying experimental conditions. Toxicol Appl Pharmacol 23:541-552.

Challen PJR, Hickish DE, Bedford J. (1958) Chronic chloroform intoxication. Br J Ind Med 15:243-249.

Chenoweth MB, Robertson DN, Erley DS, Golhke R. (1962) Blood and tissue levels of ether, chloroform, halothane and methoxyflurane in dogs. Anesthesiology 23:101-106.

Chiou WL. (1975) Quantitation of hepatic and pulmonary fist-pass effect and its implication in pharmacokinetic study. I. Pharmacokinetics of chloroform in man. J Pharmacokinet Biopharm 3:193-201.

Chu I, Secours V, Marino I, Villeneuve DC. (1980) The acute toxicity of four trihalomethanes in male and female rats. Toxicol Appl Pharmacol 52: 351-353.

Chu I, Villeneuve DC, Secours VE, Becking GC, Valli VE. (1982a) Trihalomethanes: II Reversibility of toxicological changes produced by chloroform, bromodichloromethane, chlorodibromomethane and bromoform in rats. J. Environ. Sci. Health B17:225-240.

Chu I, Villeneuve DC, Secours VE, Becking GC, Valli VE. (1982b) Toxicity of trihalomethanes: I The acute and subacute toxicity of chloroform, bromodichloromethane, chlorodibromomethane and bromoform in rats. J Environ Sci Health B17:205-224.

Clemens TL, Hill RN, Bullock LP, Johnson WD, Sultatos LG, Vesell ES. (1979) Chloroform toxicity in the mouse: role of genetic factors and steroids. Toxicol Appl Pharmacol 48:117-130.

Cohen EN, Hood N. (1969) Application of low temperature autoradiography to studies of the uptake and metabolism of volatile anesthetics in the mouse. Anesthesiology 30:306-314.

Condie LW, Smallwood CL, Laurie RD. (1983) Comparative renal and hepatoxicity of halomethanes: bromodichloromethane, bromoform, chloroform, dibromochloromethane and methylene chloride. Drug Chem Toxicol 6:563-578.

Conkle JP, Camp BJ, Welch BE. (1975) Trace composition of human respiratory gas. Arch Environ Health 30:290-295.

Cornish HH, Ling BP, Barth ML. (1973) Phenobarbital and organic solvent toxicity. Am Ind Hyg Assoc J 34:487-492.

Cowlen MS, Hewitt WR, Schroeder F. (1984) Mechanisms in 2-hexanone potentiation of chloroform hepatotoxicity. Toxicol Lett 22:293-299.

Cresteil T, Beaune P, Leroux JP, Lange M, Mansuy D. (1979) Biotransformation of chloroform by rat and human liver microsomes; in vitro effect on some enzyme activities and mechanism of irreversible binding to macromolecules. Chem Biol Interact 24:153-165.

Daniel MR, Richold M, Allen J, Jones E, Roe FJC, Uttley M, van Abbe NJ. (1980) Bacterial mutagenicity and cell transformation studies with chloroform. Toxicol Lett 6:247.

Danielsson BRG, Ghantous H, Dencker L. (1986) Distribution of chloroform and methyl chloroform and their metabolites in pregnant mice. Biol Res Pregnancy Perinatol 7:77-83.

Danni O, Brossa O, Burdino E, Milillo P, Ugazio G. (1981) Toxicity of halogenated hydrocarbons in pretreated rats - An experimental model for the study of integrated permissible limits of environmental poisons. Int Arch Occup Environ Health 49:165-176.

Davidson IWF, Sumner DD, Parker JC. (1982) Chloroform: A review of its metabolism, teratogenic, mutagenic, and carcinogenic potential. Drug Chem Toxicol 5:1-87.

Davis NC, Whipple GH. (1919) The influence of drugs and chemical agents on the liver necrosis of chloroform anesthesia. Arch Intern Med 23:636- 654.

Davis NC, Whipple GH. (1919) The influence of fasting and various diets on the liver injury effected by chloroform anesthesia. Arch Intern Med 23:612-635.

DeRouen TA, Diem JE. (1977) Relationships between cancer mortality in Louisiana drinking water source and other possible causative agents. In: Origins of Human Cancer. Cold Spring Harbor Laboratory. pp. 331-345.

De Salva S, Volpe A, Leigh G, Regan T. (1975) Long term safety studies of a chloroform-containing dentifrice and mouth-rinse in man. Fd Cosmet Toxicol 13:529-532.

de Serres FJ, Ashby J, eds. (1981) Progress in Mutation Research. V.1. Evaluation of Short-term Tests for Carcinogens. Elsevier/North Holland. New York.

Defalque RJ. (1968) The first delayed chloroform poisoning. Anesth Analg 47:374-375.

Deml E, Oesterle D. (1987) Dose-response of promotion by polychlorinated biphenyls and chloroform in rat liver foci bioassay. Arch Toxicol 60: 209-211.

Diaz Gomez MI, Castro JA. (1980a) Nuclear activation of carbon tetrachloride and chloroform. Res Commun Chem Pathol Pharmacol 27:191- 194.

Diaz Gomez MI, Castro JA. (1980b) Covalent binding of chloroform metabolites to nuclear proteins-no evidence for binding to nucleic acids. Cancer Lett 9:213-218.

Dilley JV, Chernoff N, Kay D, Winslow N, Newell GW. (1977) Inhalation teratology studies of five chemicals in rats [abstract]. Toxicol Appl Pharmacol 41:196.

DiRenzo AB, Gandolfi AJ, Sipes IG. (1982) Microsomal bioactivation and covalent binding of aliphatic halides to DNA. Toxicol Lett 11:243-252.

DiRenzo AB, Gandolfi AJ, Sipes IG, Brendel K, Byard JL. (1984) Effect of O_2 tension on the bioactivation and metabolism of aliphatic halides by primary rat-hepatocyte cultures. Xenobiotica 14:521-525.

Docks EL, Krishna G. (1976) The role of glutathione in chloroform- induced hepatotoxity. Exp Mol Pathol 24:13-22.

Duprat P, Delsaut L, Gradiski D. (1976) Pouvoir irritant des principaux solvants chlores aliphatiques sur la peau et les muqueuses oculaires du lapin. European Journal of Toxicology 9:171-177.

Dutta SN, Arora S, Sanyal RK. (1968) Studies on the toxicity of chloroform on the isolated rabbit hearts under different conditions of coronary circulation. Pharmacology 1:358-362.

Ebel RE, Barlow RL, McGrath EA. (1987) Chloroform hepatotoxicity in the mongolian gerbil. Fundam Appl Toxicol 8:207-216.

Ekstrom T, Hogberg J. (1980) Chloroform-induced glutathione depletion and toxicity in freshly isolated hepatocytes. Biochem Pharmacol 29:3059- 3065.

Ekstrom T, Stahl A, Sigvardsson K, Hogberg J. (1986) Lipid peroxidation in vivo monitored as ethane exhalation and malondialdehyde excretion in urine after oral administration of chloroform. Acta Pharmacol et Toxicol 58:289-296.

Enosawa S, Nakazawa Y. (1986) Changes in cytochrome P450 molecular species in rat liver in chloroform intoxication. Biochem Pharmacol 35: 1555-1560.

Eschenbrenner AB, Miller E. (1945a) Induction of hepatomas in mice by repeated oral administration of chloroform with observations on sex differences. JNCI 5:251-255.

Eschenbrenner AB, Miller E. (1945b) Sex differences in kidney morphology and chloroform necrosis. JNCI 302-303.

Fernandez JM, Bezanilla F, Taylor RE. (1982) Effect of chloroform on charge movement in the nerve membrane. Nature 297:150-152.

Fink R, Haschke RH. (1973) Anesthetic effects on cerebral metabolism. Anesthesiology 39:199-215.

Freireich EJ, Gehan EA, Rall DP, Schmidt LH, Skipper HE. (1966) Quantitative comparison of toxicity of anticancer agents in mouse, rat, hamster, dog, monkey, and man. Cancer Chemother Rep 50:219-244.

Fry BJ, Taylor T, Hathway DE. (1972) Pulmonary elimination of chloroform and its metabolite in man. Arch int Pharmacodyn 196:98-111.

Gettler AO, Blume H. (1931) Chloroform in the brain, lungs, and liver. Quantitative recovery and determination. Arch Pathol 11:554-560.

Gocke E, King MT, Eckhardt K, Wild D. (1981) Mutagenicity of cosmetics ingredients licensed by the European communities. Mutat Res 90:91-109.

Goodman A, Gilman L, eds. (1980) The Pharmacological Basis for Therapeutics. 6th edition. MacMillan and Co. New York.

Gopinath C, Ford EJH. (1975) The role of microsomal hydroxylases in the modification of chloroform hepatotoxicity in rats. Br. J. exp. Path. 56: 412-422.

Gottlieb MS, Carr JK. (1982) Case-control cancer mortality study and chlorination of drinking water in Louisiana. Environ Health Perspect 46: 169-177.

Gradiski D, Bonnet P, Raoult G, Magadur JL, Francin JM. (1978) Toxicite aigue comparee par inhalation des principaux solvants aliphatiques chlores. Archives des maladies professionnelles de medecine du travail et de Securite Sociale 39:249-257.

Graham EA. (1915) Late poisoning with chloroform and other alkyl halides in relationship to the halogen acids formed by their chemical dissociation. J Exp Med 22:48-75.

Groger WKL, Grey TF. (1979) Effect of chloroform on the activities of liver enzymes in rats. Toxicology 14:23-38.

Habs H, Kunstler K, Schmahl D, Tomatis L. (1983) Combined effects of fast-neutron irradiation and subcutaneously applied carbon tetrachloride or chloroform in C57B16 mice. Cancer Letters 20:13-20.

Hatch GG, Mamay PD, Ayer ML, Casto BC, Nesnow S. (1983) Chemical enhancement of viral transformation in Syrian hamster embryo cells by gaseous and volatile chlorinated methanes and ethanes. Cancer Research 43:1945-1950.

Heddle JA, Hite M, Kirkhart B, Mavournin K, MacGregor JT, Newell GW, Salamone MF. (1983) The induction of micronuclei as a measure of genotoxicity. A report of the U.S. Environmental Protection Agency Gene-Tox Program. Mutat Res 123:61-118.

Hewitt HB. (1956) Renal necrosis in mice after accidental exposure to chloroform. Br J Exp Pathol 37:32-39.

Hewitt WR, Miyajima H, Cote MG, Plaa GL. (1979) Acute alteration of chloroform induced hepato-and nephrotoxicity by mirex and kepone. Toxicol Appl Pharmacol 48:509-527.

Hewitt WR, Miyajima H, Cote MG, Plaa GL. (1980) Acute alteration of chloroform-induced hepato-and nephrotoxicity by n-hexane, methyl n-butyl ketone, and 2,5-hexanedione. Toxicol Appl Pharmacol 53:230-248.

Hewitt LA, Hewitt WR, Plaa GL. (1983) Fractional hepatic localization of $^{14}CHCl_3$ in mice and rats treated with chlordecone or mirex. Fundam Appl Toxicol 3:489-495.

Hewitt LA, Ayotte P, Plaa GL. (1986) Modifications in rat hepatobiliary function following treatment with acetone, 2-butanone, 2-hexanone, mirex, or chlordecone and subsequently exposed to chloroform. Toxicol Appl Pharmacol 83:465-473.

Heywood R, Sortwell RJ, Noel PRB, Street AE, Prentice DE, Roe FJC, Wadsworth PF, Worden AN. (1979) Safety evaluation of toothpaste containing chloroform III. Long-term study in beagle dogs. J Environ Pathol Toxicol 2:835-851.

Hill RN. (1977) Differential toxicity of chloroform in the mouse. Ann NY Acad Sci 298:170-176.

Hirose M, Imaida K, Ito H, Hayashi M, Ishihara Y, Ito N. (1981) Effects of chloroform and dimethylnitrosamine on renal carcinogenesis in unilaterally nephrectomized rats fed formic acid 2-[4-(5-nitro-2-furyl)- 2-thiazolyl]hydrazide. Carcinogenesis 2:703-708

Hogan MD, Chi PY, Hoel DG, Mitchell TJ. (1979) Association between chloroform levels in finished drinking water supplies and various site-specific cancer mortality rates. J Environ Pathol Toxicol 2:873-887.

Howe RB, Crump KS, Van Landingham, C. (1986) GLOBAL86. A computer program to extrapolate quantal animal toxicity data to low doses. K.S. Crump and Company.

Iijima M, Cote MG, Plaa GI. (1983) A semiquantitative morphologic assessment of chlordecone-potentiated chloroform hepatotoxicity. Toxicol Lett 17:307-314.

Ilett KF, Reid WD, Sipes IG, Krishna G. (1973) Chloroform toxicity in mice: correlation of renal and hepatic necrosis with covalent binding of metabolites to tissue macromolecules. Exp Mol Pathol 19:215-229.

International Agency for Research Cancer (IARC). (1979) Chloroform. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans 20:401-427.

Jernigan JD, Harbison RD. (1982) Role of biotransformation in the potentiation of halocarbon hepatotoxicity by 2,5-hexanedione. J Toxicol Environ Health 9:761-781.

Joas TA, Stevens WC, Eger EI. (1971) Electroencephalographic seizure activity in dogs during anaesthesia. Br J Anaesth 43:739-745.

Jones WM, Margolis G, Stephen CR. (1958) Hepatotoxicity of inhalation anesthetic drugs. Anesthesiology 19:715-723.

Jorgenson TA, Meierhenry EF, Rushbrook CJ, Bull RJ, Robinson M. (1985) Carcinogenicity of chloroform in drinking water to male Osborne-Mendel rats and female B6C3F1 mice. Fundam Appl Toxicol 5:760-769.

Kastenbaum MA, Bowman, KO. (1970) Tables for determining the statistical significance of mutation frequencies. Mutat Res 9:527-549.

Kimura ET, Ebert DM, Dodge PW. (1971) Acute toxicity and limits of solvent residue for sixteen organic solvents. Toxicol Appl Pharmacol 19: 699-704.

Kirkland DJ, Smith KL, Van Abbe NJ. (1981) Failure of chloroform to induce chromosome damage or sister-chromatid exchanges in cultured human lymphocytes and failure to induce reversion in Escherichia coli. Fd Cosmet Toxicol 19:651-656.

Klaassen CD, Plaa GL. (1969) Comparison of the biochemical alterations elicited in livers from rats treated with carbon tetrachloride, chloroform, 1,1,2-trichloroethane and 1,1,1-trichloroethane. Biochem Pharmacol 18:2019-2027.

Klaassen CD, Plaa GL. (1967) Relative effects of various chlorinated hydrocarbons on liver and kidney function in dogs. Toxicol Appl Pharmacol 10:119-131.

Klaunig JE, Ruch RJ, Pereira MA. (1986) Carcinogenicity of chlorinated methane and ethane compounds administered in drinking water to mice. Environ Health Perspect 69:89-95.

Kluwe WM, McCormack, Hook JB. (1978) Selective modification of the renal and hepatic toxicities of chloroform by induction of drug-metabolizing enzyme systems in kidney and liver. J Pharmacol Exp Ther 207:566-573.

Kluwe WM, Hook JB. (1981) Potentiation of acute chloroform nephrotoxicity by the glutathione depletor diethyl maleate and protection by the microsomal enzyme inhibitor piperonyl butoxide. Toxicol Appl Pharmacol 59:457-466.

Krus S, Zaleska-Rutczynska Z. (1970) Morphological counterparts of the genetically determined resistance of mice to chloroform poisoning. Experientia 26:101-102.

Kurtz CM, Bennett JH, Shapiro HH. (1936) Electrocardiographic studies during surgical anesthesia. JAMA 106:434-441.

Kuzma RJ, Kuzma CM, Buncher CR. (1977) Ohio drinking water sources and cancer rates. Am J Public Health 67:725-729.

Kylin B, Reichard H, Sumegi I, Yllner S. (1963) Hepatotoxicity of inhaled trichloroethylene, tetrachloroethylene and chloroform. Single exposure. Acta Pharmacol et Toxicol 20:16-26.

Land PC, Owen EL, Lindė HW. (1981) Morphologic changes in mouse spermatozoa after exposure to inhalational anesthetics during early spermatogenesis. Anesthesiology 54:53-56.

Lavigne JG, Marchand C. (1974) The role of metabolism in chloroform hepatotoxicity. Toxicol Appl Pharmacol 29:312-326.

Lavigne JG, Belanger PM, Dore F, Labrecque G. (1983) Temporal variations in chloroform-induced hepatotoxicity in rats. Toxicology 26:267-273.

Lawrence CE, Taylor PR, Trock BJ, Peilly AA. (1984) Trihalomethanes in drinking water and human colorectal cancer. J Natl Cancer Inst 72: 563-568.

Lee SL, Alto LE, Dhalla NS. (1979) Effects of some volatile anesthetic agents on rat heart sarcolemma. Life Sciences 24:1441-1446.

Lehmann KB, Hasegawa. (1910) Studien uber die Absorption chlorierter Kohlenwasserstoffe aus Luft durch Tier und Mensch. Archiv fur Hygiene 327-342.

Letteron P, Degott C, Labbe G, Larrey D, Descatoire V, Tinel M, Pessayre D. (1987) Methoxsalen decreases the metabolic activation and prevents the hepatotoxicity and nephrotoxicity of chloroform in mice. Toxicol Appl Pharmacol 91:266-273.

Liang JC, Hsu TC, Henry JE. (1983) Cytogenetic assays for mitotic poisons. The grasshopper embryo system for volatile liquids. Mutat Res 113:467-479.

Lofberg B, Tjalve H. (1986) Tracing tissues with chloroform-metabolizing capacity in rats. Toxicology 39:13-35.

Lucas GHW. (1928) A study of the fate and toxicity of bromine and chlorine containing anesthetics. J Pharmacol Exp Ther 34:223-237.

Mansuy D, Beaune P, Cresteil T, Lange M, Leroux JP. (1977) Evidence for phosgene formation during liver microsomal oxidation of chloroform. Biochem Biophys Res Commun 79:513-517.

Mason TJ, McKay FW. (1974) U.S. Cancer Mortality by County:1950-1969. United States Government Printing Office, Washington, D.C.

Masuda Y, Nakayama N. (1982) Protective effect of diethyldithiocarbamate and carbon disulfide against liver injury induced by various hepatotoxic agents. Biochem Pharmacol 31:2713-2725.

Masuda Y, Nakayama N. (1983) Protective action of diethyldithiocarbamate and carbon disulfide against renal injury induced by chloroform in mice. Biochem Pharmacol 32:3127-3135.

McConnell C, Ferguson DM, Pearson CR. (1975) Chlorinated hydrocarbons and the environment. Endeavour 34:13-18.

McMartin DN, O'Connor JA, Kaminsky LS. (1981) Effects of differential changes in rat hepatic and renal cytochrome P-450 concentrations on hepatotoxicity and nephrotoxicity of chloroform. Res Commun Chem Pathol Pharmacol 31:99-111.

Miklashevskii VE, Tugarinova VN, Rakhmanina NL, Yakovleva GP. (1966) Toxicity of chloroform administered perorally. Hygiene and Sanitation 31:320-323.

Mink FL, Brown TJ, Rickabaugh J. (1986) Absorption, distribution, and excretion of ^{14}C-trihalomethanes in mice and rats. Bull Environ Contam Toxicol 37:752-758.

Mirsalis JC, Tyson CK, Butterworth BE. (1982) Detection of genotoxic carcinogens in the in vivo-in vitro hepatocyte DNA repair assay. Environ Mutagen 4:553-562.

Moore DH, Chasseaud LF, Majeed SK, Prentice DE, Roe FJC. (1982) The effect of dose and vehicle on early tissue damage and regenerative activity after chloroform administration to mice. Fd Chem Toxic 20:951- 954.

Morgan A, Black A, Belcher DR. (1972) Studies on the absorption of halogenated hydrocarbons and their excretion in breath using ^{38}Cl tracer techniques. Ann Occup Hyg 15:273-282.

Morimoto K, Koizumi A. (1983) Trihalomethanes induce sister chromatid exchanges in human lymphocytes in vitro and mouse bone marrow cells in vivo. Environ Res 32:72-79.

Munson ES. (1973) Guest discussion [on chloroform anesthesia]. Anesth Analg 52:8-9.

Munson AE, Sain LE, Sanders VM, Kauffmann BM, White KL, Page DG, Barnes DW, Borzelleca JF. (1982) Toxicology of organic drinking water contaminants: trichloromethane, bromodichloromethane, dibromochloromethane and tribromomethane. Environ Health Perspect 46: 117-126.

Murray FJ, Schwetz BA, McBride JG, Staples RE. (1979) Toxicity of inhaled chloroform in pregnant mice and their offspring. Toxicol Appl Pharmacol 50:515-522.

National Cancer Institute. (1976) Report on carcinogenesis bioassay of chloroform. National Cancer Institute Carcinogenesis Program. Bethesda, MD.

National Research Council. (1986) Criteria and Methods for Preparing Emergency Exposure Guidance Level (EEGL), Short-term Public Emergency Guidance Level (SPEGL), and Continuous Exposure Guidance Level (CEGL) Documents. National Academy Press. Washington, D.C.

Newberne PM, Weigert J, Kula N. (1979) Effects of dietary fat on hepatic mixed-function oxidases and hepatocellular carcinoma induced by aflatoxin B1 in rats. Cancer Research 39:3986-3991.

Page T, Harris RH, Epstein SS. (1976) Drinking water and cancer mortality in Louisiana. Science 193:55-57.

Palmer AK, Street AE, Roe FJC, Worden AN, Van Abbe NJ. (1979) Safety evaluation of toothpaste containing chloroform II. Long term studies in rats. J Environ Pathol Toxicol 2:821-833.

Paul BB, Rubinstein D. (1963) Metabolism of carbon tetrachloride and chloroform by the rat. J Pharmacol Exp Ther 141:141-148.

Payne JP. (1981) Chloroform in clinical anaesthesia. Br J Anaesth 53: 11s-15s.

Pereira MA, Lin LHC, Lippitt JM, Herren SL. (1982) Trihalomethanes as initiators and promoters of carcinogenesis. Environ Health Perspect 46: 151-156.

Pereira MA, Chang LW. (1982) Binding of chloroform to mouse and rat hemoglobin. Chem-Biol Interactions 39:89-99.

Pereira MA, Savage RE, Guion C. (1983) Induction by chloroform of two forms of ornithine decarboxylase in rat liver. Biochem Pharmacol 32: 2511-2514.

Pereira MA, Chang LW, Ferguson JL, Couri D. (1984a) Binding of chloroform to the cysteine of hemoglobin. Chem-Biol Interactions 51:115-124.

Pereira MA, Savage RE, Guion CW, Wernsing PA. (1984b) Effect of chloroform on hepatic and renal DNA synthesis and ornithine decarboxylase activity in mice and rats. Toxicol Lett 21:357-364.

Pereira MA, Knutsen GL, Herren-Freund SL. (1985) Effect of subsequent treatment of chloroform or phenobarbital on the incidence of liver and lung tumors initiated by ethylnitrosourea in 15 day old mice. Carcinogenesis 6:203-207.

Perocco P, Prodi G. (1981) DNA damage by haloalkanes in human lymphocytes cultured in vitro. Cancer Lett 13:213-218.

Perocco P, Bolognesi S, Alberghini W. (1983) Toxic activity of seventeen industrial solvents and halogenated compounds on human lymphocytes cultured in vitro. Toxicol Lett 16:69-75.

Phoon WH, Goh KT, Lee LT, Tan KT, Kwok SF. (1983) Toxic jaundice from occupational exposure to chloroform. Med J Malaysia 38:31-34.

Piersol GM, Tumen HJ, Kau LS. (1933) Fatal poisoning following the ingestion of chloroform. Med Clin of North Am 17:587-601.

Plaa GL, Evans EA, Hine CH. (1958) Relative hepatotoxicity of seven halogenated hydrocarbons. J Pharmacol 123:224-229.

Pohl LR, Bhooshan B, Whittaker NF, Krishna G. (1977) Phosgene: a metabolite of chloroform. Biochem Biophys Res Commun 79:684-691.

Pohl LR, George JW, Martin JL, Krishna G. (1979) Deuterium isotope effect in in vivo bioactivation of chloroform to phosgene. Biochem Pharmacol 28:561-563.

Pohl LR, Martin JL, George JW. (1980) Mechanism of metabolic activation of chloroform by rat liver microsomes. Biochem Pharmacol 29:3271-3276.

Pohl LR, Branchflower RV, Highet RJ, Martin JL, Nunn DS, Monks TJ, George JW, Hinson JA. (1981) The formation of diglutathionyl dithiocarbonate as a metabolite of chloroform, bromotrichloromethane, and carbon tetrachloride. Drug Metab Dispos 9:334-339.

Pohl LR, George JW, Satoh H. (1984) Strain and sex differences in chloroform-induced nephrotoxicity. Different rates of metabolism of chloroform to phosgene by the mouse kidney. Drug Metab Dispos 12:304- 308.

Pohl LR, Gillette JR. (1984-85) Determination of toxic pathways of metabolism by deuterium substitution. Drug Metab Rev 15:1335-1351.

Poobalasingham N, Payne JP. (1978) The uptake and elimination of chloroform in man. Br J Anaesth 50:325-329.

Raabe OG. (1986) Inhalation Uptake of Selected Chemical Vapors at Trace Levels. Prepared for the California Air Resources Board. Sacramento, CA.

Raabe OG. (1988) Inhalation Uptake of Xenobiotic Vapors by People. Prepared for the California Air Resources Board. Sacramento, CA.

Ramsey JC, Andersen ME. (1984) A physiologically based description of the inhalation pharmacokinetics of styrene in rats and humans. Toxicol Appl Pharmacol 73:159-175.

Reitz RH, Quast JF, Stott WT, Watanabe PG, Gehring PJ. (1980) Pharmacokinetics and macromolecular effects of chloroform in rats and mice: Implications for carcinogenic risk estimation. In: Water Chlorination, Environmental Impact and Health Effects. Vol. 3. Jolley, RL, Brungs, WA, Cumming RB, eds. Ann Arbor Science, Ann Arbor.

Reitz RH, Fox TR, Quast JF. (1982) Mechanistic considerations for carcinogenic risk estimation:chloroform. Environ Health Perspect 46:163-168.

Reitz RH. (1987) Role of cytotoxicity in the carcinogenic process. In: Nongenotoxic Mechanisms in Carcinogenesis. Banbury Report 25. Butterworth, BE, Slaga TJ, eds. Cold Spring Harbor Laboratory, New York.

Reuber MD. (1979) Carcinogenicity of chloroform. Environ Health Perspect 31:171-182.

Reynolds ES, Yee AG. (1967) Liver parenchymal cell injury. V. Relationships between patterns of chloromethane-C^{14} incorporation into constituents of liver in vivo and cellular injury. Lab Invest 16:591-603.

Reynolds ES, Treinen RJ, Farrish HH, Moslen MT. (1984a) Relationships between the pharmacokinetics of carbon tetrachloride conversion to carbon dioxide and chloroform and liver injury. Arch Toxicol Suppl 7:303-306.

Reynolds ES, Treinen RJ, Farrish HH, Moslen MT. (1984b) Metabolism of [^{14}C]carbon tetrachloride to exhaled, excreted and bound metabolites. Biochem Pharmacol 33:3363-3374.

Roe FJC, Palmer AK, Worden AN, Van Abbe NJ. (1979) Safety evaluation of toothpaste containing chloroform. I. Long-term studies in mice. J Environ Pathol Toxicol 2:799-819.

Rogers AE, Newberne PM. (1975) Dietary effects on chemical carcinogenesis in animal models for colon and liver tumors. Cancer Research 35:3427-3431.

Roghani M, DaSilva C, Castagna M. (1987) Tumor promoter chloroform is a potent protein kinase c activator. Biochem Biophys Res Commun 142:738-744.

Rubinstein D, Kanics L. (1964) The conversion of carbon tetrachloride and chloroform to carbon dioxide by rat liver homogenates. Can J Biochem 42:1577-1585.

Rudali G. (1967) A propos de l'activite oncogene de quelques hydrocarbures halogenes utilises en therapeutiques. Potential carcinogenic hazards from drugs. UICC Monograph Series, Volume 7. Springer-Verlag Berlin Heidelberg New York.

Ruddick JA, Villeneuve DC, Chu I. (1983) A teratological assessment of four trihalomethanes in the rat. J Environ Sci Health B18:333-349.

Sagai M, Tappel AL. (1979) Lipid peroxidation induced by some halomethanes as measured by in vivo pentane production in the rat. Toxicol Appl Pharmacol 49:283-291.

San Agustin J, Lim-Sylianco CY. (1978) Mutagenic and clastogenic effects of chloroform. Bull Phil Biochem Soc 1:17-23.

Sato A, Nakajima T. (1979) A structure-activity relationship of some chlorinated hydrocarbons. Arch Environ Health 34:69-75.

Savage RE, Westrich C, Guion C, Pereira MA. (1982) Chloroform induction of ornithine decarboxylase activity in rats. Environ Health Perspect 46: 157-162.

Schauer A, Kunze F. (1976) Tumors of the liver. In: Pathology of Tumours in Laboratory Animals. Vol 1, Part 2. pp. 41-72. Turosov, VS, ed. International Agency for Research on Cancer, IARC Scientific Publication No. 6, Lyon.

Schonfeld A. (1932) On chloroform addiction. Medizinische Klinik 28: 1272-1273.

Schroeder HG. (1965) Acute and delayed chloroform poisoning. Brit J Anaesth 37:972-975.

Schwetz BA, Leong BKJ, Gehring PJ. (1974) Embryo- and fetotoxicity of inhaled chloroform in rats. Toxicol Appl Pharmacol 28:442-451.

Selye H. (1951) The general adaptation syndrome and the diseases of adaptation. Am J Med 10:549-555.

Semmens JM. (1974) Chlorodyne dependence. Brit Med J 2:277.

Simmon VF, Kauhanen K, Tardiff RG. (1977) Mutagenic activity of chemicals in drinking water. Progress in Genetic Toxicology 2:249-258.

Sipes IG, Krishna G, Gillette JR. (1977) Bioactivation of carbon tetrachloride, chloroform and bromotrichloromethane: role of cytochrome P-450. Life Sciences 20:1541-1548.

Smith AA, Volpitto PP, Gramling ZW, DeVore MB, Glassman AB. (1973) Chloroform, halothane, and regional anesthesia: a comparative study. Anesth Analg 52:1-11.

Smith JH, Maita K, Sleight SD, Hook JB. (1984) Effect of sex hormone status on chloroform nephrotoxicity and renal mixed functions oxidases in mice. Toxicology 30:305-316.

Smith JH, Maita K, Sleight SD, Hook JB. (1983) Mechanism of chloroform nephrotoxicity I. Time course of chloroform toxicity in male and female mice. Toxicol Appl Pharmacol 70:467-479.

Smith JH, Hook JB. (1983) Mechanism of chloroform nephrotoxicity II. In vitro evidence for renal metabolism of chloroform in mice. Toxicol Appl Pharmacol 70:480-485.

Smith JH, Hook JB. (1984) Mechanism of chloroform nephrotoxicity III. Renal and hepatic microsomal metabolism of chloroform in mice. Toxicol Appl Pharmacol 73:511-524.

Smith JH, Hewitt WR, Hook JB. (1985) Role of intrarenal biotransformation in chloroform induced nephrotoxicity in rats. Toxicol Appl Pharmacol 79:166-174.

Stevens JL, Anders MW. (1979) Metabolism of haloforms to carbon monoxide. III. Studies on the mechanism of the reaction. Biochem Pharmacol 28:3189-3194.

Stevens JL, Anders MW. (1981a) Effect of cysteine, diethyl maleate, and phenobarbital treatments on the hepatotoxicity of [^{1}H]- and [^{2}H]chloroform. Chem-Biol Interactions 37:207-217.

Stevens JL, Anders MW. (1981b) Metabolism of haloforms to carbon monoxide. IV. Studies on the reaction mechanism in vivo. Chem-Biol Interactions 37:365-374.

Steward A, Allott PR, Cowles AL, Mapleson WW. (1973) Solubility coefficients for inhaled anaesthetics for water, oil and biological media. Brit J Anaesth 45:282-293.

Stewart RD, Dodd HC, Erley DS, Holder BB. (1965) Diagnosis of solvent poisoning. JAMA 193:115-118.

Storms WW. (1973) Chloroform parties. JAMA 225:160.

Sturrock J. (1977) Lack of mutagenic effect of halothane or chloroform on cultured cells using the azaguanine test system. Br J Anaesth 49:207- 210.

Symons JM, Bellar TA, Carswell JK, DeMarco J, Kropp KL, Robeck GG, Seeger DR, Slocum CJ, Smith BL, Stevens AA. (1975) National organics reconnaisance survey for halogenated organics. J Am W W Assoc 67:634-647

Taylor DC, Brown DM, Keeble R, Langley PF. (1974) Metabolism of chloroform II. A sex difference in the metabolism of [^{14}C] chloroform in mice. Xenobiotica 4:165-174.

Taylor GJ, Drew RT, Lores EM, Clemmer TA. (1976) Cardiac depression by haloalkane propellants, solvents, and inhalation anesthetics in rabbits. Toxicol Appl Pharmacol 38:379-387.

Terao K, Nakano M. (1974) Cholangiofibrosis induced by short-term feeding of 3'-methyl-4-(dimethylamino)azobenzene: An electron microscopic observation. Gann 65:249-260.

Testai E, Vittozzi L. (1986) Biochemical alterations elicited in rat liver microsomes by oxidation and reduction products of chloroform metabolism. Chem-Biol Interact 59:157-171.

Testai E, Gramenzi F, Di Marzio S, Vittozzi L. (1987) Oxidative and reductive biotransformation of chloroform in mouse liver microsomes. Arch Toxicol Suppl 11:42-44.

Theiss JC, Stoner GD, Shimkin MB, Weisburger EK. (1977) Test for carcinogenicity of organic contaminants of United States drinking waters by pulmonary tumor response in stain A mice. Cancer Research 37:2717- 2720.

Thompson DJ, Warner SD, Robinson VB. (1974) Teratology studies on orally administered chloroform in the rat and rabbit. Toxicol Appl Pharmacol 29:348-357.

Timms RM, Moser KM. (1975) Toxicity secondary to intravenously administered chloroform in humans. Arch Intern Med 135:1601-1603.

Tomasi A, Albano E, Biasi F, Slater TF, Vannini V, Dianzani MU. (1985) Activation of chloroform and related trihalomethanes to free radical intermediates in isolated hepatocytes and in the rat in vivo as detected by the ESR-spin trapping technique. Chem-Biol Interactions 55:303-316.

Torkelson TR, Oyen F, Rowe VK. (1976) The toxicity of chloroform as determined by single and repeated exposure of laboratory animals. Am Ind Hyg Assoc J 37:697-705.

Tsuruta H. (1975) Percutaneous absorption of organic solvents 1) Comparative study of the in vivo percutaneous absorption of chlorinated solvents in mice. Ind Health 13:227-236.

Tumasonis CF, McMartin DN, Bush B. (1985) Lifetime toxicity of chloroform and bromodichloromethane when administered over a lifetime in rats. Ecotoxicol Environ Safety 9:233-240.

Uehleke H, Hellmer KH, Tabarelli S. (1973) Binding of ^{14}C-carbon tetrachloride to microsomal proteins in vitro and of $CHCl_3$ by reduced liver microsomes. Xenobiotica 3:1-11.

Uehleke H, Werner T. (1975) A comparative study on the irreversible binding of labeled halothane trichlorofluoromethane, chloroform, and carbon tetrachloride to hepatic protein and lipids in vitro and in vivo. Arch Toxicol 34:289-308.

Uehleke H, Werner T, Greim H, Kramer M. (1977) Metabolic activation of haloalkanes and tests in vitro for mutagenicity. Xenobiotica 7:393-400.

Ulmer JB, Braun PE. (1987) Chloroform markedly stimulates the phosphorylation of myelin basic proteins. Biochem Biophys Res Commun 146:1084-1088.

United States Environmental Protection Agency. (1985a) Health Assessment Document for Chloroform. Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office. Research Triangle Park, NC.

United States Environmental Protection Agency. (1985b) Survey of Chloroform Emission Sources. Emission Standards and Engineering Division. Office of Air Quality Planning and Standards. Research Triangle Park, NC.

United States Environmental Protection Agency. (1987) Integrated Risk Information System. Risk Estimates for Carcinogens: Chloroform. Environmental Criteria and Assessment Office. Research Triangle Park, North Carolina. Preparation date:08/11/87.

Upton AC. (1988) Are there thresholds for carcinogenesis? Ann N Y Acad Sci 354:863-884.

Van Abbe NJ, Green TJ, Jones E, Richold M, Roe FJC. (1982) Bacterial mutagenicity studies on chloroform in vitro. Fd Chem Toxic 20:557-561.

Van Dyke RA, Chenoweth MB, Van Poznak A. (1964) Metabolism of volatile anesthetics I. Conversion in vivo of several anesthetics to $^{14}CO_2$ and chloride. Biochem Pharmacol 13:1239-1247.

von Oettingen WF, Powell CC, Sharpless NE, Alford WC, Pecora LJ. (1949) Relation between the toxic action of chlorinated methanes and their chemical and physicochemical properties. National Institutes of Health Bulletin No. 191. Unites States Government Printing Office, Washington, D.C.

von Oettingen WF, Powell CC, Sharpless NE, Alford WC, Pecora LJ. (1950) Comparative studies of the toxicity and pharmacodynamic action of chlorinated methanes with special reference to their physical and chemical characteristics. Arch Int Pharmacodyn LXXXI:17-34.

Wallace CJ. (1950) Hepatitis and nephrosis due to cough syrup containing chloroform. California Medicine 73:442-443.

Wallace L. (1988) Chloroform: Available on Tap. Health Environ Digest 2:3-4.

Waters RM. (1951) Chloroform. A Study after 100 Years. University of Wisconsin Press. Madison, WI.

Watrous WM, Plaa GL. (1972) Effect of halogenated hydrocarbons on organic ion accumulation by renal cortical slices of rats and mice. Toxicol Appl Pharmacol 22:528-543.

Whipple GH. (1912) Pregnancy and chloroform anesthesia. J Exp Med 15: 246-258.

White AE, Takehisa S, Eger EI, Wolff S, Stevens WC. (1979) Sister chromatid exchanges induced by inhaled anesthetics. Anesthesiology 50: 426-430.

Wilkins JR III, Reiches NA, Kruse CW. (1979) Organic chemical contaminants in drinking water and cancer. Am J Epidemiol 110:420-448.

Wilkins JR III, Comstock GW. (1981) Source of drinking water at home and site-specific cancer incidence in Washington County, Maryland. Am J Epidemiol 114:178-190.

Winslow SG, Gerstner HB. (1978) Health aspects of chloroform- A review. Drug Chem Toxicol 1:259-275.

Withey JR, Collins BT. (1980) Chlorinated aliphatic hydrocarbons used in the foods industry: The comparative pharmacokinetics of methylene chloride, 1,2 dichloroethane, chloroform and trichloroethylene after I.V. administration in the rat. J Environ Pathol Toxicol 3:313-332.

Withey JR, Collins BT, Collins PG. (1983) Effect of vehicle on the pharmacokinetics and uptake of four halogenated hydrocarbons from the gastrointestinal tract of the rat. J Appl Toxicol 3:

Wolf CR, Mansuy D, Nastainczyk W, Deutschmann G, Ullrich V. (1977) The reduction of polyhalogenated methanes by liver microsomal cytochrome P450. Mol Pharmacol 13:698-705.

Wood-Smith FG, Stewart HC. (1962) Drugs in anaesthetic practice. Butterworths. London.

Yannai S. (1983) Adrenocortical response to single and repeated doses of chloroform in rats. Arch Toxicol 54:145-156.

Young TB, Kanarek MS, Tsiatis AA. (1981) Epidemiologic study of drinking water chlorination and Wisconsin female cancer mortality. JNCI 67:1191-1198.

Zeller A. (1883) On the fate of iodoforms and chloroform in the organism. Hoppe-Seyler's Z Physiol Chem 8:78-79.

Appendix A

Review of Epidemiological Studies

A.1. Drinking Water and Cancer - Ecological Studies

Page et al. (1976) performed unweighted multivariate regression analysis of age-adjusted 20-year mortality rates (obtained from Mason and McKay, 1974) (dependent variable) from cancers of the gastrointestinal and urinary tract versus the percent of county populations drinking Mississippi River water in Louisiana. Other independent variables included occupation, percentage of population living in a rural or urban area (rurality), and income. The least squares estimate of the coefficient for the source of drinking water in the regression analysis for gastrointestinal cancer was significant ($p<0.01$) for white and nonwhite males and females. Regression coefficients for source of drinking water were significant for urinary cancer in white males and nonwhite females, and for total cancer in white males, nonwhite males, and nonwhite females. Rurality was negative in sign and also significant in many cases. Confounders such as tobacco use, alcohol consumption, and air pollution were not considered in the analysis by Page et al., and may have compromised the findings.

DeRouen and Diem (1977) reanalyzed the data of Page et al. (1976) to examine other potentially causative factors, in particular ethnic (regional) differences between northern and southern Louisiana. They found that while mortality rates associated with kidney, liver, and bladder cancer were higher in southern Louisiana than in the northern parishes, the sex-, race-, and site-specific mortality rates for these cancers within southern Louisiana parishes did not correlate with source of drinking water. However, within the southern parishes, higher rates of mortality from gastrointestinal cancer (stomach, large intestine, and rectum) were observed in parishes drinking water from the Mississippi river than in nonriver parishes. Page et al. showed that there was no association between drinking water source and cancer of the prostate and breast, presumed to be biologically implausible sites. DeRouen and Diem, in their reanalysis of the data, however, showed that there was a significant association between lung cancer, also presumed to be a biologically implausible site, and drinking water from the Mississippi river. Based on this finding, DeRouen and Diem suggested that the effect observed by Page et al. may be nonspecific. However, they concluded that their findings do not disprove a causal role of drinking water in cancer.

Kuzma et al. (1977) compared average age-adjusted cancer mortality rates for the period from 1950-1969 (Mason and McKay, 1974) in 88 Ohio counties classified according to whether surface water or ground water served as the primary drinking water source. Analysis of covariance was used to test the hypothesis that cancer mortality rates were no different in the counties with ground water sources than in counties with surface water sources. The alternative hypothesis, that there was a difference in cancer rates in populations served by ground water than those served by surface water, is based on the findings by U.S.EPA (National Organics Reconnaisance Survey (NORS), Symons et al. (1975), and Region V survey, 1975) that surface water tends to have more organic contaminants than ground water. Covariables included per cent urbanization, median income, population, and occupational indices for white males (manufacturing and agriculture-forestry-fishery). Mortality rates for stomach, bladder, and all neoplasms were higher for white males in counties which used surface water than in counties which used ground water sources for drinking water. After adjustment for covariables, the mortality rate for stomach cancer was significantly ($p = 0.038$) higher

for white females living in surface water counties than for those living in ground water counties. These results tend to implicate drinking water as a contributing factor in the etiology of cancer in this population. However, the relevance of these findings to chloroform content or chlorination of the drinking water is unclear.

Other studies have concentrated on chlorination of water and concomitant production of halogenated carcinogens as a causative factor in human cancers. Cantor et al. (1978) compared age-adjusted cancer mortality rates by site and sex for whites in the years 1968-71 to measures of trihalomethanes (THM) in the drinking water. A weighted (by square-root of the person-year's at risk) linear regression model was used to predict cancer rates in 923 U.S. counties which were over 50% urban in 1970. Variables included in the regression model were per cent urban (1970), median school years completed by persons over age 25, population size, ratio of 1970 population to 1950 population, per cent workforce in manufacturing, and per cent in each of 10 ethnic groups, as well as a regional variable for county location within the U.S. The residual mortality rate (difference between observed and predicted) was compared to measures of THM (from U.S.EPA NORS and Region V survey) using the bivariate correlation coefficient as a measure of association. Only 76 counties could be used in this portion of the analysis. In these counties, more than half the population was served by a water supply which had been analyzed for THM content at one point in time. Reasonably strong associations between bladder cancer and THM levels in drinking water were found after controlling for confounding by urbanization, ethnicity, social class, and county industrialization (Table A-1). The association was not changed by controlling for occupation in certain high-risk (for bladder cancer) industries nor by lung cancer rates used as a surrogate measure for cigarette smoking. The measure of THM most associated with bladder cancer in both white males and females was that of bromine-containing trihalomethanes (BTHM). Chloroform and total trihalomethanes (TTHM) were not as well associated (Table A-1). The primary inconsistency in the association of THM with bladder cancer was that for males, the association only existed in the Northern U.S. counties. However, due to the large sample size in that portion of the country, that particular association was most statistically stable. When the counties were stratified by the percentage of the population served by a measured supply (50-64%, 65-84%, 85-100%), a positive dose-response relationship was suggested for bladder cancer for both sexes. As the percentage of the population served increased, the statistical association strengthened (Table A-1). The investigators also believe that an association of residual brain cancer mortality rates with BTHM levels is present for both sexes and deserves further study. There were inconsistent associations between other cancer sites and THM levels. However, there was some evidence of an association of chloroform in drinking water with kidney cancer in males, which Cantor et al. believed warrants further study. Lung cancer rates in females and non-Hodgkin's lymphoma in males were also weakly associated with THM levels in drinking water. Cantor et al. pointed out that many confounding variables could not be controlled, while others (e.g., ethnicity) were controlled in a crude manner. In addition, aggregate rather than individual data were used for this study. Thus some associations may have been missed or lessened due to confounding from diet and smoking, mobility of individuals (e.g., unknown

TABLE A-1

Correlation coefficients between residual cancer mortality rates in white males and females and trihalomethane (THM) levels in drinking water by percent of the county population served by the sampled supply. From: Cantor et al. (1978)

Site of Cancer	THM Indicator[b]	Sex	Correlation Coefficients[a] for counties in which percent population served was: 50-64	65-84	85-100	50-100
Bladder	$CHCl_3$	M	--	--	0.23 (0.26)	--
	TTHM	M	--	--	0.30 (0.14)	--
	BTHM	M	-0.22 (0.29)	0.29 (0.15)	0.38 (0.06)	0.19 (0.10)
	$CHCl_3$	F	--	--	0.25 (0.24)	--
	TTHM	F	--	--	0.38 (0.06)	--
	BTHM	F	-0.01 (0.97)	0.21 (0.30)	0.45 (0.02)	0.21 (0.06)
Kidney	$CHCl_3$	M	-0.16 (0.44)	-0.11 (0.60)	0.42 (0.04)	0.07 (0.55)
	$CHCl_3$	F	-0.33 (0.11)	0.19 (0.37)	-0.04 (0.83)	-0.01 (0.96)
Brain	BTHM	M	0.10 (0.65)	0.18 (0.37)	0.24 (0.25)	0.17 (0.14)
	BTHM	F	-0.07 (0.73)	-0.03 (0.90)	0.19 (0.35)	0.04 (0.72)

TABLE A-1 continued

Site of Cancer	THM Indicator[b]	Sex	Correlation Coefficients[a] for counties in which percent population served was: 50-64	65-84	85-100	50-100
Lung	TTHM	M	-0.02 (0.94)	0.02 (0.90)	0.15 (0.46)	0.07 (0.56)
	TTHM	F	0.25 (0.23)	0.28 (0.17)	0.15 (0.46)	0.22 (0.05)
Non-Hodgkin's Lymphoma	BTHM	M	-0.33 (0.11)	-0.19 (0.36)	0.36 (0.08)	-0.03 (0.81)
	BTHM	F	-0.36 (0.08)	0.26 (0.20)	-0.04 (0.83)	0.01 (0.97)

a. P value for two-tailed t is shown in parentheses.

b. $CHCl_3$ = chloroform; TTHM = total trihalomethanes; BTHM - brominated trihalomethanes

length of residence at the home listed on the death certificate), and other lifestyle factors. And finally, it is unknown how well the information on THM levels from the surveys reflected contamination in the past. Only a few grab samples were obtained from each water source at one point in time. Trihalomethanes were measured in 1975, while cancer mortality rates for 1968-71 were the basis for the study. Since cancer is characterized by a long latency between exposure and disease, it is important to consider past exposure as well as current exposure. Cantor et al. found that the levels of chloroform used for their study were consistent with measurements made one year later. All but a few of the supplies analyzed in the study had been disinfecting with chlorine since 1949.

Hogan et al. (1979) examined the potential association between chloroform levels in finished drinking water supplies and various site-specific cancer mortality rates, with emphasis on the statistical and biological problems associated with an ecological approach. The cancer mortality rates used were for the period 1950-1969 (Mason and McKay, 1974). Chloroform exposure data were taken from the NORS and Region V surveys conducted by the EPA. Besides chloroform levels, other explanatory variables included population density, urbanization, per cent nonwhite, percent foreign born, income, education, occupation in manufacturing, and regional variables. Both unweighted and weighted linear regression analyses were conducted. When the NORS data were used, the weighting factor was the size of the race-sex stratum of the 1960 county population. When Region V data were used, the weighting factor was the total county population, since initially the available data did not contain a sex-race breakdown for county population. For many of the associations made, the level of significance and sometimes the sign of the coefficient changed depending on whether or not a weighted procedure was used (Table A-2). In addition, when a different weighting scheme was used (Weighted 2 in Table A-2), some cancer mortality rates that were significant in earlier analyses dropped out. In general, the most consistent associations were between chloroform "exposure" and cancers of the bladder, rectum and large intestine. Hogan et al. stated that the results of ecological studies must be interpreted with caution, but are useful to generate hypotheses. Therefore, the association between chloroform levels in drinking water and certain types of cancer (e.g., bladder, large intestine and rectum) warrant further study.

Carlo and Mettlin (1980) analyzed 4255 cases of cancer reported in Erie County, N.Y., between 1973 and 1976 for any relationship between cancer and type of water source, THM levels in the drinking water as measured in 1978, and a variety of socioeconomic variables. Regression analyses were conducted on age-adjusted incidence rates for esophageal, stomach, colon, rectal, bladder, and pancreatic cancer versus water quality and control variables. Pearson correlations were also calculated. Pearson correlation coefficients indicated a relationship between surface water supply and esophageal and pancreatic cancers. However, the levels of THM were not correlated with any of the cancers studied. No significant association between THM and cancers were noted in the regression analyses for the total population. When regression analyses were conducted for population stratified by race-sex, a significant association was found between THM levels in drinking water and pancreatic cancer in white males ($p<0.05$). The investigators caution that the lack of association between THM and pancreatic cancer in other sex-race groups and absence of association

TABLE A-2

Estimated chloroform regression coefficients and associated p-values[a] from regression analyses of cancer mortality rates versus chloroform levels in drinking water using NORS and Region V surveys. From: Hogan et al. (1979)

Site	Chloroform Data	White Males			White Females		
		Unweighted	Weighted 1[b]	Weighted 2[c]	Unweighted	Weighted 1[b]	Weighted 2[c]
Bladder	NORS	.0033 (0.20)	.0034 (0.10)	.0030 (0.16)	.0027 (0.02)	.0017 (0.04)	.0016 (0.05)
Bladder	Region V	.0017 (0.55)	.0031 (0.63)	----	.0009 (0.50)	.0023 (0.07)	----
Rectum	NORS	.0069 (0.01)	.0032 (0.22)	.0031 (0.23)	.0033 (0.02)	.0031 (.004)	.0029 (0.05)
Rectum	Region V	.0036 (0.15)	.0062 (0.03)	----	-.0020 (0.29)	.0007 (0.75)	----
Large Intestine	NORS	.0011 (0.80)	.0114 (0.01)	.0102 (0.01)	.0028 (0.49)	.0102 (0.01)	.0084 (0.03)
Large Intestine	Region V	.0018 (0.62)	.0115 (0.01)	----	.0022 (0.54)	-.0019 (0.60)	----
Stomach	NORS	.0024 (0.65)	-.0096 (0.04)	----	-.0007 (0.82)	-.0069 (0.01)	----
Stomach	Region V	-.0050 (0.27)	-.0023 (0.65)	----	.0039 (0.32)	.0016 (0.63)	----

a. Two-sided p value shown in parentheses, based on a standard t test or partial F test for individual regression coefficients.

b. For NORS data, weighting factor was the size of the race-sex stratum of the 1960 county population. For Region V, the weighting factor was the total county population.

c. A compromise weighting scheme was used with some of the data wherein the weighting factor = $(10^{-6} + n_i^{-1})^{-1}$.

between THM and other cancers raises doubts as to the validity of this finding. Carlo and Mettlin discussed other statistical problems with an ecological study of this nature, such as population mobility, variability in site-specific age-adjusted incidence rates by census tract, and extrapolating THM measurements backwards in time.

A.2. Case-control studies

Brenniman et al. (1980) conducted a case-control study in Illinois to determine whether an association exists between chlorination of drinking water and gastrointestinal and urinary tract cancers. Cases (3208) and controls (43,666) were classified according to residence in chlorinated and unchlorinated groundwater communities. Communities receiving chlorinated water were matched with those receiving unchlorinated water based on rural vs. urban characteristics. Mantel-Haenszel stratified contingency table analyses were used to analyze the data. Control variables included age, sex, and residence (urban vs. rural, Standard Metropolitan Statistical Area (SMSA) vs. non-SMSA). Elevated risk was found for cancers of the gallbladder, large intestine, total gastrointestinal, and urinary tract for women. However, the investigators considered the results tenuous because, when the data were subclassified according to several control variables, the associations were not strengthened. In addition rural (nonSMSA) nonchlorinating communities had a higher incidence of some cancers than did the matched chlorinating communities. Many confounding factors were not controlled including smoking, diet, ethnicity, and occupation. Brenniman and colleagues concluded that chlorination of groundwater does not appear to contribute to the etiology of gastrointestinal and urinary tract cancers "in a major way".

Alavanja et al. (1980) conducted a case control study on all gastrointestinal and urinary tract cancer deaths occurring from January 1, 1968 through December 31, 1970 in 7 counties in New York. Several independent variables were examined including urban or rural residence, chlorination of water, and surface vs. ground water supply to individual residences. In addition, occupation of the decedent was ranked as either high risk (steel, metal processing, mining, printing, chemical, furniture manufacturing, automobile fabrication, rubber and electrical cable manufacture) or low risk. The usual place of residence listed on the death certificate was used to classify water source for cases and controls. Controls were picked from noncancer deaths occurring in the same year, and were matched for age, sex, race, foreign vs. U.S. born, and county of usual residence. The data were analyzed by the Chi-square test. There was a statistically significant excess in urinary tract (UT) and gastrointestinal (GI) cancers among women in chlorinated water areas of Erie (Odds Ratio (OR) = 3.09) and Schenectady (OR = 3.25) counties, and among men in Erie (OR = 3.20), Rensselaer (OR = 2.13) and Schenectady (OR = 5.45) counties. These odds ratios were greater than those associated with urban residence in these counties. Upon comparison of urban chlorinated vs. nonchlorinated and rural chlorinated vs. rural nonchlorinated water areas, the excess of UT and GI cancer mortalities persisted in chlorinated water areas (urban chlorinated OR = 2.88; rural chlorinated OR = 1.77). Analysis by primary anatomic site revealed a significant excess risk of stomach cancer in females, and of stomach, esophagus, large intestine, rectum, liver and kidney, pancreas, and urinary bladder in males residing in chlorinated water areas in the 7 counties studied. Alavanja et al. found no association between cancer and

concentrations of arsenic, beryllium, cadmium, lead, nickel, and nitrate in the drinking water of the study areas. The investigators concluded that the excess risk was associated with living in chlorinated areas of certain counties and was not due to a disparity in the age, race, or ethnic distribution, or to urban/rural classification, hazardous occupation, or a surface vs. ground water difference. Several confounding factors were not controlled including cigarette smoking and diet.

The association between site-specific cancer mortality and trihalomethane exposure, as estimated by chlorine dose, was investigated for Wisconsin white females by use of a death-certificate based case-control study design (Young et al. 1981). Cases were obtained from death certificates provided by the Wisconsin Bureau of Health Statistics and consisted of all white female deaths that occurred 1972-77 within 28 counties due to malignant neoplasms of esophagus, stomach, colon, rectum, liver, bile ducts, pancreas, urinary bladder, kidney, lung, breast, and brain. Controls consisted of white female noncancer deaths matched to each case by sex, race, year of death, county of residence, and nearest birthdate. Water treatment and other characteristics were obtained for 202 waterworks and linked to the decedent based on which waterworks serviced the usual place of residence (city, village, or town) listed on the death certificate. It was assumed that residents not served by private or municipal waterworks had private wells. Confounding variables considered in the analysis included urbanization, occupation, and marital status. Exposure was broken into 3 categories based on the chlorine "dose" (amount of chlorine added); low (0.01 to 0.99 ppm), medium (1.00 to 1.70 ppm), and high (1.71 to 7.00 ppm). Multinomial logistic regression was used to compute maximum likelihood estimates and 95% confidence limits of the regression coefficients. Only death from colon cancer was associated with chlorine dose ($p<0.05$). The risk of colon cancer, calculated as odds ratios, was over twice as great when the water source was affected by rural runoff. Young et al. tested this variable because of the assumption that rural runoff increased the organic precursors to trihalomethanes. For colon cancer, odds ratios of 1.51 (95% Confidence Intervals (CI) = 1.06-2.14), 1.53 (95% CI = 1.08-2.00), and 1.53 (95% CI = 1.11-2.11) were calculated for high, medium, and low chlorine dose, respectively ($p<0.02$). For colon cancer cases and controls whose water source was affected by rural runoff, odds ratos of 3.30 (95% CI = 1.45-7.48), 3.60 (95% CI = 1.57-8.26), and 2.74 (95% CI = 1.10-6.88) were calculated for high, medium, and low chlorine dosages ($p<0.025$). Young et al. discussed several possible confounders in this study and stated that while the association of colon cancer with chlorination and rural runoff factors is provocative, the findings of this study must be considered inconclusive due to the possible underestimation of risk associated with misclassification error and spurious contribution from unknown colon cancer risk factors.

Wilkins and Comstock (1981) conducted a nonconcurrent prospective study to investigate possible relationships between products of water chlorination and human cancer. Site and sex-specific incidence rates for malignant neoplasm of liver, biliary passages, kidney, and bladder were constructed from hospital records, a cancer registry, and death certificates in Washington County, Maryland for the period July 16, 1963 through July 15, 1975. Variables compared to cancer incidences were age, marital status, education, smoking history, frequency of church attendance (as a measure of disability), adequacy of housing, persons per room, year of residence in the

1963 household, and source of home drinking water. The exposed population was stratified into high exposure consisting of Hagerstown residents drinking chlorinated surface water (average chloroform concentration was 107 ug/l), low exposure consisting of users of deep wells (not chlorinated, low organic concentration and presumably low chloroform concentration), and indeterminate exposure consisting of small town residents whose drinking water came from chlorinated surface and ground water sources. Binary variable multiple regression was used to calculate adjusted rates for each risk factor at each exposure level. Relative risks were then calculated. Incidence rates for cancer of the bladder among men and cancer of the liver among women were highest among persons using water from the chlorinated surface supply. However, the increase was not significant relative to the other exposure groups after adjustment for the effects of other variables. Relative risks for these cancers ranged from 1.5 to 1.8, but the 95% confidence limits for all risk ratios contained 1.0. When the length of residence was considered to estimate exposure duration, males living in Hagerstown greater than 12 years had a significantly higher incidence of bladder cancer (RR = 6.46, 95% CI = 1.00->100) than their deep-well counterparts. While the results were only weakly suggestive, Wilkins and Comstock noted that bladder cancer has been suggestively linked with chloroform and other indices of trihalomethane in drinking water in other studies.

Gottlieb and Carr (1982) took a case-control approach to study the potential relationship between chlorination of drinking water and cancer in south Louisiana parishes. Twenty parishes were grouped according to similarities in industrial characteristics. Each group contained parishes which used either ground or surface water as primary drinking water sources. Cases were cancer deaths which occurred between 1960 and 1975. Controls were randomly selected from noncancer deaths in the parish grouping in which the case death occurred, and matched to cases by age, sex, race, and year of death. Location of the residence at the time of death was used to identify the water source. However, the length of time which each case or control lived at that residence was unknown. The total study population was 22,698, half cases and half controls. A multiway contingency table analysis was conducted, based on a log-linear model and using maximum likelihood estimations of effects and interactions. Chlorinated surface water was associated with a significant risk for rectal cancer ($p = 0.012$). The odds ratio for rectal cancer in groups receiving high chlorination level (>1.09 ppm chlorine) to groups with no chlorination is 1.53 (95% CI = 1.15 - 2.04) in surface water supplied areas, and less in areas supplied with ground water. It was methodologically impossible to separate the effects of heavy chlorination from surface water. However, both appeared necessary for increasing the risk of rectal cancer in these parishes (Gottlieb and Carr, 1982). Although an effect of chlorine on breast cancer was observed, Gottlieb and Carr attributed this to possible confounding by small family size and urbanization. Brain cancer was also associated with chlorination, but doubts are raised because the association was only significant in white males living in the Baton Rouge area where there are many chemical and petroleum industries. Association between chlorination and other sites of cancer were heavily dependent on matching variables of race, age, sex, and year of death and were not indicative of a true drinking water effect. Of interest was the lack of association of chlorination or water source with bladder cancer. Gottlieb and Carr concluded that there appears to be some cancer risk associated with water chlorination, but definitive studies are

needed with respect to the role of industrial confounders and the importance of co-contaminants.

Lawrence et al. (1984) used a case-control approach to study the association of chloroform exposure via drinking water to colorectal cancer in white women teachers in upstate New York. Sources of exposure were ascertained through exhaustive tracing of each individual's places of residence and work for the 20 years prior to death. Chloroform exposure and cumulative chloroform dose were estimated by a statistical model based on water treatment parameters. Analysis was based on 395 cases of colon and rectal cancer and 395 control noncancer deaths matched with respect to age and year of death. McNemar's matched-pair analysis and the one-sided Wilcoxon signed rank statistic were used to assess the association between chloroform exposure and colorectal cancer. Both matched and unmatched linear logistic regression was used to examine the role of other variables, including population density, marital status, age, and year of death, in cancer risk. The probability of exposure to a surface water source containing chloroform was no greater for cases than for controls, and the distribution of chloroform exposure was not different among cases and controls. No effect of cumulative chloroform exposure on incidence of colorectal cancer deaths was observed in the logistic analysis controlling for the variables mentioned above. This study was well controlled with respect to occupation, socioeconomic factors, and health care because only data from white women teachers in the central corridor of upstate New York were analyzed. Misclassification of cancer site was minimized by using histologically confirmed cases of colorectal cancer. Extensive review of records for past places of employment and residence minimized misclassification in terms of water source.

Cantor et al. (1987) examined the association between use of chlorinated drinking water and bladder cancer by a case-control study design. The investigators interviewed 2,982 cases and 5,782 controls in 10 geographic areas of the U.S. All cases consisted of persons 21 to 84 years of age with newly diagnosed histologically confirmed bladder cancer in a 1-year period beginning in December 1977. Controls, randomly selected from the general population, were matched to cases with respect to sex, 5 year age group, and geographic area, with a 2:1 matching ratio in most areas. Trained interviewers asked questions about demographic background, use of tobacco and artificial sweeteners, coffee consumption, use of hairdyes, lifetime occupational history, relevant medical history, and levels of tap water and other beverage consumption. Data on water treatment was collected for sources serving the cases and controls since 1900. Lifetime exposure scenarios to different water sources were developed for each case and control. Logistic regression for unmatched data was used to obtain the unconditional maximum likelihood point estimate and confidence intervals of the odds ratios and to adjust for potential confounding by selected variables (e.g., sex, age, study area, cigarette use, high risk occupation, urbanization, population density). Risk of bladder cancer was primarily associated with use of tap water rather than nontap beverages. Among white males, the coefficients for tap and nontap beverages were 0.176 ($p<0.001$) and 0.037 ($p=0.42$), and among white females, the coefficients were 0.123 ($p=0.09$) and 0.089 ($p=0.39$), respectively. It was suggested that nonvolatile components of tap water may be associated with risk of bladder cancer since both heated and nonheated tap water beverages were significantly associated with bladder cancer risk among males. The

regression coefficients for the heated portions (0.227, X^2=15.3) was higher than that for the nonheated portion (0.139, X^2=8.14). However, among women, the contribution from the nonheated tapwater was larger than that from heated tapwater. The relative risk increased with increasing tap water intake. The odds ratios for the highest vs. the lowest intake quintiles were 1.47 among men and 1.29 among women. The Chi-square for trend was highly significant for both sexes combined and for men alone ($p<0.0001$), but not for women alone. Bladder cancer risk among women increased with duration of surface water use, with a significant trend (Chi-square = 4.1, p = 0.04). This increase in risk was not seen among men alone or when both sexes were combined. Those with intake of chlorinated surface water of 40-59 and >60 years duration had odds ratios of 1.7 and 2.0, respectively, for the highest vs. lowest consumption quintiles (test for trend, Chi-square = 7.51 and 6.09, respectively; p = 0.006 and 0.14, respectively). Overall, odds ratios increased with duration of chlorinated surface water use among nonsmoking subjects who reported tap water consumption above the median (Chi-square trend = 6.32, p = 0.01). The lack of concordance of the findings for men and women was partially explained by the difference in smoking habits. If undetected bias or effects of another risk factor cannot explain the findings of this study, the proportion of bladder cancers in the cases attributable to ingestion of tap water from chlorinated surface sources was 12%. Among nonsmokers, the attributable risk was 27%. Cantor et al. stated that while this investigation was quite thorough in many respects, there is a need for confirmation of these findings. The relationship between these findings and the concentration of chloroform and other carcinogens in the drinking water remains untested. Moreover, heated beverages were more strongly associated with bladder cancer among men than nonheated beverages. This may suggest that the contribution of chloroform in the etiology of human bladder cancer in men, may be overshadowed by other nonvolatile chemicals present in the drinking water. On the other hand, this association was reversed in women where the nonheated portion of tapwater consumption contributed more heavily to bladder cancer risk.

Appendix B

Results of GLOBAL86

Computer printouts from GLOBAL86 and dose adjustment of the cancer potency slope.

```
9/29/88,NCI male mouse, hepatocellular carcinoma
3 0 0 2 3 3 1 1 0
18 50 45
0 83 167
1 18 44
1E-6 2 1E-6 2 1E-7 2
.001 2 .01 2 .1 2
```

Transforming q_1^* animal $\longrightarrow$ q_1^* human

(1) adjust for less than lifetime Le

$$(L/Le)^3 \quad (104/93)^3 = 1.3985$$

$$q_1^* (1.3985) = 2.423 \times 10^{-3} (1.3985) = 3.4 \times 10^{-3}$$

$\therefore$ adjust for dose by surface area scaling

$$q_1^*_{human} = q_1^*_{mouse} (70\,kg/0.035)^{1/3}$$

$$\therefore 4.3 \times 10^{-2}$$

using bw mid-exposure = 35 g

DATE: 09/29/1988 TIME: 12:45:01

GLOBAL 86 (MAY 1986)

BY RICHARD B. HOWE AND CYNTHIA VAN LANDINGHAM

CLEMENT ASSOCIATES
1201 GAINES STREET
RUSTON, LA 71270
(318) 255-4800

9/29/88,NCI male mouse, hepatocellular carcinoma

POLYNOMIAL DEGREE SELECTED BY PROGRAM, (POLY-DEGREE=0)
MONTE CARLO TEST USED IN SELECTION

GROUP	DOSE	#RESPONSES OBSERVED/#ANIMALS	#RESPONSES PREDICTED
1	.000000	1/ 18	.66
2	83.0000	18/ 50	23.72
3	167.000	44/ 45	41.27

CHI-SQUARE GOODNESS OF FIT STATISTIC IS 4.9834

P-VALUE FOR THE MONTE CARLO TEST IS .5000000000E-02

FORM OF PROBABILITY FUNCTION:
P(DOSE) = 1 - exp(-Q0 - Q1 * D - Q2 * D^2)

MAXIMUM LIKELIHOOD ESTIMATES OF DOSE COEFFICIENTS

Q(0) = 3.762379779299E-02
Q(1) = .000000000000
Q(2) = 8.792100922775E-05

MAXIMUM VALUE OF THE LOG-LIKELIHOOD IS -44.2444150722

CALCULATIONS ARE BASED UPON EXTRA RISK

GLOBAL 86 LOWER CONFIDENCE LIMITS ON DOSE FOR FIXED RISK

LOWER BOUND CONFIDENCE COEFFICIENTS FOR

RISK	MLE DOSE	ON DOSE	LIMIT SIZE	CONFIDENCE LIMIT
1.00000E-06	.10665	4.11782E-04	95.0%	Q(0) = 3.51078E-02 Q(1) = 2.42844E-03 Q(2) = 6.87044E-05
1.00000E-06	.10665	4.11782E-04	95.0%	Q(0) = 3.51078E-02 Q(1) = 2.42844E-03 Q(2) = 6.87044E-05
1.00000E-07	3.37251E-02	4.11787E-05	95.0%	Q(0) = 3.51078E-02 Q(1) = 2.42844E-03 Q(2) = 6.87043E-05

GLOBAL 86 UPPER CONFIDENCE LIMITS ON RISK FOR FIXED DOSE
****

DOSE	MLE RISK	UPPER BOUND ON RISK	CONFIDENCE LIMIT SIZE	COEFFICIENTS FOR CONFIDENCE LIMIT
1.00000E-03	8.79210E-11	2.42851E-06	95.0%	Q(0) = 3.51077E-02 Q(1) = 2.42844E-03 Q(2) = 6.87044E-05
1.00000E-02	8.79210E-09	2.42910E-05	95.0%	Q(0) = 3.51073E-02 Q(1) = 2.42844E-03 Q(2) = 6.87055E-05
.10000	8.79210E-07	2.43501E-04	95.0%	Q(0) = 3.51028E-02 Q(1) = 2.42844E-03 Q(2) = 6.87156E-05

NORMAL COMPLETION!

9/29/88,female mouse,NCI,hepatocellular carcinoma
3 0 0 2 3 3 1 1 0
20 45 41
0 143 287
0 36 39
1E-5 2 1E-6 2 1E-7 2
.001 2 .01 2 .1 2

① Adjust for less-than-lifetime exposure

$(4/_{16})^3 \quad (104/_{93})^3 = 1.3985$

$(\quad)(1.3985) = 1.3894 \times 10^{-2} \; (1.3985) = 1.94 \times 10^{-2}$

② Adjust for dose by SA scaling

$q_{1\,human}^{*} = q_{1\,mouse}^{*} \left(70\ kg/_{0.028}\right)^{1/3}$

$= 1.94 \times 10^{-2} \; (13.572) = 2.6 \times 10^{-1}$

BW mice/rats = 28 g

DATE: 09/29/1988 TIME: 13:26:42

GLOBAL 86 (MAY 1986)

BY RICHARD B. HOWE AND CYNTHIA VAN LANDINGHAM

CLEMENT ASSOCIATES
1201 GAINES STREET
RUSTON, LA 71270
(318) 255-4800

9/29/88,female mouse,NCI,hepatocellular carcinoma

POLYNOMIAL DEGREE SELECTED BY PROGRAM, (POLY-DEGREE=0)
MONTE CARLO TEST USED IN SELECTION

GROUP	DOSE	#RESPONSES OBSERVED/#ANIMALS	#RESPONSES PREDICTED
1	.000000	0/ 20	.00
2	143.000	36/ 45	35.61
3	287.000	39/ 41	39.23

CHI-SQUARE GOODNESS OF FIT STATISTIC IS 5.29483E-02

P-VALUE FOR THE MONTE CARLO TEST IS .5550000000

FORM OF PROBABILITY FUNCTION:
P(DOSE) = 1 - exp(-Q0 - Q1 * D - Q2 * D^2)

MAXIMUM LIKELIHOOD ESTIMATES OF DOSE COEFFICIENTS

Q(0) = .000000000000
Q(1) = 1.095872392443E-02
Q(2) = .000000000000

MAXIMUM VALUE OF THE LOG-LIKELIHOOD IS -30.5352996346

CALCULATIONS ARE BASED UPON EXTRA RISK

GLOBAL 86 LOWER CONFIDENCE LIMITS ON DOSE FOR FIXED RISK

LOWER BOUND CONFIDENCE COEFFICIENTS FOR

RISK	MLE DOSE	ON DOSE	LIMIT SIZE	CONFIDENCE LIMIT
1.00000E-05	9.12520E-04	7.20256E-04	95.0%	Q(0) = .00000 Q(1) = 1.38840E-02 Q(2) = .00000
1.00000E-06	9.12515E-05	7.20253E-05	95.0%	Q(0) = .00000 Q(1) = 1.38840E-02 Q(2) = .00000
1.00000E-07	9.12515E-06	7.20252E-06	95.0%	Q(0) = .00000 Q(1) = 1.38840E-02 Q(2) = .00000

GLOBAL 86 UPPER CONFIDENCE LIMITS ON RISK FOR FIXED DOSE
****

DOSE	MLE RISK	UPPER BOUND ON RISK	CONFIDENCE LIMIT SIZE	COEFFICIENTS FOR CONFIDENCE LIMIT
1.00000E-03	1.09587E-05	1.38839E-05	95.0%	Q(0) = .00000 Q(1) = 1.38840E-02 Q(2) = .00000
1.00000E-02	1.09581E-04	1.38831E-04	95.0%	Q(0) = .00000 Q(1) = 1.38840E-02 Q(2) = .00000
.10000	1.09527E-03	1.38744E-03	95.0%	Q(0) = .00000 Q(1) = 1.38840E-02 Q(2) = .00000

NORMAL COMPLETION!

1/24/89, Jorgenson, male rat renal adeno/carcinoma, Christopher's doses
5 0 0 2 3 3 1 1 0
301 313 148 48 50
0 18 38 79 155
4 4 4 3 7
1E-5 2 1E-6 2 1E-7 2
.001 2 .01 2 .1 2

1) Adjustment for $(L/L_c)^3$ not necessary as $L = L_c$

2) Adjustment for 3/4 law scaling

$$q^*_{human} = 8.076 \times 10^{-4} \, (70/0.5)^{1/3}$$

$$= 8.076 \times 10^{-4} \, (5.1924)$$

$$= 4.2 \times 10^{-3}$$

DATE: 01/24/1989 TIME: 15:17:44

GLOBAL 86 (MAY 1986)

BY RICHARD B. HOWE AND CYNTHIA VAN LANDINGHAM

CLEMENT ASSOCIATES
1201 GAINES STREET
RUSTON, LA 71270
(318) 255-4800

1/24/89, Jorgenson, male rat renal adeno/carcinoma, Christopher's doses

POLYNOMIAL DEGREE SELECTED BY PROGRAM, (POLY-DEGREE=0)
MONTE CARLO TEST USED IN SELECTION

GROUP	DOSE	#RESPONSES OBSERVED/#ANIMALS	#RESPONSES PREDICTED
1	.000000	4/301	3.62
2	18.0000	4/313	4.90
3	38.0000	4/148	3.51
4	79.0000	3/ 48	2.55
5	155.000	7/ 50	7.39

CHI-SQUARE GOODNESS OF FIT STATISTIC IS .38499

P-VALUE FOR THE MONTE CARLO TEST IS .8650000000

FORM OF PROBABILITY FUNCTION:
P(DOSE) = 1 - exp(-Q0 - Q1 * D - Q2 * D^2)

MAXIMUM LIKELIHOOD ESTIMATES OF DOSE COEFFICIENTS

Q(0) = 1.211224035657E-02
Q(1) = 1.053984007007E-04
Q(2) = 5.474884445119E-06

MAXIMUM VALUE OF THE LOG-LIKELIHOOD IS -92.7238876023

CALCULATIONS ARE BASED UPON EXTRA RISK

GLOBAL 86 LOWER CONFIDENCE LIMITS ON DOSE FOR FIXED RISK

RISK	MLE DOSE	LOWER BOUND ON DOSE	CONFIDENCE LIMIT SIZE	COEFFICIENTS FOR CONFIDENCE LIMIT
1.00000E-05	9.44155E-02	1.23819E-02	95.0%	Q(0) = 8.36327E-03 Q(1) = 8.07636E-04 Q(2) = 2.49074E-08
1.00000E-06	9.48314E-03	1.23818E-03	95.0%	Q(0) = 8.36332E-03 Q(1) = 8.07636E-04 Q(2) = 2.47446E-08
1.00000E-07	9.48734E-04	1.23818E-04	95.0%	Q(0) = 8.36332E-03 Q(1) = 8.07636E-04 Q(2) = 2.47283E-08

GLOBAL 86 UPPER CONFIDENCE LIMITS ON RISK FOR FIXED DOSE

****

DOSE	MLE RISK	UPPER BOUND ON RISK	CONFIDENCE LIMIT SIZE	COEFFICIENTS FOR CONFIDENCE LIMIT
1.00000E-03	1.05404E-07	8.07636E-07	95.0%	Q(0) = 8.36332E-03 Q(1) = 8.07636E-04 Q(2) = 2.47411E-08
1.00000E-02	1.05453E-06	8.07633E-06	95.0%	Q(0) = 8.36328E-03 Q(1) = 8.07636E-04 Q(2) = 2.48726E-08
.10000	1.05945E-05	8.07606E-05	95.0%	Q(0) = 8.36291E-03 Q(1) = 8.07636E-04 Q(2) = 2.61898E-08

NORMAL COMPLETION!

```
9/30/88, Roe, male mouse renal adenoma/adenocarcinoma, Expt. I
3 0 0 2 3 3 1 1 0
72 37 37
0 12 43
0 0 8
1E-5 2 1E-6 2 1E-7 2
.001 2 .01 2 .1 2
```

① Adjustment by $(L/L_c)^3 = (104/96)^3 = 1.2714$

$(3.05 \times 10^{-3})(1.2714) = 3.8777 \times 10^{-3}$

② SA scaling

$q_1^* \text{ Human} = (3.8777 \times 10^{-3})(70/0.05)^{1/3}$

$= 4.3 \times 10^{-2}$

bw mid exposure = 50 g

DATE: 10/27/1988 TIME: 13:02:20

GLOBAL 86 (MAY 1986)

BY RICHARD B. HOWE AND CYNTHIA VAN LANDINGHAM

CLEMENT ASSOCIATES
1201 GAINES STREET
RUSTON, LA 71270
(318) 255-4800

9/30/88, Roe, male mouse renal adenoma/adenocarcinoma, Expt. I

POLYNOMIAL DEGREE SELECTED BY PROGRAM, (POLY-DEGREE=0)
MONTE CARLO TEST USED IN SELECTION

GROUP	DOSE	#RESPONSES OBSERVED/#ANIMALS	#RESPONSES PREDICTED
1	.000000	0/ 72	.00
2	12.0000	0/ 37	.00
3	43.0000	8/ 37	8.00

CHI-SQUARE GOODNESS OF FIT STATISTIC IS 4.25814E-03

P-VALUE FOR THE MONTE CARLO TEST IS .5300000000

FORM OF PROBABILITY FUNCTION:
P(DOSE) = 1 - exp(-Q0 - Q1 * D - Q2 * D^2 - ... - Q 6 * D^ 6)

MAXIMUM LIKELIHOOD ESTIMATES OF DOSE COEFFICIENTS

Q(0) = .000000000000
Q(1) = .000000000000
Q(2) = .000000000000
Q(3) = .000000000000
Q(4) = .000000000000
Q(5) = .000000000000
Q(6) = 3.851888326604E-11

MAXIMUM VALUE OF THE LOG-LIKELIHOOD IS -19.3211081232

CALCULATIONS ARE BASED UPON EXTRA RISK

GLOBAL 86 LOWER CONFIDENCE LIMITS ON DOSE FOR FIXED RISK
****

RISK	MLE DOSE	LOWER BOUND ON DOSE	CONFIDENCE LIMIT SIZE	COEFFICIENTS FOR CONFIDENCE LIMIT
1.00000E-05	7.9871	3.27662E-03	95.0%	Q(0) = .00000
				Q(1) = 3.05195E-03
				Q(2) = .00000
				Q(3) = .00000
				Q(4) = .00000
				Q(5) = .00000
				Q(6) = 1.77585E-11
1.00000E-06	5.4415	3.27660E-04	95.0%	Q(0) = .00000
				Q(1) = 3.05195E-03
				Q(2) = .00000
				Q(3) = .00000
				Q(4) = .00000
				Q(5) = .00000
				Q(6) = 1.77585E-11
1.00000E-07	3.7073	3.27660E-05	95.0%	Q(0) = .00000
				Q(1) = 3.05195E-03
				Q(2) = .00000
				Q(3) = .00000
				Q(4) = .00000
				Q(5) = .00000
				Q(6) = 1.77585E-11

GLOBAL 86 UPPER CONFIDENCE LIMITS ON RISK FOR FIXED DOSE
****

DOSE	MLE RISK	UPPER BOUND ON RISK	CONFIDENCE LIMIT SIZE	COEFFICIENTS FOR CONFIDENCE LIMIT
1.00000E-03	3.85189E-29	3.05194E-06	95.0%	Q(0) = .00000
				Q(1) = 3.05195E-03
				Q(2) = .00000
				Q(3) = .00000
				Q(4) = .00000
				Q(5) = .00000
				Q(6) = 1.77585E-11
1.00000E-02	3.85189E-23	3.05190E-05	95.0%	Q(0) = .00000
				Q(1) = 3.05195E-03
				Q(2) = .00000
				Q(3) = .00000
				Q(4) = .00000
				Q(5) = .00000
				Q(6) = 1.77585E-11

.10000	3.85189E-17	3.05148E-04	95.0%	Q(0) = .00000 Q(1) = 3.05195E-03 Q(2) = .00000 Q(3) = .00000 Q(4) = .00000 Q(5) = .00000 Q(6) = 1.77585E-11

NORMAL COMPLETION!

9/30/88, Roe,male mouse,expt.II, renal adenoma/adenocarcinoma
2 0 0 2 3 3 1 1 0
237 49
0 40
9
1E-5 2 1E-6 2 1E-7 2
.001 2 .01 2 .1 2

(1) Adjustment $(L/Le)^3 = 104/104$

$q_1^* = 7.776 \times 10^{-3}$

(2) Adjustment dose scaling by SA

$q_1^* H = 7.776 \times 10^{-3} (70/0.05)^{1/3}$

$= 8.7 \times 10^{-2}$

DATE: 09/30/1988 TIME: 09:13:21

GLOBAL 86 (MAY 1986)

BY RICHARD B. HOWE AND CYNTHIA VAN LANDINGHAM

CLEMENT ASSOCIATES
1201 GAINES STREET
RUSTON, LA 71270
(318) 255-4800

9/30/88. Roe,male mouse,expt.II, renal adenoma/adenocarcinoma

POLYNOMIAL DEGREE SELECTED BY PROGRAM, (POLY-DEGREE=0)
MONTE CARLO TEST USED IN SELECTION

GROUP	DOSE	#RESPONSES OBSERVED/#ANIMALS	#RESPONSES PREDICTED
1	.000000	6/237	6.00
2	40.0000	9/ 49	9.00

CHI-SQUARE GOODNESS OF FIT STATISTIC IS 3.09081E-20

P-VALUE FOR THE MONTE CARLO TEST IS 1.000000000

FORM OF PROBABILITY FUNCTION:
P(DOSE) = 1 - exp(-Q0 - Q1 * D - Q2 * D^2)

MAXIMUM LIKELIHOOD ESTIMATES OF DOSE COEFFICIENTS

Q(0) = 2.564243061287E-02
Q(1) = 2.216230167442E-03
Q(2) = 5.540575418604E-05

MAXIMUM VALUE OF THE LOG-LIKELIHOOD IS -51.3502007500

CALCULATIONS ARE BASED UPON EXTRA RISK

GLOBAL 86 LOWER CONFIDENCE LIMITS ON DOSE FOR FIXED RISK

RISK	MLE DOSE	LOWER BOUND ON DOSE	CONFIDENCE LIMIT SIZE	COEFFICIENTS FOR CONFIDENCE LIMIT

1.00000E-05	4.51168E-03	1.28596E-03	95.0%	Q(0) = 2.38696E-02 Q(1) = 7.77632E-03 Q(2) = .00000
1.00000E-06	4.51212E-04	1.28596E-04	95.0%	Q(0) = 2.38696E-02 Q(1) = 7.77632E-03 Q(2) = .00000
1.00000E-07	4.51216E-05	1.28596E-05	95.0%	Q(0) = 2.38696E-02 Q(1) = 7.77632E-03 Q(2) = .00000

GLOBAL 86 UPPER CONFIDENCE LIMITS ON RISK FOR FIXED DOSE

****

DOSE	MLE RISK	UPPER BOUND ON RISK	CONFIDENCE LIMIT SIZE	COEFFICIENTS FOR CONFIDENCE LIMIT
1.00000E-03	2.21628E-06	7.77629E-06	95.0%	Q(0) = 2.38696E-02 Q(1) = 7.77632E-03 Q(2) = .00000
1.00000E-02	2.21676E-05	7.77602E-05	95.0%	Q(0) = 2.38696E-02 Q(1) = 7.77632E-03 Q(2) = .00000
.10000	2.22152E-04	7.77330E-04	95.0%	Q(0) = 2.38696E-02 Q(1) = 7.77632E-03 Q(2) = .00000

NORMAL COMPLETION!

```
9/30/88, Roe,male mouse,expt.III,ao, renal adenoma/adenocarcinoma
2 0 0 2 3 3 1 1 0
50 48
0 42
1 12
1E-5 2 1E-6 2 1E-7 2
.001 2 .01 2 .1 2
```

(1) $(L/L_e)^3 = (104/97)^3$ $(L/L_e)^3 = (104/99)^3$ $\bar{X} = 1.19534$

$(1.02 \times 10^{-2})(1.19534) = 1.2192 \times 10^{-2}$

(2) Surface area adjustment

$q_1^*{}_H = 1.2192 \times 10^{-2} \quad (70/0.05)^{1/3}$

$= 1.36 \times 10^{-1}$

DATE: 09/30/1988 TIME: 09:19:20

GLOBAL 86 (MAY 1986)

BY RICHARD B. HOWE AND CYNTHIA VAN LANDINGHAM

CLEMENT ASSOCIATES
1201 GAINES STREET
RUSTON, LA 71270
(318) 255-4800

9/30/88, Roe,male mouse,expt.III,ao, renal adenoma/adenocarcinoma

POLYNOMIAL DEGREE SELECTED BY PROGRAM, (POLY-DEGREE=0)
MONTE CARLO TEST USED IN SELECTION

GROUP	DOSE	#RESPONSES OBSERVED/#ANIMALS	#RESPONSES PREDICTED
1	.000000	1/ 50	1.00
2	42.0000	12/ 48	12.00

CHI-SQUARE GOODNESS OF FIT STATISTIC IS 4.53015E-25

P-VALUE FOR THE MONTE CARLO TEST IS 1.000000000

FORM OF PROBABILITY FUNCTION:
P(DOSE) = 1 - exp(-Q0 - Q1 * D - Q2 * D^2)

MAXIMUM LIKELIHOOD ESTIMATES OF DOSE COEFFICIENTS

Q(0) = 2.020270731751E-02
Q(1) = 3.184278156361E-03
Q(2) = 7.581614658002E-05

MAXIMUM VALUE OF THE LOG-LIKELIHOOD IS -31.8940426057

CALCULATIONS ARE BASED UPON EXTRA RISK

GLOBAL 86 LOWER CONFIDENCE LIMITS ON DOSE FOR FIXED RISK

RISK	MLE DOSE	LOWER BOUND ON DOSE	CONFIDENCE LIMIT SIZE	COEFFICIENTS FOR CONFIDENCE LIMIT

1.00000E-05	3.14021E-03	9.77052E-04	95.0%	Q(0) = 1.55966E-02 Q(1) = 1.02349E-02 Q(2) = .00000
1.00000E-06	3.14041E-04	9.77048E-05	95.0%	Q(0) = 1.55966E-02 Q(1) = 1.02349E-02 Q(2) = .00000
1.00000E-07	3.14043E-05	9.77047E-06	95.0%	Q(0) = 1.55966E-02 Q(1) = 1.02349E-02 Q(2) = .00000

GLOBAL 86 UPPER CONFIDENCE LIMITS ON RISK FOR FIXED DOSE

****

DOSE	MLE RISK	UPPER BOUND ON RISK	CONFIDENCE LIMIT SIZE	COEFFICIENTS FOR CONFIDENCE LIMIT
1.00000E-03	3.18435E-06	1.02349E-05	95.0%	Q(0) = 1.55966E-02 Q(1) = 1.02349E-02 Q(2) = .00000
1.00000E-02	3.18499E-05	1.02344E-04	95.0%	Q(0) = 1.55966E-02 Q(1) = 1.02349E-02 Q(2) = .00000
.10000	3.19135E-04	1.02297E-03	95.0%	Q(0) = 1.55966E-02 Q(1) = 1.02349E-02 Q(2) = .00000

NORMAL COMPLETION!

9/30/88, Roe, male mouse renal adenoma/adenocarcinoma, Expt. III, t
2 0 0 2 3 3 1 1 0
49 47
0 42
1 5
1E-5 2 1E-6 2 1E-7 2
.001 2 .01 2 .1 2

① Adjustment by $(L/L_e)^3 \sim 1$

② Dose scaling by surface area

$$q_1^*{}_{human} = 4.753 \times 10^{-3} (70/0.05)^{1/3}$$

$$= 5.3 \times 10^{-2}$$

DATE: 10/27/1988 TIME: 13:09:28

GLOBAL 86 (MAY 1986)

BY RICHARD B. HOWE AND CYNTHIA VAN LANDINGHAM

CLEMENT ASSOCIATES
1201 GAINES STREET
RUSTON, LA 71270
(318) 255-4800

9/30/88, Roe, male mouse renal adenoma/adenocarcinoma, Expt. III, t

POLYNOMIAL DEGREE SELECTED BY PROGRAM, (POLY-DEGREE=0)
MONTE CARLO TEST USED IN SELECTION

GROUP	DOSE	#RESPONSES OBSERVED/#ANIMALS	#RESPONSES PREDICTED
1	.000000	1/ 49	1.00
2	42.0000	5/ 47	5.00

CHI-SQUARE GOODNESS OF FIT STATISTIC IS 1.33779E-27

P-VALUE FOR THE MONTE CARLO TEST IS 1.000000000

FORM OF PROBABILITY FUNCTION:
P(DOSE) = 1 - exp(-Q0 - Q1 * D - Q2 * D^2)

MAXIMUM LIKELIHOOD ESTIMATES OF DOSE COEFFICIENTS

Q(0) = 2.061928720274E-02
Q(1) = 1.093555907428E-03
Q(2) = 2.603704541495E-05

MAXIMUM VALUE OF THE LOG-LIKELIHOOD IS -20.8091698341

CALCULATIONS ARE BASED UPON EXTRA RISK

GLOBAL 86 LOWER CONFIDENCE LIMITS ON DOSE FOR FIXED RISK

RISK	MLE DOSE	LOWER BOUND ON DOSE	CONFIDENCE LIMIT SIZE	COEFFICIENTS FOR CONFIDENCE LIMIT

1.00000E-05	9.14254E-03	2.10389E-03	95.0%	Q(0) = 1.43789E-02 Q(1) = 4.75313E-03 Q(2) = .00000
1.00000E-06	9.14429E-04	2.10388E-04	95.0%	Q(0) = 1.43789E-02 Q(1) = 4.75313E-03 Q(2) = .00000
1.00000E-07	9.14446E-05	2.10388E-05	95.0%	Q(0) = 1.43789E-02 Q(1) = 4.75313E-03 Q(2) = .00000

GLOBAL 86 UPPER CONFIDENCE LIMITS ON RISK FOR FIXED DOSE
****

DOSE	MLE RISK	UPPER BOUND ON RISK	CONFIDENCE LIMIT SIZE	COEFFICIENTS FOR CONFIDENCE LIMIT
1.00000E-03	1.09358E-06	4.75312E-06	95.0%	Q(0) = 1.43789E-02 Q(1) = 4.75313E-03 Q(2) = .00000
1.00000E-02	1.09381E-05	4.75302E-05	95.0%	Q(0) = 1.43789E-02 Q(1) = 4.75313E-03 Q(2) = .00000
.10000	1.09610E-04	4.75200E-04	95.0%	Q(0) = 1.43789E-02 Q(1) = 4.75313E-03 Q(2) = .00000

NORMAL COMPLETION!

```
9/30/88,Tumasonis,female rat cholangiocarcinoma
2 0 0 2 3 3 1 1 0
18 40
0 220
0 34
1E-6 2 1E-6 2 1E-7 2
.001 2 .01 2 .1 2
```

① $L/L_e \sim 1$

② Surface area adjustment

$$q_1^*{}_{Human} = (1.19 \times 10^{-2}) \left(\frac{70}{1.8}\right)^{1/3}$$

$$= 8.7 \times 10^{-2}$$

DATE: 09/30/1988 TIME: 14:35:57

GLOBAL 86 (MAY 1986)

BY RICHARD B. HOWE AND CYNTHIA VAN LANDINGHAM

CLEMENT ASSOCIATES
1201 GAINES STREET
RUSTON, LA 71270
(318) 255-4800

9/30/88,Tumasonis,female rat cholangiocarcinoma

POLYNOMIAL DEGREE SELECTED BY PROGRAM, (POLY-DEGREE=0)
MONTE CARLO TEST USED IN SELECTION

GROUP	DOSE	#RESPONSES OBSERVED/#ANIMALS	#RESPONSES PREDICTED
1	.000000	0/ 18	.00
2	220.000	34/ 40	34.00

CHI-SQUARE GOODNESS OF FIT STATISTIC IS 5.58390E-29

P-VALUE FOR THE MONTE CARLO TEST IS 1.000000000

FORM OF PROBABILITY FUNCTION:
P(DOSE) = 1 - exp(-Q0 - Q1 * D - Q2 * D^2)

MAXIMUM LIKELIHOOD ESTIMATES OF DOSE COEFFICIENTS

Q(0) = .000000000000
Q(1) = 4.311636329286E-03
Q(2) = 1.959834695130E-05

MAXIMUM VALUE OF THE LOG-LIKELIHOOD IS -16.9083635122

CALCULATIONS ARE BASED UPON EXTRA RISK

GLOBAL 86 LOWER CONFIDENCE LIMITS ON DOSE FOR FIXED RISK

RISK	MLE DOSE	LOWER BOUND ON DOSE	CONFIDENCE LIMIT SIZE	COEFFICIENTS FOR CONFIDENCE LIMIT

1.00000E-06	2.31930E-04	8.42496E-05	95.0%	Q(0) = .00000 Q(1) = 1.18695E-02 Q(2) = .00000
1.00000E-06	2.31930E-04	8.42496E-05	95.0%	Q(0) = .00000 Q(1) = 1.18695E-02 Q(2) = .00000
1.00000E-07	2.31931E-05	8.42495E-06	95.0%	Q(0) = .00000 Q(1) = 1.18695E-02 Q(2) = .00000

GLOBAL 86 UPPER CONFIDENCE LIMITS ON RISK FOR FIXED DOSE
****

DOSE	MLE RISK	UPPER BOUND ON RISK	CONFIDENCE LIMIT SIZE	COEFFICIENTS FOR CONFIDENCE LIMIT
1.00000E-03	4.31165E-06	1.18694E-05	95.0%	Q(0) = .00000 Q(1) = 1.18695E-02 Q(2) = -6.22744E-22
1.00000E-02	4.31174E-05	1.18688E-04	95.0%	Q(0) = .00000 Q(1) = 1.18695E-02 Q(2) = .00000
.10000	4.31267E-04	1.18625E-03	95.0%	Q(0) = .00000 Q(1) = 1.18695E-02 Q(2) = .00000

NORMAL COMPLETION!

9/30/88,Reuber,female rat cholangiocarcinoma and cholangiofibroma
3 0 0 2 3 3 1 1 0
20 39 39
0 50 100
0 3 11
1E-6 2 1E-6 2 1E-7 2
.001 2 .01 2 .1 2

① $\left(\frac{L}{L_e}\right)^3 = 1$

② Surface area adjustment

q_1^* human $= 3.6 \times 10^{-3} \left(70/0.525\right)^{1/3}$

(5.10865

$= 1.8 \times 10^{-2}$

DATE: 09/30/1988 TIME: 15:00:48

GLOBAL 86 (MAY 1986)

BY RICHARD B. HOWE AND CYNTHIA VAN LANDINGHAM

CLEMENT ASSOCIATES
1201 GAINES STREET
RUSTON, LA 71270
(318) 255-4800

9/30/88,Reuber,female rat cholangiocarcinoma and cholangiofibroma

POLYNOMIAL DEGREE SELECTED BY PROGRAM, (POLY-DEGREE=0)
MONTE CARLO TEST USED IN SELECTION

GROUP	DOSE	#RESPONSES OBSERVED/#ANIMALS	#RESPONSES PREDICTED
1	.000000	0/ 20	.00
2	50.0000	3/ 39	3.00
3	100.000	11/ 39	11.00

CHI-SQUARE GOODNESS OF FIT STATISTIC IS 2.53215E-29

P-VALUE FOR THE MONTE CARLO TEST IS 1.000000000

FORM OF PROBABILITY FUNCTION:
P(DOSE) = 1 - exp(-Q0 - Q1 * D - Q2 * D^2 - Q3 * D^3)

MAXIMUM LIKELIHOOD ESTIMATES OF DOSE COEFFICIENTS

Q(0) = .000000000000
Q(1) = 6.301488251860E-04
Q(2) = 1.199398778781E-05
Q(3) = 1.484023755578E-07

MAXIMUM VALUE OF THE LOG-LIKELIHOOD IS -33.7767154620

CALCULATIONS ARE BASED UPON EXTRA RISK

GLOBAL 86 LOWER CONFIDENCE LIMITS ON DOSE FOR FIXED RISK

RISK	MLE DOSE	LOWER BOUND ON DOSE	CONFIDENCE LIMIT SIZE	COEFFICIENTS FOR CONFIDENCE LIMIT
1.00000E-06	1.58688E-03	2.77759E-04	95.0%	Q(0) = .00000 Q(1) = 3.60024E-03 Q(2) = .00000 Q(3) = .00000
1.00000E-06	1.58688E-03	2.77759E-04	95.0%	Q(0) = .00000 Q(1) = 3.60024E-03 Q(2) = .00000 Q(3) = .00000
1.00000E-07	1.58692E-04	2.77759E-05	95.0%	Q(0) = .00000 Q(1) = 3.60024E-03 Q(2) = .00000 Q(3) = .00000

GLOBAL 86 UPPER CONFIDENCE LIMITS ON RISK FOR FIXED DOSE
****

DOSE	MLE RISK	UPPER BOUND ON RISK	CONFIDENCE LIMIT SIZE	COEFFICIENTS FOR CONFIDENCE LIMIT
1.00000E-03	6.30161E-07	3.60023E-06	95.0%	Q(0) = .00000 Q(1) = 3.60024E-03 Q(2) = .00000 Q(3) = .00000
1.00000E-02	6.30267E-06	3.60017E-05	95.0%	Q(0) = .00000 Q(1) = 3.60024E-03 Q(2) = .00000 Q(3) = .00000
.10000	6.31330E-05	3.59959E-04	95.0%	Q(0) = .00000 Q(1) = 3.60024E-03 Q(2) = .00000 Q(3) = .00000

NORMAL COMPLETION!

9/30/88,Tumasonis,male rat cholangiocarcinoma
2 0 0 2 3 3 1 1 0
22 28
0 160
0 17
1E-6 2 1E-6 2 1E-7 2
.001 2 .01 2 .1 2

1) $(L/le)^3 - 1$

2) Surface area adjustment

$$q_{1\,Human}^{*} = (8.63 \times 10^{-3})\left(70/.3\right)^{1/3}$$

$$= 5.3 \times 10^{-2}$$

DATE: 09/30/1988 TIME: 14:57:11

GLOBAL 86 (MAY 1986)

BY RICHARD B. HOWE AND CYNTHIA VAN LANDINGHAM

CLEMENT ASSOCIATES
1201 GAINES STREET
RUSTON, LA 71270
(318) 255-4800

9/30/88,Tumasonis,male rat cholangiocarcinoma

POLYNOMIAL DEGREE SELECTED BY PROGRAM, (POLY-DEGREE=0)
MONTE CARLO TEST USED IN SELECTION

GROUP	DOSE	#RESPONSES OBSERVED/#ANIMALS	#RESPONSES PREDICTED
1	.000000	0/ 22	.00
2	160.000	17/ 28	17.00

CHI-SQUARE GOODNESS OF FIT STATISTIC IS 4.72473E-31

P-VALUE FOR THE MONTE CARLO TEST IS 1.000000000

FORM OF PROBABILITY FUNCTION:
P(DOSE) = 1 - exp(-Q0 - Q1 * D - Q2 * D^2)

MAXIMUM LIKELIHOOD ESTIMATES OF DOSE COEFFICIENTS

Q(0) = .000000000000
Q(1) = 2.919716366803E-03
Q(2) = 1.824822729252E-05

MAXIMUM VALUE OF THE LOG-LIKELIHOOD IS -18.7602514352

CALCULATIONS ARE BASED UPON EXTRA RISK

GLOBAL 86 LOWER CONFIDENCE LIMITS ON DOSE FOR FIXED RISK

RISK	MLE DOSE	LOWER BOUND ON DOSE	CONFIDENCE LIMIT SIZE	COEFFICIENTS FOR CONFIDENCE LIMIT

1.00000E-06	3.42498E-04	1.15837E-04	95.0%	Q(0) = .00000 Q(1) = 8.63281E-03 Q(2) = .00000
1.00000E-06	3.42498E-04	1.15837E-04	95.0%	Q(0) = .00000 Q(1) = 8.63281E-03 Q(2) = .00000
1.00000E-07	3.42499E-05	1.15837E-05	95.0%	Q(0) = .00000 Q(1) = 8.63281E-03 Q(2) = .00000

GLOBAL 86 UPPER CONFIDENCE LIMITS ON RISK FOR FIXED DOSE
****

DOSE	MLE RISK	UPPER BOUND ON RISK	CONFIDENCE LIMIT SIZE	COEFFICIENTS FOR CONFIDENCE LIMIT
1.00000E-03	2.91973E-06	8.63277E-06	95.0%	Q(0) = .00000 Q(1) = 8.63281E-03 Q(2) = .00000
1.00000E-02	2.91986E-05	8.63243E-05	95.0%	Q(0) = .00000 Q(1) = 8.63281E-03 Q(2) = .00000
.10000	2.92111E-04	8.62908E-04	95.0%	Q(0) = .00000 Q(1) = 8.63281E-03 Q(2) = .00000

NORMAL COMPLETION!

DATE: 07/11/1990 TIME: 13:55:29

GLOBAL 86 (MAY 1986)

BY RICHARD B. HOWE AND CYNTHIA VAN LANDINGHAM

CLEMENT ASSOCIATES
1201 GAINES STREET
RUSTON, LA 71270
(318) 255-4800

7/11/90 NCI male rat renal

POLYNOMIAL DEGREE SELECTED BY PROGRAM, (POLY-DEGREE=0)
MONTE CARLO TEST USED IN SELECTION

GROUP	DOSE	#RESPONSES OBSERVED/#ANIMALS	#RESPONSES PREDICTED
1	.000000	0/ 19	.00
2	45.0000	4/ 38	4.83
3	90.0000	12/ 27	11.32

CHI-SQUARE GOODNESS OF FIT STATISTIC IS .23283

P-VALUE FOR THE MONTE CARLO TEST IS .3700000000

FORM OF PROBABILITY FUNCTION:
P(DOSE) = 1 - exp(-Q0 - Q1 * D - Q2 * D^2)

MAXIMUM LIKELIHOOD ESTIMATES OF DOSE COEFFICIENTS

Q(0) = .000000000000
Q(1) = .000000000000
Q(2) = 6.707783515062E-05

MAXIMUM VALUE OF THE LOG-LIKELIHOOD IS -31.4553750584

CALCULATIONS ARE BASED UPON EXTRA RISK

GLOBAL 86 LOWER CONFIDENCE LIMITS ON DOSE FOR FIXED RISK

LOWER BOUND CONFIDENCE COEFFICIENTS FOR

RISK	MLE DOSE	ON DOSE	LIMIT SIZE	CONFIDENCE LIMIT
1.00000E-05	.38611	2.16875E-03	95.0%	Q(0) = .00000 Q(1) = 4.61097E-03 Q(2) = 7.95205E-06
1.00000E-06	.12210	2.16874E-04	95.0%	Q(0) = .00000 Q(1) = 4.61097E-03 Q(2) = 7.95184E-06
1.00000E-07	3.86110E-02	2.16874E-05	95.0%	Q(0) = .00000 Q(1) = 4.61097E-03 Q(2) = 7.95181E-06

GLOBAL 86 UPPER CONFIDENCE LIMITS ON RISK FOR FIXED DOSE
****

DOSE	MLE RISK	UPPER BOUND ON RISK	CONFIDENCE LIMIT SIZE	COEFFICIENTS FOR CONFIDENCE LIMIT
1.00000E-03	6.70778E-11	4.61096E-06	95.0%	Q(0) = .00000 Q(1) = 4.61097E-03 Q(2) = 7.95192E-06
1.00000E-02	6.70778E-09	4.61094E-05	95.0%	Q(0) = .00000 Q(1) = 4.61097E-03 Q(2) = 7.95291E-06
1.00000E-01	6.70778E-07	4.61070E-04	95.0%	Q(0) = .00000 Q(1) = 4.61097E-03 Q(2) = 7.96284E-06

NORMAL COMPLETION!

$$q_1^* H = (4.61 \times 10^{-3}) \left(\frac{70}{.35}\right)^{1/3}$$
$$= 2.6 \times 10^{-2}$$

Appendix C

Health Risk Assessment of Chloroform in Drinking Water (abridged version)

by Bogen et al. 1989 (Draft)

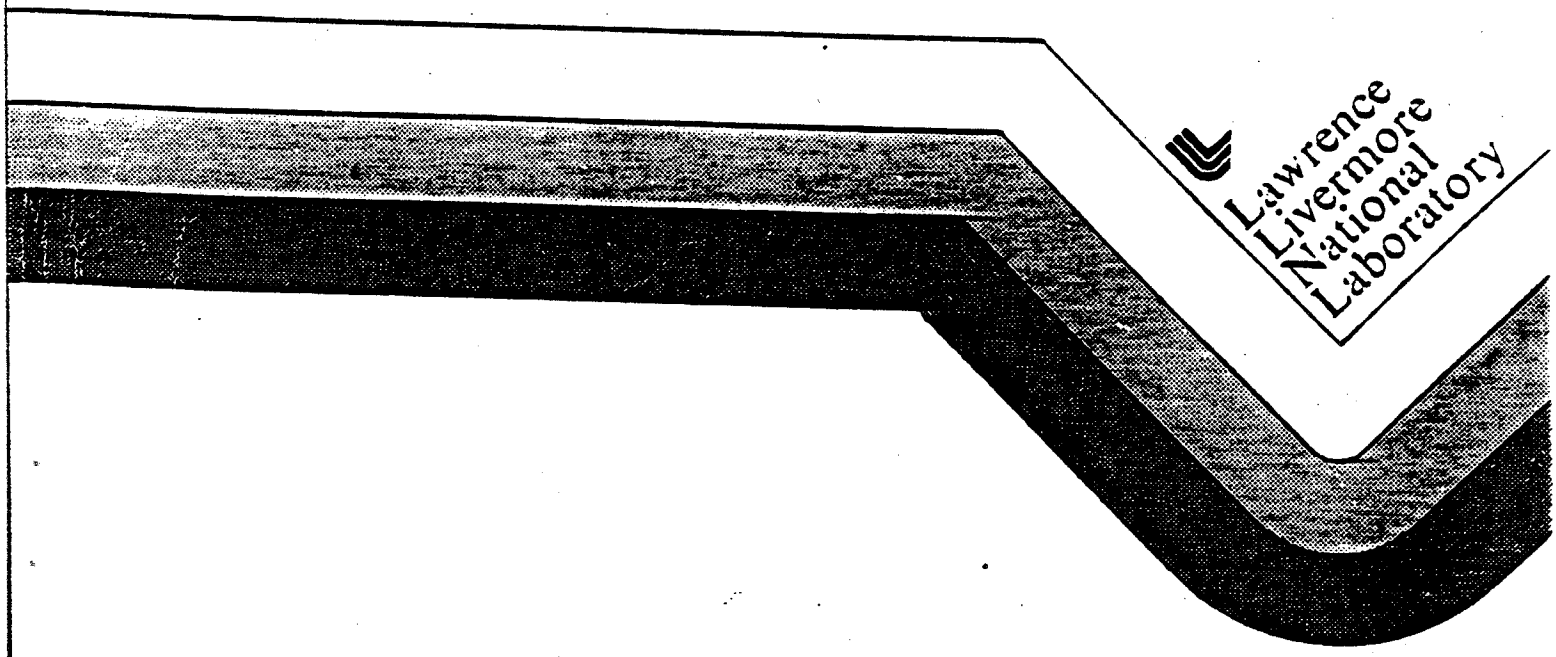
UCRL-21170

DRAFT

Health Risk Assessment of Chloroform in Drinking Water

K. T. Bogen
L. C. Hall
T. E. McKone

March 22, 1989



Work performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract W-7405-Eng-48.

DRAFT

UCRL-21170

Health Risk Assessment of Chloroform in Drinking Water

K. T. Bogen, L. C. Hall, and T. E. McKone

Environmental Sciences Division
Lawrence Livermore National Laboratory
University of California
P. O. Box 5507
Livermore, CA 94550

March 22, 1989

Prepared under Interagency Master Agreement No. 85-87088 between the Regents of the University of California, and the Department of Health Services, California Health and Welfare Agency

Acknowledgments

The health risk assessment for chloroform was prepared for the California Department of Health Services (CDHS) under contract IMA 85-87008. This document has undergone peer-review by staff scientists at Lawrence Livermore National Laboratory (LLNL) as well as by staff scientists at CDHS. The document was also peer-reviewed by an independent panel of three anonymous scientists knowledgeable in the field of regulatory toxicology, selected by CDHS. The comments and criticisms of each of these reviewers were very thoughtful and helpful, and they are gratefully acknowledged. Responses to all of the substantive points raised in this scientific review process were incorporated into the final version of this document.

This document was prepared by the above-listed LLNL authors under the editorial supervision of Dr. Kenneth T. Bogen. Sections 1 through 4 were written primarily by Linda C. Hall, Section 5 was written primarily by Dr. Thomas E. McKone, and Dr. Bogen contributed to each section and was the sole author of the second part of Section 6, Section 7, and Appendices B and C. We wish also to thank Carol Loethen, Martha Maser, Susan MacLean, and Gretchen Gallegos who provided technical and editorial assistance in preparing this document.

TABLE OF CONTENTS

ABBREVIATIONS USED IN THE TEXT[a]

Atpase	Adenosine triphosphatase
ATSDR	Agency for Toxic Substances and Disease Registry
BD	Below level of detection
BW	Body weight
CC	Cholangiocarcinoma
CDHS	California Department of Health Services
CF	Cholangiofibroma
CKM	Cell-kinetic multistage
CO	Carbon monoxide
CO_2	Carbon dioxide
CW	Concentration in water
DEN	Diethylnitrosamine
ENU	Ethylnitrosourea
ESR	Electron spin resonance
GGT	Gammaglutamyl transferase
GI	Gastrointestinal
GSCOSG	Diglutathionyl dicarbonate
GSH	Glutathione
GTPase	Guanosine triphosphatase
HA	Hepatocellular adenoma
HAF	Hepatic adenofibrosis
HCC	Hepatocellular carcinoma
HF	Hepatic fraction
HGPRT	Hypoxanthine-guanine phosphoribosyl transferase
^3H-T	Tritiated thymidine
IARC	International Agency for Research on Cancer
i.p.	Intraperitoneal
IPE	Integrated population exposure
LM	Linearized multistage
LOAEL	Lowest-observed-adverse-effect-level
3MC	3-Methylcholanthrene

MFO	Mixed function oxygenases
ML	Malignant lymphoma
MTD	Maximum tolerated dose
NADPH	Nicotinamide adenine dinucleotide phosphate
NAS	National Academy of Sciences
NB	Nephroblastoma
NCI	National Cancer Institute
NOAEL	No-observed-adverse-effect-level
NORS	National Organics Reconnaissance Survey
NRC	National Research Council
NTP	National Toxicology Program
NYSTRS	New York State Teachers Retirement System
ODC	Ornithine decarboxylase
OTZ	2-Oxothiazolidine-4-carboxylic acid
OTZG	N-(2-oxothiazolidine-4-carbonyl)-glycine
PB	Phenobarbital
PBPK	Physiologically based pharmacokinetics
PCB	Polychlorinated biphenyls
ppm	Parts per million
ppmv	Parts per million volume
RA	Renal adenoma
RC	Renal carcinoma
RH	Renal hypernephroma
SA	Surface area
s.c.	Subcutaneous
SCE	Sister chomatid exchange
SGOT	Serum glutamic oxaloacetic transaminase
SGPT	Serum glutamic pyruvic transaminase
S-phase	Synthesis phase
TCDD	Tetrachlorodibenzodioxin (dioxin)
TCE	Trichloroethylene
THM	Trihalomethane
TWA	Time-weighted average
UCL	Upper confidence limit

UDS	Unscheduled DNA synthesis
UNSCEAR	United Nations Committee on the Effects of Atomic Radiation
U.S. EPA	United States Environmental Protection Agency
USP	United States Pharmaceutical
VOCs	Volatile organic compounds

[a] Other abbreviations used in the text are in accordance with the International Standard Index.

1. INTRODUCTION

This document presents an assessment of potential health risks associated with exposure to chloroform dissolved in California drinking waters, focusing primarily on information relevant to a determination of potential cancer risk that may be associated with such exposures to chloroform. This assessment is being provided to the California Department of Health Services (CDHS) for the development of drinking-water standards to manage the health risks of chloroform exposures. Other assessments required in the risk-management process include analyses of the technical and economic feasibilities of treating water supplies contaminated with chloroform. The primary goal of this health-risk assessment is to evaluate scientifically plausible dose-response relationships for observed and potential chloroform-induced cancer in order to define dose rates that can be used to establish standards that will protect members of the general public from this chronic toxicity endpoint resulting solely from water-based exposures to chloroform. We also analyze the extent of human exposures attributable to chloroform-contaminated groundwater in California. A separate health-risk assessment for potential toxic endpoints other than cancer that may be associated with water-based exposure to chloroform has been prepared by CDHS (1989).

The document consists of seven sections, plus three supporting appendices. Each section provides information that risk managers at the CDHS can use to develop chloroform drinking-water standards that will safeguard human health. Our assessment begins in Section 2 with a brief review of the uses of chloroform, its chemical and physical properties, and its prevalence in California ground water (data on concentrations of chloroform in California ground water are summarized in Appendix A). The next section provides an overview of published studies on the absorption, distribution, metabolism, and elimination of chloroform, emphasizing those studies that have defined the rate and extent of these processes in rodents and humans. Also included in Section 3 is a review of pertinent physiologically based pharmacokinetic models and a discussion of our adaptation of these models for use in the description and prediction of chloroform metabolism in animals and humans.

In Section 4, we review data on the genetic toxicity of chloroform, and discuss data from bioassays conducted to evaluate chloroform's

carcinogenicity. We also provide an overview of relevant epidemiological studies.

In Section 5, we describe our procedure for calculating human chloroform exposures attributable to contaminated groundwater supplies. This section takes an integrated approach to the exposure assessment. A household consisting of two adults and two children uses approximately 1000 L/d of water from wells or surface water. Our approach considers how chloroform contained in this amount of water can result in human exposure through ingestion, inhalation, and dermal absorption. For each pathway we develop pathway-dose factors that translate a unit concentration in mg/L in tap water into a lifetime equivalent dose rate in mg/kg-d. We use the pathway-dose factors and data from AB1803 surveys (CDHS, 1986) to determine the magnitude and distribution of human-lifetime dose rates attributable to chloroform in California groundwater supplies.

We provide a quantitative dose-response assessment for predicted carcinogenic potential of chloroform for humans in Section 6, using, first, a "linearized" multistage dose-response extrapolation model along with several sets of animal cancer-bioassay data as input to that model, under the assumption that some component of the process or products of chloroform metabolism (the "effective dose") is directly able to induce tumor formation by causing critical discrete events, such as somatic mutations, in direct proportion to effective dose. In this quantitative carcinogenic potency assessment, a relationship between the doses applied in the animal bioassays and the corresponding effective or metabolized doses is derived using available data on chloroform metabolism in rodents and pharmacokinetic relationships discussed in Section 3. Our calculated carcinogenic potencies of chloroform to animals based on different sets of bioassay data are then extrapolated to humans using two different methods of interspecies extrapolation. This yields a set of alternative potency values based on different assumptions that might be applied to humans exposed to chloroform in the context of regulatory risk assessment. Finally, we discuss methods applicable to calculating chloroform concentrations in water associated with given, predicted cancer-risk levels using pharmacokinetic relationships derived in Section 3, and other information provided in Sections 5 and 6. In the second part of Section 6 we undertake an alternative dose-response assessment of chloroform's potential carcinogenicity to humans based on a new

"cell-kinetic" multistage (CKM) model, under the assumption that chloroform's observed ability to increase tumor incidence in laboratory animals is due solely to a capacity of sufficiently large effective doses of this compound to promote (or speed up) the occurrence of spontaneously occurring tumors, and not through any ability to induce the formation of new tumors. This alternative cancer-risk assessment for chloroform is based on information concerning CKM models, presented in Appendix B, and the application of these models to cancer risk extrapolation, presented in Appendix C.

The last section addresses some of the key uncertainties associated with the health-risk assessment and also presents some research recommendations for reducing those uncertainties.

3. PHARMACOLOGY AND METABOLISM

Chloroform is readily absorbed through the lungs and gastrointestinal tract. Administration of chloroform by ingestion, inhalation, and intraperitoneal (i.p.) or subcutaneous (s.c.) injection has produced renal and hepatic toxicity in humans and laboratory animals. Although some chloroform can be absorbed percutaneously, the rate and extent are limited in contrast with absorption by other routes.

Chloroform has been detected in post-mortem analyses of human tissue (Gettler, 1934; Gettler and Blume, 1931; McConnell et al., 1975). The greatest quantities have been measured in adipose tissue, but substantial quantities have also been found in the brain, liver, kidneys, and blood. Whole-body autoradiography studies of rodents indicate that chloroform preferentially distributes to the liver and kidneys (Ilett et al., 1973; Taylor et al., 1974; Danielsson et al., 1986). Chloroform has also been detected in lesser amounts in virtually all body tissues. Chloroform crosses the placenta of rats, mice, and rabbits, and has been detected in cows milk (Schwetz et al., 1974; Thompson et al., 1974; Dowty et al., 1975; Dilling, 1977; Murray et al., 1979; Danielsson et al., 1986). The concentration of chloroform in a given tissue depends on the route of exposure, the partition coefficient of chloroform in each tissue, and on the rate of metabolism and elimination. Once in the blood, chloroform is metabolized to phosgene, carbon dioxide (CO_2), carbon monoxide (CO), diglutathionyl dicarbonate (GSCOSG), N-(2-oxothiazolidine-4-carbonyl)-glycine (OTZG), and 2-oxothiazolidine-4-carboxylic acid (OTZ).

In this section, we first briefly review what is known about the absorption, distribution, metabolism, and elimination of chloroform. No attempt is made here to provide a comprehensive survey of relevant published studies. For this, the reader is referred to the U.S. EPA (1985a), and California Department of Health Services (CDHS) (1989). Instead, our emphasis is on studies that have addressed the rate and extent of these processes, particularly those that have examined sex-, strain-, or species-specific differences. This discussion is referred to in later sections that address the mechanism(s) of toxicity and carcinogenicity of chloroform.

Following the discussion of metabolism and excretion kinetics, a review of physiologically based pharmacokinetic (PBPK) models is also presented.

These models are now routinely used to predict the absorption, distribution, metabolism, and elimination of volatile organic compounds in rodents and humans. In Section 6, PBPK models are used to estimate the predicted relationship between applied dose and metabolized dose for bioassay-exposure scenarios relevant to the interpretation and assessment of chloroform's carcinogenicity in rodents, and to extrapolate the results of the latter analysis in an assessment of chloroform's potential carcinogenic potency for humans.

ABSORPTION

In this section we review data on the absorption of chloroform following dermal exposure, ingestion, and inhalation in animals and humans.

Dermal Absorption

Percutaneous absorption of pure, liquid chloroform occurs slowly, and is limited by the thickness of the epidermis and by the moderate lipophilicity of chloroform (U.S. EPA, 1985a). The extent of uptake of chloroform through the skin under conditions of environmental exposure to low aqueous concentrations is unknown.

Tsurata (1975) measured the *in vivo* rate of absorption of liquid chloroform (0.5 mL) across the shaved skin of mice at 329 μmol/min/cm^2. Over the 15-min period of exposure, approximately 1718 μg of chloroform were absorbed, or 0.2% of the applied dose (CDHS, 1989). From the measurements made with mouse skin, Tsurata estimated that a human with both hands immersed in liquid chloroform would absorb 19.7 mg/min, a quantity calculated by Tsurata to be equivalent to that retained following a 1-min inhalation exposure to 2429 ppmv of chloroform.

Oral Absorption

Animal mass-balance studies with orally administered radiolabeled chloroform have typically recovered most of the radioactivity (> 90%) in expired air as CO_2 or unchanged parent compound. Relatively small amounts of radiolabel have also been recovered in the urine, feces, or carcass (see Table 3-1) (Paul and Rubenstein, 1963; Van Dyke et al., 1964; Fry et al., 1972;

Table 3-1. Metabolism of chloroform (CCl_3) in rodents after oral or i.p. exposure.

Species (strain): sex (no. animals), body weight, kg	Applied dose	Exposure protocol	Recovery period (h)	Percent of applied dose recovered in expired air as: $^{14}CCl_3$	$^{14}CO_2$	excreta	carcass	% of dose recovered as metabolites	Reference
Mouse (CF/LP): Male (6), 0.025	60 mg/kg	single, oral	48	6.0	84.2	2.1	2.3	88.6	Brown et al., 1974a
Mouse (CBA): Male (7), 0.025	60 mg/kg	single, oral	48	7.1	83.9	2.8	1.2	87.9	ibid.
Mouse (C57): Male (6), 0.025	60 mg/kg	single, oral	48	5.2	87.2	3.0	2.0	92.2	ibid.
Mouse (CF/LP): Male (7), 0.025	60 mg/kg	single, oral	48	0.4	82.8	4.9[a] 0.6[b]	N/A[c]	88.3	Taylor et al., 1974
Mouse (CF/LP): Female (7), 0.025	60 mg/kg	single, oral	48	1.8	81.2	5.1[a] 0.5[b]	N/A[c]	86.8	ibid.
Mouse (B6C3F1): N/A[c] (20), 0.020	150 mg/kg	single, oral	8	26.0	50.0	4.9[a]	13.5	68.4	Mink et al., 1986
Rat (SD): Male (4), 0.225	11.9 mg/kg	single, oral	8 to 12	5.0	67.0	N/A[c]	N/A[c]	67.0	Reynolds et al., 1984
Rat (SD): Male (4), 0.225	35.8 mg/kg	single, oral	8 to 12	12.0	68.0	N/A[c]	N/A[c]	68.0	ibid.
Rat (SD): Male (6), 0.250	60 mg/kg	single, oral	72 to 96	19.7	66.1	7.6	N/A[c]	73.7	Brown et al., 1974a

Table 3-1. (Continued).

Species (strain): sex (no. animals), body weight, kg	Applied dose	Exposure protocol	Recovery period (h)	Percent of applied dose recovered in expired air as: $^{14}CCl_3$	$^{14}CO_2$	excreta	carcass	% of dose recovered as metabolites	Reference
Rat (SD): N/A[c] (6), 0.250	100 mg/kg	single, oral	8	64.8	6.5	2.6[a]	3.6	12.7[d]	Mink et al., 1986
Rat (N/A)[c]	1 mL/kg	single, i.p.	18	70.0	4.0	N/A[c]	N/A[c]	4.0	Paul and Rubenstein, 1963

[a] Percent of radiolabel recovered in urine

[b] Percent of radiolabel recovered in feces

[c] N/A = not available

[d] Total recovery of radiolabel = 78.2%

Taylor et al., 1974; Brown et al., 1974a; Reynolds et al., 1984; Mink et al., 1986). Values reported from mass-balance studies of orally administered chloroform are based on the assumption that the radiolabel recovered in urine, feces, carcass, and cage air represents material that has been absorbed through the stomach and intestines prior to elimination. This assumption may not be correct. For example, the chloroform measured in cage air (which presumably came from exhalation) may be derived in part from chloroform retained in material that passed through the digestive tract without systemic absorption. Mass-balance studies of chloroform have typically recovered 1 to 3% (and as much as 13.6%) of the radiolabel in the carcass (see discussion under Metabolism and Excretion Kinetics). These reports have not provided data on the specific disposition of the radioactivity in the carcass, i.e., whether or not radioactivity remained unabsorbed in the gastrointestinal (GI) tract. It is therefore possible that some carcass radioactivity is due to such unabsorbed material.

In mice and monkeys given 60 mg/kg of chloroform by gavage, and in humans who ingested 500 mg/kg of chloroform, peak blood levels of chloroform were measured 1 h after dosing (Brown et al., 1974a; Taylor et al., 1974; Fry et al., 1972). However, in these experiments chloroform was administered in olive oil, which may act to inhibit the rate of absorption of chloroform across the GI tract (see discussion of Withey et al., 1983). Furthermore, administration of chloroform in drinking water or by gavage has produced a broad spectrum of adverse effects in laboratory animals within minutes of exposure. Rapid onset of toxicity has also been documented in humans following accidental ingestion of chloroform (U.S. EPA, 1985a).

Withey et al. (1983) have shown that the vehicle may have pronounced effects on the rate and extent of GI absorption of chloroform. Fasted male Wistar rats were given a single 75 mg/kg dose of chloroform by gavage, either in aqueous solution or in corn oil. Blood samples were taken approximately every two minutes for a 60-min post-dosing period. Sampling was continued every half hour thereafter until the concentration of chloroform became undetectable. When chloroform was administered in corn oil, the blood concentration profile was characterized by three closely spaced but separate peaks, resulting in a relatively constant infusion of chloroform over a 30-min period. The blood concentration of chloroform declined exponentially over a period of 200 min, after which time chloroform was no longer detectable. In

contrast, when chloroform was given in water, the blood concentration of chloroform reached an almost instantaneous peak, which was 6.5 times higher than that observed when corn oil was the vehicle. Chloroform was eliminated in a biexponential manner over the next 5 h. Calculation of the area under the blood concentration-time curve demonstrated that the uptake of chloroform was 8.7 times greater when chloroform was administered in aqueous solution, compared to administration in corn oil. Withey et al. speculated that the corn oil vehicle may have acted to sequester the chloroform in immiscible globules which did not come into immediate contact with the GI tract. Although the data of Withey et al. (1983) indicate that administration of chloroform in a corn oil vehicle can substantially decrease the proportion of a dose that is absorbed, this has not been corroborated by the data of mass-balance studies. In several instances, when radiolabeled chloroform was administered orally in a corn oil vehicle, investigators were able to recover nearly all of the radiolabel in urinary metabolites, respired CO_2, or respired chloroform (Brown et al., 1974a; Taylor et al., 1974; Reynolds et al., 1984; Mink et al., 1986).

Pulmonary Absorption

Chloroform is a volatile, low molecular-weight, lipophilic compound that was widely used as a surgical inhalation anesthetic until World War II (U.S. EPA, 1985a). The human blood/air partition coefficient of chloroform has been estimated to be between 8.0 and 10.3, and chloroform diffuses rapidly across the pulmonary epithelia into the capillaries of the alveoli (Feingold and Holaday, 1977; Sato and Nakajima, 1979; Steward et al., 1973). Pulmonary uptake of chloroform depends on its concentration in air, the duration of exposure, the solubility of chloroform in blood and tissue, the volume and rate of perfusion of tissues, the rate of elimination, and the rate of alveolar ventilation (i.e., that fraction of total respiratory ventilation from which volatile organic compounds may be cleared by absorption into alveolar capillary blood (Astrand, 1975; U.S. EPA, 1985a). Under nonequilibrium conditions, the rate of pulmonary uptake of chloroform is initially rapid, but decreases as the concentration of chloroform in tissues approaches equilibrium (Lehman and Hasegawa, 1910; Raabe, 1988).

An early study of three human volunteers found that approximately 64% of the chloroform inhaled through the lungs (4200 to 7200 ppmv) is retained (Lehmann and Hasegawa, 1910). A similar value, 67% retention, was calculated by the U.S. EPA (1985a) from the data of Smith et al. (1973). This data was obtained from observations of patients anesthetized with chloroform. Human volunteers who inhaled 7 to 25 ppbv chloroform for 2 h through their nose or mouth retained 45.6 or 49.6%, respectively (Raabe, 1988). Assuming that alveolar ventilation represented 66.7% (U.S. EPA, 1988) or 70% (Raabe, 1988) of total respiratory volume, the data of Raabe (1988) imply that the retained percentage of chloroform that is alveolarly respired ranges from 65 to 74%.

DISTRIBUTION

Chloroform is a small, lipophilic molecule which readily distributes throughout the body. In humans as well as experimental animals, the highest concentrations of chloroform have been measured in adipose tissue, where it tends to accumulate (Chenoweth et al., 1962; Steward et al., 1973; Brown et al., 1974a; Taylor et al., 1974; McConnell et al., 1975).

Humans

Most of the information on the distribution of chloroform in the human body comes from analysis of tissues obtained from patients who died while under chloroform anesthesia. Use of chloroform as an anesthetic was discontinued over fourty y ago, and the reported tissue concentrations have little relevance to those that might result from low level environmental exposures. However, the measurements of chloroform concentrations in blood and tissue reported by Gettler and Blume (1931), Gettler (1934), and Smith et al. (1973) have demonstrated that chloroform partitions most readily to those tissues with a high lipid content. These data, and the partition coefficients for human tissue published by Steward et al. (1973) indicate that for a given exposure concentration, the relative tissue concentration of chloroform would be; adipose tissue > brain > liver > kidney > blood. Chloroform can cross the human placenta. Dowty et al. (1975) reported that in humans, chloroform was present in placental cord blood in concentrations that were equal to or greater than that in maternal blood.

McConnell et al. (1975) analyzed post-mortem tissue samples from eight residents of the United Kingdom. Although none of these individuals had been occupationally exposed to chloroform, detectable quantities of chloroform were measured in the liver (1 to 10 µg/kg), kidneys (2 to 5 µg/kg), brain (2 to 4 µg/kg), and adipose tissue (19 to 68 µg/kg).

Animals

In animals, chloroform preferentially distributes to adipose tissue, and to a lesser extent, the liver and kidney. Within these two organs, radioactivity from ^{14}C-labeled chloroform is typically concentrated in the region immediately surrounding the centrilobular vein of the liver and in the cortex of the kidney. The distribution of chloroform is not restricted to these tissues however, and it has been detected in virtually every tissue in the body of rodents (Lavigne and Marchand, 1974; Brown et al., 1974a; Taylor et al., 1974; Lofberg and Tjalve, 1986). In dogs exposed to an "anesthetic concentration" of chloroform for 2.5 h, the greatest concentrations were measured in fat, followed by the adrenal glands. The amounts of chloroform detected in blood, brain, liver, and kidney were approximately equal (Chenoweth et al., 1962).

Lofberg and Tjalve (1986) used a combination of whole body autoradiography and in vivo techniques to demonstrate that chloroform distributes to, and is metabolized at a large number of extrahepatic sites, including the renal cortex, nasal olfactory and respiratory mucosa, cheek, esophagus, tongue, larynx, trachea, and lungs. There is no evidence that adipose tissue has any capacity to metabolize chloroform, and Cohen and Hood (1969) showed that the radiolabel present in this tissue was primarily volatile (i.e., unmetabolized chloroform). In contrast, the radiolabel present in the liver was volatile shortly after exposure, but became largely nonvolatile (metabolites) over a 2-h post-exposure period.

Sex-specific differences in chloroform-induced renal toxicity in mice have led to speculation that there may be sex-related differences in the distribution of chloroform in this species. Taylor et al. (1974) observed distinct differences in the distribution of radioactivity between males and females of three separate strains of mice after animals were given a single gavage dose of ^{14}C-labeled chloroform. In males, chloroform was widely

distributed, with radiolabel detectable in the stomach, intestine, bladder, liver, and kidney. Within the kidney, considerably more radioactivity was associated with the cortex than the medulla. Females exhibited the same general distribution of chloroform, except that "much less" radioactivity was present in the kidney, and there was little difference in the concentration of radioactivity between the cortex and medulla. In concurrent quantitative experiments, Taylor et al. found that the amounts of radioactivity present in the kidneys of male mice of all three strains (CF/LP, CBA, C57BL) were consistently greater than those present in the kidneys of female mice. This difference was highly significant ($p < 0.0005$). The ratio of the difference in renal radioactivity between males and females varied from 2.6 (C57BL) to 3.8 (CBA). Within male mice, there were also marked strain-dependent differences in the concentration of radioactivity in the kidney. Male CBA mice had significantly ($p < 0.0005$) more radioactivity in their kidneys than males from the other two strains. There were no significant strain-dependent differences in the kidneys of female mice. Quantification of radioactivity in other tissues (liver, fat, blood) did not reveal any significant sex-dependent differences in the strains. However, when data from all three strains was combined, the livers of females had significantly ($0.01 < p < 0.02$) more radioactivity than those from males.

Following oral administration of ^{14}C-chloroform to rats (16 μCi/kg) and mice (32 μCi/kg), Mink et al. (1986) noted an apparent species-specific difference in the percentage of radiolabel contained in the organs (bladder, brain, kidneys, liver, lungs, stomach, pancreas, thymus). However, the difference in average total organ content of ^{14}C (3.6% rats, 13.46% mice) may have been due simply to the relatively poor total recovery of ^{14}C in rats compared to mice (78.2 and 94.5%, respectively).

Chloroform crosses the placenta, and has been detected in fetal blood and tissue of mice, rats, and rabbits following maternal inhalation or ingestion of chloroform (Schwetz et al., 1974; Thompson et al., 1974; Dilley et al., 1977; Murray et al., 1979). Analysis of dairy products from the United Kingdom has found chloroform in cows milk (McConnell et al., 1975). Danielsson et al. (1986) used low-temperature autoradiography and liquid scintillation counting of fetal tissue samples to demonstrate that chloroform crosses the placenta of mice at all stages of gestation. Minor differences in the distribution of radioactivity were observed, depending on the age of the

fetus. In "early gestation", metabolites of chloroform (i.e., nonvolatile radioactivity) reportedly accumulated in the brain. In "midgestation", radioactivity was evenly distributed in fetal tissues, while by "late gestation" there was some evidence of accumulation in the liver, blood, respiratory tract, oral mucosa, and esophagus. Withey and Karpinski (1985) exposed rats on d 17 of gestation to 250, 500, 1000, 1500, or 2000 ppmv of chloroform for 5 h. They found a linear relationship between the mean maternal tissue concentration of chloroform and the mean fetal tissue concentration of chloroform when plotted against the maternal exposure concentration. The ratio of fetal chloroform concentration to maternal blood level was 0.36.

METABOLISM

Chloroform is metabolized by the cytochrome p450-dependent mixed function oxygenases (MFO) to phosgene, a highly reactive, electrophilic compound. Trichloromethanol is believed to be an intermediate in the formation of phosgene, but its extremely short half-life has made it difficult to isolate (Ilett et al., 1973; Uehleke and Warner, 1975; Mansuy et al., 1977; Sipes et al., 1977; Pohl et al., 1977, 1979, 1981). Phosgene is removed by a number of different mechanisms (Figure 3-1). Because of its inherent reactivity, it readily forms covalent bonds with cellular macromolecules such as lipids and proteins (Ilett et al., 1973; Brown et al., 1974a; Hill et al., 1975; Uehleke and Werner, 1975; Docks and Krishna, 1976; Sipes et al., 1977). Alternatively, phosgene can undergo spontaneous hydrolysis to CO_2, forming H^+ and Cl^- in the process (Paul and Rubenstein, 1963; Van Dyke et al., 1964; Fry et al., 1972; Brown et al., 1974a; Taylor et al., 1974; Smith and Hook, 1984; Mink et al., 1986; Raabe, 1988). In rodents, a small percentage of this CO_2 is incorporated into the endogenous carbon pool, and is eliminated in the urine as bicarbonate, urea, and various amino acids (Brown et al., 1974a). Glutathione-S-transferase mediates the formation of several separate phosgene-derived metabolic products, including CO, GSCOSG, OTZG, and OTZ (Wolf et al., 1977; Ahmed et al., 1977; Anders et al., 1978; Stevens and Anders, 1981; Pohl et al., 1981; Branchflower et al., 1984). There is some evidence that the dichloromethyl radical, $\bullet CHCl_2$ is formed by the reductive dehalogenation of chloroform (Tomasi et al., 1985).

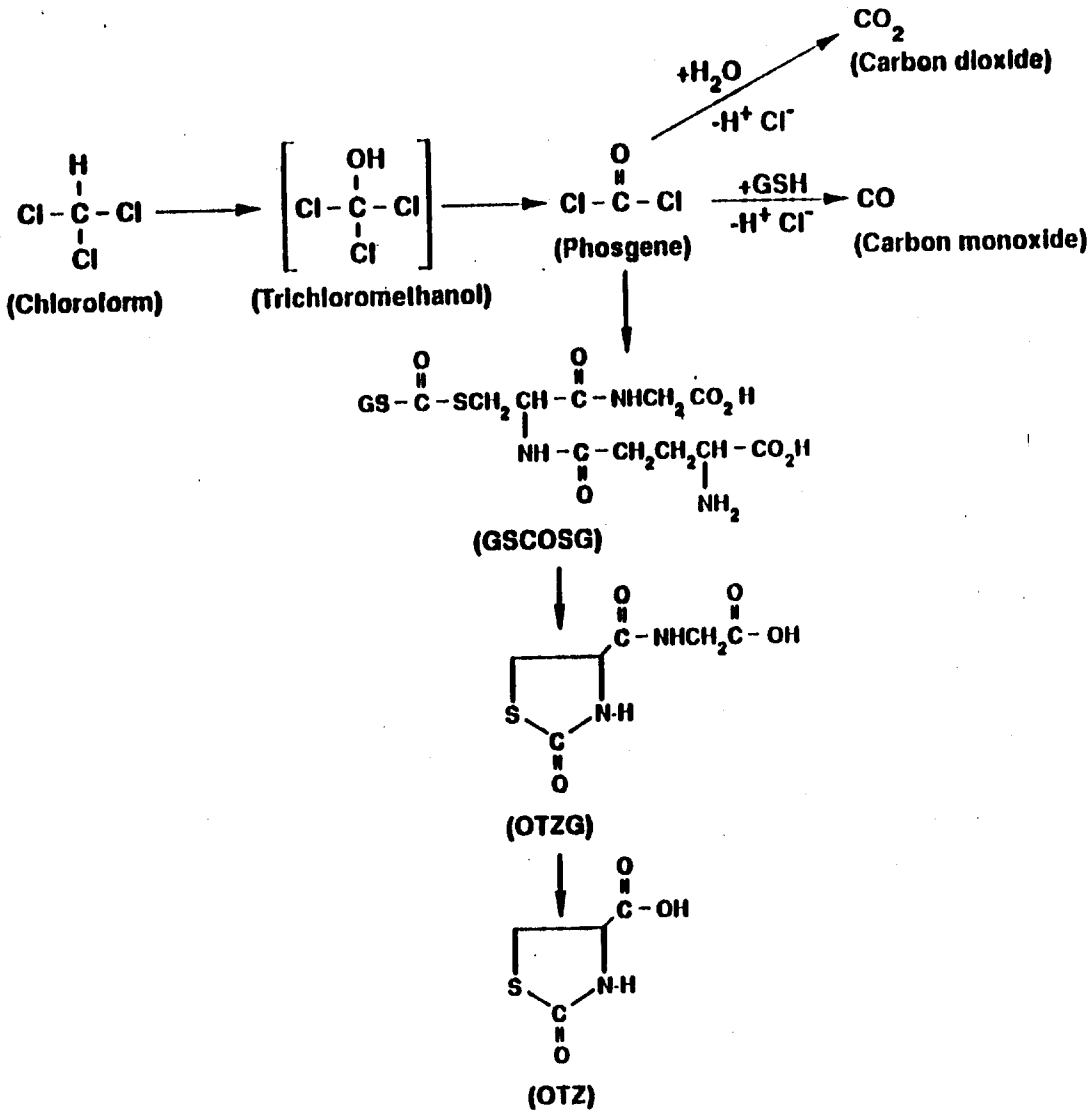


Figure 3-1. Metabolic pathways of chloroform (adapted from Stevens and Anders, 1981; Branchflower et al., 1984; Pohl et al., 1981).

Metabolite Formation

Chloroform is initially oxidized to trichloromethanol, an unstable compound that spontaneously dechlorinates to form phosgene (Ilett et al., 1973; Uehleke and Werner, 1975; Sipes et al., 1977; Gram et al., 1986). There is a considerable body of indirect evidence that phosgene is one of the principal intermediates in the metabolism of chloroform. Perhaps the most compelling data are those of Pohl et al. (1977) who showed that when rat liver microsomes were incubated with chloroform in the presence of cysteine, the reaction product (identified by gas chromatography-mass spectometry) was identical to that formed by the reaction of phosgene and cysteine, OTZ. Subsequently, Pohl et al. (1980) demonstrated that covalent binding of ^{14}C to rat liver microsomes, and the in vitro production of $^{14}CO_2$ from ^{14}C-chloroform was markedly inhibited by the addition of cysteine. The reaction of phosgene with two molecules of GSH leads to the formation of GSCOSG (Branchflower and Pohl, 1981; Pohl et al., 1981). Branchflower et al. (1984) speculated that GSCOSG is metabolized to OTZG via an intermediate, glutathionyl cysteinylglycinyl dithiocarbonate; OTZG is subsequently hydrolyzed to OZT.

The formation of phosgene from chloroform is mediated by the MFO, and is dependent on O_2 and NADPH. Phosgene production was diminished by the addition of the MFO inhibitors CO and SKF 525-A to rat liver microsomes incubated with chloroform (Mansuy et al., 1977; Pohl and Krishna, 1978).

Although the liver is the principal site of chloroform metabolism, and therefore the primary site of phosgene production, in vitro studies indicate that phosgene is also a metabolic intermediate in the renal metabolism of chloroform in rodents. When Paul and Rubenstein (1963) and Smith et al. (1985) incubated rat kidney slices with chloroform, CO_2, a known metabolic product of phosgene, was produced. Renal homogenates from DBA/2J mice incubated with GSH metabolized chloroform to phosgene, and ultimately, to OTZ (Branchflower et al., 1984). Kluwe and Hook (1981) showed that in vivo, chloroform caused depletion of renal GSH in mice, presumably by the direct reaction of phosgene with GSH to produce OTZ. The kidneys of rabbits also metabolize chloroform to phosgene and OTZG. Renal metabolism of ^{14}C-chloroform was enhanced by pretreatment with phenobarbital, which produced a four to five-fold increase in metabolites, compared to controls (Bailie et al., 1984).

Carbon dioxide is the major tertiary metabolite of chloroform in laboratory animals, and is formed by the hydrolysis of phosgene. Hydrogen and chloride ions (H^+, Cl^-) are generated as a consequence (Paul and Rubenstein, 1963; Brown et al., 1974a; Taylor et al., 1974; Reynolds et al., 1984; Mink et al., 1986). Humans are also known to metabolize chloroform to CO_2, presumably by the same route (Fry et al., 1972; Raabe, 1988). Most of the CO_2 formed by the metabolism of chloroform is eliminated through the lungs. However, radiolabeled urea, bicarbonate, and methionine were detected in the urine of mice following a 60 mg/kg oral dose of ^{14}C-chloroform, apparently from incorporation of ^{14}C (from $^{14}CO_2$) into the endogenous carbon pool.

Van Dyke et al. (1964) measured an increase in the excretion of ^{36}Cl in the urine of rats given ^{36}Cl-chloroform by i.p. injection. Of the radiolabeled chlorine excreted in the urine (the exact quantity was not specified), 27% did not precipitate as $Ag^{36}Cl$, and was presumably covalently bound to an organic molecule. The chlorine-containing metabolite was not identified.

To a limited extent, rat liver microsomes are capable of metabolizing chloroform to CO (Ahmed et al., 1977; Wolf et al., 1977). Consistent with this finding are several reports of elevated carboxyhemoglobin levels in rats exposed to chloroform (1 to 4 mmol/kg, i.p.) (Ahmed et al., 1977; Anders et al., 1978; Bellar et al., 1978; Stevens and Anders, 1981). In vitro, the production of CO from chloroform required NADPH, was enhanced by O_2, but also took place under anaerobic conditions. Pretreatment of animals with phenobarbital stimulated production of CO, while pretreatment with SKF 525A or phosgene inhibited the reaction (Ahmed et al., 1977). Anders et al. (1978) and Stevens and Anders (1979, 1981) provided evidence that CO is formed in vivo from chloroform by the sequential reaction of phosgene with two separate molecules of GSH to form GSCOSG, which is subsequently hydrolyzed to CO, H^+, and Cl^-. It has also been proposed that CO is formed by the reductive dechlorination of chloroform via a carbene (CCl_2) intermediate (Wolf et al., 1977).

The metabolism of chloroform proceeds anaerobically as well as aerobically (Pohl et al., 1980; Uehleke and Warner, 1975). Chloroform and other halogenated methanes compete with oxygen for electrons transferred through the MFO, and Tomasi et al. (1985) noted that this favors metabolic reduction of chloroform at low oxygen tension. Tomasi et al. used electron

spin resonance techniques (ESR) and the spin trap phenyl-t-butyl nitrone to demonstrate that a free radical metabolite of chloroform, possibly the dichloromethyl radical, $\cdot CHCl_2$, was produced in isolated rat hepatocytes incubated under anaerobic conditions. Similar incubations carried out in the presence of air also revealed evidence of free radical formation, but the ESR signal was much less intense (eight-fold less) than that observed under anaerobic conditions. The MFO inhibitors SKF 525A, metyrapone, and CO decreased production of the free-radical metabolite.

The possibility that chloroform may be metabolized anaerobically to a reactive intermediate was also examined by Testai and Vittozzi (1984). When rat liver microsomes were incubated under aerobic conditions with chloroform, loss of 70% of the cytochrome p450 occurred. Destruction of cytochrome p450 was completely prevented by the MFO inhibitor SKF 525A, and, to a lesser extent, by cysteine or GSH. Anaerobic incubation of microsomes resulted in a marked increase in the extent of cytochrome p450 loss, and addition of GSH had no effect. These preliminary observations indicated that oxygen-independent metabolism may contribute to the toxicity of chloroform. Subsequently, Testai and Vittozzi (1986) and Testai et al. (1987) demonstrated that covalent binding of a chloroform metabolite to lipid and protein took place when microsomes from phenobarbital-induced rats were incubated anaerobically. However, substantially fewer adducts were formed anaerobically than when incubations were conducted in an oxygen-rich atmosphere.

METABOLISM AND EXCRETION KINETICS

Much of the chloroform which has been absorbed into the body is metabolized prior to its elimination. The extent of this metabolism is variable and is species-dependent (Brown et al., 1974a; Taylor et al., 1974; Reynolds et al., 1984; Mink et al., 1986). In experimental animals, there is some indication that metabolism may be sex-dependent as well (Taylor et al., 1974; Smith et al., 1984a; Smith and Hook, 1984). In humans, and in all other animals studied to date, CO_2 is the predominant end product of metabolism. Although a number of other metabolites of chloroform have been identified, quantification of metabolite production has largely been restricted to measurements of $^{14}CO_2$ in expired breath after administration of radiolabeled parent compound. Even when urinary excretion of metabolites has been

followed, the identity of these metabolites has not been ascertained. Some chloroform is eliminated through the lungs without undergoing metabolic transformation (see Tables 3-1 and 3-2).

Humans

Fry et al. (1972) established that humans who ingested chloroform eliminated parent compound and the metabolite CO_2 through the lungs. When male and female volunteers were given a single oral dose of ^{13}C-chloroform in an olive oil vehicle, neither chloroform or CO_2 were detectable in expired breath until 20 to 30 minutes after ingestion. This period of time probably reflects a delay in absorption due to the action of the olive oil vehicle. Pulmonary elimination of chloroform and CO_2 were followed for 8 h, but peak concentrations in exhaled breath were measured between 40 min and 2 h after administration. Although most of the administered dose was recovered within the 8-h period, Fry et al. noted that chloroform was detectable in the breath of some subjects up to 24 h after dosing. Over the course of the recovery period, an average of 44.3% of a 500-mg dose was recovered as unmetabolized chloroform in males (Table 3-2). This value exhibited substantial individual variability however; of the five subjects, values ranged from 17.8 to 66.6%. Females given the same quantity of chloroform eliminated an average of 33.6% through the lungs as unmetabolized parent compound. Again, there was a considerable range in reported values (25.6 to 40.4%). A single male given a 1000-mg dose of chloroform exhaled 64.7% through the lungs unchanged. In a concurrent experiment, Fry et al. found that 48.5 to 50.6% of a single 500-mg dose was respired as $^{13}CO_2$. Although elimination of chloroform was not documented, data from the two separate phases of this study indicate that approximately half of an orally administered dose of chloroform is metabolized to CO_2, and approximately half is eliminated without undergoing metabolism. No attempt was made to measure elimination of urinary metabolites.

In a study sponsored by the State of California and cited in CDHS (1989), Raabe (1988) examined the disposition of inhaled chloroform in humans. Eight volunteers were exposed to 7 to 25 ppbv of ^{14}C-chloroform for 2 h; elimination of the radiolabel in expired air was followed over the course of the exposure period and for 30 min thereafter. Among four individuals who inhaled chloroform through the mouth only, 38.2% of the retained dose was

Table 3-2. Metabolism of chloroform (CCl_3) in humans after oral or inhalation exposure.

Sex (no. individuals)	Body weight, kg	Applied dose or concentration	Exposure protocol	Recovery period (h)	Percent of applied dose recovered in expired air as: $^{14}CCl_3$	$^{14}CO_2$	Urine	Reference
M, F (4)	N/A[a]	15.5 ppbv	inhalation,[b] 2.0 h	2.5	0.29(0.6)[c]	17.4(38.2)[c]	0.29(0.6)[c]	Raabe, 1988
M, F (4)	N/A	10.0 ppbv	inhalation,[d] 2.0 h	2.5	0.29(0.6)[c]	15.8(31.9)[c]	0.30(0.6)[c]	ibid.
M (1)	66.0	100 mg (1.5 mg/kg)	single, oral	8	80[e]	N/A	N/A	Fry et al., 1972
M (1)	66.0	250 mg (3.8 mg/kg)	single, oral	8	12.4	N/A	N/A	ibid.
M (1)	61.8	500 mg (8.1 mg/kg)	single, oral	8	66.0	N/A	N/A	ibid.
M (1)	64.6	500 mg (7.7 mg/kg)	single, oral	8	51.0	N/A	N/A	ibid.
M (1)	70.9	500 mg (7.1 mg/kg)	single, oral	8	50.0	N/A	N/A	ibid.
M (1)	80.0	500 mg (6.3 mg/kg)	single, oral	8	35.9	N/A	N/A	ibid.
M (1)	74.6	500 mg (6.7 mg/kg)	single, oral	8	17.8	N/A	N/A	ibid.
F (1)	62.7	500 mg (8.0 mg/kg)	single, oral	8	40.4	N/A	N/A	ibid.

Table 3-2. (Continued).

Sex (no. individuals)	Body weight, kg	Applied dose or concentration	Exposure protocol	Recovery period (h)	Percent of applied dose recovered in expired air as: $^{14}CCl_3$	$^{14}CO_2$	Urine	Reference
F (1)	59.0	500 mg (8.5 mg/kg)	single, oral	8	34.8	N/A	N/A	ibid.
F (1)	58.0	500 mg (8.6 mg/kg)	single, oral	8	25.6	N/A	N/A	ibid.
M (1)	61.8	500 mg (8.1 mg/kg)	single, oral	8	N/A	50.6	N/A	ibid.
F (1)	62.7	500 mg (8.0 mg/kg)	single, oral	8	N/A	48.5	N/A	ibid.
M (1)	65.0	1000 mg (15.4 mg/kg)	single, oral	8	64.7	N/A	N/A	ibid.

[a] N/A = not available
[b] Inhalation through the mouth only
[c] Values in parentheses represent the percent of retained dose recovered
[d] Inhalation through the nose only
[e] BD = below level of detection

recovered as $^{14}CO_2$. Only 0.6% of the radiolabel was recovered as chloroform in expired breath or as urinary metabolites. Nearly identical values were obtained from four volunteers who inhaled chloroform through the nose (Table 3-2). At the end of the recovery period, it was estimated that approximately 28.2% of the absorbed chloroform remained in the body. Consequently, the reported percentage of dose recovered as metabolites probably underestimates the actual extent of metabolism. Nonetheless, the elimination profile for chloroform in humans is consistent with the data of Fry et al. (1972) as far as the relative importance of respiratory excretion of chloroform metabolites. Raabe (1988) used a one-compartment model to calculate that the elimination half-life of chloroform and its metabolites in respired breath and urine were 3.5 h and 5.2 h, respectively.

Animals

Mice metabolize virtually all of an orally administered dose of chloroform. Males of three separate strains of mice eliminated 83.9 to 87.2% of a 60 mg/kg dose of ^{14}C-chloroform as $^{14}CO_2$ over a 48-h recovery period (Table 3-1). An additional 4.0 to 5.0% of the dose was recovered in the urine, feces, and carcass. Relatively minor amounts (5.2 to 7.1%) were eliminated as parent compound through the lungs (Brown et al., 1974a). To a great extent, these data were corroborated by Taylor et al. (1974), who also distinguished the relative importance of urinary and fecal elimination of chloroform in mice. Of the 5.5 and 5.6% of a 60-mg/kg oral dose recovered in excretory products, almost all of it (4.9 to 5.1% of the applied dose) was due to recovery of radiolabel in the urine. Less than 2% of the dose was eliminated as unchanged chloroform. Taylor et al. noted that although the percentage of administered dose eliminated as parent compound was relatively small in both male and female mice (0.31 and 1.8%, respectively), the difference was statistically significant ($p < 0.001$, Student's t-test)

The data of Mink et al. (1986) are consistent with the hypothesis that metabolism of chloroform in mice is saturable at high doses (U.S. EPA, 1985a; Gargas et al., 1988). Although their results were compromised by the short recovery period (8 h), 26% of a single 150-mg/kg oral dose of chloroform was eliminated as unmetabolized parent compound, and only 50% of the dose was metabolized to CO_2. A relatively large percentage (13.5%) of the applied dose

was recovered in the carcass, perhaps as a direct consequence of the abbreviated recovery period used.

Based on somewhat limited data, it appears that rats metabolize a smaller percentage of an orally administered dose of chloroform than mice. Reynolds et al. (1984) and Brown et al. (1974a) recovered 66.1 to 68.0% of a single oral dose of ^{14}C-chloroform to rats as $^{14}CO_2$ in respired air (Table 3-1). Reynolds et al. found that the peak elimination of chloroform occurred within the first 30 min after oral dosing, while the peak elimination of $^{14}CO_2$ was measured at 30 to 45 min post-dosing (11.9 mg/kg), and between 60 to 105 min post-dosing in animals given 35.8 mg/kg. Neither Reynolds et al. or Brown et al. specified the quantity of radiolabel remaining in the carcass (if any), although Brown et al. recovered 7.6% of a 60 mg/kg dose in the urine and feces. Elimination of chloroform through the lungs accounted for 5.2 to 6.0% of the dose in Brown et al., while the data of Reynolds et al. indicate that elimination of chloroform is dose-dependent. Rats that received 11.9 mg/kg eliminated 5.0% of the dose as chloroform. This value increased to 12.0% when the dose was raised to 35.8 mg/kg. By incorporating pulmonary elimination data into a linear, two-compartment model, Reynolds et al. estimated that for animals that received 11.9 mg/kg of chloroform, the half-times for oral absorption, pulmonary elimination of chloroform, and pulmonary elimination of CO_2 were 0.08, 3.83, and 2.1 h. The corresponding values for animals that received 35.8 mg/kg of chloroform were 0.13, 2.27, and 5.6 h.

It is difficult to reconcile the data of Mink et al. (1986) with those of earlier investigations. The 6.5% of the applied dose recovered as $^{14}CO_2$ from rats in that study is substantially lower than other reported values for orally administered chloroform (Table 3-1). Interpretation of the data of Mink et al. is complicated by the short follow-up period (8 h) and by the poor recovery of radiolabel (78.2%). Nevertheless, neither of these factors adequately explain the difference between the results reported by Mink et al. and those of Reynolds et al. (1984) and Brown et al. (1974a). One possible explanation may be that the 100-mg/kg dose used in this study exceeded the capacity of Sprague Dawley rats to metabolize chloroform, and that this is reflected in the relatively large percentage of applied dose eliminated as unmetabolized parent compound. This interpretation appears to be consistent with the trend exhibited in other reported percentages of applied dose eliminated as chloroform (Table 3-1) in that as the applied dose increases

from 11.9 mg/kg to 60 mg/kg, the percent eliminated as chloroform increases from 5.0 to 19.7%. It should be noted, however, that this apparent trend could also be explained simply on the basis of the substantially longer recovery period of Brown et al. (1974a).

When Paul and Rubenstein (1963) tracked the recovery of $^{14}CO_2$ expired by rats given radiolabeled chloroform intraperitoneally, they reported that only 4.0% of the dose was metabolized. The U.S. EPA (1985a) attributed this low value to the insensitivity of the analytical techniques available at the time, rather than to differences in metabolism as a result of the route of administration.

Withey and Collins (1980) gave intrajugular injections of chloroform to rats (3, 6, 9, 12, or 15 mg/kg), and followed the blood-concentration profile of chloroform over time. Regardless of dose, chloroform disappeared from the blood in an exponential manner and was reasonably described by a three-compartment model. The rate constant for elimination of chloroform from the blood (primarily through the lungs) was dose-dependent and varied from 3.6 to 6.2 min^{-1}.

Dogs that inhaled 0.393 to 0.594 ppmv of radiolabeled chloroform excreted 14.9% of the retained dose as $^{14}CO_2$, and 20% as chloroform or alcohol-soluble metabolites in a 21-h post-exposure period. An additional 9% of the retained dose was eliminated in the urine, and 2% in the feces over a period of 69 h (Raabe, 1986).

Squirrel monkeys metabolized approximately 20% of a 60 mg/kg oral dose of ^{14}C-chloroform; 18% was recovered as $^{14}CO_2$, and 2% as urinary or fecal metabolites. Whole-body autoradiographic techniques revealed the presence of radiolabel in the bile. The maximum concentration of radioactivity in the bile duct occurred 6 h after dosing (Brown et al., 1974a).

Organ-specific differences in metabolism. Chloroform is metabolized in a number of different tissues and organs, including the liver, kidneys, lungs, and gastrointestinal tract (Ilett et al., 1973; Lavigne and Marchand, 1974; McMartin et al., 1981; Smith and Hook, 1983; Branchflower et al., 1984; Smith and Hook, 1984; Rush et al., 1984; Smith et al., 1985; Lofberg and Tjalve, 1986). Of these, the liver metabolizes the greatest amount of chloroform. An increasing body of evidence indicates that the kidney may also metabolize significant quantities of chloroform, as discussed below.

One or more metabolites of chloroform are believed to be responsible for its hepatic and renal toxicity (Ilett et al., 1973; Smith and Hook, 1983; Smith and Hook, 1984; Branchflower et al., 1984; U.S. EPA, 1985a; Smith et al., 1985). In the course of examining this hypothesis, it has become apparent that there are organ-specific differences in the extent of chloroform metabolism in mice and rats. Mice appear to have sex-specific differences in the renal metabolism of chloroform as well.

There has been a long-standing controversy over whether the reactive metabolite presumed to be responsible for chloroform-induced renal toxicity is produced in the kidney, or whether it originates in the liver and reaches the kidney via the systemic circulation. Evidence has been presented that renal homogenates or renal microsomes from male mice oxidize chloroform to trichloromethanol, which spontaneously dechlorinates to phosgene--the same reactive substance as is formed in the hepatic metabolism of chloroform. This reaction is dependent on cytochrome p450 (Branchflower et al., 1984). Similarly, Smith and Hook (1984) found that the metabolism of ^{14}C-chloroform to $^{14}CO_2$ in mouse kidney homogenates required NADPH and O_2. The reaction was inhibited by CO, SKF 525A, and metyrapone, indicating that the renal metabolism of chloroform in mice is probably mediated by cytochrome p450-dependent MFO. The data of Smith et al. (1985) raise the possibility, however, that there may be qualitative differences in the renal biotransformation of chloroform between mice and rats. Incubation of rat kidney cortical slices in an atmosphere of CO did not alter chloroform-induced toxicity, whereas in mice, a similar protocol markedly reduced the toxic response to chloroform. Furthermore, the severity of the renal toxicity caused by chloroform did not differ between F344 and Sprague Dawley rats, even though the renal cytochrome p450 content of the F344 strain is approximately two times greater than that in the Sprague Dawley strain.

The metabolism of chloroform by rodent liver and kidney appears to differ with respect to the induction or inhibition of xenobiotic metabolism (Ilett et al., 1973; Kluwe et al., 1978). For example, Kluwe et al. (1978) established that the MFO in mouse kidney and liver respond differently to inducing agents. Polychlorinated biphenyls (PCB), 3-methylcholanthrene (3MC), and dioxin (TCDD) increased renal and hepatic microsomal activity, whereas phenobarbital (PB) increased only hepatic microsomal activity. The hepatotoxicity of chloroform was potentiated by pretreatment with PB,

diminished by TCDD, and was not affected by 3MC or PCB. Chloroform-induced renal toxicity was unaltered by pretreatment with PB, and was inhibited by 3MC, TCDD, or PCB. Kluwe and Hook (1981) have also reported that the renal damage induced in mice by chloroform was inhibited by pretreatment with piperonyl butoxide but not by SKF 525A (the latter is a potent inhibitor of hepatic MFO).

There are fundamental differences, which extend across many species, in the ability of the liver and kidney to metabolize xenobiotics. Although cytochrome p450-dependent MFO are present in the kidneys, the specific activity of these enzymes there is usually much less than that of the liver (Litterst et al., 1975, 1977; Fry et al., 1978). In general, the renal cytochrome p450 content is only 10 to 20% of that in the liver (Jones et al., 1980). McMartin et al. (1981) reported an even greater disparity in the metabolic capacity of these two organs. In rats, they found that the mean cytochrome p450 content of liver was 0.87 nmol/mg protein, compared to 0.071 nmol/mg protein in the kidney (approximately a 12-fold difference). When Smith and Hook (1984) evaluated the cytochrome p450 content in mouse liver and kidney, they determined that the renal cortex had an average of 0.453 nmol/mg protein, and the liver had 1.627 nmol/mg protein.

Paul and Rubenstein (1963) measured the in vitro production of $^{14}CO_2$ by rat liver and kidney slices incubated with ^{14}C-chloroform. Renal metabolism was only 11 to 24% of that in the liver. Pronounced differences in the in vitro metabolism of chloroform were also reported by Smith et al. (1985), who found that hepatic slices from naive rats produced 24 times as much $^{14}CO_2$ from ^{14}C-chloroform as did renal cortical slices.

Renal cortical and hepatic microsomes from male mice metabolized ^{14}C-chloroform to $^{14}CO_2$ and to covalently bound metabolites (Smith and Hook, 1984). The cytochrome p450 content per mg of microsomal protein was approximately four times greater in liver than in the renal cortex; this was paralleled by the extent of metabolism of chloroform, which was approximately two times greater in the hepatic microsomes.

To estimate the relative proportion of metabolism that takes place in each of these two organs, we evaluated three separate factors that contribute to the total metabolic capacity of each: tissue weight, the enzyme concentration in tissue, and the specific activity of the MFO enzymes. As noted above, McMartin et al. (1981) measured a 12-fold greater concentration

of cytochrome p450 in the livers of rats compared to that in the kidneys. In mice, Smith and Hook (1984) measured a 3.6 fold difference. There are also substantial differences in weight between the two organs which contribute to their total ability to metabolize xenobiotics. In rats, the kidneys comprise 0.94% and the liver 3.7 to 4.0% of total body weight (Harrison and Gibaldi, 1977, as cited in U.S. EPA, 1988; Lutz, 1977; Ramsey and Anderson, 1984). In mice, the kidneys make up 1.5% of total body weight and the liver 5.9%, again representing a four-fold difference in tissue weight between the two organs (Bischoff et al., 1971, as cited in U.S. EPA, 1988). Litterest et al. (1975) compared the in vitro metabolic activity of liver and kidney enzymes in several species of laboratory animals, including Sprague Dawley rats and CDF mice. They measured a 7.7-fold greater specific activity of cytochrome p450 and a 3.6-fold greater specific activity of NADPH-cytochrome-c-reductase in the liver than was present in the kidneys of rats. With regard to mice, the specific activity of NADPH-cytochrome-c- reductase and cytochrome p450 varied from 1.4 to 3 times greater, respectively, in the liver than in the kidneys. Fry et al. (1978) used suspensions of isolated viable rat hepatocytes and renal tubule fragments to compare the metabolic activity of the liver and kidney. Of the three substrates used to compare oxidative metabolism (measured as pmol product/mg cell protein/min) only 7-ethoxycoumarin was metabolized to any extent by the kidney. Even so, the specific activity of renal MFOs was only 3.2% of that in hepatocytes (a 31-fold difference). Considered together, these data indicate that liver contributes much more significantly to total chloroform metabolism than kidney. In rats, liver may be responsible for 370 times as much total oxidative metabolism as the kidney, while in mice the analagous factor may be between 43 and 450 depending on whether the data of Fry et al. (1978) are included.

Chloroform causes renal damage in both male and female rats, but in mice only males are susceptible (Eschenbrenner and Miller, 1945; Ilett et al., 1973; Smith and Hook, 1983, 1984; Ahmadizadeh et al., 1984). The sex-specific sensitivity of mice to chloroform-induced renal damage appears to be due in large part to marked differences in the metabolic activity of the kidney between the two sexes. In the presence of radiolabeled chloroform, NADPH, and O_2, microsomes from the renal cortex of male mice metabolized chloroform to $^{14}CO_2$ and covalently bound metabolites. Renal cortical microsomes from female mice were inactive (Smith and Hook, 1983). In an extension of this work,

Smith et al. (1984) found that the sensitivity of male mice and the insensitivity of female mice to the nephrotoxic effects of chloroform correlated well with the level of renal cytochrome p450-dependent MFO, which were three to five times higher in males than in females (Smith et al., 1984). It is also apparent that testosterone mediates these differences in some manner, as immature or castrated male mice were not susceptible to chloroforms nephrotoxic action, while female mice and castrated males injected with testosterone sustained kidney damage after exposure to chloroform (Smith et al., 1984).

PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELS

Physiologically based pharmacokinetic (PBPK) models mathematically describe the rate of uptake, distribution, metabolism, and elimination of xenobiotics in humans and experimental animals. These multicompartment models are based on physiologically realistic descriptions of tissue volume, cardiac output, lung capacity, and metabolism, and are based as much as possible on actual experimental measurements.

Feingold and Holaday (1977) used two separate pharmacokinetic models to study the metabolism and elimination of chloroform in humans, under exposure conditions designed to mimic clinical use as an anesthetic. In their models, the body was divided into five compartments; vessel-rich, vessel-poor, muscle, fat, and liver (the compartment responsible for metabolism). Extrahepatic metabolism of chloroform was not considered. They assumed that alveolar ventilation occurred at a rate of 4.8 L/min (288 L/h) and that cardiac output was equal to 6 L/min (360 L/h). Based on the data compiled by Steward et al. (1973), the blood/air partition coefficient was set at 8.0. The tissue/blood partition coefficients used in the models were: fat (31.0), muscle (1.9), vessel-rich (1.9), vessel-poor (1.0), and liver (2.0).

The first of the pharmacokinetic models applied by Feingold and Holaday was based on the assumption that human metabolism of chloroform is a linear, first-order process, and that a constant fraction of the chloroform delivered to the liver (the hepatic fraction, HF) is metabolized. The model was evaluated for three separate HF values (0.02, 0.06, 0.18) and for three different periods of exposure (1, 4, or 8 h). Feingold and Holaday also tested a model which assumed that there is a specific, but nonlinear relation

between the hepatic arterial concentration of chloroform and the HF metabolized (for details, see Appendix B of Feingold and Holaday, 1977). The model also assumed that for a given molar arterial concentration, chloroform was metabolized to the same extent as halothane. The basis for this approach was not explained. Rather than modeling the initial rate of uptake of chloroform, Feingold and Holaday set the alveolar concentration equal to the minimum blood arterial concentration attained during anesthesia (24.0 mg/mL). This arterial concentration was maintained for the duration of the exposure.

Results of the computer simulation of chloroform metabolism based on the linear model are summarized in Table 3-3. The percentage of dose metabolized (defined as the ratio of the amount metabolized to the amount of chloroform taken up) predicted by this model depended on the value of HF and on the duration of exposure, i.e., as the period of exposure and the HF increased, so did the amount metabolized. A similar relation was apparent when the data were expressed as absolute quantities (e.g., mmol of chloroform metabolized). The nonlinear model predicted that the percentage of chloroform metabolized would not be substantially affected by the duration of exposure, varying from 33% after a 1-h exposure to 29% following an 8-h exposure.

Table 3-3. Predicted relative amount of chloroform metabolized based on the linear pharmacokinetic model of Feingold and Holaday (1977). (See text for definition of HF and percentage metabolized.)

Percentage of chloroform metabolized when HF=								
0.02			0.06			0.18		
Duration of anesthesia, h								
1	4	8	1	4	8	1	4	8
6	8	10	18	22	26	40	48	54

The Ramsey-Andersen PBPK Model

The National Research Council (NRC) evaluated a PBPK model developed by Ramsey and Andersen (1984) which describes the uptake, metabolism, and excretion of styrene in rats and humans (NRC, 1986). The NRC analysis also considered the application of PBPK models to dose-route extrapolation from rats to humans for noncarcinogenic toxicity resulting from exposure to trichloroethylene (TCE) and benzene. This type of model has been applied to the study and prediction of animal and human pharmacokinetics for a number of volatile organic compounds (VOCs) including TCE, tetrachloroethylene, benzene, methylene chloride, and 1,1,1-trichloroethane (NRC, 1986; Gargas et al., 1986; Andersen et al., 1987; Reitz and Nolan, 1986; Hattis et al., 1987; Bogen et al., 1987; Ward et al., 1988; Bogen, 1988; Bogen and McKone, 1988; Bogen and Hall, 1989).

The Ramsey-Andersen PBPK model consists of a series of differential equations that define the rates of change of the amount of absorbed chemical present in each of four physiologically realistic tissue compartments, which are assumed to be ideally well mixed at any given time. Metabolism is presumed to occur solely in the liver through a saturable enzymatic process with Michaelis-Menten kinetics. The structure of the model is shown in Figure 3-2 and its parameter definitions are given in Table 3-4. For further discussion of the model and for details regarding implementation, see Bogen (1988), Bogen and McKone (1988), and Bogen and Hall (1989).

Application of the Ramsey-Andersen Model to Chloroform

Physiological and pharmacokinetic information is available allowing a parameterization of a Ramsey-Andersen PBPK model for chloroform uptake, distribution, metabolism, and (respiratory) excretion in rodents and humans. Here, we first describe this parameterization. We next compare results of rat and mouse studies and a selected human study of chloroform metabolism with those predicted by our parameterized Ramsey-Andersen PBPK models for these species. Under the assumption that the values for physiological parameters and K_m that we initially selected are ones that are reasonable and not likely to account for differences revealed by the latter comparison, we then adjust the values of V_{max} initially used for mice, rats, and humans so that the

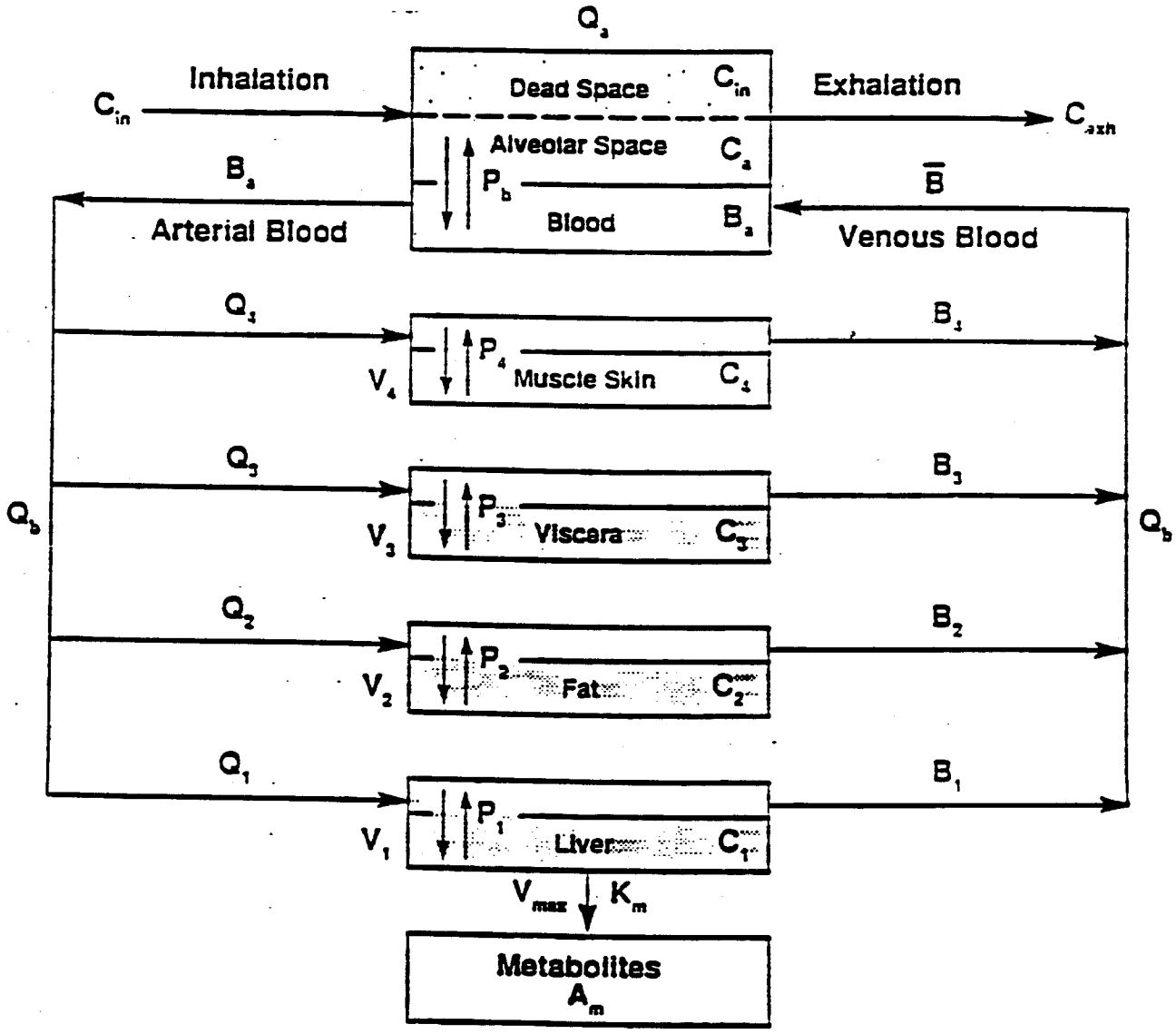


Figure 3-2. Schematic diagram of physiologically based pharmacokinetic (PBPK) model for inhalation of volatile organic compounds. The model assumes that four "well-stirred" compartments or tissue groups collect inhaled compound at rates governed by air concentration (C_{in}), air and blood flows (Q), blood concentrations (B), compartment volumes (V), tissue/blood partition coefficients (P), and metabolic parameters (V_{max}, K_m).

Table 3-4. Compartment and parameter definitions for the Ramsey-Andersen PBPK model.

Abbrev.	Definition	Unit
C_{in}	Concentration in air inhaled	mg/L air
C_a	Concentration in alveolar air	mg/L air
C_{exh}	Measured concentration in expired breath	mg/L air
Q_a	Alveolar ventilation rate	L air/h
Q_b	Cardiac output	L blood/h
P_b	Blood/air partition coefficient	L air/L blood
B_a	Arterial blood concentration	mg/L blood
$\bar{B}$	Venous blood concentration	mg/L blood
A_m	Amount metabolized in liver	mg
Q_i	Blood flow rate to compartment i	L blood/h
V_i	Volume of compartment i	L (≡ kg)
C_i	Concentration in compartment i	mg/L
B_i	Concentration in venous blood leaving compartment i	mg/L blood
A_i	Amount in compartment i	mg
P_i	Tissue/blood partition coefficient for compartment i	L blood/L tissue i
V_{max}	Maximum metabolic rate	mg/h
K_m	Apparent Michaelis constant = $\{B_1 \mid (dA_m/dt = V_{max}/2)\}$	mg/L blood

Compartmental subscripts:

i = 1	Liver (metabolizing tissue group)
2	Fat tissue (very poorly perfused)
3	Richly perfused tissues (brain, kidney, viscera)
4	Poorly perfused tissues (muscle, skin)

corresponding PBPK models predict amounts of chloroform metabolism that were actually observed in studies we selected, in the absence of more detailed data on chloroform metabolism, as the most appropriate for use as the basis for extrapolation. In Section 6, we shall use the latter "V_{max}-validated" PBPK models to estimate (a) the metabolized (toxicologically effective) doses received by animals in rodent bioassays used in that section as the basis for estimating chloroform's carcinogenic potency, and (b) the effective doses to humans environmentally exposed to chloroform. This approach is taken in Section 6 because there is evidence, reviewed in that section and in Section 4, that chloroform's toxicity and carcinogenicity are more likely due to the production of metabolite(s) or to the metabolism of chloroform than to the parent compound itself.

Our PBPK model for chloroform in rats used blood/air and tissue/air partition coefficients taken from Gargas et al. (1989), who determined these values using gas-phase vial equilibration between chloroform and tissues of F344 rats. We also used a value for V_{max} (7.0 mg/h) and K_m (0.25 mg/L) derived separately for F344 rats by Gargas et al. (1988). Values for the physiological parameters Q_a and Q_b were based on those pertaining to a reference 0.25-kg rat (U.S. EPA, 1988). The mass-balance studies of Brown et al. (1974a), Reynolds et al. (1984) and Mink et al. (1986) (see Table 3-1) provide the only available estimates of the amount of chloroform metabolized by rats dosed by gavage with a corn oil vehicle. To compare the results of the model to these data, tissue volumes were scaled directly to the relevant experimental rat body weight, flow-rate parameter values (in units of L/h) were scaled to the 0.74 power of body weight, and V_{max} was scaled to the 0.70 power of body weight (Ramsey and Andersen, 1984; Gargas et al., 1986; Paustenbach et al., 1988). Oral absorption kinetics of chloroform were presumed to be similar to those observed by Withey et al. (1983). In that study, 400-g Wistar rats were administered chloroform in 10 mL/kg of corn oil, which yielded a roughly constant, 30-min absorptive pulse equivalent to clearance of compound from the vehicle at a rate of about 0.33 mL vehicle/kg-min. We presumed that the latter clearance rate scales to the -0.26 power of body weight (see Appendix C).

With regard to the data of Mink et al.(1986) and one of the data sets (the 11.9-mg/kg dose group) of Reynolds et al. (1984), the model provided reasonable predictions of the total amount of chloroform metabolized: the

ratio of actual to predicted values ranged from 0.94 to 1.0. However, the data of Mink et al. (1986) were compromised by poor recovery of radiolabel (78.2%), and by an abbreviated recovery period (8 h). (Both factors would contribute to an underestimation of the total amount of chloroform metabolized, but to an unknown degree.) At higher experimentally administered doses in the Reynolds et al. (1984) and Brown et al. (1974a) studies, the experimentally determined metabolized dose differed more substantially from that predicted by the PBPK model; at the highest administered dose (60 mg/kg) for which we had metabolism data, the model underestimated the amount of chloroform metabolized by a factor of 2.0. The data of Brown et al. (1974a) represent the most comprehensive estimate of chloroform metabolism in rats and include measurements of respiratory elimination of the metabolite CO_2 as well as urinary and fecal elimination of metabolites over a 72- to 96-h recovery period. For this reason, these data were selected by us to serve as the basis for "correcting" the value of V_{max} originally used to yield a PBPK estimate of metabolism in accordance with experimental data. The resulting V_{max} value, determined by iterative optimization and scaled to a 1.0-kg rat, is listed in Table 3-5.

We evaluated how well the PBPK model predicted chloroform metabolism in mice as observed in the studies by Brown et al. (1974a) and Taylor et al. (1974), in which chloroform was administered by gavage in corn oil (Table 3-1). For purposes of comparing these data with the values predicted by our PBPK model, the experimental results from Brown et al. (1974a) and Taylor et al. (1974) were averaged. This approach was taken because production of chloroform metabolites by mice in these studies was nearly identical in each of the five separate sets of data (Table 3-1). The blood/air partition coefficient for chloroform in mice we used was determined by Gargas et al. (1989); in the absence of specific values for tissue/blood partition coefficients, it was assumed that those in mice were identical to the values determined for rats (Gargas et al., 1989). The metabolic constants for the mouse were obtained by setting the mouse K_m value equal to that of the rat and scaling the rat V_{max} value to the 0.7 power of body weight, as described above. Other physiological parameters for mice were taken from U.S. EPA (1988), and scaled as described above. The rate of vehicle clearance from corn oil for mice was based on that calculated from the data of Withey et al. (1983) for rats, scaled to the -0.26 power of body weight as described above.

Table 3-5. Parameter values used in PBPK model for chloroform.

Parameter		Unit	Mouse[a]	Rat[a]	Reference Human male[b]
W (body weight)		kg	0.025	1.0	70.0
Q_a		L/h	1.50	19.6	378.0
Q_b		L/h	1.02	13.9	372.0
P_b			21.3	20.8	8.0
Q_i/Q_b	i = 1		0.25	0.25	0.26
	2		0.09	0.09	0.05
	3		0.51	0.51	0.44
	4		0.15	0.15	0.25
V_i/W	i = 1		0.055	0.04	0.026
	2		0.10	0.07	0.19
	3		0.05	0.05	0.05
	4		0.70	0.75	0.62
P_i	i = 1		1.01	1.01	2.1
	2		9.76	9.76	35.0
	3		1.01	1.01	1.9
	4		0.668	0.668	1.5
V_{max}		mg/h	4.7[c]	37.2[c]	560[c]
K_m		mg/L	0.25	0.25	0.25

[a] Rat partition coefficients, body weight, and K_m are from Gargas et al. (1988, 1989). The values for Q_a and Q_b in the rat were derived as described in the text. Other rat physiological parameters are from U.S. EPA (1988). With the exception of the mouse blood/air partition coefficient for which we had a specific value (Gargas et al., 1988), it was assumed that the partition coefficients for chloroform in mice were identical to those for rats. Mouse body weight and other physiological parameters are from U.S. EPA (1988).

[b] Reference physiological parameters (except Q_a) for adult males are from the U.S. EPA (1988). The value for Q_a is from Salzano et al. (1984, as reviewed in U.S. EPA, 1988). The blood/air partition coefficient represents the median of published values (Gargas et al., 1989; Sato and Nakajima, 1979; Steward et al., 1973). Human tissue/blood partition coefficients for liver and fat are from Steward et al. (1973); the value for the vessel-rich group was taken directly from Feingold and Holaday (1977), while the partition coefficient for the vessel-poor group is an average of the values for the "muscle" and "vessel-poor" groups of Feingold and Holaday (1977).

[c] See text for derivation of V_{max}.

Compared to the averaged experimental results of Brown et al. (1974a) and Taylor et al. (1974), the PBPK model underestimated the amount of chloroform observed to be metabolized by mice by a factor of 2.2. Therefore, we again used a process of iterative optimization to calculate a "corrected" value of V_{max} for mice. This value is listed in Table 3-5.

Data on the metabolism and elimination of chloroform in humans is largely limited to that of Fry et al. (1972), who observed that an average of 49.5% of a single 500-mg oral dose of ^{14}C-chloroform was metabolized to $^{14}CO_2$ within a postexposure observation period of 8 h. Volunteers in the Fry et al. study received chloroform in 1 mL of olive oil, a quantity expected to be cleared in approximately 0.005 h based on the data of Withey et al. (1983) for rats, and scaled as described above. The Michaelis constant, K_m, was assumed to be equal to that used for rats. By iterative numerical optimization, we estimated the value of V_{max} for chloroform required to allow our PBPK model for humans to predict the extent of chloroform metabolism observed by Fry et al. (1972). This value, scaled to a reference 70-kg human as described above, was found to be 560 mg/h (Table 3-5). We note that this value is approximately four times greater than that obtained by scaling the rat V_{max} of Gargas et al. (1988) to the 0.7 power of body weight.

A recent abstract by Corley et al. (1989) described a PBPK model for chloroform reportedly validated by in vivo methods for rats and mice and by in vitro methods for humans. Because this was not a full-length peer-reviewed article, sufficient detail was not provided to evaluate the methodology used to paramaterize the model. However, we note that our values of V_{max} for mice and humans were 2.5- and 1.8-fold greater, respectively, than those derived by Corley et al., while our rat V_{max} was 16-fold greater than their corresponding value. Similarly, our V_{max}/K_m ratios were 3.5- or 3.3-fold greater than the corresponding ratios for mice and humans, respectively, and 12-fold greater than the corresponding ratio for rats than those used in the model of Corley et al. (1989).

Using the human physiological and metabolic parameters listed in Table 3-5, the metabolized fraction of the total quantity of chloroform potentially available for absorption and metabolism can be predicted. Under conditions of purely ingestive exposure, the corresponding quantity of interest is the fraction, f_{mo}, of the maximum plausible metabolic rate, i.e., the metabolic clearance fraction given a continuous rate of ingestive

6. CARCINOGENIC POTENCY

The term carcinogenic "potency" here refers to the quantitative expression of increased tumorigenic response per unit dose rate at very low dose levels. Estimated potencies for chloroform are first predicted here using the PBPK models for mice, rats, and humans discussed in Section 3 and the "linearized multistage" (LM) dose-response extrapolation model that has been adopted for regulatory purposes by the California Department of Health Services (CDHS) and the U.S. Environmental Protection Agency (U.S. EPA, 1986; Anderson et al., 1983; CDHS, 1985). This potency assessment is then followed by an alternative assessment of chloroform's potential to cause cancer in humans based on the PBPK models referred to above in combination with the "cell-kinetic multistage" (CKM) models for extrapolating cancer dose-response that are discussed in Appendices B and C of this report, under the assumption that chloroform is only a promoter, and not an initiator, of carcinogenesis. As explained further below, these two types of cancer-risk extrapolation are based on different assumptions regarding chloroform's mechanism of carcinogenic action. For chloroform, the LM-based approach proceeds from an assumption that any applied dose of chloroform results in metabolism and/or metabolite(s) which in turn result in cancer-causing somatic mutations, the occurrence of which is directly proportional to effective (metabolized) dose at very low doses. In contrast, the CKM-based approach used here presumes that chloroform is not mutagenic <u>in vivo</u>, and that its observed carcinogenicity in laboratory animals is due solely to its ability, at relatively high doses which are cytotoxic, to "promote" or enhance the growth rate of cancers that arise spontaneously (i.e., as events that are independent of exposure to chloroform). As such, the two approaches taken here to quantify chloroform's potential carcinogenicity in humans, on the basis of extrapolation from animal bioassay data, are quite different and the highly divergent risk estimates they yield are likely to be the extremes of the range of scientifically plausible estimates that can be made at present.

POTENCY ASSESSMENT BASED ON THE "LINEARIZED" MULTISTAGE MODEL

The following carcinogenic potency assessment proceeds in three steps: (1) selection of bioassay data sets indicative of chloroform carcinogenicity suitable for dose-response assessment, (2) derivation or adjustment of the appropriate response and dose information to use in dose-response assessment for the animal bioassay data, and (3) quantification of potency in terms of human applied dose based on the latter data, taking chloroform metabolism into account. Finally, a note is made on methods to calculate chloroform concentration in water associated with predicted cancer-risk levels, using information provided in this section and in Section 5.

A carcinogenic potency assessment for chloroform was included in recent health risk assessment documents on chloroform prepared by the U.S. EPA (1985a) and by the CDHS (1989). Both of these potency assessments were based on smaller sets of animal bioassay data than are considered in the present analysis, and the method used to convert applied chloroform doses to equivalent doses was different from the physiologically based pharmacokinetic (PBPK) approach used here. For comparative purposes, we point out these differences in the following subsections.

SELECTION OF BIOASSAY DATA INDICATIVE OF CHLOROFORM CARCINOGENICITY

The criteria we used for selecting particular bioassay data sets from among those available (Tables 4-1 through 4-3) are contained in regulatory guidelines available for this purpose (CDHS, 1985; U.S. EPA, 1986). Specifically, to determine tumor-incidence-rate numerators we used data sets reflecting a statistically significant increase in the incidence of malignant tumors of a given histologically defined type within animals of a given sex from a given study. In addition, we used data sets that, along with such malignant tumors, included benign tumors that were histologically related to, and had the potential to become like, the corresponding observed malignant tumors. The use of these selection criteria resulted in a total of 17 data sets on which carcinogenic potency analyses could be based. These data sets consist of data on hepatocellular carcinomas in male and female B6C3F1 mice (NCI, 1976); data on hepatocellular carcinomas and malignant lymphomas in male and female B6C3F1 mice (Reuber, 1979); data on renal hypernephromas or

adenomas in male ICI mice (Roe et al., 1979); data on renal adenomas or carcinomas in male Osborne-Mendel rats (NCI, 1976); data on renal adenomas or carcinomas in male Osborne-Mendel rats and data on cholangiofibromas or cholangiocarcinomas in female Osborne-Mendel rats (Reuber, 1979); data on hepatic adenofibrosis in male and female Wistar rats (Tumasonis et al., 1985, 1987); and data on renal adenomas or carcinomas in male Osborne-Mendel rats (Jorgenson et al., 1985). These data are summarized in Table 6-1 which appears at the end of the first part of this section (p. 129) along with corresponding dose, tumor-incidence, and potency information explained below. These bioassay data sets are used here for potency assessment because they represent the only lifetime-exposure studies with well-defined, exposure-response data that indicate a positive carcinogenic response for chloroform in animals and because comparable human epidemiologic data are not available.

We emphasize that our inclusion of all 17 of these data sets in this analysis is not intended to imply that all reflect studies that were conducted with equal scientific merit or that yielded results of equal biological significance. In particular, the validity and significance of the data sets from Reuber (1979) are unclear. These data, which are based on Reuber's reevaluation of the NCI (1976) tumor incidence data in mice and rats are substantially different from the (original) conclusions of the NCI pathologists. As noted in the review of this report (Section 4), the disparate results have been attributed to a difference of opinion between pathologists.

In its health-risk assessment of chloroform, the U.S. EPA (1985a) did not consider the data of Tumasonis et al. (1985, 1987), apparently because they were not available at the time. The incidence data resulting from Reuber's reevaluation of NCI (1976) were also excluded, although no specific reasons for this omission were given. The decision of the U.S. EPA (1985a) to incorporate only one of five data sets of Roe et al. (1979) in the potency assessment was not explained.

The recent health risk assessment of chloroform undertaken by the State of California (CDHS, 1989) includes potency calculations based on the data of the NCI (1976), Jorgenson et al. (1985), and Tumasonis et al. (1985, 1987). This assessment also considered the data of Reuber (1979) regarding the

incidence of cholangiofibromas and cholangiocarcinomas in female Osborne-Mendel rats, and four data sets from Roe et al. (1979).

ADJUSTMENT OF ANIMAL BIOASSAY DOSE-RESPONSE DATA

Brief reviews are given below of the administered dose and tumor-respon information for the NCI gavage study with mice and rats, the different interpretation of this information by Reuber (1979), and the drinking-water studies with rats of Tumasonis et al. (1985, 1987) and Jorgenson et al. (1985). This information is summarized in Table 6-1. From the administere doses, A, used in those bioassays, we derived corresponding lifetime, time-weighted average (TWA) metabolized doses using pharmacokinetic assumptions and relationships discussed in Section 3. This approach is take because chloroform is a volatile, lipophilic compound that is extensively metabolized in mammals (see Sections 3 and 4), and evidence exists demonstrating that the products or the process of this metabolism, rather th the presence of chloroform itself, are responsible for most forms of chloroform's subchronic and chronic toxicity, and for its carcinogenicity i particular, in laboratory animals (Ilett et al., 1973; Cornish et al., 1973 Brown et al., 1974b; Lavigne and Marchand, 1974; Kluwe and Hook, 1981; Letteron et al., 1987). Thus, in regulatory risk analysis for this type o compound, carcinogenic potencies can be estimated on the basis of metabolize (or "effective") dose, rather than administered or "applied" dose if adequa metabolic data are available (U.S. EPA, 1985b, 1985c, 1986; CDHS, 1985). F chloroform in particular we have adopted, in this first part of Section 6, the following rationale used by the U.S. EPA for calculating the dose-respon relationship for chloroform-induced tumorigenesis based on the total amount chloroform metabolized as the effective dose (U.S. EPA, 1985a, p. 8-98):

> The use of the amount of chloroform undergoing biotransformation as the effective dose may not eliminate all the uncertainties associated with the low-dose extrapolation, however, because the dose at the receptor sites may not be linearly proportional to the total amount metabolized. Thus, the true shape of the dose-response relationship would still be unknown. However, it seems reasonable to expect that the uncertainty with regard to the low-dose extrapolation would be somewhat reduced by considering the metabolized dose as the effective dose, that the amount of chloroform metabolized better reflects the

dose-response relationship, since the toxicity and carcinogenicity of chloroform is generally considered to be due to reactive intermediate metabolites.

Futhermore, we believe the available experimental data and modeling techniques reviewed in Section 3 are adequate to support a PBPK approach to estimating the metabolized fraction of an applied chloroform dose. For this reason, tumor-incidence data from bioassay studies are modeled in the following assessment as a function of metabolized dose (rate) of chloroform, which shall hereafter be denoted D, expressed in mg/kg-d.

The use of metabolized rather than applied dose as the basis for potency assessment adds new sources of uncertainty to our dose-response analysis. These include: uncertainty in parameter estimates based on animal or human experimental data; uncertainty involved in extrapolating kinetic constants between species, and uncertainty regarding the extent to which interindividual variability pertains to pharmacokinetic parameters, particularly for humans. The latter source of uncertainty is, of course, common to many problems in predictive regulatory toxicology, not just pharmacokinetic analysis. For toxic endpoints other than mutation and carcinogenesis, this uncertainty is generally dealt with by incorporating a safety factor to a final risk prediction, but the same approach could easily be adopted for these particular endpoints as well, to the extent that interindividual variability in mutation- or cancer-related metabolic capacity is of particular regulatory concern. Thus, even accounting for uncertainty in interspecies extrapolation of metabolic and other pharmacokinetic parameters, uncertainty remains that is difficult to quantify in the absence of relevant exploratory data. However, the only alternative to using estimates of metabolized chloroform dose in this dose-response analysis is to rely on some gross measure of the amount of chloroform that is potentially available for uptake and metabolism, such as the applied dose. But the latter measure, in light of chloroform's apparent nonreactivity without enzymatic attack (see Section 3), almost certainly does not correspond to the effective chloroform dose capable of covalently binding to critical cellular macromolecules in the "independent hit" fashion presumed to be necessary by the "linearized" multistage dose-response extrapolation model that has been adopted for use in this first potency assessment in this section. Thus, the decision of whether or not to account for chloroform metabolism involves a trade-off between the additional parameter uncertainties

associated with a pharmacokinetic model and the probable systematic error associated with some alternative model that does not consider chloroform metabolism. The optimum approach therefore must hinge on scientific judgments concerning the quality of data underlying (1) a decision regarding the intrinsic reactivity of the parent chloroform compound and (2) pharmacokinetic parameter estimates. As mentioned above, for the purpose of the present assessment, it was judged that the data base on chloroform pharmacokinetics in animals and humans is sufficient to support a PBPK approach for estimating metablized chloroform doses from applied doses to animals and humans. The application of this approach, based on the information presented in Section 3, is described below for each bioassay data set considered here for the assessment of chloroform's carcinogenic potency in animals.

NCI (1976) Study of Mice Dosed With Chloroform by Gavage

In this study, male and female B6C3F1 mice were dosed with chloroform by gavage 5 d/wk. Initially, male mice received 100 or 200 mg/kg of chloroform and females received 200 or 400 mg/kg. After 18 wk, doses of chloroform were increased to 150 and 300 mg/kg (males) and 200 and 500 mg/kg (females); the resulting time-weighted average (TWA) doses were 138 and 277 mg/kg (males) and 238 and 477 mg/kg (females). Treatment began when animals were 5 wk old, and continued for 78 weeks. Terminal sacrifice was at 92 wk after the start of treatment, when animals were 97 wk of age. The incidence of hepatocellular carcinoma (HCC) was significantly increased in treated animals of both sexes, with the first HCC appearing in wk 54 and 67 for males and females, respectively. Time-of-death information was available for this study, so the incidence-rate denominators appearing in Table 6-1 for this study represent animals surviving at least until the appearance of the first observed malignant tumor, to adjust for the effect of competing mortality risks on observed tumor incidence.

We used the validated PBPK model for chloroform, described in Section 3, to calculate the estimated experimental metabolized chloroform dose, D, received by animals in this study. The oral absorption kinetics of chloroform in a corn oil vehicle were modeled from the data of Withey et al. (1983), as discussed previously.

However, to represent D in terms of a lifetime TWA equivalent dose of the type used as input for cancer-risk extrapolation, this relationship needs to be modified to account for the interrupted (5 d/wk for 78 out of 92 wk) and partial lifetime (approximately 92 wk out of a theoretical 104-wk mouse lifespan) exposure pattern of this bioassay. For regulatory purposes, an adjustment is typically made whenever bioassay duration is different from the animals's lifespan. This adjustment is made by assuming that cumulative age-specific cancer rates increase as the third power of age (Anderson et al., 1983; CDHS, 1985). Thus, given a bioassay duration of L_e and a natural test-species standard lifespan of L, the low-dose potency (i.e., dose-response slope value) calculated using a multistage-risk- extrapolation model is increased by the factor $(L/L_e)^3$ to yield the potency expected if the bioassay had been continued for the full lifespan L at the same TWA dose level. Equivalently, the lifetime TWA dose that would yield the corrected potency value, based on risk extrapolation with the same model and observed tumor-incidence data, would be the actual bioassay TWA dose multiplied by the factor $(L_e/L)^3$. The latter approach is used in our potency assessment.

Combining the adjustments referred to above, we derived lifetime TWA-equivalent values for total metabolized chloroform dose, M, in the NCI (1976) study from corresponding values of estimated experimental metabolized dose, D, using the following relation

$$M = D \times \frac{5}{7} \times \frac{78}{92} \times \left(\frac{92}{104}\right)^3 \qquad (6\text{-}1)$$

in which D represents an estimated experimental metabolized dose listed in Table 6-1. Values of M thus calculated appear in Table 6-1.

Reuber (1979) Reevaluation of NCI (1976) Mouse Study

In 1979, Reuber published the results of his reexamination of histology slides from the NCI (1976) bioassay of chloroform. He agreed with the original NCI interpretation that male and female B6C3F1 mice developed a significant increase in HCC; however, his analysis resulted in higher tumor-incidence numerators than reported by the NCI (Table 6-1). Although time-of-death information was available for these animals, it is not clear

whether this information was incorporated into the incidence-rate denominators published by Reuber (the denominators published by Reuber differ from both the adjusted and unadjusted denominators of the NCI report). In addition to the increased incidence of HCC, Reuber reported a statistically significant increase in the incidence of malignant lymphoma (ML) in mice of both sexes and both dosage groups.

Our calculation of the estimated experimental metabolized dose, D, and the lifetime TWA metabolized dose, M, was identical to that described above for the NCI (1976) data. These values are listed in Table 6-1.

Roe et al. (1979) Study of Mice Dosed With Chloroform by Gavage

Roe et al. conducted a series of experiments in which mice were dosed with chloroform by gavage in toothpaste or arachis oil. Two of the three experiments used ICI mice, and one compared the carcinogenicity of chloroform among ICI, CBA, C57BL, and CF/1 mice. In the first experiment, male and female ICI mice were dosed with chloroform 6 d/wk for 80 wk, with terminal sacrifice at wk 96. Renal adenoma (RA) was first observed in a high-dose male at wk 88, and renal hypernephroma (RH) at wk 92. Although Roe et al. did not provide any statistical analysis of data, the U.S. EPA (1985a) determined that the incidence of RH and the combined incidence of RA and RH was significantly different from controls. A second experiment which utilized a single dose level of chloroform (60 mg/kg) also resulted in a significantly elevated incidence of RH, as well as in the combined incidence of RA and RH in male ICI mice. These mice received chloroform 6 d/wk for 80 wk, and were sacrificed at wk 104. The final experiment compared the effects of 60 mg/kg of chloroform administered by gavage in toothpaste (four strains of mice) or arachis oil (ICI mice only), 6 d/wk for 80 wk. Terminal sacrifice took place at 98 wk. None of the mice that received chloroform in toothpaste had a statistically significant increase in the incidence of tumors of any type. Administration of chloroform in arachis oil was associated with a significant increase in the combined incidence of RA and RH.

Our PBPK model was used to predict the estimated experimental metabolized dose of chloroform (Table 6-1) for mice in this study. Because specific information was not available regarding the oral absorption kinetics of chloroform from toothpaste or from arachis oil, it was assumed that the

clearance rate was similar to that from corn oil (see discussion in Section 3 regarding the implications of data reported by Withey et al., 1983).

The lifetime TWA metabolized dose for the mice in the first experiment was calculated using the relation

$$M = D \times \frac{6}{7} \times \frac{80}{96} \times \left(\frac{96}{104}\right)^3 . \qquad (6\text{-}2)$$

For mice in the second experiment in which sacrifice took place at wk 104 (the theoretical natural lifespan of the animals) the lifetime TWA metabolized dose was calculated by

$$M = D \times \frac{6}{7} \times \frac{80}{104} . \qquad (6\text{-}3)$$

For animals in the third experiment, the lifetime TWA metabolized dose was calculated by the relation

$$M = D \times \frac{6}{7} \times \frac{80}{98} \times \left(\frac{98}{104}\right)^3 . \qquad (6\text{-}4)$$

NCI (1976) Study of Rats Dosed With Chloroform by Gavage

In this study, male and female Osborne-Mendel rats were dosed with chloroform by gavage 5 d/wk for 78 wk. Treatment began when animals were 52 d old, with terminal sacrifice 111 wk after dosing began. Females initially received 125 or 250 mg/kg of chloroform; this was reduced to 90 and 180 mg/kg after 22 wk, resulting in TWA doses of 100 and 200 mg/kg. Males received 90 or 180 mg/kg of chloroform throughout the study. A statistically significant increase in the combined incidence of renal tubular cell adenomas (RA) and carcinomas (RC) was observed in male rats. Treated females had an elevated incidence of thyroid gland tumors, but because these tumors were derived from two different cell types, neither the NCI (1976) and the U.S. EPA (1985a) considered the increase to be biologically significant. Consequently, we have not included these data in our potency calculations.

The validated PBPK model for chloroform was used to calculate the metabolized dose, D, of chloroform received by rats in this study, and was

based on the parameter values listed in Table 3-5. The clearance rate of chloroform from the corn oil vehicle was derived from the data of Withey et al. (1983) as previously described.

We calculated the lifetime TWA metabolized dose of chloroform for the male rats of this study using the relation

$$M = D \times \frac{5}{7} \times \frac{78}{111} \times \left(\frac{111}{104}\right)^3 \quad (6\text{-}5)$$

Reuber (1979) Reevaluation of NCI (1976) Rat Study

In his reexamination of the NCI bioassay data, Reuber included an analysis of the histological slides from the Osborne-Mendel rats. He determined that male rats developed RA or RC in significantly greater numbers than controls. This interpretation of data is in agreement with the NCI pathology report, although Reuber's incidence-rate denominators are larger than those reported by the NCI. In addition, Reuber found that female rats had a significant increase in the incidence of cholangiofibromas or cholangiocarcinomas as well as thyroid adenomas or carcinomas. As noted previously, both the NCI (1976) and the U.S. EPA (1985a) discounted the significance of the thyroid tumors because they originated from different cell types.

Our calculation of the estimated experimental metabolized dose, D, and the lifetime TWA metabolized dose, M, was identical to that described for the NCI (1976) rat data. These values are listed in Table 6-1.

Jorgenson et al. (1985) Study of Rats Dosed With Chloroform in Water

Jorgenson et al. (1985) administered chloroform in drinking water to male Osborne-Mendel rats and female B6C3F1 mice, at 200, 400, 900, or 1800 mg/L. Water was available ad libitum. Treatment with chloroform began when animals were approximately 7 wk old, and continued for 104 wk, at which time animals were sacrificed. No evidence was found that chloroform was carcinogenic to mice (see Section 4). In rats, the combined incidence of RA and RC was significantly greater among animals from the highest dose group (1800 mg/L) compared to matched controls.

To use our PBPK model with the rat data of Jorgenson et al. (1985), it was necessary to make several approximations concerning the pattern of dosing. To model ingestion of chloroform, we assumed that the pattern of water ingestion by the rats in this study was similar to that described by Armstrong (1980; as cited by NTP, 1987). Armstrong noted that when rats were maintained on a cycle of 12 h light followed by 12 h dark, 86 % of the water was consumed during the dark period and 14 % during the light period. Water consumption was bimodal during the dark period, but changed to a pattern of frequent ingestion of small amounts during the period of light. To model a pattern of chloroform dosing that corresponds to this pattern of water ingestion, we assumed that intermittent water consumption also occurred during the night, amounting to 14% of the total dose. With this pattern of ingestion, we assumed that rats drank once every hour and that each dose was completely absorbed at a constant rate within 15 min. The remaining 72% of the dose was separated between two periods of ingestion, evenly spaced within the dark cycle.

By using the PBPK model and the dosing protocol just described, we derived the estimated experimental metabolized dose of chloroform, D, given in Table 6-1. Because dosing was continuous and took place throughout the theoretical average lifespan of the animals, the relation M = D applies to this data set.

Tumasonis et al. (1985, 1987) Study of Rats Dosed With Chloroform in Water

Tumasonis et al. (1985, 1987) administered chloroform to male and female Wistar rats in drinking water, supplied ad libitum. Treatment began when animals were weanlings and continued for 180 wk. The time of terminal sacrifice was not specified, but was assumed to have been coincident with the end of dosing. Initially, chloroform was supplied to animals at a concentration of 2.9 g/L, but this was decreased at wk 72 to 1.45 g/L to offset the increased water consumption of test animals. Based on data published by Tumasonis et al., we calculated that animals received TWA doses of 200 mg/kg (males) and 240 mg/kg (females). Both sexes of rats developed a significantly increased incidence of hepatic adenofibrosis (HAF) compared to controls.

To derive the estimated experimental metabolized dose of chloroform for animals in this study (Table 6-1), drinking water consumption patterns and oral absorption kinetics of chloroform were modeled as described for Jorgenson et al. (1985). Because dosing was continuous (7 d/wk) and continued beyond the theoretical 104-wk lifespan of a rat, the lifetime TWA metabolized dose received by animals in this study was calculated from the relation

$$M = D \times \left(\frac{180}{104}\right)^3 . \qquad (6\text{-}6)$$

CARCINOGENIC POTENCY EXTRAPOLATION BASED ON ANIMAL BIOASSAY-DATA

Recall that carcinogenic "potency" here refers to a quantitative expression of increased tumorigenic response per unit dose at very low dose levels. Recall also that our carcinogenic potency assessment is based on a quantitative analysis of animal-bioassay data sets under the assumption that chloroform is carcinogenic to both animals and humans at the low environmental dose levels of regulatory concern. The rationale for using this assumption for chloroform is discussed in detail elsewhere (U.S. EPA, 1980, 1986; Anderson et al., 1983; CDHS, 1985). Arguments against using this assumption for chloroform in dose-response extrapolation focus on the possibility that observed carcinogenicity of compounds like chloroform in bioassays conducted at high doses may be caused primarily by increased cellular proliferation (i.e., by tumor promotion or some epigenetic mechanism, rather than by initiation or some genotoxic mechanism); and that this induced cell behavior has a threshold-type dose-response relationship implying a carcinogenic potency of zero at very low dose levels (Reitz et al., 1980, 1982, 1987; Moore et al., 1981; Pereira et al., 1984).

Low-dose potency extrapolation, from dichotomous tumor-response information in given animal-bioassay data sets, was performed using a computer program (Crump and Watson, 1979) to numerically fit parameters to the multistage dose-response extrapolation model:

$$\text{Increased Risk} = R = 1 - e^{-\sum_{i=1}^{q} q_i d^i} . \qquad (6\text{-}7)$$

in which g is the number of exposed groups in the bioassay, d is the dose level in mg/kg-d at which the risk function is evaluated, and q_i, in units of $(mg/kg\text{-}d)^{-i}$, are the multistage parameters (q_1 being the low-dose "potency" parameter) that are estimated by using an iterative maximum-likelihood procedure (U.S. EPA, 1980, 1986b; Anderson et al., 1983; CDHS, 1985).

The input to this program for each bioassay data set consisted of the values for total lifetime TWA metabolized dose, M (in mg/kg-d), and corresponding tumor-incidence data given in Table 6-1. For each data set, we used GLOBAL79 to calculate a one-tailed 95% upper confidence limit (UCL) for the linear parameter, q_1, of the multistage model, denoted $q_1^*(M)$. Note that, according to the multistage model implemented in GLOBAL79, the value of q_1 in Eq. (6-7) must be greater than or equal to 0, whereas the value of $q_1^*(M)$ is restricted to be greater than 0. At any given very low-level dose, the multistage model predicts an increased UCL risk that is approximately equal to the product of $q_1^*(M)$ and dose. Calculated values of $q_1^*(M)$ corresponding to each of the bioassay data sets considered here are given in Table 6-1.

CARCINOGENIC POTENCY OF HUMAN APPLIED DOSE

If it is assumed that the above calculated values of $q_1^*(M)$ are adequate to approximate expected tumor incidence among animals subjected to very low levels of effective dose M, then the application of these values in human cancer risk assessment raises the questions of whether or not these calculated potency values should be considered valid only for the exposure route (ingestion) actually used in the corresponding bioassay, and whether or not a given value of M, or "effective" dose, should be considered equipotent for different species. These questions are addressed below.

Exposure-Route Extrapolation

Various physiological processes, such as the "first pass effect" involving liver-mediated toxification or detoxification, may result in differing values of effective dose, given the same applied dose via different exposure routes. However, in the case of chloroform, carcinogenicity is for the present analysis hypothesized to be mediated by a suspected reactive metabolite whose rate of production (according to the "oncogene mutation"

theoretic basis of the multistage model of carcinogenesis used here) should be linearly related to increased tumor risk at low levels of increased risk, i.e., at levels of increased risk $R << 0.01$ such that $(1 - e^{-R}) \cong R$. Thus, according to this risk-prediction model, it is reasonable to assume that a given effective dose, M, is equipotent regardless of exposure route, as long as the distribution of this effective dose within susceptible target tissues is not affected by exposure route.

Interspecies Dose-Equivalence Extrapolation

Following the suggestion of Mantel and Schneiderman (1975), the U.S. Environmental Protection Agency and the California Department of Health Services assume that mg/(surface area) is an equivalent measure of lifetime TWA dose among different species for carcinogens (U.S. EPA, 1980, 1986; Anderson et al., 1983; CDHS, 1985). Specifically, for purposes of carcinogen-risk assessment, it is assumed that a daily, TWA lifetime dose expressed in units of $mg/kg^{2/3}$ is equivalent among different species, because to a close approximation, surface area is proportional to the 2/3rd power of body weight, as would be the case for perfect spheres of equal uniform density. Given this assumption and given a daily lifetime TWA effective dose for an animal of M_a mg/kg-d, then the equivalent human TWA dose would be $M_a f$, where the dose-equivalence factor f is given by

$$f = \left(\frac{w_a}{w_h}\right)^{1/3}, \qquad (6\text{-}6)$$

in which w_h and w_a are the weights of humans (assumed to be 70 kg) and the test animal, respectively.

Analyses of available bioassay data indicate that extrapolation of lifetime-animal-cancer-risk estimates based on either a mg/(surface area) or a mg/kg dosage scale may lead to reasonable projections of corresponding predicted human cancer risk (Crouch and Wilson, 1979; Hogan and Hoel, 1982; Crouch, 1983). However, the basis upon which interspecies potency correlations were made on either dosage scale in these particular studies has been called into question (Bernstein et al., 1985). The Mantel and Schneiderman (1975) suggestion, relied on by the U.S. EPA, explicitly offered

only a "first approximation...[where the] basic assumption is that the locus of action of any drug is on some surface area"--an approximation that was suggested for use because no "suitable data exist on the quantitative extrapolation of carcinogenic effects from animal to man." But following the logic of the original Mantel-and Schneiderman suggestion, it would be reasonable to use a mg/kg dose-equivalence assumption whenever a chemical's carcinogenic effect could reasonably be attributed to steady-state action at potential sites distributed throughout a target-tissue-mass.

In the absence of definitive empirical data, however, it is simply not known which interspecies dose-extrapolation assumption better reflects reality in the context of extrapolating tumor-response data in animals to anticipated response in humans. Existing data do not rule out either approach. Thus, extrapolation of potency values from animals to humans is carried out here using both a dose-per-body-weight (BW) and a dose-per-body-surface-area (SA) extrapolation method.

Human Metabolism

We used a PBPK approach to estimate the extent of chloroform metabolism in humans exposed to low environmental levels of chloroform in air and water. Under these exposure conditions, in accordance with the estimates presented in Section 3, we assume that humans metabolize 98.6% of all ingested chloroform and 67.2% of all chloroform respired in alveolar air. Thus, the UCL carcinogenic potency, $q_1^*(\bar{A}_i)$, of an applied lifetime TWA ingested dose, $\bar{A}_i$, of chloroform to humans is estimated to be equal to $0.986\ q_1^*(M)$ based directly on the bioassay data for chloroform-exposed animals considered in Table 6-1; and the potency, $q_1^*(\bar{A}_r)$, of a respired or dermally absorbed dose, $\bar{A}_r$, of chloroform to humans is estimated to be equal to $0.672 q_1^*(M)$; where M is in mg/kg total body weight per day (or mg/m^2 total body surface area per day). The latter metric for effective dose was selected for use in this risk assessment for the following reasons. Although the animal tumor incidence data that serve here as a basis for potency extrapolation primarily involve liver tumors, other tumor sites (bile duct, kidney, and lymphoid tissue) are also reflected in these bioassay data, so a single tissue-specific metric for effective dose is not possible for all relevant data sets on chloroform-induced cancer in animals. While it is most desirable when using a

PBPK approach to risk assessment to employ a dose metric that corresponds to a particular target tissue at risk (Andersen et al., 1987), it is not clear how to use this strategy in the case of a compound that may exhibit toxicity in multiple unknown target tissues in the species to which one wants to extrapolate a dose-response relationship. While it would be possible to specify several different plausible alternative target tissues for which to extrapolate chloroform's carcinogenic potential in humans based on animal bioassay data, and to generate a matrix of corresponding alternative potency values, we feel that the uncertainties currently inherent in high- to low-dose potency extrapolation so greatly outweigh those involved in the identification of the most suitable target tissue(s) and PBPK models incorporating these specific compartments as to recommend the simpler approach adopted here based on total metabolized dose per kg of total body weight (or per m^2 total body surface area). Of course, if toxicity is presumed to occur only at the site of metabolism, then the latter approach to interspecies extrapolation yields results identical to those of a tissue-specific approach for any metabolizing tissue comprising a fraction of body weight (or surface area) that is equal in both humans and the animal species from which a dose-response relation is being extrapolated. For example, liver comprises about 3 to 4% of total body weight in both rats and humans (NRC, 1986; Ward et al., 1988; U.S. EPA, 1988), so effective dose per kg (or m^2) liver is approximately equivalent, as far as interspecies dose-response extrapolation is concerned, to that per kg (or m^2) total body weight for this tissue in these two species. By comparison, liver weights of common laboratory mice are about 3% to 5% of total body weight (Foster et al., 1983; U.S. EPA, 1988).

Potential Human Cancer Potency of Chloroform

Based on the bioassay data we have considered, 34 alternative values of $q_1^*(M)$ for chloroform are provided in Table 6-1 that are based on the body-weight (BW) and surface-area (SA) methods of interspecies dose-equivalence extrapolation. The potencies in Table 6-1 based on the BW method, which apply to animals and humans alike, range from 0.00094 to 0.052 $(mg/kg\text{-}d)^{-1}$ (a 55-fold range), and have an arithmetic mean value of 0.012 $(mg/kg\text{-}d)^{-1}$. The potencies in Table 6-1 based on the SA method of interspecies dose-equivalence extrapolation, which apply only to humans, range

from 0.0048 to 0.69 $(mg/kg-d)^{-1}$ (a 144-fold range), and have an arithmetic mean of 0.14 $(mg/kg-d)^{-1}$. The highest potency values obtained using either the BW- and SA-extrapolation method are based on Reubers' reevaluation of the NCI (1976) data for HCC in female B6C3F1 mice exposed to chloroform by gavage in corn oil. The complete set of 34 alternative potency values for chloroform has a 730-fold range.

We empnasize that *caution should be used in the selection or weighting of potency values from Table 6-1 for predicting human cancer risk, conditional on the assumptions used in this potency assessment, because all the corresponding bioassay data may not be equally valid or relevant*. In particular, as we have previously noted, Reubers' (1979) reevaluation of the NCI (1976) results for B6C3F1 mice and Osborne-Mendel rats resulted in a number of instances in which his interpretation of data differed substantially from the original NCI interpretation. If the potencies in Table 6-1 based on these data are removed from consideration, the arithmetic mean of the remaining 22 potencies based on the BW- and SA-extrapolation methods are 0.0094 (range: 0.00094 to 0.038 = 40-fold) and 0.11 (range: 0.0048 to 0.50 = 104-fold) $(mg/kg-d)^{-1}$, respectively. The corresponding maximum values are both based on the NCI (1976) data on HCC incidence in female B6C3F1 mice dosed with chloroform by gavage. Together, these 22 potency values have about a 530-fold range.

Comparison with Earlier Potency Estimates

Potency values for the NCI (1976), Roe et al. (1979), and Jorgenson et al. (1985) bioassay data sets listed in Table 6-1 may be compared to corresponding values calculated by the U.S. EPA (1985a) listed in Table 6-2 (p. 134), which appears after Table 6-1 (p. 129). Note that the U.S. EPA relied only on the SA approach to interspecies extrapolation of equipotent doses, whereas we report results based on the BW and the SA approaches. Our SA-based potencies for NCI (1976), Roe et al. (1979), and Jorgenson et al. (1985) range from 0.0048 to 0.50, while those of the U.S. EPA (1985a) range from 0.0044 to 0.20, $(mg/kg-d)^{-1}$.

The potencies for chloroform calculated by the U.S. EPA (1985a) (Table 6-2, p. 134) did not address the issue of exposure-route specificity and the degree to which these potencies are applicable to a given route of exposure. In 1987, the U.S. EPA published revised potency estimates for

chloroform which distinguished between oral and inhalation exposure. To calculate the oral potency value for chloroform of 0.0061, the U.S. EPA (1987) relied on the drinking water study of Jorgenson et al. (1985) as the most relevant for human oral exposure (Cogliano, 1989). However, the incidence values used as the basis of this calculation were from the data set of "all kidney tumors" in male Osborne-Mendel rats. This grouping includes renal tubular cell adenomas and carcinomas as well as nephroblastomas. Because the latter tumor type is histologically unrelated to the adenomas and adenocarcinomas, it is unclear why the U.S. EPA (1987) chose to base potency calculations on these data. We also note that this approach is different from that taken by the U.S. EPA (1985a) to calculate a potency based on Jorgenson et al. (1985). As noted above and in Table 6-2, the earlier potency estimate was based solely on the combined incidence of the histologically related tubular cell adenomas and adenocarcinomas. To calculate an inhalation-potency value for chloroform, the U.S. EPA (1987) took the geometric mean, 0.081 $(mg/kg\text{-}d)^{-1}$, of the UCL potencies calculated separately based on the incidence of hepatocellular carcinoma in male and female B6C3F1 mice from the NCI (1976) bioassay, using the rationale that the latter data sets provided a relatively conservative potency estimate (Cogliano, 1989).

The potency values for the NCI (1976), Reuben (1979), Roe et al. (1979), Jorgenson et al. (1985), and Tumasonis et al. (1985, 1987) bioassay data sets calculated by CDHS (1989) and listed in Table 6-3 (p. 136) may be compared with our potency values in Table 6-1. The principal difference in our derivation of potency values is that we based our calculations on the "effective" LTWA metabolized dose of chloroform, whereas CDHS made no adjustments for metabolism. Our BW-based potencies for the bioassay data sets noted above range from 0.00094 to 0.038 $(mg/kg\text{-}d)^{-1}$, while the corresponding potencies from CDHS range from 0.00079 to 0.019 $(mg/kg\text{-}d)^{-1}$. SA-based potencies calculated by CDHS range from 0.0041 to 0.26, while our SA-potencies range from 0.0048 to 0.50, $(mg/kg\text{-}d)^{-1}$.

CALCULATION OF CHLOROFORM CONCENTRATIONS TO LIMIT POTENTIAL CANCER RISKS

Here we show how to calculate alternative drinking-water concentrations, C_w, associated with different potency estimates (described earlier), exposure scenarios (e.g., ingestion alone or total, water-based exposure to

chloroform), and alternative predicted lifetime cancer risks. Predicted cancer risk is assumed to be equal to a lifetime TWA applied dose rate, $\bar{A}$, times a potency as a function of that dose rate, $q_1(\bar{A})$, where $\bar{A}$ is equal to the product of the chloroform concentration in water, C_w, and a pathway-exposure factor, as defined in Section 5. Alternative exposure factors, F, such as those appearing in Table 5-6, can be used to calculate C_w, depending on the assumptions that are adopted concerning the exposure scenario of the target population at risk. Similarly, a carcinogenic potency, $q_1^*(\bar{A})$, based on those alternative values of $q_1^*(M)$ that appear in Table 6-1 may be selected depending on the assumptions that are adopted concerning the relevant bioassay data set, exposure route(s), and interspecies dose-equivalence factor.

Concentrations of chloroform associated with some specified level of acceptable increased individual cancer risk (e.g., 10^{-6}), denoted R_a, can be calculated using the formula

$$C_w = \frac{R_a}{F^* q_1^*(M)} \quad . \tag{6-8}$$

in which the integrated exposure factor F^* is an adjustment of the factor F referred to above, where F is defined as the sum, $F_1 + F_2 + F_3$, of ingestive, respiratory, and dermal factors, respectively. The latter adjustment is made to (1) reflect the difference between M and $\bar{A}$ for humans (explained in the previous subsection) and (2) incorporate an alveolar ventilation rate of 378 L/h for a reference 70-kg human (from Table 3-5), where the latter value is in contrast with the assumption of a 1200 L/h respiration rate for a 70-kg adult with 50 or 100% chloroform uptake used to derive F_2 (the respiratory portion of the integrated exposure factor F) in Section 5. The proper adjustment is therefore given by the relation

$$F^* = (0.986)\, F_1 + \left(\frac{0.662 \times 378}{1200}\right) F_2 + (0.662) F_3 \quad . \tag{6-9}$$

in which F_1, F_2, and F_3 are given as "best estimator" and "upper bound" values in Table 5-6, except that the "best" F_2 value of 0.045 listed in that table should be increased by a factor of 2 to the new value, $F_2 = 0.090$, to reflect a "100% availability for uptake" assumption compatible with the PBPK approach

discussed in Section 3 and used in this potency assessment. Using the latter values yields 0.058 L/kg-d and 0.097 L/kg-d as the corresponding "best" and "upper bound" estimates, respectively, for the adjusted integrated dose factor, F^* (equal to 58% and 36%, respectively, of the corresponding unadjusted values).

Predicted increased population risk or number of additional cancer cases, I, in the exposed population considered in Section 5, using an estimated carcinogenic potency $q_1^*(M)$ of chloroform from this section, is given by

$$I = IPE \times q_1^*(M) \quad , \tag{6-10}$$

in which IPE is integrated population exposure (in person-mg/kg-d) and where estimates for IPE are given in Table 5-8. Because IPE is defined in terms of F (see Eq. (5-19)), adjustments to IPE analogous to those for F discussed above are required for proper use of Eq. (6-9) to predict increased population risk in a way consistent with the PBPK approach used in this potency assessment.

Table 6-1. Dose-response data and corresponding estimates of carcinogenic potency for chloroform.

Study Species Strain	Sex, Weight[a]	Daily Dose (mg/kg-d) Experimental applied dose, A	Estimated experimental metabolized dose[b], D	LIWA metabolized dose[c], M	Tumor Type[d]	Tumor Incidence[e]	95% UCL Potency[f] of metabolized dose = q_1^*(M) in (mg M/kg d)$^{-1}$ BW[g]	SA[h]
NCI (1976)	M	0	0	0	HCC	1/18		
Mouse	34	138	91.1	38.2		18/47	0.017	0.22
B6C3F1		277	147	61.6		44/45		
	F	0	0	0	HCC	0/19		
	30	238	134	56.2		36/45	0.038	0.50
		477	204	85.5		39/41		
Reuber (1979)[i]	M	0	0	0	HCC	0/17		
Mouse	34	138	91.1	38.2		20/46	0.021	0.27
B6C3F1		277	147	61.6		44/44		
		0	0	0	HL	0/17		
		138	91.1	38.2		14/46	0.0086	0.11
		277	147	61.6		10/44		
	F	0	0	0	HCC	0/19		
	30	238	134	56.2		40/45	0.052	0.69
		477	204	85.5		40/40		
		0	0	0	HL	0/19		
		238	134	56.2		9/45	0.0036	0.048
		477	204	85.5		4/40		

Table 6-1. Continued.

Study Species Strain	Sex, Weight[a]	Daily Dose (mg/kg-d) Experimental applied dose, A	Estimated experimental metabolized dose[b], D	LIWA metabolized dose[c], M	Tumor type[d]	Tumor incidence[e]	95% UCL Potency[f] of metabolized dose = q_1^*(M) in (mg M/kg·d)$^{-1}$ BW[g]	SA[h]
Roe et al. (1979)	M 35	0	0	0	RH	0/72		
		17	16.9	9.49		0/37	0.0040	0.050
		60	52.8	29.7		3/38		
House ICI		0	0	0	RA or RH	0/72		
		17	16.9	9.49		0/37	0.0057	0.072
		60	52.8	29.7		8/38		
	M 39	0	0	0	RH	0/237		
		60	52.0	34.3		2/49	0.0032	0.039
		0	0	0	RA or RH	6/237		
		60	52.0	34.3		9/49	0.0091	0.11
	M[j] 41	0	0	0	RA or RH	0/50		
		60	52.0	30.4		12/47	0.014	0.17

Table 6-1. Continued.

Study Species Strain	Sex, Weight[a]	Daily Dose (mg/kg·d) Experimental applied dose, A	Estimated experimental metabolized dose[b], D	LTWA metabolized dose[c], M	Tumor Type[d]	Tumor Incidence[e]	95% UCL Potency[f] of metabolized dose = q_1^*(M) in (mg M/kg·d)$^{-1}$ BW[g]	SA[h]
NCI (1976) Rat Osborne-Mendel	M 375	0 90 180	0 57.2 88.8	0 34.9 54.2	RA or RC	0/19 4/38 12/27	0.0062	0.035
Reuber (1979)[i] Rat Osborne-Mendel	M 375	0 90 180	0 57.2 88.8	0 34.9 54.2	RA or RC	0/19 8/50 14/49	0.0078	0.045
	F 500	0 100 200	0 60.6 93.1	0 37.0 56.8	CF or CC	0/20 3/39 11/39	0.0054	0.028
Iumasonis et al. (1985) Rat Wistar	M 230	0 200	0 147	0 762	HAF	0/22 17/28	0.0018	0.012

Table 6-1. Continued.

Study Species Strain	Sex, Weight[a]	Daily Dose (mg/kg-d): Experimental applied dose, A	Estimated experimental metabolized dose[b], D	LIWA metabolized dose[c], M	Tumor: Type[d]	Tumor: Incidence[e]	95% UCL Potency[f] of metabolized dose = q_1^*(M) in (mg M/kg·d)$^{-1}$: BW[g]	SA[h]
Tumasonis et al. (1985) cont.	F 180	0	0	0	HAF	0/18		
		240	172	892		34/40	0.0029	0.019
Jorgenson et al. (1985) Rat Osborne-Mendel	M 521	0	0	0	RA or RC	1/50		
		18[k]	17.8	17.8		4/313		
		38	35.6	35.6		4/148	0.00094	0.0048
		79	64.6	64.6		3/48		
		155	116	116		7/50		

Footnotes to Table 6-1.

[a] Terminal body weight, grams.

[b] See text for derivation of D as a function of A.

[c] Lifetime, time-weighted average metabolized dose, M, in mg/kg-d. See text for derivation as a function of D.

[d] HCC = hepatocellular carcinoma, HAF = hepatic adenofibrosis, ML = malignant lymphoma, RA = renal adenoma, RH = renal hypernephroma, RC = renal carcinoma, TA = thyroid adenoma, TC = thyroid carcinoma, CF = cholangiofibroma, CC = cholangiocarinoma.

[e] For the NCI data sets tumor-incidence denominators are adjusted so as not to include animals dying before the occurrence of the first tumor of the type counted in the corresponding data set.

[f] "Potency" here means the low-dose dose-response slope expressed by an upper-bound linear multistage coefficient such that at very low doses, risk = (potency x dose), according to a multistage (or, with time-to-tumor data as input, a time-dependent multistage) risk prediction model (U.S. EPA, 1980; Anderson et al., 1983; Crump and Howe, 1984). 95% UCL = one-tailed 95% upper confidence limit.

[g] BW = Body weight interspecies dose-extrapolation method; equivalent doses assumed to be in mg/kg, so $M_{human} = M_{animal}$.

[h] SA = Surface Area interspecies dose-extrapolation method; equivalent doses assumed to be in $mg/kg^{2/3}$, so $M_{human} = M_{animal}[(\text{animal weight})/70\ kg]^{1/3}$.

[i] Data cited under Reuber (1979) are based on a reevaluation of the NCI (1976) data.

[j] Arachis oil vehicle

[k] TWA doses are from CDHS (1988b).

Table 6-2. U.S. EPA estimates of carcinogenic potency for chloroform.[a]

Study Species Strain	Sex	Daily Dose (mg/kg·d) Experimental applied dose, A	Daily Dose (mg/kg·d) LIWA metabolized dose[b], M	Tumor Type[c]	Tumor Incidence[d]	95% UCL Potency[e] of metabolized dose = $q_1^*(M)^f$
NCI (1976)	M	0	0		1/18	
Mice		138	78.6	HCC	18/50	0.033
B6C3F1		277	157.7		44/45	
	F	0	0		0/20	
		238	133.6	HCC	36/45	0.20
		477	271.8		39/41	
NCI (1976)	M	0	0	RA or	0/19	
Rats		90	36.1	RC	4/50	0.024
Osborne-Mendel		180	72.8		12/50	
Roe et al (1979)	M	0	0	RA or	0/50	
Mice		60	41.4	RH	9/48	0.10
ICI						
Jorgenson et al (1985)	M[g]	0	0		4/301	
Rats		19	17.8	RA or	4/313	
Osborne-Mendel		38	35.6	RC	4/148	0.0044
		81	75.8		3/48	
		160	150.0		7/50	
	M[h]	0	0	RA,	1/50	
		19	17.8	RC, and	6/313	
		38	35.6	NB	7/148	0.0061
		81	75.8		3/48	
		160	150.0		7/50	

Footnotes for Table 6-2.

[a] Information derived from U.S. EPA (1985a), Tables 8-20, 8-21, 8-22, 8-23, and 8-24.

[b] Lifetime time-weighted average (LTWA) metabolized dose, M, in mg/kg-d, except that the values for Jorgenson et al. (1985) were based on their assumption that, under the conditions of this study, 100% of the administered dose was metabolized. See text for discussion.

[c] HCC = hepatocellular carcinoma, RA = renal adenoma, RC = renal carcinoma, RH = renal hypernephroma, NB = nephroblastoma.

[d] Tumor-incidence denominator appears not to have been adjusted by EPA for animals dying before the occurrence of the first corresponding tumor type observed in the NCI (1976) study.

[e] "Potency" here means the low dose, dose-response slope expressed by an upper-bound linear multistage coefficient such that at very low doses, risk = (potency x dose), according to a multistage risk prediction model (U.S. EPA, 1980; Andersen et al., 1983). 95% UCL = one-tailed 95% upper confidence limit.

[f] The unit of potency is assumed to be $(mg\ M/kg\text{-}d)^{-1}$. The U.S. EPA's intended unit for these reported potency values is ambiguous, but it is clear that these potency values are equivalent to ones based on a surface-area method for interspecies extrapolation of equipotent doses under the assumption that humans would metabolize 100% of very small ingested doses.

[g] Tumor-type and incidence data used by the U.S. EPA (1985a).

[h] Tumor-type and incidence data used by the U.S. EPA (1987). See text for discussion.

Table 6-3. CDHS estimates of carcinogenic potency for chloroform.[a]

Study Species Strain	Sex	Daily Dose (mg/kg-d) Experimental applied dose, A	LTWA dose[b], $\bar{A}$	Tumor Type[c]	Tumor Incidence[d]	95% UCL Potency[e] of dose = $q_1^*(\bar{A})$ BW[f]	95% UCL Potency[e] of dose = $q_1^*(\bar{A})$ SA[g]
NCI (1976)	M	0	0		1/18		
Mice		138	83	HCC	18/50	0.0034	0.043
B6C3F1		277	167		44/45		
	F	0	0		0/20		
		238	143	HCC	36/45	0.019	0.26
		477	287		39/41		
NCI (1976)	M	0	0	RA or	0/19		
Rats		90	45	RC	4/50	0.0036	0.021
Osborne-Mendel		180	90		12/50		
Reuber (1979)	F	0	0	CF or	0/20		
Rats		100	50	CC	3/39	0.0036	0.018
Osborne-Mendel		200	100		11/39		
(based on NCI, 1976)							
Roe et al. (1979)	M	0	0	RA or	0/72		
Mice		17	12	RH	0/37	0.0039	0.043
ICI		60	43		8/37		
	M	0	0	RA or	6/237		
		60	40	RH	9/49	0.0078	0.087
	M	0	0	RA or	1/49		
		60	42	RH	5/47	0.0047	0.053
	M	0	0	RA or	1/50		
		60	42	RH	12/48	0.012	0.14

Footnotes for Table 6-3

[a] Information derived from CDHS (1989), Tables 8-A and 8-B.

[b] Lifetime time-weighted average (LTWA) dose in mg/kg-d, calculated using the relation: LTWA = D x d/7 x l_e/L_e where D = administered dose, mg/kg-d, d = number of days dosed per wk, l_e = length of exposure, wk, and L_e = length of experiment, wk.

[c] HCC = hepatocellular carcinoma, RA = renal adenoma, RC = renal carcinoma, CF = cholangiofibroma, CC = cholangiocarcinoma, RH = renal hypernephroma.

[d] Tumor-incidence denominator is unadjusted for animals dying before the occurrence of the first corresponding tumor type observed in the NCI (1976) study.

[e] "Potency" here means the low-dose, dose-response slope expressed by an upper-bound linear multistage coefficient such that at very low doses, risk = (potency x dose), according to a multistage risk prediction model (U.S. EPA, 1980; Andersen et al., 1983). 95% UCL = one-tailed 95% upper confidence limit.

[f] BW = Body weight interspecies dose-extrapolation method; equivalent doses assumed to be in mg/kg, so $dose_{human} = dose_{animal}$.

[g] SA = Surface area dose-extrapolation method; equivalent doses assumed to be in $mg/kg^{2/3}$, so $dose_{human} = dose_{animal}$ [(animal weight)/70 kg]$^{1/3}$.