United States
Environmental Protection
Agency

Office of Health and
Environmental Assessment
Washington DC 20460

EPA-600/8-84-004A
March 1984
External Review Draft

Research and Development

EPA

Health Assessment Document for Chloroform

Review Draft

(Do Not Cite or Quote)

Part 1 of 2

NOTICE

This document is a preliminary draft. It has not been formally released by EPA and should not at this stage be construed to represent Agency policy. It is being circulated for comment on its technical accuracy and policy implications.

ERRATA

The attached pages are to be substituted for the corresponding pages in the Health Assessment Document for Chloroform (September 1985) in which certain key individuals' names were inadvertently omitted. Most notably, that of one of the principal authors, Dr. Jean C. Parker, was not included.

In addition, several typographical errors have been corrected in the front matter of the document.

PREFACE

The Office of Health and Environmental Assessment has prepared this health assessment to serve as a "source document" for EPA use. This health assessment document was developed for use by the Office of Air Quality Planning and Standards to support decision-making regarding possible regulation of chloroform as a hazardous air pollutant. However, the scope of this document has since been expanded to address multimedia aspects.

In the development of the assessment document, the scientific literature has been inventoried, key studies have been evaluated and summary/conclusions have been prepared in order to quantitatively identify the toxicity of chloroform and related characteristics. Observed effect levels and other measures of dose-response relationships are discussed, where appropriate, to place the nature of the health responses in perspective with observed environmental levels.

Any information regarding sources, emissions, ambient air concentrations, and public exposure has been included only to give the reader a preliminary indication of the potential presence of this substance in the ambient air. While the available information is presented as accurately as possible, it is acknowledged to be limited and dependent in many instances on assumption rather than specific data. This information is not intended, nor should it be used, to support any conclusions regarding risks to public health.

If a review of the health information indicates that the Agency should consider regulatory action for this substance, a considerable effort will be undertaken to obtain appropriate information regarding sources, emissions, and ambient air concentrations. Such data will provide additional information for drawing regulatory conclusions regarding the extent and significance of public exposure to this substance.

TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

TABLE OF CONTENTS (continued)

TABLE OF CONTENTS (continued)

TABLE OF CONTENTS (continued)

TABLE OF CONTENTS (continued)

LIST OF TABLES

LIST OF TABLES (continued)

LIST OF TABLES (continued)

LIST OF TABLES (continued)

LIST OF FIGURES

LIST OF FIGURES (continued)

AUTHORS, CONTRIBUTORS, AND REVIEWERS

The EPA Office of Health and Environmental Assessment (OHEA) is responsible for the preparation of this health assessment document. The OHEA Environmental Criteria and Assessment Office (ECAO/RTP) Research Triangle Park, NC 27711, had overall responsibility for coordination and direction of the document production effort (Si Duk Lee, Ph.D., Project Manager, ECAO/RTP, 919-541-4159).

AUTHORS

Larry Anderson, Ph.D. — Chapter 8
Carcinogen Assessment Group
Office of Health and Environmental Assessment
U.S. Environmental Protection Agency
Washington, DC

David Bayliss, M.S. — Chapter 8
Carcinogen Assessment Group
Office of Health and Environmental Assessment
U.S. Environmental Protection Agency
Washington, DC

Chao W. Chen, Ph.D. — Chapter 8
Carcinogen Assessment Group
Office of Health and Environmental Assessment
U.S. Environmental Protection Agency
Washington, DC

Joan P. Coleman, Ph.D. — Chapter 5
Syracuse Research Corporation
Syracuse, NY

I.W.F. Davidson, Ph.D. — Chapter 4
Bowman Gray School of Medicine
Winston-Salem, NC

D. Anthony Gray, Ph.D. — Chapter 3
Syracuse Research Corporation
Syracuse, NY

Si Duk Lee, Ph.D. — Chapter 2
Environmental Criteria and Assessment Office
Office of Health and Environmental Assessment
U.S. Environmental Protection Agency
Research Triangle Park, NC

Jean C. Parker, Ph.D. — Chapters 4,8
Carcinogen Assessment Group
Office of Health and Environmental Assessment
U.S. Environmental Protection Agency
Washington, DC

Sheila Rosenthal, Ph.D. — Chapter 7
Reproductive Effects Assessment Group
Office of Health and Environmental Assessment
U.S. Environmental Protection Agency
Washington, DC

Carol Sakai, Ph.D. — Chapter 6
Reproductive Effects Assessment Group
Office of Health and Environmental Assessment
U.S. Environmental Protection Agency
Washington, DC

Sharon B. Wilbur, M.A. — Chapter 5
Syracuse Research Corporation
Syracuse, NY

U.S. Environmental Protection Agency Peer Reviewers

Karen Blanchard
Office of Air Quality Planning and Standards
Research Triangle Park, NC

Lester D. Grant, Ph.D.
Office of Health and Environmental Assessment
Environmental Criteria and Assessment Office
Research Triangle Park, NC

Joseph Padgett
Office of Air Quality Planning and Standards
Research Triangle Park, NC

Jerry F. Stara, D.V.M.
Office of Health and Environmental Assessment
Environmental Criteria and Assessment Office
Cincinnati, OH

Environmental Criteria and Assessment Office Support Staff

F. Vandiver Bradow
Allen Hoyt

The OHEA Carcinogen Assessment Group (CAG) was responsible for preparation of the sections on carcinogenicity. Participating members of the CAG are listed below (principal authors of present carcinogenicity materials are designated by an asterisk [*]).

Participating Members of the Carcinogen Assessment Group

Roy E. Albert, M.D. (Chairman)
Elizabeth L. Anderson, Ph.D.
Larry D. Anderson, Ph.D.*
Steven Bayard, Ph.D.
David L. Bayliss, M.S.*
Robert P. Beliles, Ph.D.*
Chao W. Chen, Ph.D.*
Margaret M.L. Chu, Ph.D.
James C. Cogliano, Ph.D.*
Herman J. Gibb, B.S., M.P.H.
Bernard H. Haberman, D.V.M., M.S.
Charalingayya B. Hiremath, Ph.D.
Robert E. McGaughy, Ph.D.
Jean C. Parker, Ph.D.*
Charles H. Ris, M.S., P.E.
Dharm V. Singh, D.V.M., Ph.D.
Todd W. Thorslund, Sc.D.

The OHEA Reproductive Effects Assessment Group (REAG) was responsible for preparation of the sections on mutagenicity, teratogenicity, and reproductive effects. Participating members of the REAG are listed below (principal authors of present sections are designated by an asterisk [*]).

Participating Members of the Reproductive Effects Assessment Group

Eric D. Clegg, Ph.D.
John R. Fowle, III, Ph.D.
David Jacobson-Kram, Ph.D.
K.S. Lavappa, Ph.D.
Sheila L. Rosenthal, Ph.D.*
Carol N. Sakai, Ph.D.*
Lawrence R. Valcovic, Ph.D.
Vicki Vaughan-Dellarco, Ph.D.
Peter E. Voytek, Ph.D. (Director)

External Peer Reviewers

Dr. Karim Ahmed
Natural Resources Defense Fund
122 East 42nd Street
New York, NY 10168

Dr. Eula Bingham
Graduate Studies and Research
University of Cincinnati (ML-627)
Cincinnati, OH 45221

Dr. James Buss
Chemical Industry Institute of Toxicology
Research Triangle Park, NC 27709

Dr. I.W.F. Davidson
Wake Forest University
Bowman Gray School of Medicine
Winston-Salem, NC

Dr. Larry Fishbein
National Center for Toxicological Research
Jefferson, AR 72079
(501) 542-4390

Dr. Derek Hodgson
Department of Chemistry
University of North Carolina
Chapel Hill, NC 27514

Dr. Marshall Johnson
Thomas Jefferson Medical College
Department of Anatomy
1020 Locust Street
Philadelphia, PA 19107

Dr. Trent Lewis
National Institute for Occupational Safety and Health
26 Columbia Parkway
Cincinnati, OH 45226
(513) 684-8394

Dr. Richard Reitz
Dow Chemical, USA
Toxicological Research Laboratory
1803 Building
Midland, MI 48640

Dr. Bernard Schwetz
National Institute of Environmental Health Sciences
Research Triangle Park, NC 27709

Dr. James Selkirk
Oak Ridge National Laboratory
Oak Ridge, TN 37820
(615) 624-0831

Dr. Samuel Shibko
Food and Drug Administration
Division of Toxicology
200 C Street, SW
Washington, DC 20204

Dr. Robert Tardiff
1423 Trapline Court
Vienna, VA 22180
(703) 276-7700

Dr. Norman M. Trieff
University of Texas Medical Branch
Department of Pathology
Galveston, TX 77550
(409) 761-1895

Dr. Benjamin Van Duuren
Institute of Environmental Medicine
New York University Medical Center
New York, NY 10016
(212) 340-5629

Dr. James Withey
Health and Protection Branch
Department of National Health & Welfare
Tunney's Pasture
Ottawa, Ontario KIA 01Z Canada

Mr. Matthew Van Hook
Consultant
1133 North Harrison Street
Arlington, VA 22205

SCIENCE ADVISORY BOARD
ENVIRONMENTAL HEALTH COMMITTEE
CHLORINATED ORGANICS SUBCOMMITTEE

The content of this health assessment document on chloroform was independently peer-reviewed in public session by the Chlorinated Organics Subcommittee of the Environmental Health Committee of the Environmental Protection Agency's Science Advisory Board.

ACTING CHAIRMAN, ENVIRONMENTAL HEALTH COMMITTEE

Dr. John Doull, Professor of Pharmacology and Toxicology, University of Kansas Medical Center, Kansas City, Kansas 66207

EXECUTIVE SECRETARY, SCIENCE ADVISORY BOARD

Dr. Daniel Byrd III, Executive Secretary, Science Advisory Board, A-101 F, U.S. Environmental Protection Agency, Washington, DC 20460

MEMBERS

Dr. Seymour Abrahamson, Professor of Zoology and Genetics, Department of Zoology, University of Wisconsin, 500 Highland Avenue, Madison, Wisconsin 53706

Dr. Ahmed E. Ahmed, Associate Professor of Pathology, Pharmacology, and Toxicology, The University of Texas Medical Branch, Galveston, Texas 77550.

Dr. George T. Bryan, Professor of Human Oncology, K4/528 C.S.C Clinical Sciences, University of Wisconsin, 500 Highland Avenue, Madison, Wisconsin 53792.

Dr. Ronald D. Hood, Professor and Coordinator, Cell and Developmental Biology Section, Department of Biology, The University of Alabama, and Principal Associate, R.D. Hood and Associates, Consulting Toxicologists, P.O. Box 1927, University, Alabama 35486.

Dr. K. Roger Hornbrook, Department of Pharmacology, P.O. Box 26901, University of Oklahoma, Oklahoma City, Oklahoma 73190.

Dr. Thomas Starr, Chemical Industry Institute of Toxicology, P.O. Box 12137, Research Triangle Park, North Carolina 27709.

EPA-600/8-84-004A
March 1984
External Review Draft

Review
Draft

(Do Not
Cite or Quote)

Health Assessment Document for Chloroform

Part 1 of 2
External Review Draft

U.S. ENVIRONMENTAL PROTECTION AGENCY
Office of Research and Development
Office of Health and Environmental Assessment
Environmental Criteria and Assessment Office
Research Triangle Park, NC 27711

March 1984

PREFACE

The Office of Health and Environmental Assessment has prepared this health assessment to serve as a "source document" for EPA use. This health assessment document was developed for use by the Office of Air Quality Planning and Standards to support decision-making regarding possible regulation of chloroform as a hazardous air pollutant.

In the development of the assessment document, the scientific literature has been inventoried, key studies have been evaluated and summary/conclusions have been prepared so that chemical's toxicity and related characteristics are qualitatively identified. Observed effect levels and other measures of dose-response relationships are discussed, where appropriate, so that the nature of the adverse health response are placed in perspective with observed environmental levels.

This document will be subjected to a thorough copy editing and proofing following the revision based on the EPA's Scientific Advisory Board review comments.

TABLE OF CONTENTS

TABLE OF CONTENTS (cont.)

TABLE OF CONTENTS (cont.)

TABLE OF CONTENTS (cont.)

LIST OF TABLES

LIST OF TABLES (cont.)

LIST OF TABLES (cont.)

LIST OF TABLES (cont.)

LIST OF FIGURES

LIST OF FIGURES (cont.)

The Office of Health and Environmental Assessment (OHEA), U.S. EPA, is responsible for the preparation of this health assessment document. The Environmental Criteria and Assessment Office (ECAO/RTP), OHEA, had the overall responsibility for coordination and the document production effort.

Project Manager

Si Duk Lee, Ph.D.
Environmental Criteria and Assessment Office
U.S. EPA, Research Triangle Park, N.C. 27711
(919) 541-4159

Authors and Reviewers

The principal authors of this document are:

Larry Anderson Ph.D.
Carcinogen Assessment Group
U.S. EPA, Washington, D.C.

David Baylis, M.S.
Carcinogen Assessment Group
U.S., EPA, Washington, D.C.

Chao W. Chen, Ph.D.
Carcinogen Assessment Group
U.S., EPA, Washington, D.C.

Carol Sakai, Ph.D.
Reproductive Effects Assessment Group
U.S., EPA, Washington, D.C.

Sheila Rosenthal, Ph.D.
Reproductive Effects Assessment Group
U.S., EPA, Washington, D.C.

I.W.F. Davidson, Ph.D.
Bowman Gray School of Medicine
Winston Salem, N.C.

D. Anthony Gray, Ph.D.
Syracuse Research Corp.
Syracuse, N.Y.

Sharon B. Wilbur, M.A.
Syracuse Research Corp.
Syracuse, N.Y.

Joan P. Coleman, Ph.D.
Syracuse Research Corp.
Syracuse, N.Y.

The following individuals provided peer-review of this draft or earlier drafts of this document:

U.S. Environmental Protection Agency

Joseph Padgett
Office of Air Quality Planning and Standards
U.S. EPA

Karen Blanchard
Office of Air Quality Planning and Standards
U.S. EPA

Jerry F. Stara, D.V.M.
Office of Health and Environmental Assessment
Environmental Criteria and Assessment Office
U.S. EPA

Lester D. Grant, Ph.D.
Office of Health and Environmental Assessment
Environmental Criteria and Asssessment Office
U.S. EPA

Participating Members of the Carcinogen Assessment Group

Roy E. Albert, M.D. (Chairman)
Elizabeth L. Anderson, Ph.D.
Larry D. Anderson, Ph.D.
Steven Bayard, Ph.D.
David L. Bayliss, M.S.
Chao W. Chen, Ph.D.
Margaret M. L. Chu, Ph.D.
Bernard H. Haberman, D.V.M., M.S.
Charalingayya B. Hiremath, Ph.D.
Robert E. McGaughy, Ph.D.
Dharm W. Singh, D.V.M., Ph.D.
Todd W. Thorslund, Sc.D.

Participating Members of the Reproductive Effects Assessment Group

Peter E. Voytek, Ph.D. (Director)
John R. Fowle, III, Ph.D.
Carol Sakai, Ph.D.
Ernest Jackson, M.D.
K.S. Lavappa, Ph.D.
Sheila Rosenthal, Ph.D.
Vicki Vaughn-Dellarco, Ph.D.

External Peer Reviewers

Dr. Karim Ahmed
Natural Resources Defense Fund
122 E. 42nd Street
New York, N.Y. 10168

Dr. Eula Bingham
Graduate Studies and Research
University of Cincinnati (ML-627)
Cincinnati, Ohio 45221
(513) 475-4532

Dr. James Buss
Chemical Institute of Industrial Toxicology
Research Triangle Park, N.C. 27709

Dr. I.W.F. Davidson
Wake Forest University
Bowman Gray Medical School
Winston Salem, N.C.

Dr. Larry Fishbein
National Center for Toxicological Research
Jefferson, Arkansas 72079
(501) 542-4390

Dr. Derek Hodgson
Department of Chemistry
University of North Carolina
Chapel Hill, N.C. 27514

Dr. Marshall Johnson
Thomas Jefferson Medical College
Department of Anatomy
1020 Locust Street
Philadelphia, Pennsylvania 19107

Dr. Trent Lewis
National Institute of Occupational Safety and Health
26 Columbia Parkway
Cincinnati, Ohio 45226
(513) 684-8394

Dr. Richard Reitz
Dow Chemical, USA
Toxicology Research Laboratory
1803 Building
Midland, Michigan 48640

Dr. Marvin A. Schneiderman
Clement Associates, Incorporated
Arlington, Virginia 22209
(703) 276-7700

Dr. Bernard Schwetz
National Institute of Environmental Health
Research Triangle Park, NC 27709
(919) 541-7992

Dr. James Selkirk
Oak Ridge National Laboratory
Oakridge, Tennessee 37820
(615) 624-0831

Dr. Samuel Shibko
Food and Drug Administration
Division of Toxicology
200 C Street, S.W.
Washington, D.C. 20204
Telephone:

Dr. Robert Tardiff
1423 Trapline Court
Vienna, Virginia 22180
(703) 276-7700

Dr. Norman M. Trieff
University of Texas Medical Branch
Department of Pathology, UTMB
Galveston, Texas 77550
(409) 761-1895

Dr. Benjamin Van Duuren
Institute of Environmental Medicine
New York University Medical Center
New York, New York 10016
(212) 340-5629

Dr. James Withey
Health and Protection Branch
Department of National Health & Welfare
Tunney's Pasture
Ottawa, Ontario
CANADA, KIA 01Z

1. SUMMARY AND CONCLUSIONS

Chloroform is a dense, colorless, volatile liquid used primarily in the production of chlorodifluoromethane (90%) and for export (5%). Non-consumptive uses (5%) include use as a solvent, as a cleaning agent, and as a fumigant ingredient. Direct United States production of chloroform in 1981 was 184 million kg, while indirect production is estimated at 13.2 million kg (≈193 million kg overall). The amount of chloroform emitted to air is estimated to be 7.2 million kg, emissions to water are 2.6 million kg, and emissions to land are 0.6 million kg. Total United States emissions are estimated to be 10.4 million kg.

Chloroform is ubiquitous in the environment, having been found in urban and non-urban locations. The northern hemisphere background average has been determined to be 14 ppt (10^{-12} v/v), while the southern hemisphere has been determined to be <3 ppt. The global average is 8 ppt. For the most part, urban ambient air concentrations remain <1000 ppt, and rural or remote locations can be <10 ppt. There are some notable exceptions, however, the reasons for this are not readily apparent. The highest values reported were in Rutherford, New Jersey, (31,000 ppt) and Niagara Falls, New York (21,611 ppt).

Hydroxyl radical oxidation is the primary atmospheric reaction of chloroform. Based on the rate constant for reaction with chloroform, a half-life of 11.5 weeks is expected. The principal products from this reaction are HCl and CO_2. It has been estimated that roughly 1% of the tropospheric chloroform will diffuse into the stratosphere, based on a lifetime of 0.2 to 0.3 years and a troposphere-to-stratosphere turnover time of 30 years. An EXAMS model of chloroform in water confirms other data that the major removal process for chloroform in water is evaporation.

The best analytical method for detection of chloroform appears to be gas chromatography with electron capture or electrolytic conductivity detector. This gives a detection limit of <5 ppt.

The pharmacokinetics and metabolism of chloroform have been studied in both humans and experimental animals. Chloroform is rapidly and extensively absorbed through the respiratory and gastrointestinal tracts. Absorption through the skin would make a significant contribution to body burden only in instances of contact of the skin with liquid chloroform.

The available data suggest that, for resting human at least 2 hours of exposure are required to reach an apparent body equilibrium with the inhaled chloroform concentration. The percentage of the inhaled chloroform concentration retained in the body at "equilibrium" would be ≈65%, and is independent of the inhaled concentration. The magnitude of chloroform uptake into the body (dose or body burden) is directly proportional to the concentration of chloroform in the inspired air, the duration of exposure, and the respiratory minute volume, and can be estimated by multiplying the percent retention by the total volume of air breathed during exposure and by the exposure concentration.

The absorption of chloroform from the gastrointestinal tract appears to be virtually complete, judging from recovery of unchanged chloroform and metabolites in the exhaled air of humans and in the exhaled air, urine, feces, and carcass of experimental animals. Peak blood levels occurred at ≈1 hour after oral administration of chloroform in olive oil to humans or animals.

After inhalation or ingestion, highest concentrations of chloroform are found in tissues with higher lipid contents. Results from the administration of ^{14}C-labeled chloroform to animals indicate that the distribution of radioactivity (reflecting both chloroform and its metabolites) may be affected by the route of exposure. Oral administration appeared to result in the accumulation of a

greater proportion of radioactivity in the liver than did inhalation exposure, but differences in experimental protocols make this interpretation tentative. Sex differences in the distribution of chloroform and its metabolites were found only in mice and not in rats or squirrel monkeys. The kidneys of male mice accumulated strikingly more radioactivity than did those of female mice. Other than the renal accumulation of radioactivity in male mice, the tissue distribution of radioactivity after oral administration was similar in mice, rats, and squirrel monkeys.

Chloroform has been detected in fetal liver. Chloroform would be expected to appear in human milk, because it has been found in cow's milk, cheese, and butter.

Chloroform is metabolized via microsomal cytochrome P-450 oxidation to a reactive intermediate. The primary end product of chloroform metabolism is CO_2, but small amounts of the reactive intermediate bind covalently to tissue macromolecules or conjugate with cysteine and glutathione. Covalent binding of the reactive intermediate to macromolecules is considered to be responsible for the hepato- and nephrotoxicity of chloroform.

The initial product of cytochrome P-450 oxidation of cnloroform is trichloromethanol, which spontaneously dehydrochlorinates to produce phosgene. Phosgene is thought to be the toxic reactive intermediate produced during the metabolism of chloroform. Phosgene reacts with water to yield CO_2, with protein to form a covalently bound product, and with cysteine and glutathione. While the liver is the primary site for chloroform metabolism, other tissues, including the kidney, can also metabolize chloroform to CO_2.

Interspecies comparisons of the magnitude of chloroform metabolism have been made only for the oral route. Mice, rats, and squirrel monkeys metabolized 85, 66, and 18%, respectively, of a 60 mg/kg body weight dose of ^{14}C-chloroform

to $^{14}CO_2$(measured in the expired air). Most of the remainder was exhaled as unchanged chloroform; small amounts of radioactivity (2 to 8% of the dose) were excreted in the urine and feces. Human subjects (1 or 2/dose) who ingested 0.1, 0.5, and 1.0 g of ^{13}C-chloroform (≈1.4, 7, and 14 mg/kg body weight) metabolized all of the low dose, 50% of the intermediate dose, and only ≈35% of the high dose, judging from the excretion of unchanged chloroform and $^{13}CO_2$ through the lungs. This difference indicates that the fraction of the dose metabolized is dose-dependent.

Regardless of the route of entry into the body, chloroform is excreted unchanged through the lungs and eliminated via metabolism, with the primary stable metabolite, CO_2, also being excreted through the lungs. High concentrations of unchanged chloroform have been found in the bile of squirrel monkeys after oral administration, but not in the urine or feces. The inorganic chloride generated from chloroform metabolism is excreted via the urine.

Decay curves for the pulmonary excretion of unchanged chloroform in humans appear to consist of three exponential components. The terminal component, thought to correspond to elimination from adipose tissue, had a half-time of 36 hours. This long half-time, together with the relatively high levels of chloroform found in adipose tissues of humans known to have been exposed only to ambient levels of the chemical and of animals exposed experimentally, indicates a potential for bioaccumulation.

The adverse health effects of exposure to chloroform include neurological, hepatic, renal, and cardiac effects. These effects have been documented in humans as well as in experimental animals. In addition, studies with animals indicate that chloroform is carcinogenic and may be teratogenic.

Evidence of chloroform's effects on humans has been obtained primarily during the use of this chemical as an inhalation anesthetic. In addition to depression of the central nervous system, chloroform anesthesia was associated

with cardiac arhythmias (and some cases of cardiac arrest), hepatic necrosis and fatty degeneration, polyuria, albuminuria, and in cases of severe poisoning, renal tubular necrosis. When used for obstetrical anesthesia, chloroform was likely to produce respiratory depression in the infant. Experimental exposures of humans to chloroform have focused only on subjective responses. Humans exposed experimentally to chloroform for 20 to 30 minutes have reported dizziness, headache, giddiness, and tiredness at concentrations >1000 ppm, and light intoxication at concentrations above 4000 ppm.

Similar symptoms occurred in workers employed in the manufacture of lozenges containing chloroform; exposure concentrations ranged from 20 to 237 ppm, with occasional brief exposure to ≈1000 ppm. Additional complaints were of gastrointestinal distress, and frequent and scalding micturition. The only other report of adverse effects stemming from occupational exposure to chloroform was of enlargement of the liver; this report was compromised by the apparent lack of suitable controls.

Acute inhalation experiments with animals revealed that single exposures to 100 ppm were sufficient to produce mild hepatic effects in mice. The exposure level that would produce mild renal effects is not known, but frank toxic effects occurred in the kidneys of male mice exposed to 5 mg/ℓ (1025 ppm). In subchronic inhalation experiments, histological evidence of mild hepato- and nephrotoxicity occurred in rats with exposures to as low as 25 ppm, 7 hours/day for 6 months. The effects were reversible if exposure was terminated, and did not occur when exposure was limited to 4 hours/day.

Information on the effects of acute and long-term oral exposure to chloroform is available primarily from experiments with animals. Human data are mainly in the form of case reports and involve the abuse of medications containing not only chloroform, but other potentially toxic ingredients as well;

however, a fatal dose of as little as 1/3 ounce was reported. As with inhalation exposure, the primary effects of oral exposure were hepatic and renal damage. Narcosis also occurred with high doses, but this effect was not usually a focus of concern in these experiments. Subchronic and chronic toxicity experiments with rats, mice, and dogs did not clearly establish a no-effect level of exposure for systemic toxicity. Although a dose level of 17 mg/kg/day of chloroform produced no adverse effect in four strains of mice, the lowest dosage tested, 15 mg/kg/day, elevated some clinical chemistry indices of hepatic damage in dogs and appeared to affect a component of the reticuloendothelial system (histiocytes) in their livers.

No controlled studies have been performed to define dose-response thresholds for neurological or cardiac effects of ingested or inhaled chloroform. It is not known whether subtle impairment of neurological or cardiac function might occur at levels as low as or lower than those which affect the liver.

Several substances, which are of interest because of accidental or intentional human exposure, have been shown to modify the systemic toxicity of chloroform, usually by modifying the metabolism of chloroform to the reactive intermediate. Examples of substances that potentiate chloroform-induced toxicity are ethanol, PBBs, ketones and steroids. Factors that appear to protect against toxicity include disulfiram and high carbohydrate diets.

Chloroform appears to have teratogenic potential in laboratory animals when inhaled. Chloroform was selectively more toxic to the fetuses than to the dams when pregnant rats and mice were exposed to the vapor. Delayed fetal development occurred at an exposure level (30 ppm) that produced minimal maternal effects. Embryotoxic effects and low but statistically significant incidences of teratogenic effects occurred at an exposure level that produced mild to moderate maternal effects (i.e., 100 ppm). When chloroform was administered

orally (via gavage) to pregnant rats and rabbits, however, toxic effects on fetal development occurred only at dosage levels that produced severe toxic effects in the dams; no teratogenic effects were observed at any dosage level in these animals.

It has been demonstrated that chloroform can be metabolized *in vivo* and *in vitro* to a substance(s) (presumably phosgene) that interacts with protein and lipid. However, the sole experiment measuring interaction of metabolically activated chloroform with DNA yielded a negative result. This result was judged as inconclusive, because the specific activity of the $^{14}CHCl_3$ may have been too low.

The majority of the assays for mutagenicity and genotoxicity have also yielded negative results; however, many of these results are inconclusive because of various inadequacies in the experimental protocols used. The major problem is with those bacterial, sister chromatid exchange, and chromosome aberration studies that used reconstituted exogenous activation systems (i.e., S-9 mix). In none of these studies was it shown that chloroform was activated or metabolized by the activation system used. Metabolism of 2-aminoanthracene or vinyl compounds (used as positive controls) is probably an inadequate indication that the activation system can metabolize chloroform because these substances are not halogenated alkanes and are therefore not metabolized like them. A better indication that an activation system is sufficient for metabolism of chloroform may be to show that it metabolizes $^{14}CHCl_3$ to intermediates that bind to macromolecules. A second problem with experimental protocols utilizing exogenous activation systems relates to the possiblity that any reactive metabolic intermediates formed may react with microsomal or membrane lipid or protein before reaching the DNA of the test organism. A third potential problem occurs in those *in vitro* protocols in which precautions were not taken to prevent escape of volatilized $CHCl_3$.

Studies in which endogenous activation systems were used include those in yeast, in Drosophila (sex-linked recessive lethal), and in mice (bone-marrow micronucleus, sperm head abnormalities and host-mediated assay). The results from several of these studies suggest that chloroform may be a weak mutagen.

In summary, with the present data, no definitive conclusions can be reached concerning the mutagenicity of chloroform. However, there is some indication (from the binding studies and from the mutagenicity tests that utilized endogenous or in vivo metabolism) that chloroform may have the potential to be a weak mutagen. In order to substantiate this, only certain well-designed in vivo mutagenicity studies or studies with organisms possessing endogenous activation systems are recommended.

Chloroform in corn oil administered at maximally and one-half maximally tolerated doses by gavage for 78 weeks produced a statistically significant increase in the incidence of hepatocellular carcinomas in male and female B6C3F1 mice and renal epithelial tumors (malignant and benign) in male Osborne-Mendel rats; a carcinogenic response of female Osborne-Mendel rats to chloroform was not apparent in this study.

A statistically significant increase in the incidence of renal tumors (benign and malignant) was found in another study in male ICI mice treated with chloroform in either toothpaste or arachis oil by gavage for 80 weeks; however, treatment with a gavage dose of chloroform in toothpaste for 80 weeks did not produce a carcinogenic response in female ICI mice and male mice of the CBA, C57BL, and CF/1 strains. A carcinogenic response was not observed in male and female Sprague-Dawley rats given chloroform in toothpaste by gavage for 80 weeks, but early mortality was high in control and treatment groups. Gavage doses of chloroform in toothpaste did not show a carcinogenic effect in male and female beagle dogs treated for 7 years; however, the treatment period was short

in relation to the lifespan of the beagle dog. Results of preliminary toxicity tests and the carcinogenicity studies indicate that doses of chloroform in toothpaste given to mice, rats, and dogs in the carcinogenicity studies approached maximally tolerated doses. However, doses of chloroform in toothpaste given to mice and rats were lower than those given in corn oil.

Hepatomas were found in NLC mice given chloroform twice weekly for an unspecified period of time and in female strain A mice given chloroform once every 4 days for a total of 30 doses at a level which produced liver necrosis; however, small numbers of animals were examined in pathology, the duration of studies was below the lifetime of the animals, and no control group of NLC mice was apparent. Although a carcinogenic effect of chloroform was not evident in newborn (C57 x DBA2 - F1) mice given single or multiple subcutaneous doses during the initial 8 days of life and observed for their lifetimes, the dose levels used appeared well below a maximum tolerated dose and the period of treatment after birth was quite short compared to lifetime treatment. Chloroform was ineffective at maximally tolerated and lower doses in a pulmonary adenoma bioassay in Strain A mice. Although an ability of chloroform to promote growth and spread of Lewis lung carcinoma, Erlich ascites, and B16 melanoma cells in mice has been shown, the mechanism by which chloroform produced this effect is uncertain, and the relevance of this study to the evaluation of the carcinogenic potential of chloroform is presently not clear.

There are no epidemiologic studies of cancer and chloroform per se. There appears to be an increased risk of cancer of the bladder, rectum, and large intestine from chlorinated drinking water and, by inference, possibly from chloroform, the predominate contaminant.

In conclusion, evidence that chloroform has carcinogenic activity is based on increased incidences of hepatocellular carcinomas in male and female B6C3F1 mice, renal epithelial tumors in male Osborne-Mendel rats, kidney tumors in male ICI mice, and hepatomas in NLC and female strain A mice. Applying the International Agency for Research on Cancer (IARC) criteria for animal studies, this level of evidence would be sufficient for concluding that chloroform is carcinogenic in experimental animals.

Although there is limited evidence in humans for the carcinogenicity of chlorinated drinking water, and by inference, possibly for chloroform, the human evidence for chloroform itself is insufficient. Considering both human and animal evidence, the overall IARC classification would be Group 2B, meaning that chloroform is probably carcinogenic in humans.

Four data sets that contain sufficient information are used to estimate the carcinogenic potency of chloroform. They are: liver tumors in female mice, liver tumors in male mice, kidney tumors in male rats, and kidney tumors in male mice. The carcinogenic potencies, calculated by the linearized multistage model on the basis of these four data sets, are comparable within an order of magnitude. The two data sets for liver tumors in female and male mice give slightly higher potency values than the two data sets for kidney tumors in male rats and male mice. The geometric mean, $q_1^* = 7 \times 10\text{-}2/(mg/kg/day)$, of the potencies calculated from liver tumors in male and female mice (the most sensitive species), is taken to represent the carcinogenic potency of chloroform. Based on this potency, the upper-bound estimate of the cancer risk due to 1 µg/m3 of chloroform in air is P = 1 x 10-5. The upper-bound estimate of the cancer risk due to 1 µg/liter in water is P = 2 x 10-6. These estimates appear consistent with the limited epidemiologic data available for humans. The estimated potency of chloroform is in the fourth quartile of 53 suspect carcinogens that have been evaluated by the Carcinogen Assessment Group.

2. INTRODUCTION

The U.S. Environmental Protection Agency is responsible under the authority of various laws for the identification, comprehensive assessment, and as appropriate regulation, of environmental substances which may be of concern to the public health. For example, under section 112 of the Clean Air Act the EPA Administrator is directed to establish standards for any air pollutant (other than those for which national ambient air quality standards are applicable) which, in his judgment, "causes, or contributes to, air pollution which may reasonably be anticipated to result in an increase in mortality or an increase in serious irreversible, or incapacitating reversible, illness."

Within EPA, the Office of Health and Environmental Assessment is responsible for providing scientific assessments of health effects for potentially hazardous air pollutants such as chloroform. These health assessment documents form the scientific basis for subsequent agency actions, including the Administrator's judgment as to whether regulations or standards may be appropriate.

This Health Assessment Document for Chloroform represents a comprehensive data base that considers all sources of chloroform in the environment, the likelihood of human exposures and the possible consequences to man and lower organisms from its absorption. This information is integrated into a format that can serve as the basis for qualitative and quantitative risk assessments, while at the same time identifying gaps in our knowledge that limit present evaluative capabilities. Accordingly, it is expected that this document may serve the information needs of many government agencies and private groups that may be involved in decision making and regulatory activities. (As with all such EPA documents, this preliminary draft is made available to the scientific community and the general public so that comments of interested individuals and organizations may be considered, and the latest scientific evidence incorporated, in the final draft. This draft will also be reviewed by the Environmental Health Advisory Committee of EPA's Science Advisory Board at a subsequently announced public meeting).

3. BACKGROUND INFORMATION

3.1. INTRODUCTION

This section provides background information supportive of the human health effects data presented in subsequent sections. It is not intended to be a comprehensive review of analytical methodology, sources, emissions, air concentrations, or environmental transport and fate information. In order to fulfill this purpose and since the literature concerning chloroform is vast, only a portion of the available literature was included. Those articles included were chosen because of their relevance to the topic at hand and because they were representative of the literature as a whole.

To provide the most complete overview possible, some non-peer reviewed information has been added, from the Chloroform Materials Balance Draft Report by Rem et al. (1982). This is an updated version of the original Level I Materials Balance: Chloroform (Wagner et al., 1980). As described by Wagner et al. (1980):

"A Level I Materials Balance requires the lowest level of effort and involves a survey of readily available information for constructing the materials balance. Ordinarily, many assumptions must be made in accounting for gaps in information; however, all are substantiated to the greatest degree possible. Where possible, the uncertainties in numerical values are given, otherwise they are estimated. Data gaps are identified and recommendations are made for filling them. A Level I Materials Balance relies heavily on the EPA's Chemical Information Division (CID) as a source of data and references involving readily available information. Most Level I Materials Balance are completed within a 3-6 week period; CID literature searches generally require a 2 week period to complete. Thus, the total time required for completion of a Level I materials balance ranges from 5-7 weeks."

Because a greater level of effort went into the 1980 and 1982 Materials Balance reports on chloroform than would normally be devoted to background (non-health effects) information in an EPA health assessment document, information in this chapter is drawn from these reports. However, because such information has not been peer-reviewed and includes some major assumptions, it should not be used to support any regulations or standards regarding risks to public health.

3.2. PHYSICAL AND CHEMICAL PROPERTIES

Chloroform ($CHCl_3$) (CAS registry number 67-66-3) is a member of a family of halogenated saturated aliphatic compounds. Synonyms for chloroform include the following:

Chloroforme (French)	Methenyl trichloride
Chloroformio (Italian)	Methyl trichloride
Formyl trichloride	NCI-C02686
Methane trichloride	Trichloromethaan (Dutch)
Methane, trichloror	Trichloroform
Methenyl chloride	Trichloromethane

Table 3-1 lists important physical properties. Cloroform is a colorless, clear, dense, volatile liquid with an ethereal non-irritating odor (DeShon, 1979). Chloroform is nonflammable; however, when hot chloroform vapors are mixed with alcohol vapors, the mixture burns with a greenish flame. At 25°C and 1 atmosphere, a 1 ppm concentration of chloroform in air is equal to 4.88 mg/m^3.

Chloroform decomposes with prolonged exposure to sunlight regardless of the presence of air (DeShon, 1979). It also decomposes in the dark in the presence

TABLE 3-1

Physical Properties of Chloroform[a]

Molecular weight	119.38
Melting point (°C)	-63.2
Boiling Point	61.3
Water-chloroform azeotrope (°C)	56.1
Specific gravity (25/4°C)	1.48069
Vapor density (101kPa, 0°C, kg/m^3)	4.36

Vapor pressure

°C	kPa	torr
-30	1.33	10.0
-20	2.61	19.6
-10	4.63	34.7
0	8.13	61.0
10	13.40	100.5
20	21.28	159.6
30	32.80	246.0
40	48.85	366.4

Solubility in water

°C	g/kg H_2O
0	10.62
10	8.95
20	8.22
30	7.76

Log octanol/water partition coefficient[b]	1.97
Conversion factors at 25°C and 1 atm[c]	
1 ppm $CHCl_3$ in air equals 4.88 mg/m^3	
1 mg/m^3 $CHCl_3$ in air equals 0.205 ppm	

[a]Source: DeShon, 1979

[b]Hansch and Leo, 1979

[c]Calculated

of air. The principal decomposition products include phosgene, hydrogen chloride, chlorine, carbon dioxide, and water. Ozone causes chloroform to decompose rapidly.

Chloroform forms a hydrate in water at 0°C ($CHCl_3 \cdot 18H_2O$, CAS registry number 67922-19-4); the hexagonal crystal decomposes at 1.6°C (DeShon, 1979). Chloroform is chemically stable to water, having a hydrolysis half-life of 3100 years in neutral water at 25°C (Mabey and Mill, 1978); the half-life of chloroform in air from hydroxyl radical reactions is 78 days (Hampson, 1980).

The hydrogen atom on chloroform can be removed in the presence of warm alkali metal hydoxide to form a trichloromethyl anion (DeShon, 1979). This anion can condense with carbonyl compounds. Both wet and dry chloroform will react with aluminum, zinc, and iron.

Small amounts of ethanol are used to stabilize chloroform from oxidation during storage (DeShon, 1979).

3.3. SAMPLING AND ANALYSIS

3.3.1. Chloroform in Air. Chloroform in air can be analyzed by a number of methods; however, the method of Singh et al. (1980) appears to be substantially free of artifact problems and completely quantitative. In this method, an air sample in a stainless steel canister at 32 psig is connected to a preconcentration trap consisting of a 4" x 1/16" ID stainless steel tube containing glass beads, glass wool, or 3% SE-30 on acid washed 100/120 mesh chromosorb w. The sampling line and trap, maintained at 90°C, are flushed with air from the canister; then the trap is immersed in liquid O_2 and air is passed through the trap, the initial and final pressure being noted (usually between 30 and 20 psig) on a high-precision pressure guage. The ideal gas law can be used to estimate the volume of air passed through the trap. The contents of the trap are desorbed onto a chromatography column by backflushing it with an inert gas while holding

the trap at boiling water temperature. An Ascarite trap may be inserted before the chromatography column to remove water. Suitable columns include 20% SP-2100 and 0.1% CW-1500 on Supelcoport (100/120 mesh, 6' x 1/8" stainless steel) and 20% DC-200 on Supelcoport (80/100 mesh, 33' x 1/8" Ni). Both columns can be operated at 45°C with a carrier gas flow of 40 mℓ/min on the former column and 25 mℓ/min on the latter. An electron capture detector operating at 325°C was found to be optimum. It should be noted that the above authors found Tenax to be unsuitable for air analyses because of the presence of artifacts in the spectrum from oxidation of the Tenax monomer. In addition, when Tenax is used as a sorbent, safe sampling volumes (i.e., that volume of air which, if sampled over a variety of circumstances, will not cause significant breakthrough) should be adhered to. Brown and Purnell (1979) determined the safe volume for chloroform per gram Tenax to be 9.3 ℓ (flow rate 5-600 mℓ/min; $CHCl_3$ conc. <250 mg/m^3 temp. up to 20°C) with a safe desorption temperature of 90°C.

The detection limits of this method were not specified and are dependent on the volume of air sampled. Analyses as low as 16 ppt have been reported using this method (Singh et al., 1980).

3.3.2. Chloroform in Water. Chloroform in water can be analyzed by the purge-and-trap method (Method 502.1) as recommended by the Environmental Monitoring and Support Laboratory of the U.S. EPA (1981a). In this method, an inert gas is bubbled through 5 mℓ of water at a rate of 40 mℓ/minute for 11 minutes, allowing the purgable organic compounds to partition into the gas. The gas is passed through a column containing Tenax GC at 22°C, which traps most of the organics removed from the water. The Tenax column is then heated rapidly to 130°C and backflushed with helium (20-60 mℓ/minute, 4 minutes) to desorb the trapped organics. The effluent of the Tenax column is passed into an analytical gas chromatography column packed with 1% SP-1000 on Carbopack-B (60/80 mesh, 8'x 0.1"

I.D.) maintained at 40°C. The column is then temperature programmed starting at 45°C for 3 minutes and increasing at 8°C/minute until 220°C is reached; it is then held there for 15 minutes or until all compounds have eluted. A halogen-specific detector (or GC-MS) having a sensitivity of 0.10 μg/ℓ with a relative standard deviation of <10% must be used.

3.3.3 Chloroform in Blood. Chloroform in blood can be analyzed by using a modified purge-and-trap method (Pellizzari et al., 1979). This method involves diluting an aliquot of whole blood (with anticoagulant) to ≈50 mℓ with prepurged, distilled water. The mixture is placed in a 100 mℓ 3-neck round bottom flask along with a teflon-lined magnetic stirring bar. The necks of the flask are equipped with a helium inlet, a Tenax trap, and a thermometer. The Tenax trap is a 10 cm x 1.5 cm i.d. glass tube containing pre-extracted (Soxhlet, methanol, 24 hrs) and conditioned (270°C, 30 mℓ/min helium flow, 20 min) 35/60 mesh Tenax (≈1.6 g, 6 cm). The sample is then heated to 50°C and purged with a helium flow rate of 25 mℓ/min for 90 min. Analysis can be performed as indicated in Section 3.3.2.

3.3.4. Chloroform in Urine. Chloroform in urine can be analyzed by using an apparatus identical to the one described in Section 3.3.3, using 25 mℓ of urine diluted to 50 mℓ instead of blood.

3.3.5. Chloroform in Tissue. Chloroform in tissue can be analyzed by using an apparatus identical to the one described in Section 3.3.3, using 5 g of tissue diluted to 50 mℓ and macerated in an ice bath instead of blood. The purge time is reduced to 30 minutes.

3.4. EMISSIONS FROM PRODUCTION AND USE

3.4.1. Emissions from Production.

3.4.1.1. DIRECT PRODUCTION -- Chloroform is produced commercially in the United States by two methods, chlorination of methane and chlorination of methyl

chloride produced from methanol and hydrogen chloride (Wagner et al., 1980; DeShon, 1979). The chemistry is summarized by the following reactions:

Methane Chlorination

$$4CH_4 + 10Cl_2 \longrightarrow CH_3Cl + CH_2Cl_2 + CHCl_3 + CCl_4 + 10HCl$$

Methanol Hydrochlorination

$$HCl + CH_3OH \xrightarrow[280 - 350°C]{\text{Catalyst}} CH_3Cl + H_2O$$

$$3CH_3Cl + 6Cl_2 \longrightarrow CH_2Cl_2 + CHCl_3 + CCl_4 + 6HCl$$

The methanol process accounts for 74% of capacity, while methane accounts for 26% of capacity (SRI International, 1983).

In the chlorination of methane, natural gas is directly chlorinated in the gas phase with chlorine at 485-510°C (Anthony, 1979). The product mixture contains all chlorinated methanes, which are removed by scrubbing and separated by fractional distillation.

In the second process, gaseous methanol and HCl are combined over a hot catalyst to form methyl chloride (Ahlstrom and Steele, 1979). The methyl chloride is then chlorinated with chlorine to produce CH_2Cl_2, $CHCl_3$, and CCl_4 (DeShon, 1979). The chlorination conditions for both processes can be adjusted to optimize chloroform production.

United States production is carried out by five manufacturers at seven sites summarized in Table 3-2.

The annual production of chloroform in the United States has risen from 35 million kg (77 million lbs) in 1960 (DeShon, 1979) to >184 million kg (405 million lbs) in 1981 (USITC, 1982) with few declines.

TABLE 3-2

Chloroform Producers, Production Sites, and Capacities*

Producer	Production Site	Capacity Millions of kg (Millions of lbs)	Process
Diamond Shamrock	Belle, WV	18(40)	Methanol
Dow Chemical	Freeport, TX	45(100)	Methane
	Plaquemine, LA	45(100)	Methanol
Linden Chemicals and Plastics, Inc.	Moundsville, WV	14(30)	Methanol
Stauffer Chemical Co.	Louisville, KY	34(75)	Methanol
Vulcan Materials Co.	Geismar, LA	27(60)	Methanol
	Wichita, KA	50(110)	Methanol and Methane
TOTAL		233(515)	

*Source: SRI International, 1983.

3.4.1.1.1. Chloroform Emissions from the Methane Chlorination Process--Rem et al. (1982) reported that air emissions could come from process vents, in-process and product storage tanks, liquid waste streams, secondary emissions (handling and disposal of process wastes), and fugitive emissions from leaks in process valves, pumps, compressors and pressure relief valves.

The emission factors they calculated were based on a typical methane chlorination facility as reported by U.S. EPA (1980a) having a total chloromethane capacity of 2×10^5 metric tons (441×10^6 lbs), operating continuously (8760 hr/yr), and having a product mix of 20% CH_3Cl, 45% CH_2Cl_2, 25% $CHCl_3$, and 10% CCl_4. The emission factor for the uncontrolled recycle methane inert gas purge vent for the above plant (0.014 kg/metric ton) was calculated from an hourly $CHCl_3$ emission rate of 0.071 kg/hr reported by Dow Chemical Company for a 46,000 metric ton/yr facility assuming continuous (8760 hr/yr) operation (Beale, n.d.). The uncontrolled emission factor for the distillation area emergency inert gas vent (0.032 kg/metric ton) was calculated from an emission factor for volatile organic compounds (VOC) of 0.20 kg/metric ton of total chloromethane production and composition data showing chloroform to be 4% of VOC (U.S. EPA, 1980a). In-process and product storage emissions (0.91 to 0.80 kg/metric ton, depending on controls) were calculated from emission equations for breathing and working losses from AP-61 (U.S. EPA, 1981b) assuming tanks to be half-full, have 95% emission controls when present, and a 12°C diurnal temperature variation (U.S. EPA, 1980b). Rem et al. (1982) calculated the total chloroform emissions to air to be 70.2 metric tons (155×10^3 lbs) by multiplying the appropriate factors by plant capacity use after including secondary emissions (0.21 kg/metric ton) and fugitive emissions (5.5 kg/hr).

Releases of chloroform to water come from scrubbers, neutralizers, and cooling water (Rem et al., 1982). Based on a 300 ppm $CHCl_3$ content in total

wastewater discharges averaging 68 ℓ/min and assuming that 90% would volatilize, Rem et al. (1982) calculated a release factor of 0.023 kg/metric ton. They then added to this the emission from indirect contact cooling water (100 ppm, 5800 ℓ cooling water/metric ton $CHCl_3$, 90% evaporation) to calculate a release rate of 3.3 metric tons of $CHCl_3$ (7.3×10^3 lbs) per year to water.

No quantifiable data were available to Rem et al. (1982) regarding release of chloroform to land from methane chlorination.

3.4.1.1.2. Chlorination Emissions from the Methanol Hydrochlorination-Methyl Chloride Chlorination Process -- Chloroform emissions to air come from process vents, in-process and product storage tank emissions, and fugitive emissions from leaks in valves, pumps, compressors, and pressure relief valves (Rem et al., 1982). Rem et al. (1982) used an uncontrolled emission factor reported by Vulcan Materials Company for process vents (Hobbs, 1978), and assumed continuous (8,760 hr/year) operation, and controls sufficient to reduce emissions 80% to obtain the emission factor for controlled process vents (0.003 kg/metric ton for controlled; 0.015 kg/metric ton for uncontrolled). Storage emissions (0.176 kg/metric ton for controlled, 0.88 kg/metric ton for uncontrolled) were calculated from Hobbs (1978) and from emission equations for breathing and working losses from AP-42 (U.S. EPA, 1981b), assuming tanks to be half-full, have 80% emission reduction controls, and a 12°C diurnal temperature variation (U.S. EPA, 1980b). Fugitive emission factors (3.32 kg/hr for uncontrolled; 1.08 kg/hr for controlled) for volatile organic compounds were used (U.S. EPA, 1980c) along with a control factor of 67.5% based on leak detection and repair (U.S. EPA, 1980b). Emission rates were then calculated to be 196 metric tons (432×10^3 lbs) based on plant capacity, capacity use (66%), the level of emission controls used at each plant, and continuous operation (8,760 hr/year).

Rem et al. (1982) assumed that releases of chloroform to water from methyl chloride chlorination resulted from contamination of cooling water and from spent acid and spent caustic streams. For cooling water contamination, they assumed minor spills and leaks resulted in contamination of 100 mg chloroform/ℓ cooling water, that 5,800 ℓ of cooling water per metric ton of chloroform produced was consumed, and that 90% of the chloroform evaporated. For spent acid and spent caustic streams they assumed that 0.04 kg of chloroform are released for every metric ton of chloromethane produced. They further stated that this release factor was not considered to be very reliable; however, in the absence of better data they used this factor to approximate emissions. In addition, they assumed that 1/3 of the chloromethane production consists of chloroform and that 90% of the released chloroform evaporates. Using these assumptions, Rem et al. (1982) calculated that 0.070 kg of chloroform is released to water per metric ton of chloroform produced, or 8.0 metric tons (17.6×10^3 lbs) of chloroform were released to water based on 1980 production levels.

Rem et al. (1982) reported that the bottoms from chloroform distillation in the methyl chloride chlorination process are the feed for carbon tetrachloride and perchloroethylene production, and that during their production a residue is formed that contains chloroform. This residue is landfilled or deep-well injected. This represents the only known release of chloroform from carbon-tetrachloride/perchloroethylene production.

Rem et al. (1982) assumed that 1.02 kg of residue is produced/metric ton of chloroform from methyl chloride production (see Wagner et al., 1980). They further assumed (as did Wagner et al., 1980) that 18.4% of the residue is chloroform and that 25% is landfilled. This results in a release factor of 0.047 kg chloroform per metric ton of chloroform produced, or 5.4 metric tons (12×10^3 lbs) based on 1980 production.

3.4.1.1.3. Summary of Direct Production -- Direct production emits some 283 metric tons into the environment on an annual basis. Greater than 95% (266.2 metric tons) of this is emitted into the air. Direct production accounts for ≈3% of all environmentally released chloroform, and ≈3.6% of all chloroform released to air. Table 3-3 summarizes chloroform discharges from direct production.

3.4.1.2. INDIRECT PRODUCTION

3.4.1.2.1. Chloroform Formation During Ethylene Dichloride Production -- Ethylene dichloride (EDC) is produced by two methods, direct chlorination and oxychlorination, and is used principally for vinyl chloride monomer (VCM) production. A combination of the two methods is used by most VCM production facilities in a process known as the balanced process since the HCl from the dehydrochlorination of EDC is used to produce more EDC from ethylene, the major products of the overall reaction being VCM and H_2O.

Direct Chlorination

$$CH_2 = CH_2 + Cl_2 \longrightarrow CH_2ClCH_2Cl$$

Oxychlorination

$$2\,HCl + 1/2\,O_2 \xrightarrow{\text{catalyst}} Cl_2 + H_2O$$

$$CH_2 = CH_2 + Cl_2 \longrightarrow CH_2ClCH_2Cl$$

Balanced Process

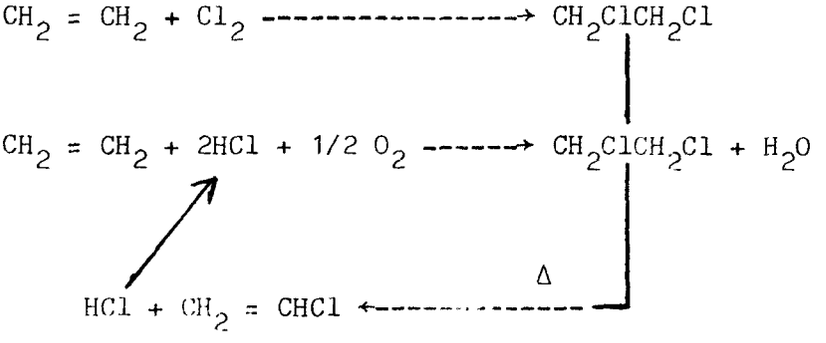

TABLE 3-3

Chloroform Discharges from Direct Sources*

Source	Environment Release (metric tons/year)			
	Air	Water	Land	Total
Methyl Chloride Chlorination	196	8	5.4	209.4
Methane Chlorination	70.2	3.3	---	73.5
Total:	266.2	11.3	5.4	282.9

*Source: Rem et al. (1982)

Chloroform is formed as a byproduct during EDC manufacture. Rem et al. (1982) estimated chloroform emissions to air from EDC production based on emission factors developed by the EPA during field studies of domestic EDC production facilities (see U.S. EPA, 1980d). Domestic production facilities are listed in Table 3-4. Emission sources (e.g., process vents, fugitive emissions, storage) for each plant, plant capacity, capacity utilization (56%), and control technology were all combined with the emission factors to determine the overall chloroform emissions at the current level of control. According to their calculations, chloroform emissions to the atmosphere are ≈760 metric tons/year ($1,675 \times 10^3$ lbs/year).

Chloroform releases to water may occur during the discharge of wastewater from the process; however, the amount of chloroform present is unknown.

Chloroform releases to land from EDC production reportedly occur when the light ends from EDC distillation are landfilled (Rem et al., 1982). An estimated 217 metric tons (478×10^3 lbs) were landfilled in 1980.

3.4.1.2.2. Chlorination of Drinking Water -- Chloroform in drinking water arises when humic substances or methyl ketones (e.g., acetone) in water react with a hypochlorite anion (Stevens et al., 1976; NAS, 1978). Hypochlorite is the principal reactant in chlorinated water above pH 5. Chloroform is produced by the haloform reaction outlined below:

$$R\text{-}COCH_3 + 3OCl^- \longrightarrow RCOCCl_3 + 3OH^-$$

$$R\text{-}COCCl_3 + OH^- \longrightarrow RCOO^- + CHCl_3$$

Rem et al. (1982) used the data from the National Organics Reconnaissance Survey (NORS) (Symons et al., 1975) and the National Organics Monitoring Survey (NOMS) (U.S. EPA, n.d.) to estimate the concentration of chloroform in drinking

TABLE 3-4

Ethylene Dichloride Producers, Production Sites and Capacities[a]

Producer	Production Site	Capacity millions of Kg (millions of lbs)	
Alantic Richfield Co.			
ARCO Chem. Co., div.	Port Arthur, TX	204	(450)
Borden Inc.			
Borden Chem. Div.			
Petrochems. Div.	Geismar, LA	231	(510)
Dow Chemical. U.S.A.	Freeport, TX	726	(1600)
	Oyster Creek, TX	476	(1050)
	Plaquemine, LA	862	(1900)
E.I. du Pont de Nemours and Co., Inc.			
Conoco Inc., subsid.			
Conoco Chems. Co. Div.	Lake Charles, LA	524	(1155)
Ethyl Corp.			
Chems. Group	Baton Rouge, LA	318	(700)
	Pasadena, TX	102	(225)
Formosa Plastics Corp. U.S.A.	Baton Rouge, LA	249	(550)
	Point Comfort, TX	386	(850)
Georgia-Pacific Corp.			
Chem. Div.	Plaquemine, LA	748	(1650)
The BF Goodrich Co.			
BF Goodrich Chem. Group	Deer Park, TX	145	(320)
	La Porte, TX	719	(1585)
Convent Chem. Corp., subsid.	Calvert City, KY	454	(1000)
	Convent, LA	363	(800)
PPG Indust., Inc.			
Chems. Group			
Chem. Div.	Lake Charles, LA	1225	(2700)

TABLE 3-4 (cont.)

Producer	Production Site	Capacity millions of Kg	(millions of lbs)
Shell Chem. Co.	Deer Park, TX	635	(1400)
	Norco, LA	544	(1200)
Union Carbide Corp.			
Ethylene Oxide Derivatives Div.	Taft, LA	68[b]	(150)
	Texas City, TX	68	(150)
Vulcan Materials Co.			
Vulcan Chems., div.	Geismar, LA	159	(350)
	TOTAL	9,206	20,295

[a]Source: SRI International, 1983

[b]Captive use only

water. These surveys provided information on chloroform concentrations in 137 U.S. cities. To determine the amount of chloroform generated, the authors multiplied the volume of water treated by each city by the chloroform concentration in the drinking water. The amount of chloroform generated by each city was then summed and divided by the volume of water generated to give a weighted average concentration of 41 $\mu g/\ell$. This was then multiplied by the estimated volume of water chlorinated annually (4.6×10^{13} ℓ/year) to yield the amount in the U.S. (1,900 metric tons, 4.2×10^{6} lbs). Rem et al. (1982) stressed that this value probably represents a minimum since NORS was conducted during the winter (hence, chloroform levels were low) and NOMS samples were iced when taken (hence, may be lower than if allowed full contact time). Thus, the actual value may be higher than this estimate.

3.4.1.2.3. Chlorination of Municipal Sewage -- Chlorination of municipal sewage results in increased chloroform concentrations (NAS, 1978). Municipal wastewater generally contains a lower concentration of chloroform precursors (humic materials) than do ambient waters; therefore, the amount of chloroform generated from wastewater chlorination is smaller (Wagner et al., 1980). Rem et al. (1982) calculated chloroform production from wastewater treatment based on analyses of the secondary effluent from 28 municipal plants published by the EPA (U.S. EPA, 1979a). These analyses showed that the average chloroform concentration increased by 9 $\mu g/\ell$, from 5 to 14 $\mu g/\ell$. Rem et al. (1982) assumed that all municipal wastewater was chlorinated and multiplied the average concentration increase by the municipal wastewater flow (9.7×10^{10} ℓ/day) listed in U.S. EPA (1981c). By this method, 320 metric tons (0.7×10^{6} lbs) was calculated to be produced annually.

3.4.1.2.4. Chlorination of Cooling Waters -- Cooling water used in electric power generating plants is treated with chlorine as a biocide to prevent fouling

intake screens and condensers in both once-through and closed cycle systems (U.S. EPA, 1980e). Rem et al. (1982) calculated chloroform production based on the size of the average power plant (hence, the volume of water required), the type of cooling system used (once-through or recirculating), and the fact that 65% of all power plants chlorinate cooling water. They calculate that 72 metric tons of chloroform (160 x 10^3 lbs) are discharged directly into water by once-through systems and 190 metric tons of chloroform (420 x 10^3 lbs) are emitted into the air by recirculating systems. A total of 262 metric tons of chloroform (580 x 10^3 lbs) are produced annually from cooling water chlorination.

3.4.1.2.5. Chlorination in the Pulp and Paper Industry -- Pulp and paper mills emit more chloroform to the environment than any other single source. Chloroform is produced during the bleaching of wood pulp, a process that whitens the final paper product. Rem et al. (1982) based their estimation on information contained in a number of documents concerned with the pulp and paper industry (U.S. EPA, 1980f; NCASI, 1977; Metcalf and Eddy Inc., 1972; TAPPI, 1963). From the operating conditions, analytical data, and production steps detailed in these documents, Rem et al. (1982) determined the quantity of chloroform produced for each of nine different types of mills for which monitoring data existed and applied these values to all mills for which no values from sampling existed. The authors determined that chloroform is emitted at three different stages: into the air during the bleaching process itself, into the air during the detention time in wastewater treatment plants, and into the water from treatment plant effluent. The amount of chloroform produced annually was calculated to be 128 metric tons (282 x 10^3 lbs) released to air during bleaching operations, 3,985 metric tons (8.78 x 10^6 lbs) released to air from wastewater detention (4,113 metric tons to air), and 298 metric tons (657 x 10^3 lbs) discharged into water from treatment plants (4,411 metric tons or 9.72 x 10^6 lbs total).

3.4.1.2.6. Chloroform Production from Combustion of Leaded Gasoline -- Chloroforom has been reported to be a component of automobile exhaust (Harsch et al., 1977). Its presence is reportedly the result of using ethylene dichloride and ethylene dibromide as lead scavengers in leaded gasoline (Lowenbach and Schlesinger Associates, 1979). Rem et al. (1982) cite other sources which state that chloroform is not formed during the combustion of leaded fuel containing ethylene dichloride. The Emissions Testing and Characterization Branch of EPA's Environmental Sciences Research Laboratory measured chlorocarbon emissions from automobiles using leaded gasoline and found no chloroform. Rem et al. (1982) then reason that even if chloroform were present in automobile exhaust, the decrease in the usage of leaded gasoline will decrease the amount of chloroform produced.

The authors cite an EPA report (U.S. EPA, 1982a) which states that leaded gasoline consumption is expected to drop by >75%, from 34 x 10^9 gallons to 8.3 x 10^9 gallons/year. Rem et al. (1982) used the estimate of Wagner et al. (1980) that 1% of the ethylene dichloride in gasoline would be converted to chloroform, and the new lead phase-down regulations (U.S. EPA, 1982b) to calculate an annual emission rate of 180 metric tons (397 x 10^3 lbs) in 1983 and 44 metric tons (97 x 10^3 lbs) by 1990.

3.4.1.2.7. Chloroform Formation During Atmospheric Trichloroethylene Decomposition -- Trichloroethylene is a major industrial solvent used principally for vapor degreasing of fabricated metal parts (66%) (Chemical Marketing Reporter, 1981), and the majority of each year's production is used for replacement of evaporative loss to the environment (Rem et al., 1982). The postulated formation of chloroform during the atmospheric decomposition of trichloroethylene is based on laboratory experiments in which trichloroethylene, NO_2, H_2O, and a hydrocarbon mixture were irradiated with light having the

intensity and spectral distribution of the lower troposphere (U.S. EPA, 1976). Dichloroacetylchloride, phosgene, chloroform, and HCl were detected as products. Rem et al. (1982) did not describe the method they used to determine the amount of chloroform produced from this reaction; however, they estimated 780 metric tons of chloroform are produced annually.

3.4.1.2.8. Miscellaneous Indirect Source -- Wagner et al. (1980) have listed a number of other indirect sources of chloroform that are difficult if not impossible to quantify. These sources are: chlorination wastewaters from the textile industry, the food processing industry, breweries, combustion of tobacco products treated with chlorination pesticides, thermal decomposition of plastics, biological production in red marine algae, and the reaction of chlorinated pollutants with humic substances in natural waters.

3.4.1.2.9. Summary of Indirect Production -- Chloroform is produced and emitted into the environment from a variety of indirect sources. These sources account for ≈84% of all chloroform air emissions, 98% of water discharges, and 36% of all land discharges (84.6% of all environmental releases).

The environmental discharges of chloroform from indirect sources are summarized in Table 3-5.

3.4.2. Emissions from Use. Chloroform is used consumptively for the production of chlorodifluoromethane or Fluorocarbon-22 (accounts for 90% of domestic 1982 production, 60% for refrigerants, 30% for fluoropolymer) and for exports (3% in 1982) (Anonymous, 1983). Non-consumptive uses include its use as an extraction solvent; as a solvent for penicillin, alkaloids, vitamins, flavors, lacquers, floor polishes, artificial silk manufacture, resins, fats, greases, gums, waxes, adhesives, oils, and rubber; as a dry cleaning agent; as an intermediate in pesticide and dye manufacture; and as a fumigant ingredient (Rem et al., 1982; DeShon, 1979; Merck Index, 1976). The great majority of chloroform used non-

TABLE 3-5

Chloroform Discharges from Indirect Sources[a]

Source	Environmental Release (metric tons/year)			
	Air	Water	Land	Total
Pulp and Paper Mills	4113	298	0	4411
Drinking Water Chlorination	0	1900	0	1900
Ethylene Dichloride Manufacture	760	---[b]	217	977
Trichloroethylene Photodegradation	780	0	0	780
Municipal Wastewater Chlorination	0	320	0	320
Cooling Water Chlorination	190	72	0	262
Automobile Exhaust	180	0	0	180
TOTAL	6023	2590	217	8830

[a]Source: Rem et al., 1982

[b]minor releases possible

consumptively is emitted into the environment since (except for expansions) the chloroform purchased for these uses is make-up solvent used to replace that amount not recovered from processes (Wagner et al., 1980).

3.4.2.1. EMISSIONS FROM PHARMACEUTICAL MANUFACTURING -- Chloroform is used as an extraction solvent during the manufacture of some antibiotics and steroids, and during the manufacture of certain other biological and natural pharmaceuticals (Rem et al., 1982). It is also used as a chemical intermediate. Based on a Pharmaceutical Manufacturing Association (PMA) Survey, Rem et al. (1982) reported that ≈1000 metric tons of chloroform are released into the environment (no year was specified; the PMA report was dated 1978). The distribution was as follows: 57.0% (570 metric tons) to air, 4.6% (46 metric tons) to water, and 38.4% (384 metric tons) to land.

3.4.2.2. EMISSIONS FROM FLUOROCARBON-22 PRODUCTION -- The single largest use of chloroform is for Fluorocarbon-22 production (Chlorodifluoromethane, $CHClF_2$). Fluorocarbon-22 producers and production sites are listed in Table 3-6. Chloroform release to the environment can occur from process emissions, fugitive emissions, and storage emissions (Rem et al., 1982). Rem et al. (1982) reported that the first source listed does not represent a significant source of chloroform emissions based on the design of Fluorocarbon-22 production facilities and the process description.

Storage emission estimates were based on U.S. EPA (1980g) for chloroform feedstock storage in fixed roof tanks. Rem et al. (1982) reported that the Allied Chemical facility at El Segundo, California, uses a control system that results in complete capture of chloroform vapors. Du Pont (and all others by assumption) use refrigerated condensers that reduce the uncontrolled emission factor of 2.5 kg/metric ton by 66%. Fugitive emissions from leaks in valves, pumps, compressors, and relief valves was estimated to result in an uncontrolled

TABLE 3-6

Chlorodifluoromethane Producers and Production Sites*

Producer	Production Site
Allied Corp.	
Allied Chem. Co.	Baton Rouge, LA
	Danville, IL
	El Segundo, CA
E.I. du Pont de Nemours and Co., Inc.	
Petrochems. Dept.	
Freon Products Div.	Deepwater, NJ
	Louisville, KY
Pennwalt Corp.	
Chems. Group	
Fluorochemicals Div.	Calvert City, KY

*Source: SRI International, 1983

emission rate of 0.75 kg/metric ton. Total emissions to air were calculated by Rem et al. (1982) to be 139 metric tons/year by multiplying the emission factors by 1980 Fluorocarbon-22 production (97,500 metric tons).

No estimate was made for emissions to wastewater because of a lack of data. Emissions to land were based on the reported practice of landfilling spent catalyst by Allied. Rem et al. (1982) assumed a catalyst contamination level of 10% and a total emission of 2.0 metric tons/year.

3.4.2.3. EMISSIONS FROM HYPALON® MANUFACTURE -- Hypalon® is a chemically resistant synthetic rubber made by substituting chloride and sulfonyl groups onto polyethylene. The process involves dissolving polyethylene in chloroform followed by reaction with chlorine and sulfur dioxide. Based on a Du Pont report, Rem et al. (1982) estimated that 54.9 metric tons of chloroform were emitted into the air from Hypalon® manufacture in 1980, based on an emission inventory conducted by the Texas Air Control Board, Austin, Texas. No information was available for water or land emissions.

3.4.2.4. CHLOROFORM EMISSIONS FROM GRAIN FUMIGATION -- Chloroform is a registered pesticide for use on certain insects that commonly infest stored raw bulk grains and is present in only one product (Rem et al., 1982). This product, Chlorofume® FC.30 Grain Fumigant (Reg. No. 5382-15), marketed by Vulcan Materials Company, contains 72.2% chloroform, 20.4% carbon disulfide, and 7.4% ethylene dibromide. Originally registered in 1968, the EPA issued a "Notice of Presumption Against Continued Registration of a Pesticide Product -- Chloroform (Trichloromethane)" in 1976 because of oncogenic effects in rats and mice. Continued study resulted in returning it to the normal registration process (U.S. EPA, 1982c). Based on a personal communication with D. Lindsay of Vulcan Materials, Rem et al. (1980) estimated that between 10,000 and 12,000 gallons of chloroform/year were used in grain fumigants in the United States. Vulcan

reported 1981 sales of 7,000 gallons of Chlorofume® in 1981 or 5054 gallons (19,131 ℓ). Based on its density, 28,400 kg (28.4 metric tons) was released to the environment (air) in this way.

3.4.2.5. CHLOROFORM LOSSES FROM LOADING AND TRANSPORTATION -- Rem et al. (1982) estimated chloroform losses from loading ships, barges, tank cars, and tank trucks. The method was based on the degree of chloroform saturation of the air expelled from tanks during filling, temperature, vapor pressure, control efficiency, and filling methods as described by U.S. EPA (1979b) and Environment Reporter (1982). The U.S. mode of transportation was taken from Sax (1981) as follows: rail, 40.3%; barge, 47.8%; and truck, 11.9%. Loading losses were calculated to be 40.9 metric tons (90,200 lbs).

Transit losses result from temperature and barometric pressure changes. The losses were assumed to be the same for barges, tank trucks, and rail cars and were estimated from the following equation:

$$L_T = 0.1\ P\ W$$

where LT= transit loss, lb/week - 10^3 gal transported

P= true vapor pressure of transported liquid, psia

W= density of condensed vapors lb/gal

No reference or justification for the use of the formula was presented. By this method, and assuming 1 week transit time, Rem et al. (1982) calculated that 49.2 metric tons (0.11 x 10^6 lbs) chloroform were lost to the air using 1980 production values.

3.4.2.6. MISCELLANEOUS USE EMISSIONS -- Rem et al. (1982) cited the previous materials balance (Wagner et al., 1980) in predicting the emissions from chloroform contamination of methyl chloride, methylene chloride, and carbon tetrachloride. Chloroform is present to some extent in these products since they

are all made by the same process. Assuming a contamination level of 7.5 ppm, 17.5 ppm and 150 ppm for methyl chloride, methylene chloride, and carbon tetrachloride, respectively, Rem et al. (1982) estimate that releases to air, land and water would be 9.8, 0.6, and 0.15 metric tons, respectively.

Chloroform is also used in a variety of products (see Section 3.4.2) and as a general solvent. Rem et al. (1982) estimate that, while these uses are generally declining, laboratory uses in particular may account for 8.5% of production or 14,200 metric tons of chloroform. Rem et al. (1982), however, estimated the range of uncertainty to be $\pm$ 50%.

3.4.2.7. SUMMARY OF CHLOROFORM DISCHARGES FROM USE -- Chloroform discharges from manufacturing facilities that use chloroform as a process ingredient account for 12% of all chloroform emissions to air, 1.8% of water discharges, and 63% of all land discharges, or 12.7% of chloroform discharges to all media. Table 3-7 summarizes chloroform discharges to all media.

3.4.3. Summary. Chloroform is produced by direct and indirect processes. Direct production accounts for 184 million kg annually, while indirect production accounts for ≈8.8 million kg annually.

Direct production of chloroform and processes associated with its use (i.e., Fluorocarbon-22 production, Hypalon® manufacture, loading and transit losses, grain fumigation, pharmaceutical use) emit some 1.6 million kg to the environment. Virtually all of the indirectly produced chloroform is emitted into the environment; the total amount of chloroform emitted is ≈10.4 million kg. This represents ≈5.6% of direct production. The relative source contributions from all quantifiable sources are listed in Table 3-8.

3.5. AMBIENT AIR CONCENTRATIONS

Monitoring data for a number of U.S. and world locations are presented in Table 3-9. For the most part, ambient concentrations remain <1000 ppt (1 ppt =

TABLE 3-7

Chloroform Discharges from Use[a]

Source	Environmental Release (metric tons/year) Air	Water	Land	Total
Pharmaceuticals	570	46	384	1000
Chlorodifluoromethane Manufacture	139	--[b]	2	141
Loading and Transit Losses	90.1	0	0	90.1
Hypalon® Manufacture	54.9	--[b]	--[b]	54.9
Grain Fumigation	28.4	0	0	28.4
Secondary Product Contamination	9.8	0.6	0.2	10.6
Total	892.2	46.6	386.2	1325.0

[a]Source: Rem et al., 1982

[b]minor releases possible

TABLE 3-8

Relative Source Contribution for Chloroform[a]

Source	Environmental Release (metric tons/year) Air	% of Total[c]	Water	% of Total[c]	Land	% of Total[c]	Total	% of Total[c]
Pulp and Paper Mills	4113	39	298	2.9	0	0	4411	42
Drinking Water Chlorination	0	0	1900	18	0	0	1900	18
Pharmaceuticals	570	5.5	46	0.4	384	3.7	1000	10
Ethylene Dichloride Manufacuture	760	7.3	---[b]	----	217	2.1	977	9.4
Trichloroethylene Photodegradation	780	7.5	0	0	0	0	780	7.5
Municipal Wastewater Chlorination	0	0	320	3.1	0	0	320	3.1
Cooling Water Chlorination	190	1.8	72	0.7	0	0	262	2.5
Methyl Chloride Chlorination	196	1.9	8	0.1	5.4	0.1	209.4	2.0
Automobile exhaust	180	1.7	0	0	0	0	180	1.7
Chlorodifluoromethane Manufacture	139	1.3	----	----	2	0.02	141	1.4
Loading and Transit Losses	90.1	0.9	0	0	0	0	90.1	0.9
Methane Chlorination	70.2	0.7	3.3	0.03	----	----	73.5	0.7
Hypalon® Manufacture	54.9	0.5	----	----	----	----	54.9	0.5
Grain Fumigation	28.4	0.3	0	0	0	0	28.4	0.3
Secondary Product Contamination	9.8	0.1	0.6	0.006	0.2	0.002	10.6	0.1
Laboratory Usage[d]	----	----	----	----	----	----	----	----
TOTAL	7181.4	68.8	2647.9	25.4	608.6	5.8	10,438[c]	

[a]Source: Rem et al. (1982)

[b]dashed lines indicate minor releases possible

[c]values are rounded

[d]not included because of uncertainty

TABLE 3-9

Ambient Levels of Chloroform

Location	Type of Site	Date	Analytical Method	Concentration (ppt, v/v) Max.	Min.	Average	Reference
Alabama							
Tuscaloosa	urban	2/77	GC-ECD	3000	100	800	Holzer et al., 1977
Talladega Forest	rural	2/77	GC-ECD	200	NR	100	Holzer et al., 1977
Arizona							
Phoenix	urban	4-5/79	GC-coulometry	514.0	27.1	111.4	Singh et al., 1981
California							
Stanford Hills	clean[a]	11/75	GC-ECD	217	12	33	Singh et al., 1979
Point Reyes	clean marine	12/75	GC-ECD	114	15	37	Singh et al., 1979
Los Angeles	urban	4-5/76	GC-ECD	724	23	102	Singh et al., 1979
Palm Springs	urban-suburban	5/76	GC-ECD	616	20	99	Singh et al., 1979
Yosemite	remote-high altitude	5/76	GC-ECD	24	12	17	Singh et al., 1979
Mill Valley	clean marine[a]	1/77	GC-ECD	36	4	25	Singh et al., 1979
Riverside	urban-suburban	4-5/77	GC-ECD	310	24	25	Singh et al., 1979
Badger Pass	remote-high altitude	5/77	GC-ECD	38	2	16	Singh et al., 1979
Point Arena	clean marine	5/77	GC-ECD	42	8	20	Singh et al., 1979
Point Arena	clean marine	8-9/78	GC-ECD	48	12	18	Singh et al., 1979
Los Angeles	urban	4/79	GC-ECD	223.5	24.3	88.2	Singh et al., 1981
Oakland	urban	6-7/79	GC-ECD	60.1	13.1	32.1	Singh et al., 1981
Delaware							
Delaware City	NS	7/74	GC-ECD	<10	<10	NR	Lillian et al., 1975a
Kansas							
Jetmore	remote-continental	6/78	GC-ECD	34	9	16	Singh et al., 1979
Maryland							
Baltimore	urban	7/74	GC-ECD	<10	<10	NR	Lillian et al., 1975a
Montana							
Western Montana 1979	remote	3/76	GC-MS	NR	NR	9	Cronn and Harsch, 1979
Nebraska							
Reese River	remote-high altitude	5/77	GC-ECD	19	6	13	Singh et al., 1979
New Jersey							
Rutherford	urban	1978	GC-MS	31,000	NR	4600	Bozzelli and Kebbekus, 1979
Newark	urban	1978	GC-MS	7500	NR	3900	Bozzelli and Kebbekus, 1979

TABLE 3-9 (cont.)

Location	Type of Site	Date	Analytical Method	Concentration (ppt, v/v) Max.	Min.	Average	Reference
Piscataway	urban	1978	GC-MS	2900	NR	2200	Bozzelli and Kebbekus, 1979
Somerset (county)	urban	1978	GC-MS	11,000	NR	5000	Bozzelli and Kebbekus, 1979
Bridgewater Township	rural	1978	GC-MS	NR	NR	ND[b]	Bozzelli and Kebbekus, 1979
Bound Brook	urban	3/76	GC-MS	NR	NR	854	Pellizzari, 1977
Patterson	urban	3/76	GC-MS	NR	NR	768	Pellizzari, 1977
Clifton	urban	3/76	GC-MS	NR	NR	1700	Pellizzari, 1977
Fords	urban	3/76	GC-MS	NR	NR	3422	Pellizzari, 1977
Newark	urban	3/76	GC-MS	NR	NR	7582	Pellizzari, 1977
Passaic	urban	3/76	GC-MS	NR	NR	854	Pellizzari, 1977
Hoboken	urban	3/76	GC-MS	NR	NR	427	Pellizzari, 1977
Seagrit	urban	6/74	GC-ECD	60	<10	40	Lillian et al., 1975a
Seagrit	urban	6/75	GC-ECD	50	5	35	Lillian et al., 1975b
Sandy Hook	urban	7/74	GC-ECD	63	<10	30	Lillian et al., 1975a
Sandy Hook	urban	7/75	GC-ECD	55	10	25	Lillian et al., 1975b
Bayonne	urban	7/75	GC-ECD	15,000	<10	1030	Lillian et al., 1975a
New York							
Staten Island	urban	3/76	GC-MS	NR	NR	4268	Pellizzari, 1977
New York City	urban	6/74	GC-ECD	480	<10	160	Lillian et al., 1975a
New York City	urban	6/75	GC-ECD	450	10	200	Lillian et al., 1975b
White Face Mountain	remote	9/74	GC-ECD	250	<10	9	Lillian et al., 1975a
White Face Mountain	remote	9/75	GC-ECD	350	6	8	Lillian et al., 1975b
Niagara Falls	urban	NS	GC-MS	21,611	215	NR	Pellizzari et al., 1979
Ohio							
Wilmington	Air Force Base	7/74	GC-ECD	4800	<10	340	Lillian et al., 1975a
Wilmington	Air Force Base	7/75	GC-ECD	5000	20	480	Lillian et al., 1975a
Texas							
Houston	urban	6-7/77	GC-MS	11,034	Trace	NR	Pellizzari et al., 1979

TABLE 3-9 (cont.)

Location	Type of Site	Date	Analytical Method	Concentration (ppt, v/v) Max.	Min.	Average	Reference
Washington							
Pullman	rural	12/74 to 2/75	GC-MS	NR	NR	20	Grimsrud and Rasmussen, 1975
Pullman	rural	11/75	GC-ECD	NR	NR	43	Rasmussen et al., 1977
England							
Liverpool/Manchester	suburban	NS	GC-ECD	8[c]	3[c]	NR	Pearson and McConnell, 1975
Organochlorine manufacturer	urban	NS	GC-ECD	40[c]	<10.1[c]	NR	Pearson and McConnell, 1975
Moel Faman, Flintshire	urban	NS	GC-ECD	0.4[c]	<0.1[c]	NR	Pearson and McConnell, 1975
Rannoch Moor, Argyllshire	urban	NS	GC	0.5[c]	0.1[c]	NR	Murray and Riley, 1973
Rural areas	rural	NS	GC	1.2	0.82	0.82	Murray and Riley, 1973
Ireland							
Cork	urban	1974	GC-ECD	NR	NR	26.5	Cox et al., 1976
Japan							
Kobe	NS	NS	GC-ECD	9400	300		Okuno et al., 1974
Atlantic Ocean							
Northeast Atlantic (Cape Blanc to Lands End) 34°19'N 13°32'W to 49°54'N 05°54'W		7-8/72	GC	0.96	0.14	0.35	Murray and Riley, 1973

[a]Subject Urban Transport

[b]Detection Limits 10 ppt

[c]ppb by mass

NR = Not reported; NS = Not specified

ND = Not detected

10^{-12}, v/v), and some <10 ppt. There are notable exceptions, however, although the reasons for this are not readily apparent.

Singh (1977) and Singh et al. (1979) have determined northern and southern hemisphere background concentrations as well as a global average. The hemispheric values are 14 ppt for the northern hemisphere and <3 ppt for the southern hemisphere. This difference in hemispheric values suggests that the oceans are not a significant source of chloroform, but rather, that chloroform, for the most part, is anthropogenic. The global average concentration determined by Singh et al. (1979) is 8 ppt.

An interesting point not presented in Table 3-9 is that chloroform concentrations above an inversion layer are significantly lower than concentrations below it. In Wilmington, OH, above an inversion layer, the chloroform concentration was <10 ppt, whereas below it the concentration was 120 ppt (Lillian et al., 1975a).

3.6. ATMOSPHERIC REACTIVITY

The principal atmospheric reactant responsible for the removal of chloroform is probably the hydroxyl radical (Atkinson et al., 1979; Graedel, 1978; Altshuller, 1980; Singh, 1977; Crutzen and Fishman, 1977). Hydroxyl radicals are formed in the lower atmosphere in two ways, first, by the photodissociation of ozone (λ <310) into O (^{1}D) atoms (Atkinson et al., 1979). These go on to react with either water, hydrogen or methane to form hydroxyl radicals. The second important source of hydroxyl radicals is the reaction of hydroperoxyl radicals with nitric oxide.

Hydroxyl radical reactions probably follow the course outlined below (Graedel, 1978):

$$CHCl_3 + HO \longrightarrow \cdot CCl_3 + H_2O$$

$$\cdot CCl_3 + O_2 \longrightarrow \cdot O_2CCl_3$$

$$\cdot O_2CCl_3 + NO \longrightarrow \cdot OCCl_3 + NO_2$$

$$\cdot OCCl_3 \longrightarrow COCl_2 \text{ (phosgene)} + Cl\cdot$$

$$COCl_2 + H_2O \longrightarrow CO_2 + 2\ HCl$$

Pearson and McConnell (1975) found HCl and CO_2 as the only products of chloroform irradiation with UV (λ >290nm) light. The half-life reported by these workers (23 weeks) was of the same order of magnitude as that calculated from the hydroxyl radical rate constant (11.5 weeks) (Singh et al., 1981).

Chloroform will not react photolytically in the troposphere; the UV cutoff for chloroform is 175 nm (i.e., it will not absorb light >175 nm). Callahan et al. (1979) calculated that roughly 1% of the tropospheric chloroform would diffuse eventually into the stratosphere, based on a lifetime of 0.2-0.3 years and a troposphere-to-stratosphere turnover time of 30 years.

3.7. ECOLOGICAL EFFECTS/ENVIRONMENTAL PERSISTENCE

3.7.1. Ecological Effects.

3.7.1.1. TERRESTRIAL -- Data on the terrestrial ecological effects of chloroform are not available. Significant effects are not expected because chloroform is quite volatile and does not accumulate in terrestrial (or aquatic) environments, and is diluted rapidly and degraded to low concentrations in the troposphere (NAS, 1978). Conceivably, acute effects on wildlife can occur in the vicinity of major chloroform spills, but significant chronic effects from long-term exposure to low ambient levels is unlikely.

3.7.1.2. AQUATIC -- The toxicity of chloroform to aquatic organisms has been reviewed by the U.S. EPA (1980h). As summarized in Table 3-10, two freshwater fish (rainbow trout, bluegill) and one invertebrate (Daphnia magna) species have been acutely tested under standard conditions; LC_{50} concentrations ranged from 28,900 to 115,000 µg/ℓ (Bentley et al., 1975; U.S. EPA, 1978a), and

TABLE 3-10

Acute and Chronic Effects of Chloroform on Aquatic Organisms[a]

Species	Duration	Concentration (µg/ℓ)	Method	Effect	Reference
Cladoceran *Daphnia magna*	48-hr	28,900	S,U[b]	LC_{50}	U.S. EPA, 1978a
Rainbow trout *Salmo gairdneri*	96-hr	66,800	S,U	LC_{50}	Bentley et al., 1975
Rainbow trout *Salmo gairdneri*	96-hr	43,800	S,U	LC_{50}	Bentley et al., 1975
Bluegill *Lepomis macrochirus*	96-hr	115,000	S,U	LC_{50}	Bentley et al., 1975
Bluegill *Lepomis macrochirus*	96-hr	100,000	S,U	LC_{50}	Bentley et al., 1975
Pink shrimp *Penaeus duorarum*[c]	96-hr	81,500	S,U	LC_{50}	Bentley et al., 1975
Orangespotted sunfish *Lepomis humilis*	1-hr	106,890 to 152,700	NS	death	Clayberg, 1917
Goldfish *Carassius auratus*	30 to 60 min	97,000 to 167,000	NS	50% anesthetized	Cherkin and Catchpool, 1964
Threespine stickleback *Gasterosteus aculeatus*	90-min	207,648[d]	NS	anesthesia with recovery	Jones, 1947a

TABLE 3-10 (cont.)

Species	Duration	Concentration (µg/ℓ)	Method	Effect	Reference
Ninespine stickleback *Pungitius pungitius*	NS	148,320 to 296,640	NS	Avoidance	Jones, 1947b
Rainbow trout (embryo-larval) *Salmo gairdneri*	27 days[f]	2,030	F,M[e]	LC_{50} at 50 mg/ℓ hardness	Birge et al., 1979
Rainbow trout (embryo-larval) *Salmo gairdneri*	27 days[f]	1,240	F,M[e]	LC_{50} at 200 mg/ℓ hardness	Birge et al., 1979
Rainbow trout (embryo) *Salmo gairdneri*	23 days	10,600	F,M[e]	40% teratogenesis	Birge et al., 1979

[a]Source: U.S. EPA, 1980

[b]Static test, unmeasured concentration

[c]Saltwater species

[d]Corrected from vol/vol to µg/ℓ

[e]Flow-through test, measured concentration

[f]Exposures began within 20 minutes of fertilization and ended 8 days after hatching.

hr = hour; min = minutes; NS = Not stated

the trout was more sensitive than the bluegill. With stickleback, goldfish, and orangespotted sunfish, anesthetization or death occurred after exposure to 97,000 to 207,000 µg/ℓ chloroform for 30 to 90 minutes (Clayberg, 1917; Jones, 1947a; Cherkin and Catchpool, 1964). Only one test has been conducted with chloroform and saltwater organisms; the 96-hour LC_{50} for the pink shrimp was 81,500 µg/ℓ (Bentley et al., 1975).

Embryo-larval tests with rainbow trout at 2 levels of hardness provided 27-day LC_{50} values of 2030 and 1240 µg/ℓ (Birge et al., 1979). There was a 40% incidence of teratogenesis in the embryos at hatching (23 day exposure at 10,600 µg/ℓ).

Bluegills bioconcentrated radiolabeled chloroform by a factor of 6 after a 14-day exposure, and the tissue half-life was <1 day (U.S. EPA, 1978a). This degree of bioconcentration and short biological half-life suggest that chloroform residues would not be an environmental hazard to consumers of aquatic life (U.S. EPA, 1980h).

3.7.2. Environmental Persistence. A number of researchers have reported the dominance of hydroxyl radical oxidations in the fate of chloroform in the atmosphere (see Section 3.6). Singh et al. (1981) calculated an atmospheric residence time for chloroform based on the NASA reviewed rate constant reported by Hampson (1980). They reported a 116-day (16.6-week) residence time for a hydroxyl radical concentration of 10^6 molecules/cm^3. This compares well to the observed 33-week lifetime of chloroform in a sunlit flask (Pearson and McConnell, 1975). This lifetime was based on experiments conducted in northwest England, which receives less intense sunlight than most of the U.S., and may account for its longevity.

According to recent hydroxyl radical measurements, tropospheric ambient air concentrations range from $\approx 10^6$ to 10^7 molecules/mℓ (Atkinson et al., 1979);

models of the troposphere have suggested a concentration ranging between 2 to 6 x 10^5 molecules/mℓ (Crutzen and Fishman, 1977; Singh, 1977).

Table 3-11 summarizes the literature values for k_{OH}, the temperature of measurement, and the calculated lifetime based on the indicated hydroxyl radical concentration. If a hydroxyl radical concentration of 2 x 10^6 molecules/cm^3 (typical for summer months; winter concentrations are lower) (Singh et al., 1981) is assumed, most of the lifetimes calculated from the rate constants range between 0.2 to 0.5 years (69 to 181 days, 122 average).

Mabey and Mill (1978) critically reviewed hydrolysis data available in the literature. They determined that chloroform had a hydrolysis half-life of >3,000 years at pH 7 and 298 K. This is based on a base hydrolysis rate of 0.602 x 10^{-4} and a OH^- concentration of 10^{-7} in neutral water.

Dilling et al. (1975) and Dilling (1977) determined the volatilization half-life of chloroform from water. For a 1 ppm chloroform solution stirred at 200 rpm, the time for 50% removal was 21.5 minutes (average); 90% removal was accomplished in 71 minutes. The addition of dry granular bentonite clay, dolomitic limestone, or peatmoss had little effect on the evaporation rate. The rapid volatilization of chloroform was seen also by Jensen and Rosenberg (1975), who reported that 0.1-1.0 ppm solutions of chloroform in partly open sunlit aquaria lost 50-60% of the chloroform in 8 days as opposed to only 5% in closed aquaria. Pearson and McConnell (1975) suggested that the presence of chloroform in ambient waters may be from aerial transport and washout.

An EXAMS model of the fate of chloroform in a pond, a river, and an oligotrophic lake and eutrophic lake revealed the dominant process in all cases to be volatilization. Input parameters included hydrolysis (2.5 x 10^{-9} hr^{-1}), octanol/water partition coefficient (91), vapor pressure (150.5 torr at 20°C), solubility (8200 ppm), Henry's Law Constant (2.88 x 10^{-3}), reaeration rate ratio

TABLE 3-11

Values for k_{OH}

k_{OH} x 10^{14} $cm^3 molecule^{-1} sec^{-1}$	K	[OH] x 10^{-5}	Lifetime (years)	Reference
6.51	265	4	1.2	Singh et al., 1979
10.1	296			Howard and Evenson, 1976
16.8	298	10	0.19	Cox et al., 1976
11.4	298			Davis et al., 1976[a]
6.4	265	9	0.56	
7.4	273			Hampson, 1980[b]
10	298			

[a] $(4.69 \pm 0.71) \times 10^{-12} \exp - (2254 \pm 214/RT)$

[b] Evaluated by NASA

(0.583), alkoxy radical rate constant (0.7 M^{-1} hr^{-1}) (RO• = 10^{14} M), and a stream loading of 1 g/hr. No photochemical or bacterial degradation parameters were entered since chloroform has no UV absorbence >290 nm, and virtually no bacterial degradation occurs with chloroform (Pearson and McConnell, 1975; Bouever et al., 1981). Table 3-12 summarizes the EXAMS model generated fate of chloroform.

3.8. EXISTING CRITERIA, STANDARDS, AND GUIDELINES

3.8.1. Air.

The Occupational Safety and Health Administration (OSHA) currently limits occupational exposure to chloroform to a ceiling level of 50 ppm (40 CFR 1910.1000). This ceiling level is not to be exceeded in the workplace at any time. To protect against mild central nervous system depression, irritant effects, and fetal abnormalities (which were considered to occur at lower exposure levels than those causing liver injury), the National Institute for Occupational Safety and Health (NIOSH) recommended in 1974 that exposure to chloroform be limited to 10 ppm as a Time-Weighted Average (TWA) exposure for up to a 10-hour workday, 40 hour workweek. A ceiling level of 50 ppm was proposed for any 10-minute period (NIOSH, 1974). NIOSH lowered the recommended criterion to 2 ppm TWA in 1976 (NIOSH, 1977) in response to a positive NCI carcinogenesis bioassay (NCI, 1976). NIOSH recommended that exposure to halogenated anesthetic agents, including chloroform, be limited to 2 ppm because this is the lowest detectable level using the recommended sampling and analysis techniques, and not because a safe level of airborne exposure could be defined.

On the basis of recent reports of carcinogenicity and embryotoxicity, the American Conference of Governmental Industrial Hygienists (ACGIH) currently classifies chloroform as an Industrial Substance Suspect of Carcinogenic Potential for Man (ACGIH, 1981). The ACGIH recommends a Threshold Limit Value (TLV) of 10 ppm and a 15-minute Short-Term Exposure Limit (STEL) TWA of 50 ppm for chloroform.

TABLE 3-12

Summary of EXAMS Models of the Fate of Chloroform[a]

	River	Pond	Oligotrophic Lake	Eutrophic Lake
Maximum total concentrations in water column (mg/ℓ)	9.92×10^{-7}	2.50×10^{-3}	1.33×10^{-4}	1.26×10^{-4}
Maximum concentration in sediments (dissolved in pore water, mg/ℓ)	9.85×10^{-7}	1.36×10^{-3}	5.82×10^{-6}	4.57×10^{-6}
Maximum concentration in Bios				
Plankton (μg/g)	2.58×10^{-5}	6.49×10^{-2}	3.45×10^{-3}	3.27×10^{-3}
Benthos (μg/g)	2.56×10^{-5}	3.53×10^{-2}	1.51×10^{-4}	1.19×10^{-4}
Maximum total concentration in sediment (mg/kg, dry weight)	3.19×10^{-6}	6.50×10^{-3}	2.83×10^{-5}	9.35×10^{-6}
Total steady accumulation (kg)	9.15×10^{-4}	5.43×10^{-2}	0.33	0.3
% in water column	96.93	91.9	99.95	99.94
% in sediments	3.07	8.1	0.05	0.06
Disposition				
chemical transformation (%)	0.00	0.00	0.00	0.00
biotransformation (%)	0.00	0.00	0.00	0.00
volatilization (%)	1.74	93.35	94.98	95.57
Volatilization half-life	36 hours	40 hours	10 days	9 days
exported (%)	98.26	6.65	5.02	4.43
export half-life	0.65 hours	566 hours	192 days	196 days
Mass Flux from Volatilization (kg/hr)	1.74×10^{-5}	9.33×10^{-4}	2.28×10^{-3}	2.29×10^{-2}
Self-Purification Time	37 hours	31 days	65 days	56 days

[a]Based on load of 1.00 g/hr

Foreign industrial air standards for chloroform include (Utidjian, 1976): Bulgaria, 10 ppm; Czechoslovakia, 10 ppm (50 ppm for brief exposures); Finland, 50 ppm; Hungary, 4 ppm (20 ppm for brief exposures); Japan, 50 ppm; Poland, 10 ppm; Rumania, 10 ppm; Yugoslavia, 50 ppm; West Germany, 10 ppm (Utidijian, 1976).

3.8.2. Water. As discussed in the Ambient Water Quality Criteria Document for chloroform (U.S. EPA, 1980h), the EPA has proposed an amendment that would add to the National Interim Primary Drinking Water Regulations a section on the control of organic halogenated chemical contaminants. The proposed limit for total trihalomethanes in drinking water, which includes chloroform as the major constituent, is 100 μg/ℓ. Although some estimates of cancer risk were performed, this limit was set primarily on the basis of technological and economic feasibility, and initially will apply only to water supplies serving >75,000 consumers. The basis and purpose of this regulation are discussed in a report that was prepared by the Office of Drinking Water (U.S. EPA, 1978b).

The U.S. EPA (1980h) recently derived cancer-based ambient water criteria for chloroform. Since zero level concentrations of chloroform will never be attainable in chlorine-treated water, levels that may result in incremental increases of cancer risk over the lifetime were estimated at risks of 1×10^{-5}, 10^{-6}, and 10^{-7}. The corresponding recommended criteria, which were derived with the tumor incidence data from the NCI bioassay with female mice (NCI, 1976), are 1.90, 0.19, and 0.019 μg/ℓ, respectively, if exposure is assumed to be from the consumption of drinking water and fish and shellfish products and at 157 μg/ℓ, 15.7 μg/ℓ, and 1.57 μg/ℓ, respectively, if exposure is assumed to be from the consumption of aquatic organisms only.

3.8.3. Food. Chloroform has been approved by the Food and Drug Administration (FDA) as a component of articles intended for use in contact with food (i.e., an

indirect food additive). The use of chloroform in the food industry is summarized as follows:

Component of adhesives	21 CFR 175.105
Adjuvant substance required in the production of polycarbonate resins	21 CFR 177.1580

Chloroform also has been exempted from the requirement of tolerance when used as a solvent in pesticide formulations that are applied to growing crops (40 CFR 180.1001), or when used as a fumigant after harvest for barley, corn, oats, popcorn, rice, rye, sorghum (milo), or wheat (40 CFR 180.1009).

3.8.4. Drugs and Cosmetics. The positive NCI carcinogenicity bioassay of chloroform (NCI, 1976) has prompted the FDA to restrict the use of chloroform in drug (21 CFR 310-513) and cosmetic (21 CFR 700.18) products.

3.9. RELATIVE SOURCE CONTRIBUTIONS

The sum of all the environmental releases of chloroform from all sources listed in Section 3.4.3 amounts to a total of 10,438 metric tons. All sources are summarized in Table 3-8 with the percent of the total emissions. Total emissions from all sources constitute about 5.6% of production (184,000 metric tons). Table 3-8 does not include estimated emissions from laboratory use. Rem et al. (1982) suggested that these are potentially large but gave no numerical estimate.

REFERENCES

ACGIH (American Conference of Governmental Industrial Hygienists). 1980. Documentation of the Threshold Limit Values. 4th ed. Cincinnati, OH. pp. 90-91.

ACGIH (American Conference of Governmental Industrial Hygienists). 1981. Threshold Limit Values for Chemical Substances and Physical Agents in the Workroom Environment with Intended Changes for 1981. Cincinnati, OH. pp. 13, 42.

Ahlstrom, R.C., Jr. and J.M. Steele. 1979. Methylchloride. In: Kirk-Othmer Encyclopedia of Chemical Technology, 3rd ed., M. Grayson and D. Eckroth, eds. New York: John Wiley and Sons, Inc. vol. 5. pp. 677-685.

Altshuller. 1980. Lifetimes of organic molecules in the troposphere and lower stratosphere. In: Advances in Environmental Science and Technology. vol. 10. Pitts and Metcalf, eds. New York: John Wiley and Sons, Inc. p. 181-219.

Anonymous. 1983. Chemical Profile. Chloroform. Chemical Marketing Reporter, June 25, 1979, p. 54.

Anthony, T. 1979. Methylene chloride. In: Kirk-Othmer Encyclopedia of Chemical Technology, 3rd ed., M. Grayson and D. Eckroth, eds., New York: John Wiley and Sons, Inc. vol. 5. p. 686-693.

Atkinson, R., K.R. Darmall, A.C. Lloyd, A.M. Winer and J.N. Pitts, Jr. 1979. Kinetics and mechanisms of the reactions of the hydroxyl radical with organic compounds in the gas phase. In: Ado. Photochem. 11: 375-488.

Beale, J. (n.d.). Letter from J. Beale, Dow Chemical U.S.A., Midland, Michigan, to L. Evans, Emissions Standards and Engineering Division, U.S. EPA concerning Dow facility at Freeport, TX. (Cited in Rem et al., 1982).

Bentley, R.E., et al. 1975. Acute toxicity of chloroform to bluegill (Lepomis macrochirus), rainbow trout, (Salmo gairdneri), and pink shrimp (Penaeus durorarum). Contract No. WA-6-99-1414-B. U.S. Environmental Protection Agency (Cited in U.S. EPA, 1980h.)

Birge, W.J. et al. 1979. Toxicity of organic chemicals to embryo-larval stages of fish. EPA-560-79-007. U.S. Environmental Protection Agency. (Cited in U.S. EPA, 1980h.)

Bouever, E.J., B. Rittman and P.L. McCarty. 1981. Anaerobic degradation of halogenated 1- and 2-carbon organic compounds. Environ. Sci. Technol. 15: 596-599.

Bozzelli, J.W. and B.B. Kebbekus. 1979. Analysis of selected volatile organic substances in ambient air. State of New Jersey Department of Environmental Protection.

Brown, R.H. and C.J. Purnell. 1979. Collection and analysis of trace organic vapour pollutants in ambient atmospheres. The performance of a Tenax-GC adsorbent tube. J. Chromatog. 178: 79-90.

Callahan, M.A., M.W. Slimak, N.W. Gabel, I.P. May, C.F. Fowler, J.R. Freed, P. Jennings, R.L. Durfee, F.C. Whitmore, B. Maestri, W.R. Mabey, B.R. Holt and C. Gould. 1979. Water related fate of 129 priority pollutants, vol. II. U.S. EPA - 440/4-79-029B.

Chemical Marketing Reporter. 1981. Chemical Profile: Trichloroethylene. April 6, 1981.

Cherkin, A. and J.F. Catchpool. 1964. Temperature dependence of anesthesia in goldfish. Science. 144: 1460. (Cited in U.S. EPA, 1980h.)

Clayberg, H.D. 1917. Effects of ether and chloroform on certain fishes. Biol. Bull. 32: 234. (Cited in U.S. EPA, 1980h.)

Cox, R.A., R.G. Derwent, A.E.J. Eggleton and J.E. Lovelock. 1976. Photochemical oxidation of halocarbons in the troposphere. Atmosph. Environ. 10: 305-308.

Cronn, D.R. and D.E. Harsch. 1979. Determination of atmospheric halocarbon concentrations by gas chromatography-mass spectrometry. Anal. Letters. 12: 1489-1496.

Crutzen, P.J. and J. Fishman. 1977. Average concentrations of OH in the troposphere and budgets of CH_4, CO, H_2, and CH_3CCl_3. Geophys. Res. Lett. 4: 321-324.

Davis, D.D., G. Machado, B. Conaway, Y. Oh and B. Watson. 1976. A temperature dependent kinetics study of the reaction of OH with CH_3Cl, $CHCl_3$, and CH_3Br. Jour. Chem. Phys. 65: 1268-1274.

DeShon, H.D. 1979. Chloroform. In: Kirk-Othmer Encyclopedia of Chemical Technology, 3rd ed., M. Grayson and D. Eckroth, eds. New York: John Wiley and Sons, Inc. vol 5. pp. 693-703.

Dilling, W.L. 1977. Interphase transfer processes. II. Evaporation rates of chloromethanes, ethanes, ethylenes, propanes, and propylenes from dilute aqueous solutions. Comparisons with theoretical predictions. Environ. Sci. Technol. 11: 405-409.

Dilling, W.L., N.B. Tefertiller and G.J. Kallos. 1975. Evaporation rates and reactivities of methylene chloride, chloroform, 1,1,1-trichloroethane, trichloroethylene, tetrachlorocthylene, and other chlorinated compounds in dilute aqueous solutions. Environ. Sci. Technol. 9: 833-838.

Environmental Reporter. 1982. State Air Laws. Bureau of National Affairs. Washington, DC. (Cited in Rem et al., 1982).

Graedel, T.E. 1978. Chemical compounds in the atmosphere. Academic Press, NY. p. 331, 334.

Grimsrud, E.P. and R.A. Rasmussen. 1975. Survey and analysis of halocarbons in the atmosphere by gas chromatography-mass spectometry. Atmos. Environ. 9:1014-1017.

Hampson, R.F. 1980. Chemical, kinetic, and photochemical data sheets for atmospheric reactions. Washington, D.C. Department of Transportation Report FAA-EE-80-17.

Harsch, D.E., R.A. Rasmussen, and D. Pierotti. 1977. Identification of a potential source of chloroform in urban air. Chemosphere. 11: 769-775.

Harsch, D.E., D.R. Cronn and W.R. Slater. 1979. Expanded list of halogenated hydrocarbons measureable in ambient air. Jour. Air Pollut. Control Assoc. 29: 975-976.

Harsch, C. and A.J. Leo. 1979. Substituent constants for correlation analysis in chemistry and biology. New York: John Wiley and Sons. p. 172-173.

Hobbs, F.D. 1978. Trip Report for Vulcan Materials Company, Geismar, Louisiana. Prepared for U.S. Environmental Protection Agency. Emissions Standards and Engineering Division, Research Triangle Park, NC. (Cited in Rem et al., 1982).

Holzer, G., H. Shanfield, A. Zlatkis, W. Bertsch, P. Juarez, M. Mayfield and H.M. Liebich. 1977. Collection and analysis of trace organic emissions from natural sources. Jour. Chromatog. 142: 755-764.

Howard, C.J. and K.M. Evenson. 1976. Rate constants for the reactions of hydrozyl with methane and fluorine, chlorine, and bromine substituted methanes at 296° Kelvin. Jour. Chem. Phys. 64: 197-202.

Jensen, S. and R. Rosenberg. 1975. Degradibility of some chlorinated aliphatic hydrocarbons in sea water and sterilized water. water res. 9: 659-661.

Jones, J.R.E. 1947a. The oxygen consumption of Gasterosteus aculeatus L. to toxic solutions. Exp. Biol. 23: 298. (Cited in U.S. EPA, 1980h.)

Jones, J.R.E. 1947b. The reactions of Pygosteus gungitius L. to toxic solutions. Jour. Exp. Biol. 24: 110. (Cited in U.S. EPA, 1980h.)

Lant, S.G. 1979. Diamond Shamrock. Personal communications to D. Goodwin, Office of Air Quality Planning and Standards. April 3, 1978. (Cited in Wagner et al., 1980).

Lillian, D., J.B. Singh, A. Appleby, L. Lobban, R. Aruts, R. Gompert, R. Hague, J. Tommey, J. Kazazis, M. Antell, D. Hansen and B. Scott. 1975a. Atmospheric fates of halogenated hydrocarbons. Environ. Sci. Technol. 9: 1042-1048.

Lillian, D., H.B. Singh, A. Appleby, L. Lobban, R. Arnts, R. Gompert, R. Hague, J. Toomey, J. Kazazis, M. Antell, D. Hansen and B. Scott. 1975b. Fates and levels of ambient halocarbons. Amer. Chem. Soc. Symp. Series. 17: 152-158.

Lowenbach and Schlesinger Associates. 1979. Chloroform: A Preliminary Materials Balance. Prepared for the Office of Toxic Substances, U.S. Environmental Protection Agency. Washington, DC. (Cited in Rem et al., 1982).

Mabey, W. and T. Mill. 1978. Critical review of hydrolysis of organic compounds in water under environmental conditions. Jour. Phys. Chem. Ref. Data. 7: 383-415.

Merck Index: An Encyclopedia of Chemicals and Drugs. 1976. M. Windholz, ed. 9th ed. Rahway, New Jersey. Merck and Co., Inc. p. 272.

Metcalf and Eddy, Inc. 1972. Waste Water Engineering, Collection Treatment Disposal. McGraw-Hill Book Co, NY. (Cited in Wagner et al., 1980).

Murray, A.J. and J.P. Riley. 1973. Occurrence of some chlorinated aliphatic hydrocarbons in the environment. Nature. 242: 37-38.

NAS (National Academy of Sciences). 1978. Chloroform, carbon tetrachloride, and other halomethanes: An environmental assessment. National Research Council. Washington, D.C. 294 pp.

NCASI (National Council for Air and Stream Improvement). 1977. Analysis of Volatile Halogenated Organic Compounds in Bleached Pulp Mill Effluent. NCASI Technical Bulletin No. 298. New York, NY. (Cited in Rem et al., 1982).

NCI (National Cancer Institute). 1976. Report on Carcinogenesis Bioassay of Chloroform. Available from National Technical Information Service, Springfield, Virginia. (NTIS PB-264-018).

NIOSH (National Institute for Occupational Safety and Health). 1974. Criteria for a recommended standard - occupation exposure to chloroform. Publ. No. (NIOSH) 75: 114. Dept. Health, Education, and Welfare. Washington, D.C.

NIOSH (National Institute for Occupational Safety and Health). 1977. Criteria for a recommended stnadard - occupational exposure to waste anesthetic gases and vapors. Publ. No. (NIOSH) 77-140. Department of Health, Education, and Welfare, Washington, D.C.

Okuno, T., M. Tsuji and K. Shintani. 1974. On the chlorination of hydrocarbons in air. Jour. Japan. Soc. Air Pollution. 9: 211.

Pearson, C.R. and G. McConnell. 1975. Chlorinate C_1 and C_2 hydrocarbons in the marine environment. Proc. R. Soc. Land B. 189: 305-332.

Pellizzari, E.D. 1977. The measurement of carcinogenic vapors in ambient atmosphere. U.S. Environmental Protection Agency. EPA-600/7-77-055.

Pellizzari, E.D., M.D. Erickson and R.A. Zweidinger. 1979. Analytical protocols for making a preliminary assessment of halogenated organic compounds in man and environmental media. U.S. Environmental Protection Agency. EPA-560/13-79-010. Available from NTIS PB80-109168.

Rasmussen, R.A., D.E. Harsch, P.H. Sweany, J.P. Krasnec and D.R. Crown. 1977. Determination of atmospheric halocarbons by a temperature-programmed gas chromatographic freezeout concentration method. Jour. Air Pollut. Contr. Distr. 27: 579-581.

Rem, R.M., M.E. Anderson, S.A. Duletsky, D.C. Misenheimer and H.F. Rollius. (1982). Chloroform Materials Balance, Draft Report. EPA contract 68-02-3168, Task 69.

Sax, N.I. (ed.). 1981. Dangerous Properties of Industrial Materials Report. Van Nostrand Reinhold Co. New York, NY. March/April. p.46. (Cited in Rem et al., 1982).

Singh, H.B. 1977. Atmospheric halocarbons: Evidence in favor of reduced average hydroxyl radical concentration in the troposphere. Geophys. Res. Lett. 4: 101-104.

Singh, H.B., L.J. Salas, H. Shigeishi, A.J. Smith, E. Scribner and L.H. Cavanagh. 1979. Atmospheric distributions, sources and sinks of selected halocarbons, hydrocarbons, SF_6, and N_2O. U.S. Environmental Protection Agency. EPA-600/3-79-107.

Singh, H.B., L.J. Salas, A. Smith and H. Shigeishi. 1980. Atmospheric measurements of selected toxic organic chemicals-interim report- 1979. U.S. Environmental Protection Agency, EPA-600/3-80-072. Avail. NTIS PB80-198989.

Singh, H.B., L.J. Salas, A.J. Smith and H. Shigeishi. 1981. Measurements of some potentially hazardous organic chemicals in urban environments. Atmos. Environ. 15: 601-612.

SRI International. 1983. 1983 Directory of chemical producers: United States of America. Menlo Park, California. SRI International. p. 502, 503, 584.

Stevens, A.A., C.J. Slocum, D.R. Seeger and G.G. Robeck. 1976. Chlorination of organics drinking water. Jour. Amer. Water Works Assoc. 68: 615-620.

Symons, J.M., T.A. Bellar, J.K. Carswell, J. DeMarco, K.L. Kropp, G.G. Robeck, D.R. Seeger, C.J. Slocum, B.L. Smith and A.A. Stevens. 1975. National Organics Reconnaissance Survey for Halogenated Organics. J. Am. Water Works Assoc. p. 634-647.

TAPPI (Technical Association of the Pulp and Paper Industry). 1963. The Bleaching of Pulp. TAPPI Monograph No. 27. New York, NY. (Cited in Rem et al., 1982).

U.S. EPA. n.d. National Organic Monitoring Survey. Technical Support Division, Office of Water Supply. Washington, DC. (Cited in Rem et al., 1982).

U.S. EPA. 1976. Atmospheric Freons and Halogenated Compounds. EPA-600/3-78-108. Environmental Sciences Research Laboratory, Research Triangle Park, NC. (Cited in Rem et al., 1982).

U.S. EPA. 1978a. In-depth studies on health and environmental impacts of selected water pollutants. U.S. Environmental Protection Agency. Contract No. 68-01-4646. (Cited in U.S. EPA, 1980h.)

U.S. EPA. 1978b. Statement of basis and purpose for an amendment to the national-interim primary drinking water regulations on trihalomethanes. Office of Water Supply, Washinton, D.C. (Cited in U.S. EPA, 1980h.)

U.S. EPA. 1979a. Fate of Priority Pollutants in Publicly Owned Treatment Works. EPA-400/1-70-301. Office of Water Regulations and Standards, Washington, DC. (Cited in Rem et al., 1982).

U.S. EPA. 1979b. Compilation of Air Pollution Emissions Factors, Third Edition -Supplement 12. AP-42. Transportation and Marketing of Petroleum Liquids. Office of Air Quality Planning and Standards, Research Triangle Park, NC. pp. 4.4-1 to 4.4-13. (Cited in Rem et al., 1982).

U.S. EPA. 1980a. Organic Chemical Manufacturing Volume 8: Selected Processes. Report 5: Chloromethanes by Methane Chlorination Process. EPA-450/3-80-028c. Office of Air Quality Planning and Standards. Research Triangle Park, NCℓ (Cited in Rem et al., 1982).

U.S. EPA. 1980b. Organic Manufacturing Volume 8: Selected Processes. Report 6: Chloromethanes by Methanol Hydrochlorination and Methyl Chloride Chlorination Process. EPA-450/3-80-028c. Office of Air Quality Planning and Standards. Research Triangle Park, NC. (Cited in Rem et al., 1982).

U.S. EPA. 1980c. Synthetic Organic Chemical Manufacturing Industry - Background Information for Proposed Standards, VOC Fugitive Emissions. EPA-450/3-80-33a. Office of Air Quality Planning and Standards. Research Triangle Park, NC. (Cited in Rem et al., 1982).

U.S. EPA. 1980d. Organic Chemical Manufacturing Volume 8: Selected Processes. Report 1: Ethylene Dichloride. EPA-450/3-80-028c. Office of Air Quality Planning and Standards. Research Triangle Park, NC. pp. III-1 to III-9. (Cited in Rem et al., 1982).

U.S. EPA. 1980e. Development Document for Effluent Limitations Guidelines and Standards for the Steam Electric Point Source Category. EPA-440/1-80-029b. Office of Water Regulations and Standards. Washington, DC. (Cited in Rem et al., 1982).

U.S. EPA. 1980f. Development Document for Effluent Limitations Guidelines and Standards for the Pulp, Paper and Paperboard and Builders' Paper and Board Mills. EPA-440/1-80-025b. Office of Water Regulations and Standards. Washington, DC. (Cited in Rem et al., 1982).

U.S. EPA. 1980g. Organic Chemical Manufacturing Volume 8: Selected Processes. Report 3: Fluorocarbon (Abbreviated Report). EPA-450/3-80-028c. Office of Air Quality Planning and Standards, Research Triangle Park, NC. p. III-1 to III-6. (Cited in Rem et al., 1982).

U.S. EPA. 1980h. Ambient Water Quality Criteria for Chloroform. Available from: National Technical Information Service, Springfield, Virginia. (NTIS PB81-117442).

U.S. EPA. 1981a. The determination of halogenated chemical indicators of industrial contamination in water by the purge and trap method. Method 502.1. U.S. Environmental Protection Agency, EPA-600/4-81-059.

U.S. EPA. 1981b. Air Pollution Emission Factors, Third Edition Supplement 12. AP-42. Storage of Organic Liquids. Office of Air Quality Planning and Standards. Research Triangle Park, NC. (Cited in Rem et al., 1982).

U.S. EPA. 1981c. The 1980 Needs Survey. EPA-430/9-81-008. Office of Water, Washington, DC. (Cited in Rem et al., 1982).

U.S. EPA. 1982a. Projections of Total Lead Usage Under Alternative Lead Phasedown Programs. Washington, DC. (Cited in Rem et al., 1982).

U.S. EPA. 1982b. Lead Phasedown Regulations. 47 FR 49322. November 1. (Cited in Rem et al., 1982).

U.S. EPA. 1982c. Chloroform Position Document 2. Office of Pesticides and Toxic Substances. Washington, DC. pp. 1-3. (Cited in Rem et al., 1982).

USITC. 1982. Synthetic organic chemicals. United States production and sales, 1981. U.S. International Trade Commission, Washington, D.C. USITC Publication 1292. p. 245.

Utidjian, H.M.D. 1976. Jour. Occup. Med. 18: 253. (Cited in ACGIH, 1980.)

Wagner, K., H. Bryson, G. Hunt, A. Shcohet. 1980. Draft Report Level I Materials balance chloroform. U.S. Environmental Protection Agency Contract No. 68-01-5793.

4. DISPOSITION AND RELEVANT PHARMACOKINETICS

4.1. INTRODUCTION

Considering that chloroform was the major anesthetic agent in use during the hundred years from its introduction by Simpson in 1847 (Waters, 1951; Snow, 1858; Simpson, 1847) until after the Second World War, there is relatively little detailed information about its pharmacokinetics and metabolism in man. This undoubtedly is due to the fact that until recently, specific and sensitive analytical methods were unavailable for the measurement of $CHCl_3$ and its metabolites at the concentrations in which they were likely to be present in vivo. Although chloroform as an anesthetic agent has been replaced by drugs with less cardiac and hepatic toxicity, it is still widely used in large bulk as an industrial solvent, as a chemical intermediary, and as a grain fumigant. Chloroform is present in the water supplies of many United States cities in concentrations reaching 311 μg/ℓ, and also has been indentified as a contaminant of the air (U.S. Occupational Safety and Health Administration (OSHA), 1978; National Institute for Occupational Safety and Health (NIOSH), 1977b; Dowty et al., 1975; Symons et al., 1975). Ordinary exposure to chloroform, therefore, includes occupational, food, drinking water, and ambient air (NIOSH, 1977b; Dowty et al., 1975; McConnell et al., 1975), hence, exposure can be chronic by both oral and pulmonary routes, but at levels far below anesthetic concentrations (5000 to 10,000 ppm; 24.85 to 49.70 g/m^3). Nonetheless, chloroform has been detected in the breath of healthy people living in non-industrial environments (Conkle et al., 1975) and in post-mortem human tissue samples (McConnell et al., 1975).

4.2. ABSORPTION

Chloroform is rapidly and extensively absorbed through the lungs and from the gastrointestinal tract. Inhalation is considered the primary route of entrance into man for occupational exposure and air pollution. Absorption after oral ingestion is of particular interest for chloroform as a contaminating component of drinking water and foodstuffs. Significant absorption through intact skin occurs only with liquid contact or submersion.

4.2.1. Dermal Absorption. Absorption of chloroform through the skin from direct liquid contact (immersion of hands or arms) is a slow process. Early studies (Torkelson et al., 1976; Schwenkenbecher, 1904; Witte, 1874) showed that chloroform does penetrate the skin and can be absorbed into the body by this route. Tsurata (1975, 1977) has studied the percutaneous absorption of a series of chlorinated organic solvents applied to a standard area of shaved abdominal mouse skin for 15 minute periods. Absorption was quantitated by presence of compound in total mouse body plus expired air, as determined by GC. For all solvents, percutaneous absorption linearly increased with time over the short exposure period and was directly related to water solubility. For chloroform the absorption rate was 329 moles/min/cm^2 skin, third highest of 8 solvents measured. This investigator extrapolated this absorption rate to a calculation of the amount absorbed into the human body as the result of 1 min immersion of both hands (800 cm^2 area). The estimated amount absorbed, 19.7 mg/min, was equated to an inhalation exposure concentration of 2429 ppm for 1 min. Tsurata concluded that skin absorption from liquid contact could be a significant route of entry into the body for chloroform. More recently Jakobson et al. (1983) carried out similar experiments with guinea pigs for 10 chlorinated organic solvents (which

however did not include chloroform). Liquid contact (skin area, 3.1 cm^2) was maintained for up to 12 hours and solvent concentration in blood was monitored during, and for some solvents after exposure. For these solvents, the blood elimination curves following dermal exposure were non-linear, corresponding to a kinetic model involving at least two body compartments. Furthermore percutaneous absorption of these solvents, as reflected by blood concentration profiles, showed three different patterns that were related to water solubility. For solvents which were relatively hydrophilic [300 to 900 mg/100 ml water] the blood concentration increased steadily during the entire dermal exposure, indicating that absorption occurs faster than elimination by metabolism or pulmonary excretion. Chloroform with a water solubility of about 750 mg/100 ml water might be expected to be in this group.

4.2.2. Oral. The kinetics of gastrointestinal absorption of chloroform after oral ingestion have not been specifically studied; however, transmucosal diffusive passage occurs readily, as expected from its neutral and lipophilic properties (Tables 4-1 and 4-2), and as demonstrated by its biological effects produced by peroral administration of a wide range of dosages and dosing schedules for toxicity studies in rats, mice, guinea pigs, and dogs (Fishbein, 1979; Hill et al., 1975; Brown et al., 1974; Kimura et al., 1971; Klaassen and Plaa, 1967; Miklashevskii et al., 1966; Plaa et al., 1958; Eschenbrenner and Miller, 1945), teratology studies in rats and rabbits (Thompson et al., 1974), and metabolism studies in mice, rats, rabbits, monkeys, and man (Brown et al., 1974; Taylor et al., 1974; Fry et al., 1972; Rubinstein and Kanics, 1964; Paul and Rubinstein, 1963). Accidental and intentional ingestion of chloroform with rapid appearance of clinical symptoms also has been reported in man (Storms, 1973; Schroeder, 1965; Piersol et al., 1933).

TABLE 4-1

Physical Properties of Chloroform and Other Chloromethanes*

	Vapor Pressure at 25°C, torr	Ostwald Solubility, 37°C		
		Water/ air	Blood/ air	Olive Oil/ air
Dichloromethane	400	7.6	9.7	152
Chloroform	250	4.0	10.3	401
Carbon tetrachloride	100	0.25	2.4	361

*Source: Sato and Nakajima, 1979

Conversion factors:

20°C; 750 mmHg 1 ppm in air = 4.97 μg/ℓ = 4.97 mg/m^3
37°C; 760 mmHg 1 ppm in air = 4.69 μg/ℓ = 4.69 mg/m^3

TABLE 4-2

Partition Coefficients for Human Tissue at 37°C

Tissue	Coefficient	Relative to blood
Blood	8.0	
Brain		
Grey matter	16	2.0
White matter	24	3.0
Heart	8	1.0
Kidney	11	1.4
Liver	17	2.1
Lung	7	0.9
Muscle	12	1.5
Fat tissue	280	35.0

[a]Source: Steward et al., 1973

Brown et al. (1974) and Taylor et al. (1974) found that ^{14}C-chloroform in olive oil given perorally to mice, rats, and monkeys (60 mg/kg) was essentially completely absorbed by virtue of a 93 to 98% recovery of radioactivity in exhaled air, urine, and carcass (Table 4-9). Absorption was rapid, with peak blood levels at 1 hour in mice and monkeys. In man, Fry et al. (1972) observed that ^{13}C-chloroform (0.5 g) in olive oil swallowed in a gelatin capsule resulted in rapid appearance of the stable isotope in exhaled breath (Table 4-8), with peak blood levels at 1 hour.

Withey et al. (1982) have investigated the effect of dosing vehicle on the intestinal absorption of chloroform in fasting rats (400 gm) following intragastric intubation of equivalent doses (75 mg/kg) in about 4 ml of water or corn oil. The postabsorptive peak blood concentration averaged 6.5 times higher for water than corn oil (39 vs 6 μg/ml), while the time to initial peak blood concentration was essentially the same (5.6 vs. 6.0 min). Although the absorption from water vehicle exhibited one blood concentration peak, the absorption from corn oil showed two peaks in blood concentration at 6 and 40 minutes. The ratio of the areas under the blood concentration curves for 5 hours after dosing (AUC, 5 hr) was 8.7; water, corn oil. These results suggest that the absorption of chloroform with both vehicles is rapid; but, the rate and extent of absorption may be diminished, and the pattern of absorption altered by intragastric intubation of high volumes (for a rat) of corn oil vehicle. A slower partitioning of lipophilic compounds dissolved in corn oil with mucosal lipids can be expected in comparison with a water vehicle. Furthermore, in contrast to aqueous absorption into the portal system and thence to the liver, corn oil and other liquids are extensively transported via mucosal lymphatic system which slowly drains by way of the left lymphatic thoracic duct into the systemic circulation via the superior vena cava. While these considerations are

unlikely to affect the pharmacokinetics of chloroform in man in any practical way, they are of importance in relation to the modes of dosing employed in long-term carcinogenicity tests of chloroform and other lipophilic compounds.

4.2.3. Pulmonary Absorption. Chloroform has a relatively high vapor pressure (250 torr at 25°C; Table 4-1) and a high blood/air partition coefficient (8 to 10.3 at 37°C; Table 4-2), and hence, its vapor in ambient air is a primary mode of exposure and the lungs a principal route of entry into the body. The total amount absorbed via the lungs (as for all vapors) is directly proportional to: (1) the concentration of the inspired air, (2) the duration in time of exposure, (3) the blood/air Ostwald solubility coefficient, (4) the solubility in the various body tissues, and (5) physical activity, which increases pulmonary ventilation rate and cardiac output. Hence, the basic kinetic parameters of the pulmonary absorption of chloroform and its equilibration in the body are as valid for low concentrations expected in the ambient environment as for the high vapor concentrations associated with its use as an anesthetic (5000 to 10,000 ppm; 24.85 to 49.70 g/m^3) (Smith et al., 1973; Morris, 1951; Waters, 1951). These parameters have not been as well studied as they have for modern anesthetics like halothane (Fiserova-Bergerova and Holaday, 1979) or even for other common halogenated hydrocarbon solvents like trichloroethylene, methylene chloride, or methylchloroform.

The earliest attempt at controlled studies of pulmonary absorption of chloroform in man were conducted by Lehmann and Hasegawa (1910). These investigators calculated retention values for chloroform (% inspired air concentration of chloroform retained in the body) from differences between inspired and expired air concentrations (analyzed by alkali hydrolysis with chloride titration). As expected, initial retention values were high, and decreased with exposure dura-

tion as total body equilibrium with inspired air concentration was approached (Table 4-3). The rate of uptake to equilibration and the final retention value achieved is related to the solubility of chloroform in blood (blood/air partition coefficient). Figure 4-1 illustrates for chloroform and other vapors that the greater the Ostwald solubility coefficient for a vapor agent, the less rapid equilibrium occurs. From the data of Lehmann and Hasegawa (Table 4-3) and recent data of Smith et al. (1973) of blood levels during anesthesia shown in Figure 4-2, total body equilibrium with inspired chloroform concentration requires at least $\geq$2 hours in normal man at resting ventilation rate and cardiac output. The retention value at equilibrium suggested by the Lehmann and Hasegawa (1910) data is ≈65%, and is 67% as calculated from the data of Smith et al. (1973). The difference, 33 to 36%, represents body elimination of chloroform by routes other than pulmonary (primarily by metabolism). The percent retention value is independent of the inspired air concentration at equilibrium.

The magnitude of chloroform pulmonary uptake into the body (dose, body burden) is directly related to the concentration of chloroform in the inspired air and to the duration of exposure. The total amount retained in the body during inhalation exposure can be estimated by multiplying percent retention (R) by the volume of air inspired during the exposure period, or:

$$\text{Amount uptake} = (C_I - C_A) \cdot V \cdot T$$

where V is ventilation rate (ℓ/minute), T is exposure period (minute), and C_I and C_A are inspired air concentration and end alveolar air concentration, respectively. Physical activity increases uptake by increasing the ventilation rate, V, and the cardiac output which influences rate of distribution to the various tissues of the body.

TABLE 4-3

Retention and Excretion of Chloroform in Man
During and After Inhalation Exposure to
Anesthetic Concentrations*

Subject	1	2	3
Inspired air conc. (ppm)	4448	4920	4407
	Retention (%)		
Exposure period (min)			
0 to 5	74.5	68.4	80.0
5 to 10	72.4	61.6	74.2
10 to 15	68.6	51.2	76.9
15 to 20	67.6	50.2	74.6
20 to 25	NR	NR	74.2
25 to 30	NR	NR	73.8
	Excretion, mg/ℓ expired air		
Postexposure (min)			
0 to 10	NR	NR	1.70
10 to 20	NR	NR	0.97
20 to 30	NR	NR	0.85

*Source: Lehmann and Hasegawa, 1910

NR = Not reported; min = minutes

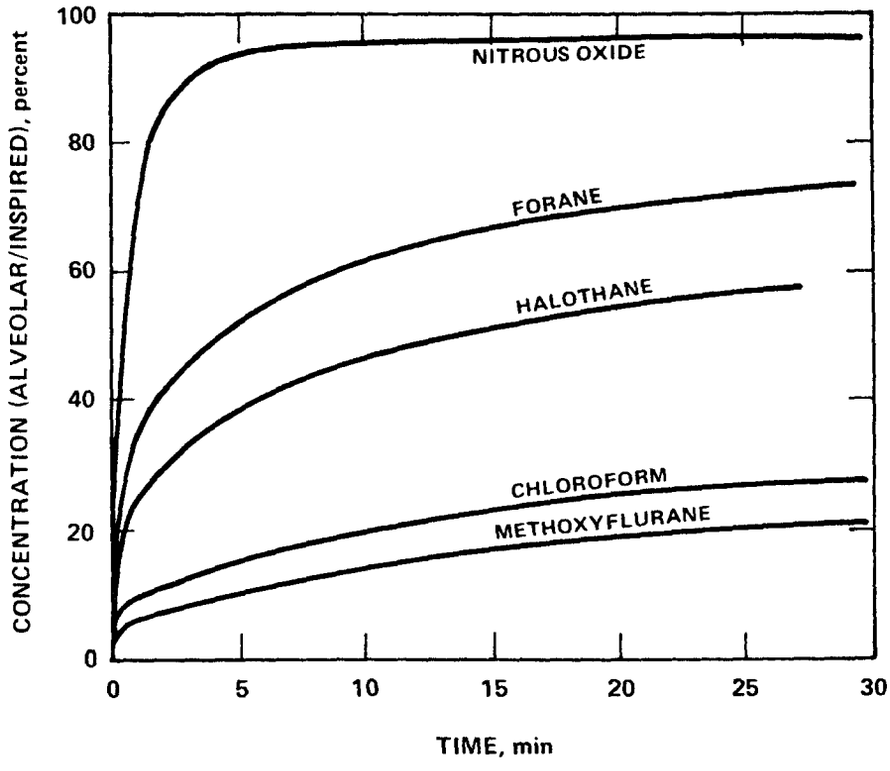

Figure 4-1. Rate of rise of alveolar (arterial) concentration toward inspired concentration for five anesthetic agents of differing Ostwald solubilities (blood/air partition coefficients): nitrous oxide, 0.47; forane, 1.4; halothane, 2.4; chloroform, 8; and methoxyflurane, 11. Note rate of alveolar chloroform rise is less than that of halothane with a smaller Ostwald coefficient and greater than that of methoxyflurane with a larger coefficient.

Source: Munson (1973)

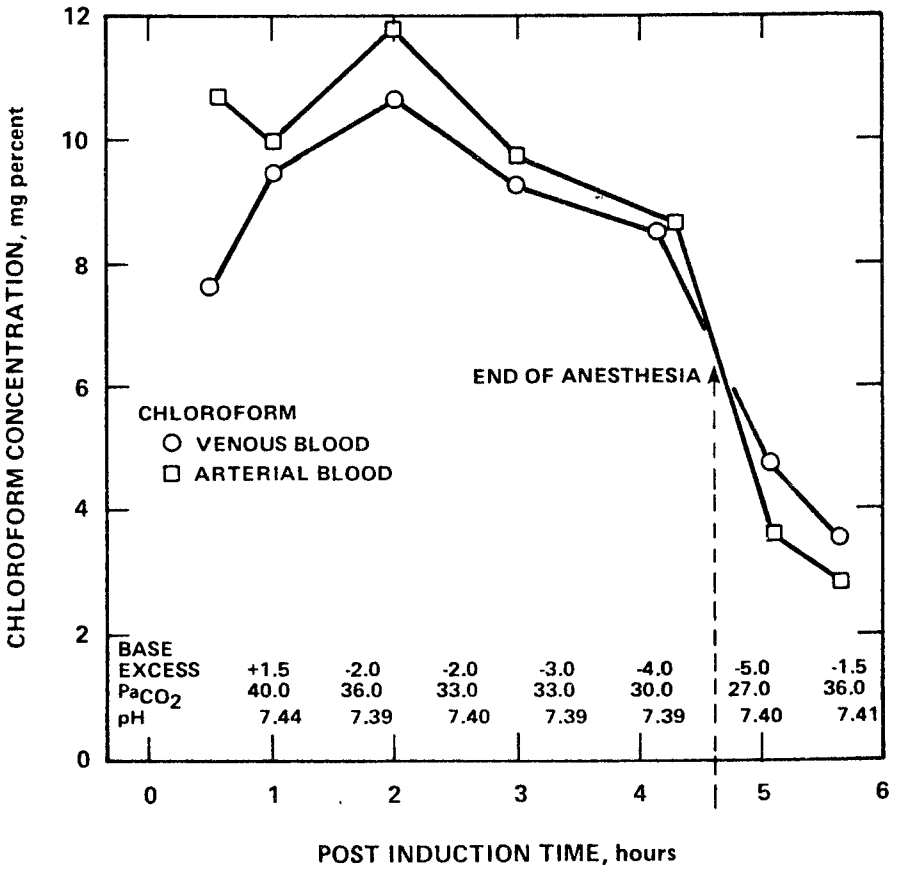

Figure 4-2. Arteriovenous blood concentrations of a patient during anesthesia with chloroform. Note anesthetic blood concentration for chloroform, the decreasing difference between arterial and venous concentrations at 2 to 3 hours, indicating whole-body equilibrium, and the rapid fall of blood concentration with termination of chloroform exposure.

Source: Smith et al. (1973).

During inhalation of chloroform (and in the post exposure elimination phase), the arterial blood concentration of chloroform is directly proportional to inspired air concentration (and end alveolar air concentration). This fixed relationship is defined by the blood/air partition coefficient in comparison to other solvents (Sato and Nakajima, 1979) (Table 4-1), and hence, for equivalent ambient air exposure concentrations, the blood concentration of chloroform is proportionally higher. For inspired air concentration required for surgical anesthesia (8,000 to 10,000 ppm; 39/76 to 49.70 g/m^3), Smith et al. (1973) observed a mean arterial blood chloroform concentration for 10 patients of 9.8 mg/dℓ with a range of 7 to 16.5 mg/dℓ (Figure 4-2), and Morris (1951) found similar values for his patients. For inspired air concentrations less than anesthetic levels, for example low vapor concentrations of 10 to 100 ppm (49.7 to 497 mg/m^3), blood chloroform concentrations are lower in direct proportion.

The amount of pulmonary absorption of chloroform is also influenced by total body weight and by the total fat content of the body (average body fat content is 8% of body weight) (Geigy Scientific Tables, 1973). The capacity of adipose tissue to absorb chloroform in vivo is determined by the product of adipose tissue weight and lipid solubility of chloroform. The lipid solubility of chloroform is relatively high for this haloalkane (olive oil/air, 401; Tables 4-1 and 4-2), and also, the adipose tissue/blood partition coefficient is high (280 at 37°C); therefore, the uptake and storage of chloroform in adipose tissue can be substantial, and it is increased with excess body weight and obesity.

4.3. TISSUE DISTRIBUTION

Chloroform, after pulmonary or peroral absorption, is distributed into all body tissues. The compound crosses the placental barrier, as indicated by

embryotoxicity and teratogenicity in mice, rats, and rabbits after oral and inhalation dosing (Murray et al., 1979; Dilley et al., 1977; Schwetz et al., 1974; Thompson et al., 1974). It has been found in fetal liver (von Oettingen, 1964). Chloroform can be expected to also appear in human colostrum and mature breast milk, since it has been found in fresh cow's milk and in high content in cheese and butter (Table 4-4).

As to be expected from its lipophilic nature and modest water solubility (Table 4-1), highest concentrations are found in tissues with higher lipid content; relative tissue concentrations are reflected by individual tissue/blood partition coefficients. Coefficients for human tissues, given in Table 4-2, indicate that relative tissue concentrations are expected in the order of adipose tissue > brain > liver > kidney > blood. The absolute amounts of chloroform found in these tissues at any given time are proportional to the body dose (i.e., to the concentration in the inspired air and duration of inhalation or to the oral dose, partition coefficient, and to the tissue compartment size).

Gettler (1934) and Gettler and Blume (1931), using a modified Fujiwara analytical method, determined the chloroform content of the brain, lungs, and liver of nine patients who died during surgical anesthesia (presumably 5000 to 10,000 ppm; 24.85 to 49.50 g/m^3 inspired air) as: brain, 120 to 182; lung, 92 to 145; and liver, 65 to 88 mg/kg tissue wet weight. Even higher values (372 to 480 mg/kg in brain tissue) were found in seven cases of death due to excessive administration of chloroform (Gettler, 1934). The blood concentration during surgical anesthesia has been recently determined (by GC) to range from 70 to 165 mg/ℓ in 10 patients (average, 98) by Smith et al. (1973). These tissue concentrations are in general agreement with the tissue/blood partition coefficients summarized from the literature by Steward et al. (1973) and given in Table 4-2.

TABLE 4-4

Chloroform Content in United Kingdom Foodstuffs
and in Human Autopsy Tissue*

Chloroform in U.K. Foodstuffs		Chloroform in Human Autopsy Tissue			
Foodstuff	Chloroform µg/kg	Age of Subject	Sex	Tissue	Chloroform µg/kg (Wet tissue)
Dairy produce		76	F	Body fat	19
Fresh milk	5			Kidney	2
Cheshire cheese	33			Liver	5
English butter	22			Brain	4
Hens eggs	1.4				
Meat		76	F	Body fat	5
English beef (steak)	4			Kidney	5
English beef (fat)	3			Liver	1
Pig's liver	1			Brain	2
Oils and Fats		82	F	Body fat	67
Margarine	3			Liver	8.7
Olive oil (Spanish)	10				
Cod liver oil	6	48	M	Body fat	67
Vegetable cooking oil	2			Liver	9.5
Beverages		65	M	Body fat	64
Canned fruit drink	2			Liver	8.8
Light ale	0.4				
Canned orange juice	9	75	M	Body fat	65
Instant coffee	2			Liver	10.0
Tea (packet)	18				
		66	M	Body fat	68
Fruit and vegetables					
Potatoes (S. Wales)	18	74	F	Body fat	52
Potates (N.W. England)	4				
Apples	5				
Pears	2				
Tomatoes	2				
Fresh bread	2				

*Source: McConnell et al., 1975

In contrast to the high tissue levels of chloroform found in response to inspired air concentrations required for anesthesia, McConnell et al. (1975) recently analyzed post-mortem tissue from eight persons, four males and four females, with an age range of 48 to 82, living in the United Kingdom in ordinary non-industrial circumstances, for chloroform and other halogenated compounds (carbon tetrachloride, trichloroethylene, perchloroethylene, hexachlorobutadiene). Significant tissue levels of three chlorinated hydrocarbons were found. Chloroform level, μg/kg wet tissue weight, were: body fat, 5 to 68 (average of 51); liver, 1 to 10 (average of 7.2); kidney, 2 to 5; and brain, 2 to 4 (Table 4-4). Presumably, these tissue levels of chloroform were derived from air, foodstuff (Table 4-4), and drinking water contamination (OSHA, 1978; Dowty et al., 1975; Symons et al., 1975).

There have been few controlled exposure studies in animals investigating the distribution of chloroform in body tissues and determining dose-dependent tissue concentrations. Chenoweth et al. (1962) determined blood and tissue concentrations of chloroform in two normal fasted dogs after 2.5 hours of surgical anesthesia. Concentration of chloroform in the inhaled stream during anesthesia was not determined, but anesthesia was judged to be satisfactory at an arterial level of 45 to 50 mg/dℓ. Blood and tissue chloroform was determined by infrared spectroscopy after tissue extraction in cold carbon disulfide and distillation. Table 4-5 shows the relative concentration of chloroform in body tissues. The highest concentrations were found in fat tissue, some 10-fold greater than blood, and in adrenals (4-fold greater than blood); the concentrations in brain, liver, and kidney were similar to blood.

Cohen and Hood (1969) used low-temperature whole-body autoradiography to study the distribution of ^{14}C-chloroform in mice. Individual mice were administered 2.4 μℓ of ^{14}C-labeled chloroform by inhalation over a 10-minute

TABLE 4-5

Concentration of Chloroform in Various Tissues of Two Dogs After 2.5 Hours Anesthesia[a]

	Dog A	Dog B
	μg/gm wet tissue weight ± 5%[b]	
Arterial blood	275	397
Brain	298	392
Adrenal (total)	1185	1305
Fat, omentum	2820	1450
Right ventricle	214	314
Skeletal muscle	189	155
Lung	147	336
Liver	282	290
Spleen	237	255
Kidney	225	226
Bile	209	205
Thyroid	460	760
Pancreas	296	350
Urine	57	73

[a]Source: Chenoweth et al., 1962

[b]Chloroform concentration was determined by infrared spectrometry after tissue extraction.

period. The animals were sacrificed 0, 15, or 120 minutes after exposure. Autoradiography of mice killed immediately after inhalation showed the highest concentration of radioactivity in body fat and liver, while lesser and relatively uniform amounts were seen in blood, brain, lung, kidney, and muscle. By 120 minutes after exposure, a considerable decrease in total radioactivity occurred, now principally confined to liver, duodenum, and fat. A mottled appearance in the liver suggested a segmental or localized distribution. Biopsy specimens were taken from selected tissues in each animal and radioactivity determined by scintillation counting. Table 4-6 shows the distribution of radioactivity (chloroform and metabolites) in these tissues, and tissue/blood concentration ratios. Following sacrifice, after 10 minutes of exposure, most tissues approach a unit concentration with blood. However, in both fat and liver, the concentration exceeds unity. By 15 minutes, the ratio of radioactivity in brown fat reaches its peak at 15 times that found in blood. The relative concentration of radioactivity in the liver continues to increase until the termination of the experiment at 120 minutes, when it reaches a final value 6.7 times in excess of that in the blood. Kidney and lung tissues also increased in relative concentration over the 2-hour period to a value of 1.53 and 1.43 of blood, respectively. The increasing ratios of liver and kidney/blood radioactivity represent a continued accumulation of metabolites within these organs. High body fat/blood concentrations shows that adipose tissue represents an important storage site, prolonging retention of chloroform in the body.

Whole-body autoradiography was also carried out by Brown et al. (1974) on male and female Sprague-Dawley rats and squirrel monkeys given ^{14}C-chloroform perorally (60 mg/kg). Male and female rats killed 3 hours after dosage showed no apparent sex difference in distribution of radioactivity. Radioactivity was greatest in body fat and liver, while lesser amounts were seen in blood, brain,

TABLE 4-6

Concentrations of Radioactivity (Chloroform Plus Metabolites) in Various Tissues of the Mouse (NMRI)[a,b]

Tissue	Total Radioactivity (counts/min/mg)			Tissue/Blood Ratio		
	0 min	15 min	120 min	0 min	15 min	120 min
Blood	260 ± 22.0	103 ± 17.5	37 ± 4.0	1.00	1.00	1.00
Brain	217 ± 16.4	112 ± 9.9	23 ± 2.7	0.84	1.12	0.63
Muscle	288 ± 44.4	110 ± 5.1	26 ± 6.9	0.87	1.07	0.70
Lung	262 ± 24.1	149 ± 12.6	53 ± 8.2	1.01	1.44	1.43
Kidney	284 ± 35.0	145 ± 21.6	56 ± 8.0	1.08	1.41	1.53
Liver	407 ± 36.9	208 ± 9.3	250 ± 17.9	1.56	2.10	6.76
Fat	1674 ± 201	953 ± 92.7	266 ± 30.1	6.42	9.25	7.18
Brown fat	3158 ± 384	1490 ± 98.4	211 ± 40.3	12.12	14.70	5.70

[a]Source: Cohen and Hood, 1969

[b]Animal sacrifices were at 0, 15, or 120 minutes following 10-minute inhalation of chloroform. Data represent duplicate determinations in each of two animals at each time sequence (± S.E.)

Min = minutes

lung, kidney, and muscle. Squirrel monkeys showed a similar distribution, with the exception that high concentrations of radioactivity were present in the bile and increased with time. Examination of bile extract by gas-liquid chromatography showed the bile radioactivity was unchanged chloroform, indicating an excretion of chloroform by the biliary route in the monkey.

Brown and his colleagues (Taylor et al., 1974) also investigated the tissue distribution of chloroform in 3 strains of mice (CF/LP, CBA, and C57) by whole-body autoradiography after oral dosing (60 mg/kg ^{14}C-chloroform). In the male mice of the three strains examined 3 hours after dosing, the greatest amounts of radioactivity appeared in liver and kidneys, and lesser amounts in renal cortex but not medulla. Female mice showed greatest radioactivity in liver, intestine, and bladder, with much less radioactivity in kidney and little differentiation between renal cortex and medulla. The same general patterns were observed 5, 7, and 24 hours after dosing. Biopsy samples of these tissues were taken, and radioactivity determined by scintillation counting. Table 4-7 shows the distribution of ^{14}C-chloroform radioactivity in male and female mice killed 5 hours after dosing. There is a 3.5-fold difference between the activity present in the male and female kidneys of each strain. Male mice had greater activity in the kidneys, but female mice showed relatively greater activity in the liver. This sex difference in distribution was abolished by castration or testosterone administration to female mice. The sex difference in tissue distribution of chloroform and its metabolites may relate to the nephrotoxic effect of chloroform that occurs in male mice but not in female mice (Bennet and Whigham, 1964; Culliford and Hewitt, 1957; Hewitt, 1956; Shubik and Ritchie, 1953; Eschenbrenner and Miller, 1945a, 1945b). The pattern of tissue distribution of chloroform in mice also depends on mode of exposure. Cohen and Hood (1969), after chloroform inhalation, found highest levels in body fat (Table 4-6), while

TABLE 4-7

Tissue Distribution of ^{14}C-Chloroform Radioactivity in CF/LP Mice After Oral Administration (60 mg/kg)[a,b]

Tissue	Mean DPM/100 mg wet weight (SEM)	
	Male (6)	Female (6)
Liver	18,157 (1898)	21,535 (2097)
Kidney	13,759 (1047)	3920 (533)
Brown fat	1011 (80)	1074 (54)
Blood	2910 (423)	2906 (457)

[a]Source: Taylor et al., 1974

[b]Similar results were obtained for CBA and C57 strains.

Brown et al. (1974) and Taylor et al. (1974) observed lower levels in fat and highest levels in liver and kidney following oral dosing (Table 4-7). The high liver levels of chloroform after oral administration may be due, in part, to first passage and extraction by the liver after this route of administration, to differences of time after exposure (2 versus 5 hours), and to metabolism and covalent binding of metabolites to cellular macromolecules (see below).

It is worth re-emphasizing that the sex difference in tissue distribution and binding of chloroform (and metabolites) in kidney and liver, noted by Brown and his colleagues (Taylor et al., 1974), appeared to be peculiar to mice and that these workers did not observe such differences in male and female rats or squirrel monkeys (Brown et al., 1974).

4.4. EXCRETION

Elimination of chloroform from the body is perforce the sum of metabolism and excretion of unchanged chloroform via pulmonary and other routes. Unmetabolized chloroform is excreted almost exclusively through the lungs; however, metabolism of chloroform is extensive, with the proportion excreted unchanged dependent on body dose. Surprisingly, considering its historical importance, its longtime use as an industrial chemical and anesthetic agent, few controlled experimental studies in man have been made on the kinetics of excretion of chloroform.

4.4.1. Pulmonary Excretion. Figure 4-3 shows the time-course of pulmonary elimination of chloroform after accidental inhalation exposure to a mixture of solvents, including chloroform, carbon tetrachloride, trichloroethylene, and perchloroethylene. Stewart et al. (1965) determined, post-exposure, the alveolar

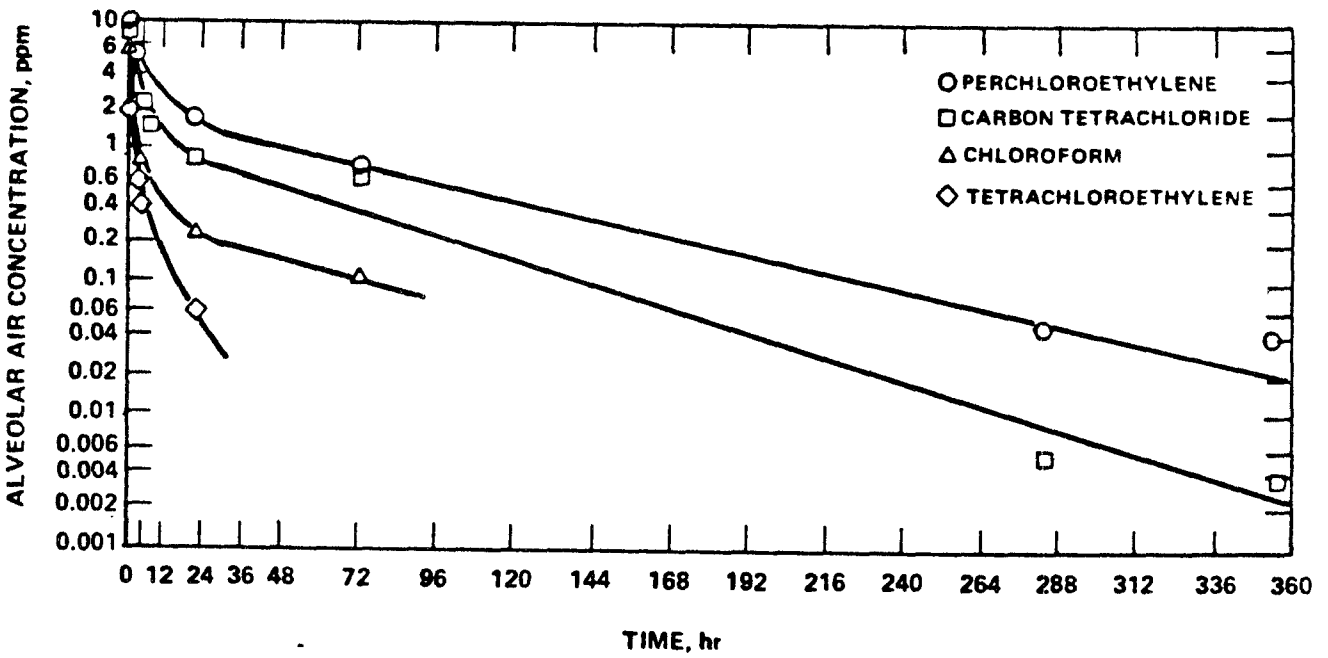

Figure 4-3. Exponential decay of chloroform, carbon tetrachloride, perchloroethylene and trichloroethylene in exhaled breath of 48 year-old male accidentially exposed to vapors of these solvents. The alveolar air (end tidal) was collected in a 50-mℓ glass pipette, and the sample was analyzed by infrared spectroscopy and gas-liquid chromatography. Initial values (30 min. post exposure) were chloroform, 7 ppm; carbon tetrachloride, 9.5 ppm; perchloroethylene, 11 ppm; and trichloroethylene, 4 ppm.

Source: Stewart et al. (1965).

air concentrations of these solvents by infrared and gas-liquid chromatography analysis. The kinetics of pulmonary excretion of the solvents are independent of one another. However, all, including chloroform, demonstrate the typical kinetics of gaseous vapor pulmonary elimination, that has been observed experimentally for relatively hydrophobic, volatile gaseous anesthetics and industrial solvents (Eger, 1963; Fiserova-Bergerova et al., 1974, 1979, 1980; Droz et al., 1977). At termination of exposure with zero concentration in inspired air, chloroform (and the other solvents) immediately begins to be eliminated from the body into the lungs, with blood and alveolar air concentrations describing parallel exponential decay curves with three major components (Figure 4-3). These exponential components have been related by many investigators (Eger, 1963; Fiserova-Bergerova et al., 1974, 1979, 1980; Droz et al., 1977) to first-order kinetics of pulmonary elimination associated with desaturation of physiological compartments in accordance with a blood flow-limited model in which the rate constants are determined predominately by tissue perfusion, volume of tissue distribution and by partition coefficients:

$$\text{Tissue uptake and desaturation of compartment, T} = F_T \cdot \lambda_{bl/air} \exp\left(- \frac{F_T}{V_T \lambda_{T/bl}} \cdot t \right)$$

where F is blood flow through tissue compartment, V is volume of the compartment, λ is partition coefficient, and exp is base of natural logarithm.

$$t_{1/2} = \frac{0.693 \; V \; \lambda_{T/bl}}{F}$$

Since three exponential components are typically observed experimentally, three physiological tissue compartments are included in the model described by a three-term exponential function of the form:

$$\text{Uptake, Desaturation} = A\, e^{-\alpha t} + B\, e^{-\beta t} + C\, e^{-\gamma t}$$

where A, B, C are macrocoefficients and α, β, γ, are hybrid constants (defined above). These terms represent three flow-limited major body compartments (1) a vessel-rich group of tissues (VRG) with high blood flow and high diffusion rate constant (VRG: brain, heart, kidneys, liver and endocrine and digestive systems), (2) lean body mass (MG: muscle and skin), and (3) adipose tissue (FG). More recently, Fiserova-Bergerova and coworkers (1974, 1979, 1980) have mathematically re-formulated this physiological, first-order model to accomodate the effect of metabolism on uptake, distribution and clearance of inhaled vapor compounds.

The half-time (t 1/2) of elimination from the physiological compartments (VRG < MG < FG) are independent of the body dose, but are dependent on tissue/blood partition coefficients and blood/air partition coefficients. Since these solvent compounds have high solubility in body fat (Table 4-1), they are eliminated slowly from fat depots with long half-time of elimination, as illustrated by Stewart's patient (Stewart et al., 1965) in Figure 4-3. From Figure 4-3, it can be estimated graphically that chloroform has a half-time of elimination from the fat compartment (FG) of ≈36 hours, with similar long half-times for the highly fat soluble compounds, perchloroethylene and carbon tetrachloride.

There is little information available for the half-times of pulmonary elimination of chloroform from the VRG and MG. From the early data of Lehmann and Hasegawa (1910) given in Table 4-3, the half-time of pulmonary elimination from the VRG appears to be ≈30 minutes. A similar estimate can be made from the data of Smith et al. (1973) and Morris (1951) at termination of anesthesia in man; these workers found that blood chloroform concentration rapidly fell exponentially from 7 to 3.5 mg/dℓ within 30 minutes (Figure 4-2).

Pulmonary elimination of chloroform was investigated by Fry et al. (1972) in male and female volunteers given ^{13}C-chloroform in olive oil orally (by gelatin capsule). Chloroform was determined in expired air by GLC. Their data, summarized in Table 4-8, show that the amount of chloroform excreted through the lungs within 8 hours (expressed as a percentage of the dose, 0.1 to 1.0 g), increased (0 to 65%) in proportion to the dose. Following a peak blood concentration (0.5 mg/dℓ for a 500 mg dose) 1 hour after oral dosage, absorption, and distribution, the blood chloroform concentration declined exponentially with three components: (1) a very rapid disappearance, with half-time of 14 minutes possibly corresponding to VRG compartment kinetics, (2) a slower disappearance, with half-time of 90 minutes corresponding to MG kinetics, and (3) a very slow disappearance, with very long half-time from adipose tissue. This half-time was undetermined, but chloroform was detected in blood and breath 24 hours later. Fry and coworkers (1972) noted a linear relationship for their subjects between pulmonary excretion of chloroform and body weight deviation from ideal, an index of excessive leanness or excessive body fat from normal. Their data in Figure 4-4 show that for both male and female subjects given a standard oral dose of chloroform, lean subjects eliminate via the lungs a greater percentage of the dose, while overweight subjects eliminate less chloroform. The different slopes of the linear relationship for men and women presumably reflect the different proportion of adipose tissue in the two sexes. The bodies of women tend to contain higher proportions of fat than those of men (Geigy Scientific Tables, 1973). These observations reinforce the role of adipose tissue as a storage site for chloroform.

Brown and his coworkers (Brown et al., 1974; Taylor et al., 1974) have demonstrated an animal species difference in the amount of pulmonary excretion of ^{14}C-chloroform from a standard oral dose (60 mg/kg body weight) given in olive

TABLE 4-8

Pulmonary Excretion of $^{13}CHCl_3$ Following Oral Dose: Percent of Dose[a]

Subjects	Dose (g)	Mean for 8 Hours[b]	Range
8 M and F	0.5	40.3	17.8 to 66.6
1	1.0	64.7	NA
1	0.25	12.4	NA
1	0.10	nil	NA

Pulmonary excretion of $^{13}CO_2$ following 0.5 g oral dose of $^{13}CHCl_3$: Cumulative percent of dose[b]

	Time after dose (hours)				
Subjects	0.5	1.75	2.5	5.5	7.5
Male (1)	2.1	24.1	35.9	49.2	50.6
Female (62.7 kg) (1)	0.5	10.7	28.3	47.5	48.5

[a]Recalculated from the data of Fry et al., 1972

[b]Within 4% of value calculated for infinite time

NA = Not applicable

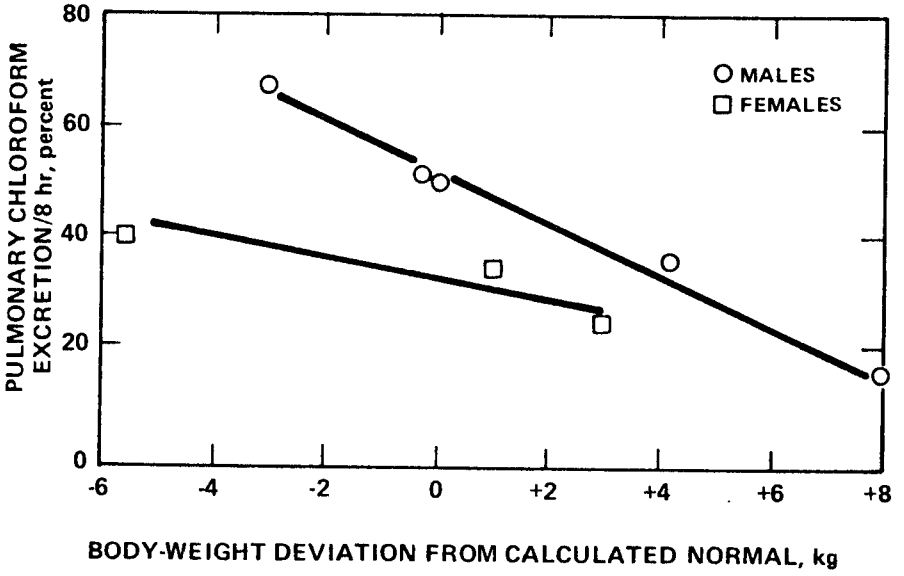

Figure 4-4. Relationship between total 8-hour pulmonary excretion of chloroform following 0.5-g oral dose in man and the deviation of body weight from ideal. The different slopes of the linear relationship for men and women reflect the different proportion of adipose tissue in the two sexes.

oil. Mice (three strains), rats, and squirrel monkeys excrete chloroform via the lungs (6, 20, and 79%, respectively, of the standard dose). This species difference is primarily related to the capacity to metabolize chloroform rather than differences in pulmonary kinetics, since, as shown in Table 4-9, the percentage of the dose metabolized to $^{14}CO_2$ is inversely proportional to that of pulmonary excretion. The mice, 48 hours after dosing, retained only 2% of chloroform radioactivity (Table 4-9).

Withey and Collins (1980) determined the kinetics of distribution and elimination of chloroform from blood of Wistar rats after intravenous administration of 3, 6, 9, 12 or 15 m/kg of chloroform given in 1 ml water intrajugularly. For all doses, the blood decay curves exhibited three components of exponential disappearance of chloroform (α, β, γ components) and "best" fitted a first-order three compartment model. Table 4-9b summarized the values obtained for the kinetic parameters. For volatile, lipophilic compounds, for which a major route of elimination is pulmonary, experiments utilizing dose administration via relatively large intravenous bolus injections (relative to rat total blood volume), and which measures only blood chloroform disappearance, provide a number of problems for data interpretation of elimination (pulmonary and metabolism) and/or tissue distribution. In these experiments, pulmonary elimination, which is rapid for organic solvents, occurs simultaneously with distribution and metabolism; in contrast, experiments in which the animal is preloaded by oral or inhalation administration, distribution is more readily separable from pulmonary elimination. After intravenous administration (Table 4-9b), the rate constant k_e (for elimination of chloroform from the central compartment blood out of the body (principally via pulmonary excretion and/or metabolism) was dose-dependent and consistent with a half-time of elimination of only 3.6 min for the lowest dose and only 6.2 min for the highest dose. Since the

TABLE 4-9

Species Difference in the Metabolism of ^{14}C-Chloroform
(Oral Dose of 60 mg/kg)*

Species	No.	^{14}C-radioactivity 48 hours after dose Mean values as percent dose				
		Expired $^{14}CHCl_3$ or metabolites	Expired $^{14}CO_2$	Urine + Feces	Carcass	Total
Mice						
CF/LP, CBA, C57 strains	19	6.1	85.1	2.6	1.8	95.6
Rats						
S-D	6	19.7	65.9	7.6	NR	93.2
Squirrel Monkeys	6	78.7	17.6	2.0	NR	98.3

*Recalculated from the data of Brown et al., 1974

NR = Not recorded

Table 4-10. Kinetic Parameters for Chloroform After I.V. Administration to Rats

Dose* mg/kg	V_d ml	α	β	γ	k_e	k_{12}	k_{21}	k_{13}	k_{31}
					min^{-1} ± S.E.				
3.0	45.07 ± 0.04	0.72 ± 0.11	0.135 ± 0.001	0.0287 ± 0.0064	0.1907 ± 0.0239	0.2346 ± 0.0651	0.2575 ± 0.0316	0.0921 ± 0.0008	0.0421 ± 0.0098
6.0	53.57 ± 12.61	0.64 ± 0.13	0.081 ± 0.01	0.0158 ± 0.0019	0.1874 ± 0.0356	0.2681 ± 0.0631	0.1862 ± 0.0188	0.0730 ± 0.0246	0.0233 ± 0.0032
9.0	64.46 ± 14.26	0.64 ± 0.084	0.095 ± 0.0001	0.0189 ± 0.0009	0.1284 ± 0.0217	0.2529 ± 0.0489	0.2421 ± 0.0064	0.0594 ± 0.0034	0.0281 ± 0.0042
12.0	80.62 ± 20.20	0.32 ± 0.20	0.060 ± 0.028	0.0074 ± 0.0056	0.1035 ± 0.0232	0.1071 ± 0.0774	0.1192 ± 0.0676	0.0395 ± 0.0253	0.0106 ± 0.0084
15.0	89.13 ± 12.83	0.35 ± 0.048	0.070 ± 0.006	0.0134 ± 0.0005	0.1124 ± 0.0110	0.1104 ± 0.0256	0.1523 ± 0.0188	0.0396 ± 0.0104	0.0193 ± 0.0018
15.0; $t_{\frac{1}{2}}$ min		2.1	9.9	51.7	6.2	6.3	4.6	17.5	35.9

*2 to 4 rats/dose . From Withey et al., 1982.

half-times for distribution into other tissue compartments from the central compartment blood have longer half-times, it is likely that, of the dose introduced into the blood, a major portion (depending on dose) was excreted within a few minutes by the lungs. Further indication that the dose and/or mode of administration influenced the distribution and elimination of chloroform was shown by the proportional increase of the apparent volume of distribution, V_d (45 ml; 3 mg/kg to 89 ml; 15 mg/kg) and the decrease with dose in values for rate constants of transfer from blood to other tissue compartments. The volume of distribution (V_d) of chloroform was 89 ml for the highest dose or about 22% b.w., surprisingly low for a lipid soluble compound that is known to diffuse into all the major organ systems (Tables 4-5 and 4-6). Adipose tissue is known to be a major tissue compartment for chloroform [Section 4.3]; the clearance of chloroform from perirenal fat was found to be slow with a half-time of 106 min, and since the rate constant, k_{31} was given as 0.0193 min^{-1} (for 15 mg/kg dose) indicating a half-time of 36 min, the adipose tissue appears to be a deep compartment. These investigators believe that their kinetic data shows no evidence in the rat of nonlinear or dose-dependent Michaelis-Menten kinetics and they suggest that a dose of 15 mg/kg is below hepatic metabolism saturation.

4.4.2. Other Routes of Excretion. Chloroform is not eliminated in significant amounts from the body by any route other than pulmonary. Studies of chlorinated compounds in the urine after chloroform inhalation exposure or peroral dosage to animals and humans have failed to detect unchanged chloroform (Brown et al., 1974; Fry et al., 1972). Brown et al. (1974) identified chloroform in high concentration in the bile of squirrel monkeys after oral ^{14}C-chloroform dosage, and suggested an active enterohepatic circulation in this species. For monkeys,

they found only 2% of dose radioactivity in combined urine and feces collected for 48 hours after dosing (Table 4-9), and only 8 and 3% for rats and mice, respectively.

4.4.3. Adipose Tissue Storage. There is no definitive experimental evidence in the literature concerning bioaccumulation after chronic or repeated daily exposure to chloroform. However, there are practical reasons to believe that extended residence in body fat occurs. In man, chloroform has a relatively high fat tissue/blood partition coefficient of 35 (Table 4-2), a long half-time of elimination from adipose tissue compartments of ≈36 hours (Figure 4-3), and it has been detected in blood and breath 24 to 72 hours after a single exposure (Stewart, 1974; Fry et al., 1972; Stewart et al., 1965.). Figure 4-5 clearly shows the slow elimination of chloroform from the adipose tissue of dogs following a 3-hour anesthesia (Chenoweth et al., 1962). Despite the rapid exponential decline of blood levels of chloroform within 3 hours after termination of anesthesia, significant levels of chloroform were still present 20 hours later.

Fry et al. (1972) has provided indirect evidence in man of the storage of chloroform in body fat (Figure 4-4), while analysis of body fat of animals given a single inhalation exposure or oral dosage demonstrated marked accumulation of chloroform in this tissue (Taylor et al., 1974; Cohen and Hood, 1969) (Tables 4-5, 4-6, 4-7). Particularly pertinent are the observations of McConnell et al. (1975), who demonstrated the occurrence of significant amounts of chloroform (and other chlorinated hydrocarbons) in autopsy tissues (highest concentrations in body fat) of humans exposed only to ordinary ambient air (Table 4-4), and of Conkle et al. (1975), who analyzed the GC-MS alveolar air of eight fasting healthy men working in a nonindustrial environment and found in three men

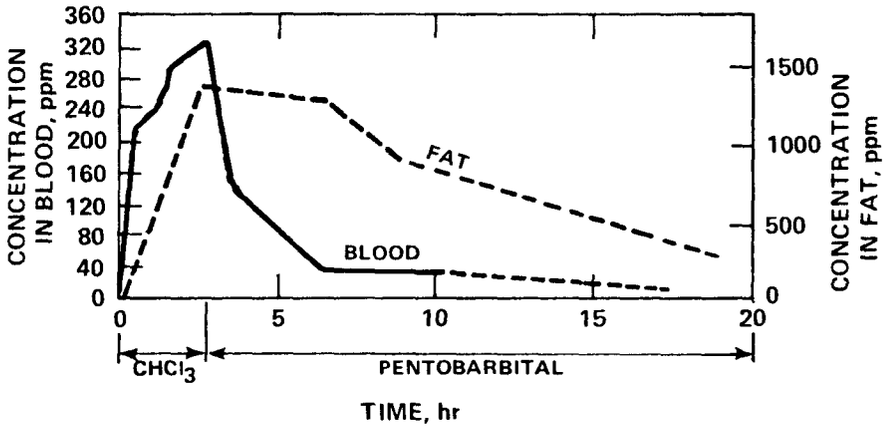

Figure 4-5. Blood and adipose tissue concentrations of chloroform during and after anesthesia in a dog. Note the high prolonged levels of chloroform in adipose tissue (broken line) for 20 hours even after rapid exponential fall in blood concentration (solid line) with termination of chloroform anesthesia after three hours.

Source: Chenoweth et al. (1962).

significant rates of pulmonary excretion of chloroform (Table 4-10), as well as other halocarbons (for example, methylene chloride, dichlorobenzene, methylchloroform) in all eight men.

4.5. BIOTRANSFORMATION OF CHLOROFORM

4.5.1. Known Metabolites. The haloforms, and chloroform in particular, long have been known to undergo extensive mammalian biotransformation. Zeller (1883) clearly demonstrated an increased daily urinary inorganic chloride excretion representing 25 to 60% of the dose in dogs given oral doses of chloroform (7 to 10 g in gelatin capsules). Eighty years later, Van Dyke et al. (1964), using ^{36}Cl-chloroform, confirmed in rats that the extra urinary inorganic chloride originated from the metabolism of chloroform. Zeller (1883) also found, in the urine of his dogs given chloroform, a levo-rotatory oxidative metabolite that he suggested to be the glucuronide of trichloromethanol, a compound only recently postulated as an intermediate of P_{450} oxidative metabolism (Figure 4-6). Van Dyke and coworkers (1964) also found evidence for ^{36}Cl-metabolites (≈2% dose) in the urine of their rats given chloroform. However, other investigators (Brown et al., 1974; Fry et al., 1972; Paul and Rubinstein, 1963; Butler, 1961) with newer methodologies have not been able to identify lesser chloromethanes in the urine or breath of mouse, rat, or man after chloroform exposure.

In addition to the chloride ion, it has been established from both in vivo and in vitro studies that the major end-product metabolite of chloroform is carbon dioxide (CO_2) (Brown et al., 1974; Fry et al., 1972; Rubinstein and Kanics, 1964; Van Dyke et al., 1964; Paul and Rubinstein, 1963), with phosgene identified as the immediate precursor metabolite from in vitro studies (Mansuy et al., 1977; Pohl et al., 1977; Ilett et al., 1973) (Figure 4-6). CO_2 from

TABLE 4-11

Levels of Chloroform in Breath of Fasted Normal Healthy Men*

Subject	Age	Chloroform excretion, μg/hr
A	34	2.0
B	28	ND
C	33	ND
D	38	ND
E	47	11.0
F	28	ND
G	38	ND
H	23	0.22

*Source: Conkle et al., 1975

ND = Not detected

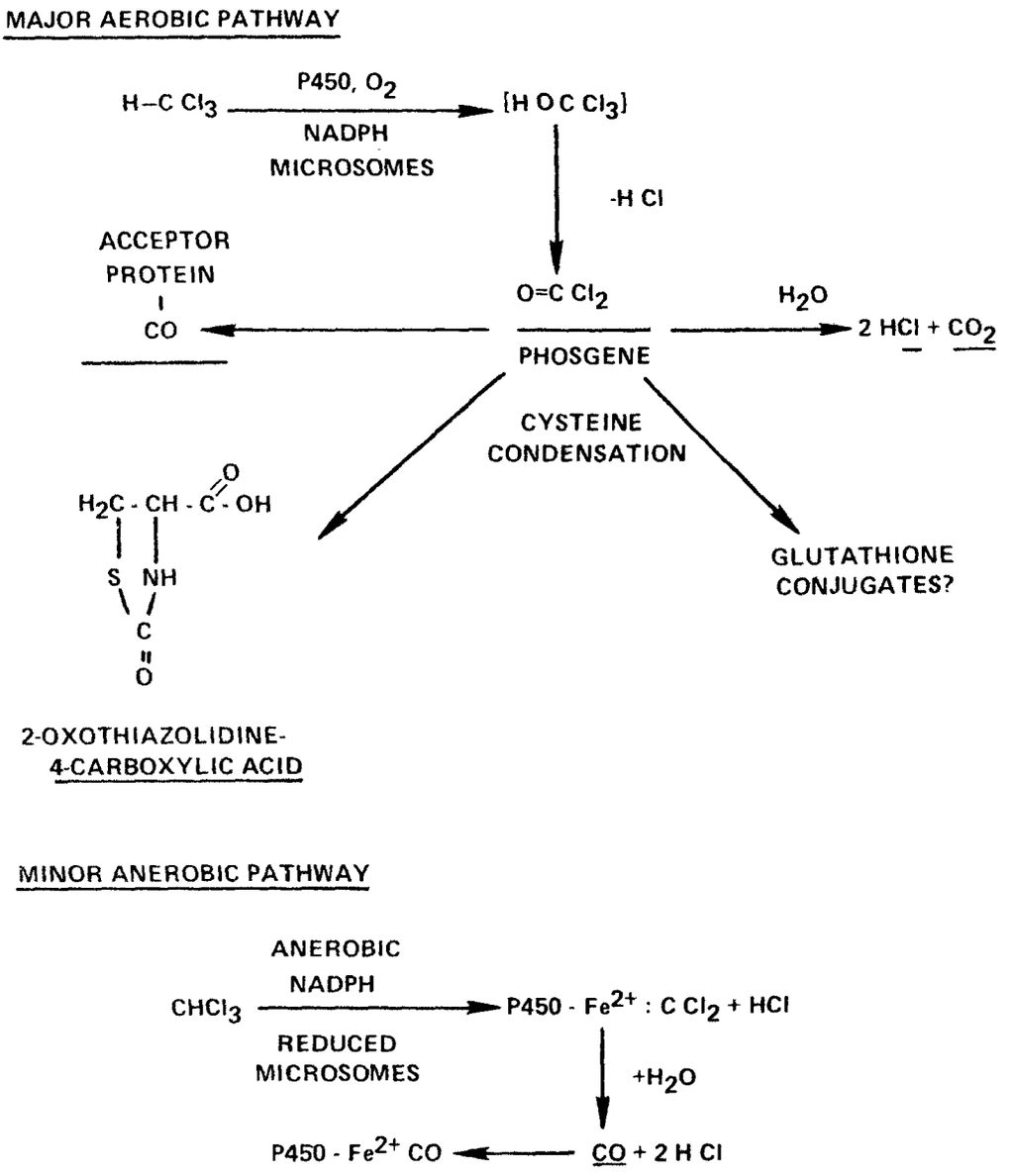

Figure 4-6. Metabolic pathways of chloroform biotransformation. (Identified $CH\ Cl_3$ metabolites are underlined.)

chloroform metabolism is primarily excreted through the lungs, but a small per centage (<7%) is incorporated into endogenous metabolites and excreted into the urine as bicarbonate, urea, methionine, and other amino acids (Brown et al., 1974). Carbon monoxide (CO) has also been identified as a very minor metabolite of anaerobic chloroform metabolism (Figure 4-6), both from in vitro studies (Ahmed et al., 1977; Wolf et al., 1977) and in vivo animal studies (Anders et al., 1978; Bellar et al., 1974).

In addition to chloroform metabolites that are excreted, phosgene and other "reactive intermediates" of chloroform metabolism interact with and covalently bind to tissue acceptors such as protein and lipids (Docks and Krisna, 1976; Uehleke and Werner, 1975; Brown et al., 1974; Ilett et al., 1973; Cohen and Hood, 1969; Reynolds, 1967; Cessi et al., 1966).

The liver is the principal site of chloroform metabolism, although Paul and Rubinstein (1963) and Butler (1961) found that rat kidney, adipose tissue, and skeletal muscle also converted chloroform to CO_2 (25, 0.8, and 8% of liver, respectively).

4.5.2. Magnitude of Chloroform Metabolism. Chloroform is metabolized to differing extents in man and other animal species. Since chloroform and other halogenated hydrocarbons are thought to produce pathological effects by metabolism in target tissues to reactive intermediates that covalently bind to macromolecules (Chapter 5), the total capacity to metabolize chloroform as well as individual tissue sites of metabolism are important determinants of expected interspecies differences in toxic susceptibility. This interdependence between intensity of toxic response and metabolism, and interspecies differences in magnitude of metabolism, are important considerations in extrapolation from experimental animal to man (Reitz et al., 1978).

Few studies have been made of the capacity of man to metabolize chloroform; virtually no studies have been made of the pharmacokinetic, endocrine, genetic, and environmental factors modifying metabolism in man. The early studies of Lehmann and Hasegawa (1910) on the retention of chloroform from inspired air inhaled by three volunteers (4500 to 5000 ppm, average of 64%) (Table 4-3) suggest that 36% of pulmonary uptake of chloroform in man is metabolized. Similarly, a retention value of 67%, calculated from the data of Smith et al. (1973) for patients inhaling 10,000 ppm chloroform during surgical anesthesia (Figure 4-2), indicates 33% chloroform metabolism during inhalation exposure. A similar estimate of the extent of chloroform metabolism during anesthesia has been made by Feingold and Holaday (1977). These workers simulated, with a computer, chloroform inhalation kinetics using a non-linear whole-body compartmental model, and found that the percent of chloroform uptake metabolized was 30%. This rate of metabolism remained constant during 8 hours of anesthesia, and continued for several days following termination of anesthesia, presumably from chloroform stored during anesthesia in adipose tissue.

Fry et al. (1972) have investigated the metabolism of chloroform in man after single oral doses. Isotopically labeled ^{13}C-chloroform dissolved in 1.0 mℓ olive oil/gelatin capsule was given to 12 healthy male and female volunteers (58 to 60 kg body weight) at doses of 0.1 to 1.0 g. For two of the volunteers, pulmonary excretion of $^{13}CO_2$ in expired air from the metabolism of chloroform was serially connected over a 7.5 hour period and analyzed by mass spectrometry. The results given in Table 4-8 show that 49 and 51% of a 0.5 g dose was metabolized to $^{13}CO_2$. Pulmonary excretion of unchanged ^{13}C-chloroform during a comparable period (8 hours) in a separate experiment with these two subjects were 67 and 40%, respectively. No metabolites other than CO_2 (e.g., methylene dichloride, tetrachloroethane) were found in expired air, and chloro-

form was not found in the urine. These results indicate that: (1) virtually all of an oral chloroform dose (0.5 g) can be accounted for by pulmonary excretion of CO_2 and unchanged chloroform, (2) metabolism of chloroform to CO_2 is ≈50% of this dose, and (3) absorption and metabolism are rapid and virtually complete within 5 hours as shown in Table 4-8, possibly because of first pass through the liver. In this respect, the kinetics of metabolism of oral doses may differ substantially from inhalation doses. Furthermore, the data of Table 4-8 suggest that the fraction of the dose metabolized is dose-dependent. Thus, an oral dose of 0.1 g was completely metabolized (100%), with no chloroform excreted unchanged through the lungs; but for a 1.0 g dose, 65% was excreted and only 35% metabolized. These results suggest that metabolism is rate-limited in man, since a diminishing proportion of dose is metabolized with increasing dose (Table 4-8).

In man, Chiou (1975) has shown that up to 38% of an oral dose of chloroform is metabolized in the liver, and up to about 17% is excreted intact from the lungs before the chloroform reaches the systemic circulation, an example of a first-pass effect.

Animal experiments demonstrate a marked species difference in the metabolism of chloroform. Early experiments in the 1960's by Paul and Rubinstein (1963), Van Dyke et al. (1964), and Cohen and Hood (1969) with mice and rats given ^{14}C-chloroform indicated a minimal metabolism of chloroform (≈4%) occurred in these species. More recent studies by Brown and his coworkers (Brown et al., 1974; Taylor et al., 1974) have shown that mice and rats metabolize chloroform to CO_2 extensively (65 to 85%), and to a greater extent than non-human primates or man. These investigators gave equivalent oral doses (60 mg/kg) of ^{14}C-chloroform to mice (3 strains), rats, and squirrel monkeys, and determined ^{14}C-labeled chloroform or volatile metabolites and $^{14}CO_2$ in expired air, ^{14}C-radioactivity in urine, and ^{14}C-radioactivity remaining in animals at sacrifice 4 to 8 hours

after dosing. Total recovery of ^{14}C-chloroform radioactivity was excellent and accounted for 93 to 98% of the administered dose. Their results are summarized in Table 4-9. Using $^{14}CO_2$ as a measure of the fraction of the chloroform dose metabolized (intermediate chloromethane metabolites were not found in breath or urine), mice metabolized 85% of the dose, rats, 66%, and squirrel monkeys, 18%. A further 2 to 8% of ^{14}C-radioactivity ($^{14}CO_2$ incorporated into urea, bicarbonate, and amino acids) were found in urine. They found no strain difference in mice, or sex difference in mice, rats, or monkeys in capacity to metabolize chloroform, or in tissue distribution and binding of metabolites, except for mice, where kidney radioactivity concentration was greater in males than females and lesser in livers of males than females (Table 4-7). These findings of Brown and coworkers of large interspecies differences for metabolism of chloroform and marked sex differences in mice (but not other species) for tissue distribution and covalent binding of intermediate metabolites to tissue macromolecules in liver and kidney emphasize the difficulties and dangers of extrapolating studies in lower animals to man (Reitz et al., 1978).

4.5.3. Enzymic Pathways of Biotransformation. It has been postulated for many years (Docks and Krishna, 1976; Uehleke and Werner, 1975; Brown et al., 1974; Ilett et al., 1973; Reynolds, 1967; Paul and Rubinstein, 1963) that a reactive metabolite of $CHCl_3$ is responsible for its liver and renal toxicity in man (von Oettingen, 1964; Conlon, 1963) and experimental animals (Bhooshan et al., 1977; Pohl et al., 1977; Ilett et al., 1973; Klaassen and Plaa, 1966), and possibly the production of liver tumors in mice (Eschenbrenner and Miller, 1945a). For example, when rats or mice are treated with ^{14}C-chloroform, the extent of hepatic necrosis parallels the amount of ^{14}C-label bound irreversibly to liver protein (Docks and Krishna, 1976; Brown et al., 1974; Ilett et al., 1973). Both necrosis

and binding are potentiated by pretreatment of animals with phenobarbital, a known inducer of liver microsomal metabolism, and inhibited by pretreatment with the inhibitor piperonyl butoxide. Chloroform administration also decreases the level of liver glutathione in rats pretreated with phenobarbital, further suggesting that a reactive metabolism is produced (Docks and Krishna, 1976; Brown et al., 1974). The results of *in vitro* studies with rat and mouse liver microsomes support the *in vivo* observations by establishing that ^{14}C-chloroform is metabolized to a reactive metabolite which binds covalently to microsomal protein (Bhooshan et al., 1977; Sipes et al., 1977; Uehleke and Werner, 1975; Ilett et al., 1973). This metabolic process is oxygen dependent and appears to be mediated by a cytochrome P_{450} which is inducible by phenobarbitol (Sipes et al., 1977; Uehleke and Werner, 1975; Ilett et al., 1973).

The demonstrations by Pohl et al. (1977) and Mansuy et al. (1977) of carbonyl chloride (phosgene) formation from chloroform by rat microsomal preparations suggest that phosgene may be the key causal agent for these toxic effects. The finding of Weinhouse and collaborators (Shah et al., 1979) that phosgene is also a reactive metabolic intermediate in the metabolism of carbon tetrachloride emphasizes basic similarities in the metabolism and toxicities of these two chloroalkanes. Figures 4-6 and 4-7 show for comparison the currently proposed pathways of metabolism of chloroform and carbon tetrachloride.

Figure 4-6 indicates that the initial step in the metabolism of chloroform involves the oxidation of the aliphatic carbon (H-C) to trichloromethanol by a phenobarbital inducible cytochrome P_{450} (Sipes et al., 1977; Uehleke and Werner, 1975; Ilett et al., 1973). This metabolic step has been suggested by Mansuy et al. (1977) and Pohl et al. (1977) as the precursor of phosgene formed by rat microsomes *in vitro* from chloroform. Phosgene was confirmed as a metabolite by reaction with cysteine to give 2-oxothiazolidine-4-carboxylic acid which was

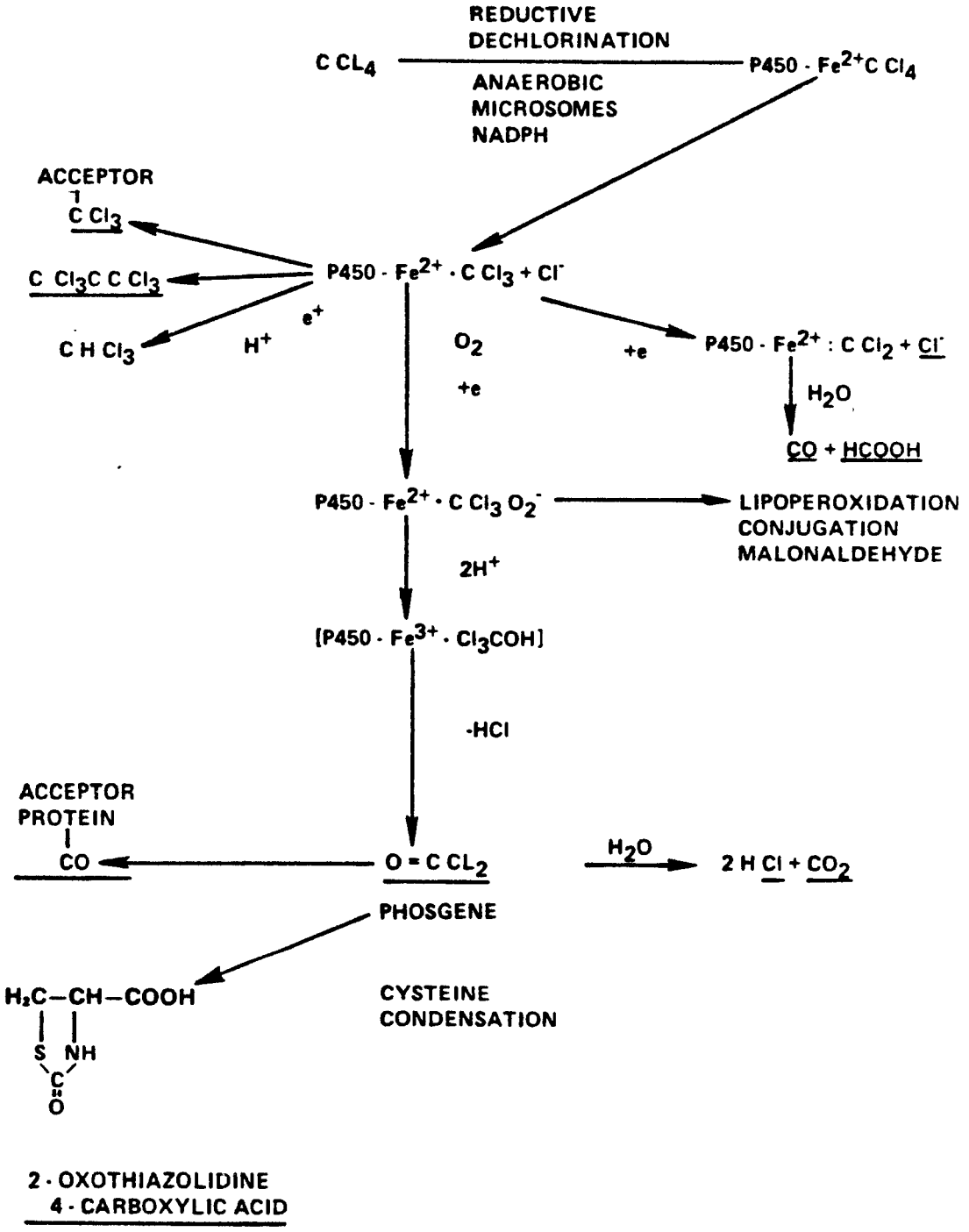

Figure 4-7. Metabolic pathways of carbon tetrachloride biotransformation. ($C Cl_4$ metabolites identified are underlined).

Source: Shah et al. (1979).

identified by GC-CIMS. Trichloromethanol is highly unstable and spontaneously dehydrochlorinates to produce phosgene (Seppelt, 1977). The electrophilic phosgene reacts with water to yield CO_2, a known metabolite of $CHCl_3$ *in vitro* (Rubinstein and Kanics, 1964; Paul and Rubinstein, 1963) and *in vivo* (Brown et al., 1974; Fry et al., 1972), with protein to form a covalently bound product (Pohl et al., 1977; Sipes et al., 1977; Uehleke and Werner, 1975; Brown et al., 1974; Ilett et al., 1973), or with cysteine (Pohl et al., 1977), and possibly with glutathione (Docks and Krishna, 1976; Brown et al., 1974). The finding that deuterated chloroform ($CDCl_3$) depletes glutathione in the livers of rats less than $CHCl_3$ supports this notion (Docks and Krishna, 1976).

The postulated oxidation of the C-H bond of chloroform by P_{450} to produce trichloromethanol which spontaneously yields phosgene is further supported by the observations of Pohl and Krishna (1978). These workers found that chloroform metabolism to phosgene by rat liver microsomes is oxygen and NADPH dependent, and inhibited by CO and SKF 525-A. Moreover, in the presence of cysteine and $^{18}O_2$ atmosphere, $^{18}O_2$ is incorporated into the 2-oxo position of 2-oxothiazolidine-4-carboxylic acid. Oxidative cleavage of the C-H bond appears to be the rate-determining step, since deuterium labeled chloroform ($CDCl_3$) is biotransformed into phosgene slower than $CHCl_3$; $CDCl_3$ appears also to be less hepatotoxic than $CHCl_3$. Pohl (1980) has further characterized the metabolism of chloroform in rat liver microsomes by measuring the covalent binding of $^{14}CHCl_3$ and C^3HCl_3 to microsomal protein. Chloroform does not appear to be activated by reductive dechlorination to the radical $\cdot CHCl_2$, because the ^3H-label does not bind to microsome protein as does the ^{14}C-label.

Figure 4-7 summarizes current knowledge of the biotransformation of carbon tetrachloride. The first step is a rapid reductive formation of the trichloromethyl ($\cdot CCl_3$) radical by complexing with one or more of the P_{450} cytochromes

(Shah et al., 1979; Poyer et al., 1978; Recknagel and Glende, 1973). This radical undergoes several reactions in addition to binding to lipids (Villarruel and Castro, 1975; Uehleke and Werner, 1975; Villarruel et al., 1975; Gordis, 1969; Reynolds, 1967) and protein (Uehleke et al., 1977; Uehleke and Werner, 1973), although not to nucleic acids (Uehleke et al., 1977; Uehleke and Werner, 1975; Reynolds, 1967). Anaerobically, the addition of a proton and electron yields chloroform (Glende et al., 1976; Uehleke et al., 1973; Fowler, 1969; Butler, 1961), dimerization to hexachloroethane (Uehleke et al., 1973; Fowler, 1969), or further reductive dechlorination to CO via the carbene, : CCl_2 (Wolf et al., 1977). Aerobically, the $\cdot CCl_3$ radical is oxidized by the P_{450} system to trichloromethanol (Cl_3COH), which is the precursor of phosgene (Cl_2CO) that Weinhouse and colleagues (Shah et al., 1979) have shown to be an intermediate in carbon tetrachloride metabolism by rat liver homogenates. Hydrolytic dechlorination of phosgene yields CO_2 (Shah et al., 1979).

Under normal physiological conditions (i.e., aerobic conditions), a minimal formation of chloroform might be expected to occur. Carbon tetrachloride yields a chloroform most readily in vitro under anaerobic conditions and its formation is inhibited by oxygen (Uehleke et al., 1977; Glende et al., 1976). Shah et al. (1979) observed that chloroform does not compete successfully with carbon tetrachloride for initial binding to P_{450} cytochrome (Sipes et al., 1977; Recknagel and Glende, 1973). Wolf et al. (1977) also found that binding of chloroform to reduced cytochrome P_{450} was very slow compared to that of carbon tetrachloride.

Anders and coworkers (Anders et al., 1978; Ahmed et al., 1977) have shown, as they have for dihalomethanes, that trihalomethanes, including chloroform, also yield CO as a metabolite. Intraperitoneal administration of haloforms (1 to 4 mmoles/kg) to rats led to dose-dependent elevations in blood CO levels. Treatment of the rats with either phenobarbital (but not 3-methyl-cholanthrene) or SKF

525-A, respectively, increased or decreased metabolism to CO. The order of yield of CO from the iodoforms was greatest for iodoform > bromoform > chloroform for the same dose. Thus, chloroform was minimally metabolized to CO (i.e., to less than one-tenth of that for iodoform or bromoform). Similar findings were made by these workers with rat liver microsomes (Ahmed et al., 1977). Metabolism of the haloforms to CO by rat liver microsomes required NADPH, could proceed anaerobically but was increased 2-fold by O_2, was increased by pretreatment with phenobarbital and inhibited by SKF 525-A or $COCl_2$ pretreatment, and was stimulated by glutathione or cysteine addition in both anaerobic (3-fold) or aerobic (8-fold) conditions. These results suggested that haloforms were metabolized to CO via a cytochrome P_{450} dependent system. However, chloroform was a poor substrate compared to iodoform or bromoform, yielding <2% of its quantity of CO as formed from equimolar concentrations of these halomethanes. Wolf et al. (1977) also found that chloroform, to a very limited extent, was metabolized to CO by reduced rat P_{450} preparations. These workers investigated the spectral and biochemical interactions of a series of halogenated methanes with rat liver microsomes under anaerobic reducing conditions. Tetra- (e.g., CCl_4) and trihalogens (e.g., $CHCl_3$) all formed complexes with reduced cytochrome P_{450} with absorption peak at 460 to 465. A shift to 454 occurred with CO formation and subsequent complexing of CO to P_{450}. CO formation required NADPH, was higher in microsomes from phenobarbital and 3-methylcholanthrene-treated rats, and was not found at high oxygen concentrations (<8%). Figure 4-8 shows the relative rates of CO formation from carbon tetrachloride and other polyhalomethanes. Chloroform, in comparison to carbon tetrachloride, was a very poor reaction substrate, and binding of chloroform to reduced cytochrome P_{450} was extremely slow compared to that of carbon tetrachloride. Wolf et al. (1977) proposed the reduction sequence shown in Figures 4-6 and 4-8 for the reductive dechlorination of chloroform and of

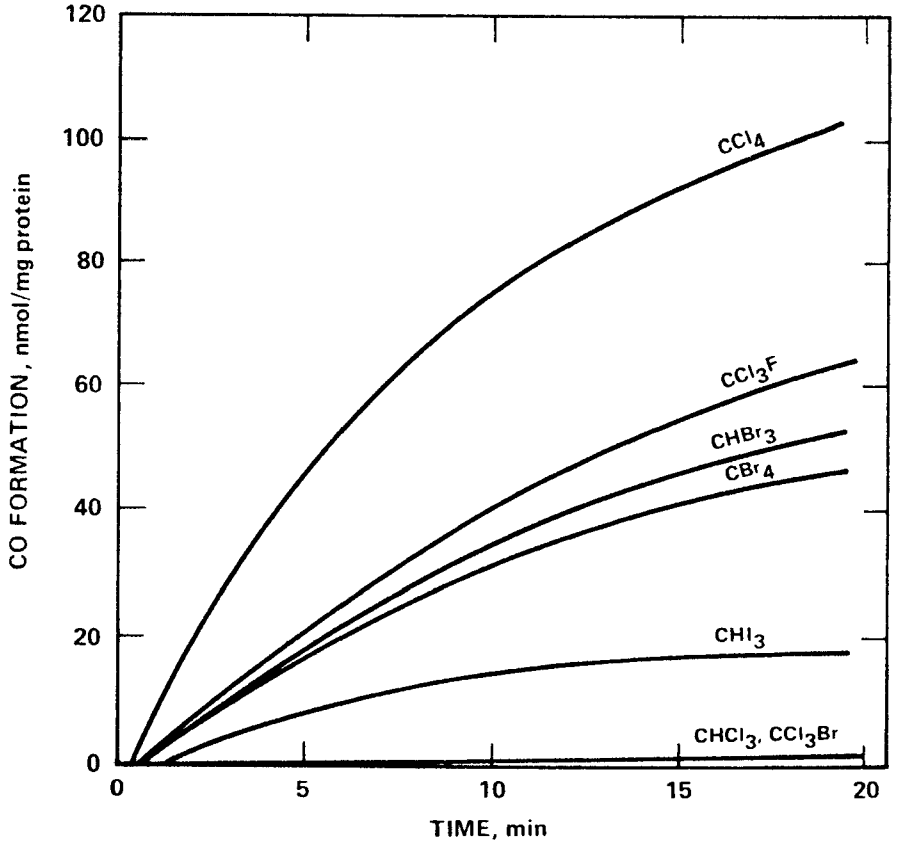

Figure 4-8. Rate of carbon monoxide formation after addition of various halomethanes to sodium dithionite-reduced liver microsomal preparations from phenobarbitol-treated rats. Note the low rate of metabolism of chloroform to CO compared to carbon tetrachloride.

Source: Wolf et al. (1977).

carbon tetrachloride to yield CO via a carbene (CCl_2) intermediate. The physiological importance of this pathway of metabolism appears to be more significant for carbon tetrachloride than for chloroform.

4.6. COVALENT BINDING TO CELLULAR MACROMOLECULES

4.6.1. Proteins and Lipids. Reactive intermediates of the metabolism of chloroform (phosgene, carbene, •Cl) and carbon tetrachloride ($•CCl_3$, phosgene, carbene, •Cl) that irreversibly bind to cellular macromolecules (covalent binding) are generally believed to result in an alteration of cellular integrity, which leads to centrolobular hepatic necrosis and renal proximal tubular epithelial damage. Chloroform, mole for mole, is generally accepted to be less hepatotoxic than carbon tetrachloride (Brown, 1972; Klaassen and Plaa, 1969; Plaa et al., 1958).

Chloroform in non-lethal doses produces renal damage in mice, dogs, and man; (Bhoosan et al., 1977; Pohl et al., 1977; Ilett et al., 1973; Klaassen and Plaa, 1966, 1967; Bennet and Whigham, 1964; von Oettingen, 1964; Conlon, 1963; Plaa et al., 1958; Culliford and Hewitt, 1957; Hewitt, 1956; Shubik and Ritchie, 1953) whereas, in experimental animals, carbon tetrachloride does not do so (Storms, 1973; Klaassen and Plaa, 1966; Plaa and Larson, 1965; Bennet and Whigham, 1964; Culliford and Hewitt, 1957; Hewitt, 1956; Shubik and Ritchie, 1953), although it does in man (New et al., 1962; Guild et al., 1958). To explain these species differences in toxicity as well as known intraspecies (Hill et al., 1975; Deringer et al., 1953; Shubik and Ritchie, 1953) and sex differences (Taylor et al., 1974; Ilett et al., 1973; Bennet and Whigham, 1964; Culliford and Hewitt, 1957; Hewitt, 1956; Deringer et al., 1953; Shubik and Ritchie, 1953; Eschenbrenner and Miller, 1945b), prevailing concepts implicate (1) differences

in the rates of metabolism and organ system capacities for metabolism, which in turn determines the amount of irreversible macromolecular binding and (2) differences in the enzyme pathways for metabolism of the two haloalkanes (Figures 4-6 and 4-7).

Carbon tetrachloride, by a reductive dechlorination via complexing with reduced P_{450}, yields the trichloromethyl free radical ($\cdot CCl_3$) (Recknagel and Glende, 1973; Slater, 1972) (Figure 4-7), which can covalently bind to lipid and protein (Shah et al., 1979; Villarruel et al., 1975; Castro and Diaz Gomez, 1972; Reynolds, 1967), and can also initiate peroxidation of polyenoic fatty acids (Slater, 1972; Recknagel and Ghoshal, 1966). Chloroform does not appear to be activated to free radicals ($\cdot CCl_3$ or $\cdot CHCl_3$), but does bind covalently to liver lipid and protein (Sipes et al., 1977; Docks and Krishna, 1976; Uehleke and Werner, 1975; Brown et al., 1974; Ilett et al., 1973), and initiates lipid peroxidation in some circumstances (Koch et al., 1974; Ilett, 1973; Brown, 1972; Slater, 1972). Several investigators have shown that diene conjugates (products of lipoperoxidation) are not increased *in vivo* in normal rats when chloroform is inhaled or injected intraperitoneally (Brown et al., 1974; Brown, 1972; Klaassen and Plaa, 1969), but only when rats are pretreated with phenobarbital and the metabolism of chloroform is greatly enhanced (Brown et al., 1974; Brown, 1972). Studies *in vitro* show diene conjugation and malonaldehyde formation (an index of lipoperoxidation) by microsomes of phenobarbital pretreated rats were not increased but decreased by the addition of chloroform (Brown, 1972; Klaassen and Plaa, 1969), suggesting that with isolated microsomes, metabolism of chloroform is too small for sufficient quantities of reactive intermediates to accumulate and initiate lipoperoxidation. However, Rubinstein and Kanics (1964) found chloroform to be more rapidly metabolized by rat microsomal fractions than carbon tetrachloride (see Table 4-11). These findings indicate that differences in

TABLE 4-12

Covalent Binding of Radioactivity From ^{14}C-Chloroform and ^{14}C-Carbon Tetrachloride in Microsomal Incubation *In Vitro*[a]

	Incubation Condition	Microsomal[b] Protein	Microsomal[b] Lipid	To Added serum albumin
		nmol/mg in 60 minutes		
$^{14}C\text{-}CCl_4$	N_2	20.0	76.0	1.4
$^{14}C\text{-}CHCl_3$	N_2	5.1	4.1	0.9
	O_2	8.5	7.0	1.7

[a]Source: Uehleke et al., 1977

[b]Microsomes from phenobarbital pretreated rabbits

metabolic activation [carbon tetrachloride to produce free radicals (Figure 4-7), but chloroform primarily to phosgene (Figure 4-6)] explain the greater potential of carbon tetrachloride for initiating lipoperoxidation. Table 4-11 shows the data of Uehleke et al. (Uehleke et al., 1977; Uehleke and Werner, 1975) for the covalent binding of rabbit microsomes following incubation with ^{14}C-labeled chloroform and carbon tetrachloride. Both protein and lipid binding of ^{14}C-radioactivity are 4-fold and 20-fold, respectively, more extensive for carbon tetrachloride than chloroform; lipids are labeled preferentially by carbon tetrachloride but are not by chloroform. Furthermore, covalent binding from chloroform metabolism occurs mainly with anaerobic conditions (a minor metabolic pathway) and is not greatly increased with aerobic metabolism, the major pathway for metabolism of chloroform, which is O_2 dependent (Figure 4-6).

Covalent binding occurs preferentially to lipids and proteins of the endoplasmic reticulum proximate to P_{450} system for metabolism. However, considerable covalent binding from chloroform metabolites occurs in other cell fractions of liver and kidney, particularly to mitochondria (Uehleke and Werner, 1975; Hill et al., 1975). Hill et al. (1975) found when C57BL male mice were injected interperitoneally with 0.07 mℓ/kg ^{14}C-chloroform in oil and sacrificed 12 hours later, that in the liver, 50% of the radioactivity was irreversibly bound to microsome, 23% to mitochondria, 25% to cytosol, and <2% to nuclei; for kidney, 38% of radioactivity was bound to microsome, 39% to mitochondria, 22% to cytosol, and <2% to nuclei. A similar distribution was found in male NMRI mice by Uehleke and Werner (1975), who observed minimal binding to microsomal RNA but significant binding to nicotine-adenine nucleotides. The data of Ilett et al. (1973), shown in Figure 4-9, demonstrates that in C57 BL/6 mice, the amount of covalent binding in liver and kidney microsomal fractions increases proportionally with the chloroform dose.

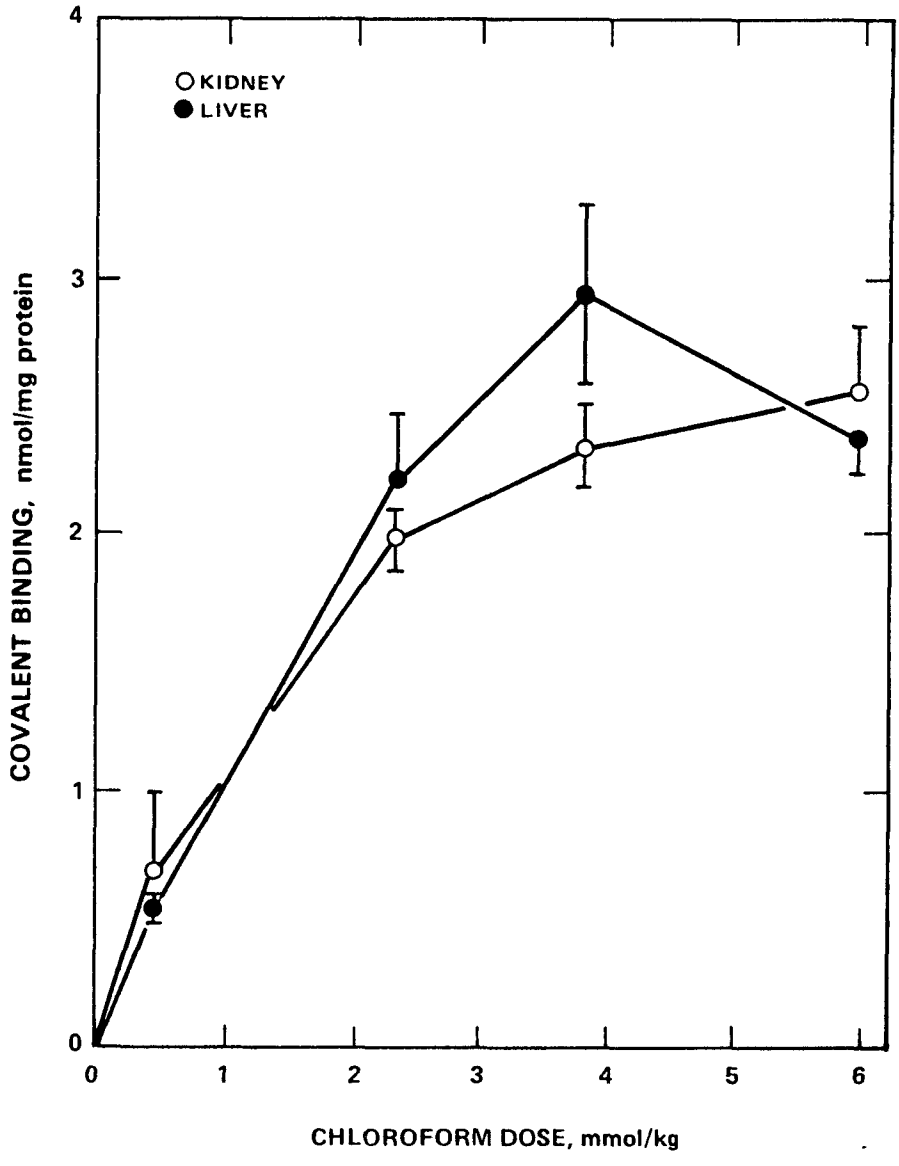

Figure 4-9. Effect of increasing dosage of i.p.-injected ^{14}C-chloroform on extent of covalent binding of radioactivity in vivo to liver and kidney proteins of male mice 6 hours after administration.

Source: Ilett et al. (1973).

4.6.1.1. GENETIC STRAIN DIFFERENCE -- Hill et al. (1975) described in mice two genetic variations in chloroform toxicity paralleling genetic differences in covalent binding in liver and kidney. In one inbred strain (DBA/2), the male animals were 4 times more sensitive to the lethal effects of oral doses of chloroform (LD_{50} of 0.08 mℓ/kg) than the second strain (C57 BL/6, LD_{50} of 0.33 mℓ/kg). Males of the F_1 hybrid strain ($B6D2F_1/J$) had an intermediate LD_{50} of 0.2 mℓ/kg, midway between those of the two parental strains. The susceptibility of DBA mice was related to a dose-dependent necrosis of the proximal convoluted renal tubules. However, mice of all three genotypes that received >0.17 mℓ/kg chloroform exhibited both renal tubular necrosis and hepatic centrolobular necrosis. Males and females of the same strain exhibited similar dose thresholds to hepatic damage, but females died of chloroform-induced hepatic damage without developing renal lesions. This sex-related absolute difference is dependent on androgen profile of the mice; testosterone-treated females become sensitive to renal toxicity (Bennet and Whigham, 1964; Culliford and Hewitt, 1957; Eschenbrenner and Miller, 1945b).

Table 4-12 shows the extent of covalent binding in liver and kidney of these three strains after a single intraperitoneal injection of ^{14}C-chloroform (0.07 mℓ/kg) to the males. Kidney homogenates from DBA/2J male mice, more sensitive to renal necrosis, contained more than 2-fold as much radioactivity as those from resistant C57BL/6J; covalent binding in the F_1 hybrid was intermediate, as expected. A significant difference was also noted in labeling of kidney subcellular fractions. While all subcellular fractions of susceptible male DBA mice were labeled to a greater extent than F_1 or C57BL strains, the greatest increase was in labeling of the mitochondrial fraction.

TABLE 4-13

Mouse Strain Difference in Covalent Binding of Radioactivity From ^{14}C-Chloroform[a,b]

Tissue	Specific Activity Relative to C57 BL		
	DBA	F_1	C57BL
		Tissue homgenates	
Liver	0.82	0.96	1.00
Kidney	2.41	1.64	1.00
		Subcellular fractions	
Liver			
Nuclei	0.67	0.76	1.00
Mitochondria	1.14	1.14	1.00
Microsomes	0.64	0.73	1.00
Cell sap	0.98	1.09	1.00
Kidney			
Nuclei	2.20	1.67	1.00
Mitochondria	3.67	1.97	1.00
Microsomes	1.74	1.44	1.00
Cell sap	1.65	1.23	1.00

[a]Source: Hill et al., 1975

[b]Adult male mice of each genotype given ^{14}C-chloroform (0.07 mℓ/kg) intraperitoneally and sacrified 12 hours later. Genotype comparisons are given as ratio of radioactivity to C57BL = 1.

In the liver, the distribution of covalent binding was generally opposite to that observed in the kidneys (Table 4-12), but neither liver homogenates nor subcellular fractions showed significant strain differences.

4.6.1.2. SEX DIFFERENCE -- Kidneys of male mice are known to covalently bind more ^{14}C-chloroform radioactivity than do those of females, but females bind more in the liver than males (Taylor et al., 1974; Ilett et al., 1973) (see Tables 4-7 and 4-13). Table 4-14 shows that pretreatment of male mice with phenobarbital increases covalent binding in the liver but not in the kidney (Ilett et al., 1973). A similar observation has been made by Kluwe et al. (1978) in male mice. They found that phenobarbital increased liver but not kidney microsomal activity; 3-methylcholanthrene, dioxin, and PCGs increased both liver and kidney microsomal enzyme activities. From the renal and hepatic toxicity profile to chloroform displayed by mice treated with these various inducers, these investigators concluded that the chloroform metabolite(s) responsible for hepatic damage is probably generated in the liver, and the metabolite(s) responsible for renal damage is generated in the kidney.

4.6.1.3. INTER-SPECIES DIFFERENCE -- In addition to intra-species strain (mice) differences in covalent binding noted above, Uehleke and Werner (1975) have also observed an apparent inter-species difference. Figure 4-10 shows the *in vitro* binding of radioactivity from ^{14}C-chloroform by microsomal preparations from rat, mouse, rabbit, and man. Human and rabbit microsomes have the highest rate of covalent binding from chloroform, with the mouse followed by the rat considerably lower. Inter-species differences in the covalent binding rates for carbon tetrachloride were small. These species differences in binding of chloroform metabolites to protein and lipid *in vitro* do not, however, parallel the

TABLE 4-14

In Vivo Covalent Binding of Radioactivity From $^{14}CHCl_3$ in Liver and Kidney of Male and Female Mice (C57BL/6)[a,b]

	Covalent Binding of ^{14}C-Chloroform nmoles/mg protein + S.E.	
	Male	Female
Liver	2.92 ± 0.35	3.66 ± 0.39
Kidney	2.34 ± 0.16	0.39 ± 0.02

[a]Source: Ilett et al., 1973

[b]Mice were sacrificed 6 hours after intraperitoneal administration of 3.72 nmoles/kg of $^{14}CHCl_3$.

TABLE 4-15

In Vitro Covalent Binding of Radioactivity from $^{14}CHCl_3$ to Microsomal Protein from Liver and Kidney of Male and Female Mice (C57BL/6)*

	Pretreatment	Covalent Binding of ^{14}C-Chloroform p moles/mg protein/5 minutes + SEM	
		Liver	Kidney
Male	NA	572 ± 54	44.6 ± 4.1
Male	Phenobarbital	1454 ± 143	41.0 ± 3.2
Female	NA	419 ± 20	14.6 ± 2.5

*Source: Ilett et al., 1973.

NA = Not applicable

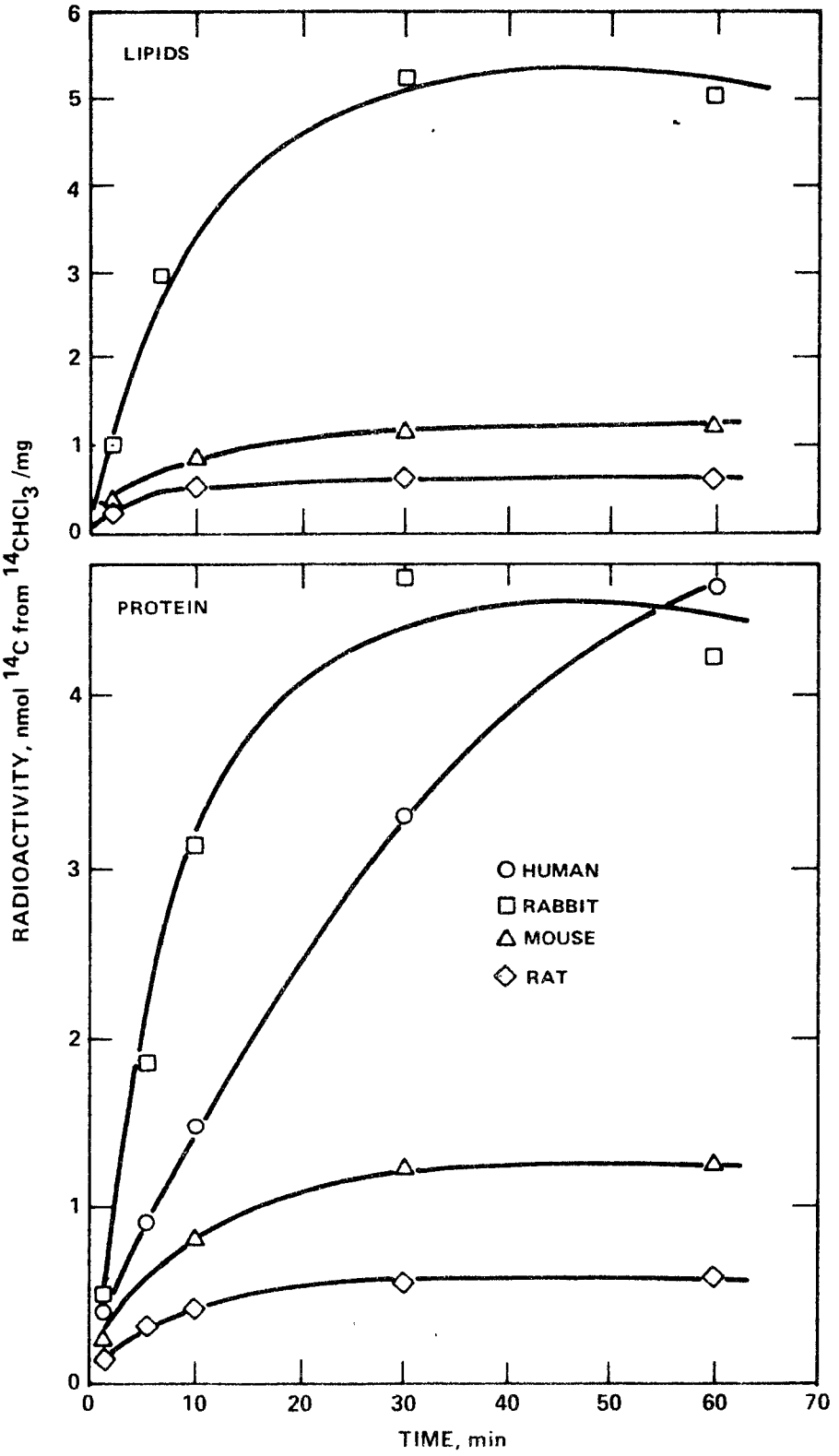

Figure 4-10. Comparison of irreversible binding of radioactivity from ^{14}C-$CHCl_3$ to protein and lipid of microsomes from normal rabbit, rat, mouse, and human liver incubated in vitro at 37°C in O_2.

Source: Uehleke and Werner (1975).

species differences in metabolism of chloroform *in vivo*, as measured by the conversion of chloroform to CO_2 (Table 4-9); *in vivo*, the mouse has the greatest capacity to metabolize chloroform (80%), followed by rats (65%), nonhuman primates (20%), and man (30 to 50%).

4.6.1.4. AGE DIFFERENCE -- Uehleke and Werner (1975) have shown that irreversible protein binding of radioactivity from ^{14}C-chloroform and ^{14}C-carbon tetrachloride to liver microsomes of newborn rats (18 hours old) is low compared to that of microsomes from adult rats (32 days); however, the binding was shown to be proportional to P_{450} content of the microsomes, which was proportionally low in microsomes from newborn rats.

4.6.2. Nucleic Acids. P_{450} systems activate chloroform and carbon tetrachloride *in vivo* and *in vitro* to reactive metabolites that extensively covalently bind to proteins and lipids, but do so only minimally to nucleic acid (Uehleke et al., 1977; Wolf et al., 1977; Uehleke and Werner, 1975; Fowler, 1969; Reynolds, 1967), unlike many other carcinogens that bind DNA. Reitz et al. (1980) measured DNA alkylation in liver and kidneys of mice after an oral dose of 240 mg/kg ^{14}C-chloroform (specific activity not given) and found values of 3×10^{-4} and 1×10^{-4} mol % for liver and kidney DNA respectively. These workers judged that chloroform has very little direct interaction with DNA when compared to known carcinogens, as reported in the literature for dimethylnitrosamine (3.5×10^{-1} mol % alkylation, liver DNA), dimethylhydralazine (2.6×10^{-2}, colon DNA) and N-methyl-N-nitrosourea (1.5×10^{-1}, brain DNA) but given by parenteral routes (Pegg and Hui, 1978; Cooper et al., 1978; Kleihues and Margison, 1974). The failure of chloroform or carbon tetrachloride reactive species to significantly bind DNA has been ascribed to their short half-life compared to epoxides, and to

their lack of nuclear penetration. Recently, however, Diaz Gomez and Castro (1980) have shown that highly purified rat liver nuclear preparations are able to anaerobically activate carbon tetrachloride, and to aerobically activate chloroform to reactive metabolites that bind to nuclear lipids and proteins. Their data, given in Table 4-15, show that activity in nuclear preparations is smaller than in microsomes, but within the same order of magnitude. These results might be relevant to the hepatocarcinogenic effects of chloroform and carbon tetrachloride in mice and rats, since the nuclear targets (DNA, RNA, nuclear proteins) are in the immediate neighborhood sites of activation, thus, making unnecessary the present assumption that the highly reactive intermediates ($\cdot CCl_3$, phosgene, malonaldehyde, or carbene), produced at the endoplasmic reticulum, must travel to the nucleus.

4.6.3. Role of Phosgene. Phosgene is a prominent intermediate of both chloroform and carbon tetrachloride metabolisms (Figures 4-6, 4-7). It is known to be highly reactive and toxic to cells and tissues (Pawlowsky and Frosolono, 1977), and its two highly reactive chlorines suggest that it could act on cellular macromolecules similar to bifunctional alkylating agents. Reynolds (1967) showed that ^{14}C-phosgene, given to intact rats, labeled liver protein (and lipids to a smaller extent). The pattern of labeling was quite different from that of ^{14}C-carbon tetrachloride and more similar to ^{14}C-chloroform. Moreover, ^{36}Cl-carbon tetrachloride radioactivity was also stably incorporated into liver lipid and protein, pointing to the $\cdot CCl_3$ radical rather than phosgene as the reactive form for carbon tetrachloride that labels lipid. Cessi et al. (1966) also reported that ^{14}C-phosgene labeled terminal amino group of polypetides in a manner similar to in vivo protein labeling produced by carbon tetrachloride.

TABLE 4-16

Covalent Binding of Radioactivity from ^{14}C-Chloroform and ^{14}C-Carbon Tetrachloride in Rat Liver Nuclear and Microsomal Incubation *In Vitro**

	Incubation Condition	Protein	Lipid
		p mol/mg ± S.D.	
$^{14}C\text{-}CCl_4$	N_2		
Nuclear		21.9 ± 2.5	147 ± 12
Microsomal		50.3 ± 4	190 ± 11
$^{14}C\text{-}CHCl_3$	O_2		
Nuclear		27.0 ± 3	20 ± 3
Microsomal		68.0 ± 9	57 ± 8

*Source: Diaz Gomez and Castro, 1980

4.6.4. Role of Glutathione. Ekstrom and Hogberg (1980) found that chloroform, in freshly isolated rat liver cells, induced depletion of cellular glutathione. Brown et al. (1974) demonstrated that exposure of rats to an atmosphere of 0.5% chloroform for 2 hours markedly decreased glutathione (GSH) in the liver when the animals were pretreated with phenobarbital to stimulate metabolism. GSH liver content of untreated rats was not decreased. Phenobarbital pretreatment has been shown to markedly potentiate toxicity of both chloroform and carbon tetrachloride in rats (Docks and Krishna, 1976; Cornish et al., 1973; McLean, 1970; Scholler, 1970). However, it has not been possible to detect a decrease in the liver glutathione levels following administration of carbon tetrachloride or trichlorobromomethane (Docks and Krishna, 1976; Boyland and Chasseaud, 1970). Sipes et al. (1977) have shown that the addition of GSH liver microsomes from phenobarbital pretreated rats incubated in vitro with ^{14}C-labeled halocarbons, chloroform, carbon tetrachloride, and trichloromomethane, inhibited covalent binding ≈80% for all three compounds. Their results, given in Table 4-16, also show the effects of anaerobic and aerobic conditions on covalent binding. The reduction in binding of chloroform by an atmosphere of N_2 suggests that its bioactivation is mediated by a cytochrome P_{450} oxidative pathway to phosgene (Figure 4-6), while the enhanced binding of carbon tetrachloride in N_2 reflects P_{450} mediated reductive pathways (Figure 4-7) and formation of free radical. These investigators suggest that in phenobarbital-treated animals, chloroform depletes liver GSH by the formation of conjugate between the reactive intermediate phosgene and GSH (Docks and Krishna, 1976; Brown et al., 1974). In the case of carbon tetrachloride, they suggest that GSH addition in vitro (Table 4-16) also conjugates with the phosgene metabolite of carbon tetrachloride produced when incubated in air, but, in addition, GSH decreases the levels of $\cdot CCl_3$ by reducing the free radical to chloroform. In vivo, it is postulated that

TABLE 4-17

Effect of Glutathione, Air, N_2 or CO: O_2 Atmospere on the *In Vitro* Covalent Binding of C Cl_4, $CHCl_3$ and C Br Cl_3 to Rat Liver Microsomal Protein[a]

Incubation Conditions	Substrate		
	CCl_4[b]	$CHCl_3$	$CBrCl_3$
	p moles ^{14}C-bound/mg microsomal protein/minute		
Air	97 ± 10	59 ± 5	1456 ± 66
N_2	310 ± 51	21 ± 1	1370 ± 143
$CO:O_2$(8:2)	18 ± 1	20 ± 1	853 ± 62
SKF 525 A (0.5 mM), Air	109 ± 5	7 ± 7	2105 ± 159
Glutathione, Air	17 ± 2	15 ± 2	218 ± 25
NADPH omitted, Air	6 ± 1	3 ± 0	65 ± 13

[a]Source: Sipes et al., 1977

[b]^{14}C-labeled substrate is a final concentration of 1 x 10^{-3}M incubated at 37°C. Microsomes were from phenobarbital pretreated rats.

the oxidized glutathione may be reduced back to reduced GSH by glutatione reductase; this would explain the lack of fall of liver GSH content with carbon tetrachloride *in vivo* (Gillette, 1972). Thus, the toxic effects of chloroform and carbon tetrachloride may be mediated through different mechanisms of covalent binding, and GSH may play different roles for these chlorocarbons in preventing covalent binding of reactive intermediates of metabolism.

Chloroform and carbon tetrachloride are known to cause greater liver damage in fasted animals than in fed animals (Diaz Gomez et al., 1975; Jaeger et al., 1975; Krishnan and Stenger, 1966; Goldschmidt et al., 1939; Davis and Whipple, 1919). For chloroform, a decreased content of hepatic GSH from fasting has been postulated to be responsible for the increased susceptibility of fasted mice (Docks and Krishna, 1976; Brown et al., 1974). Nakajima and Sato (1979) have recently offered an additional explanation. These investigators studied the metabolism of the chlorocarbons *in vitro* with microsomes from livers of fasted rats, and found that the disappearance of chloroform from incubation increased 3-fold for a 24 hour fast, although fasting produced no significant increase in the microsomal protein and cytochrome P_{450} liver contents (Table 4-17). Similar results were obtained for carbon tetrachloride. These observations suggest that the increased toxicity of chloroform and carbon tetrachloride from food deprivation may be due not only to decreased GSH, but also to a greater production of reactive intermediates and covalent binding to cellular macromolecules.

SUMMARY

At ambient temperatures, chloroform is a volatile liquid with high lipid solubility and appreciable solubility in water. Hence, chloroform is readily absorbed into the body through the lungs and intestinal mucosa; the portals of

TABLE 4-18

Effects of 24-Hour Food Deprivation on Chloroform and Carbon Tetrachloride
In Vitro Microsomal Metabolism, Protein, and P-450 Liver Contents of Rats*

	Male			Female		
	Fed	Fasted	Ratio	Fed	Fasted	Ratio
			Metabolism, nmole/g/min			
Chloroform	19.7 ± 2.6	55.1 ± 7.5	2.8	15.3 ± 6.8	39.3 ± 2.5	2.6
Carbon tetrachloride	1.9 ± 0.2	5.9 ± 0.8	3.1	1.1 ± 0.5	4.5 ± 0.3	4.1
			Protein content, mg/kg liver			
	27.7 ± 3.7	23.0 ± 2.7	NR	22.5 ± 1.5	23.7 ± 1.7	NR
			P-450, nmol/mg protein			
	0.842 ± 0.123	0.823 ± 0.03	NR	0.638 ± 0.051	0.673 ± 0.044	NR

*Source: Nakajima and Sato, 1979

NR = Not reported

entry with exposure from air, water and food. Few data are available on the pharmacokinetics of absorption and excretion of chloroform in man, particularly at the low exposure concentrations expected in ambient air and drinking water. However, studies show absorption from the gastrointestinal tract in man, monkeys, rats and mice is rapid and complete, occurring by first-order passive absorptive processes. A dose-dependent first-pass effect with pulmonary elimination of unchanged chloroform occurs with oral ingestion in man, thus decreasing the amount of chloroform reaching the systemic circulation. In rats, the kinetics of peroral absorption are also influenced by the dosing vehicle; the absorption rate is decreased for chloroform given in corn oil vehicle as compared to an aqueous solution. Pulmonary uptake and elimination occur also by first-order diffusion processes with three distinct components with rate constants corresponding to tissue loading or desaturation of at least three major body compartments. Half-times in man have been found to be approximately 14-30 minutes, 90 minutes and 24-36 hours, respectively. The longest half-time is associated with the lipids and the adipose tissue compartment. During inhalation exposure, at equilibrium with inspired air concentration, the blood/air partition coefficient is about 8 at 37°C and the adipose tissue/blood partition coefficient is 280 at 37°C. The quantity of chloroform absorbed is dependent also on body weight and fat content of the body.

Tissue distribution of chloroform is consistent with its lipophilic nature and modest water solubility. This chloroalkane readily crosses the blood brain and placental barriers and distributes into breast milk. Concentrations occurring in all major tissue organs are dose related to inspired air concentrations or to oral dosage. Relative tissue concentrations occur in the order of adipose tissue > brain > liver > kidney > blood.

Elimination of chloroform from the body occurs by two major and parallel occurring processes: 1) pulmonary elimination of unchanged chloroform by first order kinetics, and 2) metabolism of chloroform. Chloroform is metabolized in the liver, and to a lesser extent in the kidneys and other tissues. Metabolism is dose-dependent and saturable, with a greater proportion of small doses being metabolized. There are striking differences in the pharmacokinetics and quantitative metabolism of chloroform in man as compared to other animals. For large steady-state body burdens, 30-40% is metabolized by man, 20% by the nonhuman primate, > 65% by the rat, and > 85% by the mouse. Metabolism produces phosgene and other putative reactive metabolites that covalently bind extensively to cellular lipids and proteins, although not significantly to DNA or other nucleic acids. The intensity of metabolite binding and organ localization parallel the acute cellular toxicity of chloroform in liver and kidney observed in experimental animals. Both binding and toxicity are highly dependent on animal species and genetic strain, as well as on sex and age. An additional variable is the tissue level of reduced glutathione which plays an important role in protecting against both binding and toxicity. Conversely, inducers of hepatic and renal P450 metabolizing systems increase binding and toxicity.

4.8 REFERENCES

Ahmed, A.E., V.L. Kubic and M.W. Anders. 1977. Metabolism of haloforms to carbon monoxide. I. In vitro studies. Drug Metab. Dispos. 5: 198-204.

Anders, M.W., J.L. Stevens, R.W. Sprague, Z. Shaath and A.E. Ahmed. 1978. Metabolism of haloforms to carbon monoxide. II. In vivo studies. Drug Metab. Dispos. 6: 556-560.

Bellar, T.A., J.J. Lichtenburg and R.C. Kroner. 1974. Occurrence of organohalides in chlorinated drinking waters. Amer. Water Works Assoc. Jour. 66: 703-706.

Bennet, R.A. and A. Whigham. 1964. Chloroform sensitivity of mice. Nature. 204: 1328.

Bhooshan, B., L.R. Pohl and G. Krishna. 1977. A biochemical mechanism for chloroform activation. Fed. Proc. (Abstr). 36: 396.

Boyland, E. and L.F. Chasseaud. 1970. The effect of some carbonyl compounds on rat liver glutathione levels. Biochem. Pharmacol. 19: 1526-1528.

Brown, D.M., P.F. Langley, D. Smith and D.C. Taylor. 1974a. Metabolism of chloroform. I. The metabolism of ^{14}C-chloroform by different species. Xenobiotica. 4: 151-163.

Brown, B.R., K.G. Sipes and A.M. Sagalyn. 1974b. Mechanisms of acute hepatic toxicity: Chloroform, halothane and glutathione. Anesthesiol. 41: 554-561.

Brown, S.S. 1972. In Foreign compound metabolism in mammals. vol. 2. Mathway, D.C., ed. London, The Chemical Society. p. 461.

Butler, T.C. 1961. The reduction of carbon tetrachloride in vivo and reduction of carbon tetrachloride to chloroform in vitro by tissues and tissue constituents. Jour. Pharmacol. Exp. Ther. 134: 311-319.

Castro, J.A. and M.I. Diaz Gomez. 1972. Studies on irreversible binding of $^{14}C\text{-}CHCl_3$ to microsomal lipids in rats under varying experimental conditions. Toxicol. Appl. Pharmacol. 23: 541-552.

Cessi, C., C. Columbini and L. Mameli. 1966. The reaction of liver proteins with a metabolite of carbon tetrachloride. Biochem. Jour. 101: 46c.

Chenoweth, M.B., D.N. Robertson, D.S. Erly and K. Goekhe. 1962. Blood and tissue levels of ether, chloroform, halothane and methoxyflurane in dogs. Anesthesiol. 23: 101-106.

Chiou, W.L. 1975. Quantitation of hepatic and pulmonary first-pass effects and its implications in pharmacokinetic study. I. Pharmacokinetics of chloroform in man. J. Pharmac. Biopharmaceut. 3: 193-201.

Cohen, E.N. and W. Hood. 1969. Application of low-temperature autoradiography studies of the uptake and metabolism of volatile anesthetics in the mouse. Anesthesiol. 30: 306-314.

Conkle, J.P., B.J. Camp and B.E. Welch. 1975. Trace composition of human respiratory gas. Arch. Environm. Health. 30: 290-295.

Conlon M.F. 1963. Addiction to chlorodyne. Brit. Med. Jour. 2: 1177-1178.

Cooper, H.K., J. Buecheler and P. Kleihues. 1978. DNA alkylation in mice with genetically different susceptibility to 1,2-dimethylhydrazine-induced colon carcinogenesis. Cancer Res. 38: 3063-3065.

Cornish, H.H., B.P. Ling and M.L. Barth. 1973. Phenobarbital and organic solvent toxicity. Amer. Ind. Hyg. 34: 487-492.

Culliford, D. and H.B. Hewitt. 1957. The influence of sex hormone status on the susceptibility of mice to chloroform-induced necrosis of renal tubules. Jour. Endocr. 14: 381-393.

Davis, N. and C. Whipple. 1919. The influence of fasting and various diets on the liver injury effected by chloroform anesthesia. Arch. Int. Med. 23: 612-633.

Deringer, M.K., T.B. Dunn and W.E. Heston. 1953. Results of strain C3H mice to chloroform. Proc. Soc. Exp. Biol. Med. 83: 474-479.

Diaz Gomez, M.I. and J.A. Castro. 1980. Nuclear activation of carbon tetrachloride on chloroform. Res. Comm. Chem. Path. Pharmacol. 27: 191-194.

Diaz Gomez, M.I., C.R. DeCastro, E.C. DeFerreyra, N. D'Acosta, O.M. DeFerros and J.A. Castro. 1975. Mechanistic studies on carbon tetrachloride hepatotoxicity in fasted and fed rats. Toxicol. Appl. Pharmacol. 32: 101-108.

Dilley, J.V., N. Chernoff, D. Kay, N. Winslow and G.W. Newell. 1977. Inhalation teratology studies of five chemicals in rats. Toxicol. Appl. Pharmacol. 41: 196.

Docks, E.L. and G. Krishna. 1976. The role of glutathione on chloroform-induced hepatoxicity. Exp. Mol. Pathol. 24: 13-22.

Dowty, B.J., D.R. Carlisle and L.L. Laseter. 1975. New Orleans drinking water sources tested by gas chromatography-mass spectrometry. Occurrence and origin of aromatics and halogenated aliphatic hydrocarbons. Environm. Sci. Technol. 9: 762-765.

Droz, P.O. and J.G. Fernandez. 1977. Effect of physical workload on retention and metabolism of inhaled organic solvents. A comparative theoretical approach and its application with regards to exposure monitoring. Int. Arch. Occup. Environ. Hlth. 38: 231-246.

Eger, E.I. 1963. Mathematical model of uptake and distribution. In: Papper, E.M. and Kitz, R.J. eds. Uptake and Distribution of Anesthetic Agents. McGraw-Hill, New York. pp. 72-103.

Ekstrom, T. and J. Hogberg. 1980. Chloroform induced glutathione depletion and toxicity in freshly isolated hepatocytes. Biochem. Pharmacol. 29: 3059-3064.

Eschenbrenner, A.B. and E. Miller. 1945a. Induction of hepatomas in mice by repeated oral administration of chloroform with observations on sex differences. Jour. Natl. Cancer Inst. 5: 251-255.

Eschenbrenner, A.B. and E. Miller. 1945b. Sex differences in kidney morphology and chloroform necrosis. Science. 102: 302-303.

Feingold, A. and D.A. Holaday. 1977. The pharmacokinetics of metabolism of inhalation anaesthetics. Brit. Jour. Anaesth. 49: 155-162.

Fiserova-Bergerova, V., J. Vlach and J.C. Cassady. 1980. Predictable "individual differences" in uptake and excretion of gases and lipid soluble vapours simulations study. Br. J. Ind. Med. 37: 42-49.

Fiserova-Bergerova, V. and D.A. Holaday. 1979. Uptake and clearance of inhalation anesthetics in man. Drug. Metab. Rev. 9: 43-60.

Fiserova-Bergerova, V., J. Vlach and K. Singhal. 1974. Simulation and prediction of uptake, distribution, and exhalation of organic solvents. Br. J. Ind. Med. 31: 45-52.

Fishbein, L. 1979. Potential halogenated industrial carcinogenic and mutagenic chemicals. II. Halogenated saturated hydrocarbons. Sci. Total Environm. 11: 163.

Fowler, S.J.L. 1969. Carbon tetrachloride metabolism in the rabbit. Brit. Jour. Pharmacol. 37: 733-737.

Fry, J., T. Taylor and D.F. Hathaway. 1972. Pulmonary elimination of chloroform and its metabolite in man. Arch. Int. Pharmacodyn. 196: 98-111.

Geigy Scientific Tables. 1973. Diem, K. and C. Lentner, eds. 7th ed. Ciba-Ceigy, Basle.

Gettler, A.O. 1934. Medicolegal aspects of deaths associated with chloroform or ether. Amer. Jour. Surg. 26: 168-174.

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

Gillette, J.R. 1972. Pharmacology and the future of man. In: Proceeding of the 5th Intern. Cong. Pharmacol. San Francisco, Karger, Basel.

Glende, E.A. Jr., A. M. Hruszkewycz and H.O. Recknagel. 1976. Critical role of lipid peroxidation in carbon tetrachloride-induced loss of aminopyridine demethylase, cytochrome P450 and glucose 6-phosphatase. Biochem. Pharmacol. 25: 2163-2170.

Goldschmidt, S., N.M. Vars and I. Ravdin. 1939. The influence of the foodstuffs upon the susceptibility of the liver to injury by chloroform and the probable mechanism of their action. Jour. Clin. Invest. 18: 277-289.

Gordis, E. 1969. Lipid metabolites of carbon tetrachloride. Jour. Clin. Invest. 48: 203-209.

Guild, W.R., J.V. Young and J.P. Merrill. 1958. Anuria due to carbon tetrachloride intoxication. Ann. Int. Med. 48: 1221-1227.

Hewitt, H.B. 1956. Renal necrosis in mice after accidental exposure to chloroform. Brit. Jour. Exp. Path. 37: 32-39.

Hill, R.N., T.L. Clemens, D.K. Liu, E.S. Vesell and W.D. Johnson. 1975. Genetic control of chloroform toxicity in mice. Science. 190: 159-160.

Ilett, K.F., W.D. Reid, I.G. Sipes and G. Krishna. 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.

Jaeger, R.J., R.B. Connolly and S.D. Murphy. 1975. Short-term inhalation toxicity of halogenated hydrocarbons. Effects on fasting rats. Arch. Environm. Health. 30: 26-30.

Jakobson, I., J.E. Wahlberg, B. Holmberg and G. Johansson. 1982. Uptake via the blood and elimination of 10 organic solvents following epicutaneous exposure to anesthetized guinea pigs. Toxicol. Appl. Pharmacol. 63: 181-187.

Kimura, E.T., D.M. Ebert and B.W. Dodge. 1971. Acute toxicity and limits of solvent residue for sixteen organic solvents. Toxicol. Appl. Pharmacol. 19: 699-704.

Klaassen, C.D. and G.L. Plaa. 1969. Comparison of the biochemical alterations elicited in livers from rats treated with carbon tetrachloride, chloroform, and metahylchloroforms. Biochem. Pharmacol. 18: 2019-2027.

Klaassen, C.D. and G.L. Plaa. 1967. Relative effects of various chlorinated hydrocarbons on liver and kidney function in mice. Toxicol. Appl. Pharmacol. 10: 119-131.

Klaassen, C.D. and C.L. Plaa. 1966. Relative effects of various chlorinated hydrocarbons on liver and kidney function in mice. Toxicol. Appl. Pharmacol. 9: 139-151.

Kleihues, P. and G.P. Margison. 1974. Carcinogenicity of N-methyl-N-nitrosurea. Possible role of excision repair of O^6-methylguanine from DNA. J. Nat. Cancer Inst. 53: 1839-1841.

Kluwe, W.M., K.M. McCormack and J.B. Hook. 1978. Selective modification of the renal and hepatic toxicities of chloroform by induction of drug metabolizing enzyme systems in kidney and liver. Jour. Pharmacol. Exp. Therap. 207: 566-573.

Koch, R.R., E.A. Glende and R.O. Recknagel. 1974. Hepatotoxicity of bromotrichloromethane-bond dissociation energy and lipoperoxidation. Biochem. Pharmacol. 23: 2907-2915.

Krishnan, N. and R. Stenger. 1966. Effects of starvation on the hepatotoxicity of carbon tetrachloride. Amer. Jour. Pathol. 49: 239-246.

Lehman, K.B. and Hasegawa. 1910. Studies of the absorption of chlorinated hydrocarbons in animals and humans. Arch. Hyg. 72: 327-342. (Ger.)

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

McConnell, G., D.M. Ferguson and C.R. Pearson. 1975. Chlorinated hydrocarbons and the environment. Endeavor. 34: 13-18.

McLean, A.E. 1970. The effects of protein deficiency and microsomal enzyme induction by DDT and phenobarbitone on the active toxicity of chloroform. Brit. Jour. Exp. Pathol. 51: 317-321.

Miklashevskii, V.E., V.N. Tugarinova, N.L. Rakhmanina and G.P. Yakovleva. 1966. Toxicity of chloroform administered perorally. Hyg. Sanit. 31: 320-322.

Morris, L.E.V. 1951. Chloroform in blood and respired atmosphere. In: Chloroform, A Study After 100 Years. R.M. Waters, ed., Madison, University of Wisconsin Press. pp. 95-119.

Munson, E.S. 1973. Discussion: Chloroform, halothane and regional anesthesia. Anesth. Analg. 52: 8-9.

Murray, F.J., B.A. Schwetz, J.G. McBride and R.E. Staples. 1979. Toxicity of inhaled chloroform in pregnant mice and their offspring. Toxicol. Appl. Pharmacol. 50: 515-522.

Nakajima T. and A. Sato. 1979. Enhanced activity of liver drug-metabolizing enzymes for aromatic and chlorinated hydrocarbons following food deprivation. Toxicol. Appl. Pharmacol. 50: 549-556.

New, P.S., G.D. Lubash, L. Scherr and A.L. Rubin. 1962. Acute renal failure associated with carbon tetrachloride intoxication. Jour. Amer. Med. Assoc. 181: 903-906.

NIOSH (National Institute for Occupational Safety). 1977b. National Occupational Hazard Survey. Vol. III. Survey Analyses and Supplemental Tables. Cincinnati, USDHEW. pp. 2864-2866.

OSHA (U.S. Occupational Safety and Health Administration). 1978. Air contaminants. U.S. Code Fed. Regl., Title 29, part 1910" 1000. pp. 612-614.

Paul, G.G. and D. Rubinstein. 1963. Metabolism of carbon tetrachloride and chloroform by the rat. Jour. Pharmacol. Exp. Ther. 141: 141-148.

Pawlowsky, R. and M.F. Frosolono. 1977. Effect of phosgene on rat lungs after single high exposure. Arch. Environm. Health. 32: 278-283.

Pegg, A.E. and G. Hui. 1978. Formation and subsequent removal of O^6-methylguanine from deoxyribonucleic acid in rat liver and kidney after small doses of dimethylnitrosamine. Biochem. J. 173: 739-748.

Piersol, G.M., H.J. Tiemen and L.S. Kau. 1933. Fatal poisoning following the ingestion of chloroform. Med. Clin. North Amer. 17: 587-601.

Plaa, G.L. and R.E. Larson. 1965. Relative nephrotoxic properties of chlorinated methane, ethane, and D24 ethylene derivatives in mice. Toxicol. Appl. Pharmacol. 7: 37-44.

Plaa, G.L., E.A. Evans and C.H. Hine. 1958. Relative hepatotoxicity of seven halogenated hydrocarbons. Jour. Pharmacol. Exp. Ther. 123: 224-229.

Pohl, L.R. 1980. Investigations of the mechanism of activation of chloroform. Toxicol. Res. Proj. Director. 5: No. 8.0463.

Pohl, L.R., B. Bhooshan, N.F. Whitaker and G. Krishna. 1977. Phosgene: A metabolite of chloroform. Biochem. Biophys. Res. Commun. 79: 684-691.

Pohl, L.R. and G. Krishna. 1978. Deuterium isotope effect in bioactivation and hepatotoxicity of chloroform. Life Science 23: 1067.

Poyer, J.R., R.A. Floyd, P.B. McCoy, E.G. Janzen and E.R. Davis. 1978. Spin-trapping of the trichloromethyl radical produced during enzymic NADPH oxidation in the presence of carbon tetrachloride or bromotrichloroethane. Biochem. Biophys. Acta. 539: 402-409.

Recknagel, R.O. and A.K. Ghoshal. 1966. Quantitative estimation of peroxidative degeneration of rat liver microsomal and mitochondrial lipids after carbon tetrachloride poisoning. Exp. Molec. Pathol. 5: 413-426.

Rechnagel, R.O. and E.A. Glende, Jr. 1973. Carbon tetrachloride hepatotoxicity: An example of lethal cleavage. CRC Crit. Rev. Toxicol. 2: 263-297.

Reitz, R.H., J.F. Quast, W.T. Scott, P.G. Watanabe and O.J. Behring. 1980. Pharmacokinetics and macromolecular effects of chloroform in rats and mice: Implications for carcinogenic risk estimation. Water Chlorinat. Environ. Impact Health Eff. 3: 983-993.

Reitz, R.H., P.J. Gehring and C.N. Park. 1978. Carcinogenic risk estimation for chloroform: An alternative to EPA's procedures. Food Cosmet. Toxicol. 16: 511-514.

Reynolds, E.S. 1967. Liver parenchymal cell injury. IV. Pattern of incorporaton of carbon and chlorine from carbon tetrachloride into chemical constituents of liver in vivo. Jour. Pharmacol. Exp. Ther. 155: 117-126.

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

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

Scholler, K.L. 1970. Modification of the effects of chloroform on the rat liver. Brit. Jour. Anaesth. 42: 603-605.

Schroeder, H.G. 1965. Acute and delayed chloroform poisoning. A case report. Brit. Jour. Anaesth. 37: 972-975.

Schwenkenbecher. 1904. The absorption capability of the skin. Arch. Anat. Physiol. pp. 121-165.

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

Seppelt, K. 1977. Trifluromethanol, CF_3OH. Angew. Chem. Int. Ed. Engl. 16: 322-323.

Shah, H., S.P. Hartman and S. Weinhouse. 1979. Formation of carbonyl chloride in carbon tetrachloride metabolism by rat liver in vitro. Cancer Res. 39: 3942-3947.

Shubik, P. and A. L. Ritchie. 1953. Sensitivity of male DBA mice to the toxicity of chloroform as a laboratory hazard. Science. 117: 285.

Simpson, J.Y. 1847. On a new anaesthetic agent, more efficient than sulfuric ether. Lancet. 2: 549-550.

Sipes, I.G., G. Krishna and J.R. Gillette. 1977. Bioactivation of carbon tetrachloride, chloroform and bromotrichloromethane: Role of cytochrome P-450. Life Sci. 20: 1541-1548.

Slater, T.F. 1972. Free Radical Mechanisms in Tissue Injury. Pion. Ltd., London.

Smith, A.A., P.O. Volpetto, Z.W. Gremling, M.B. DeVore and A.B. Glassman. 1973. Chloroform, halothane and regional anesthesia. A comparative study. Anesth. Analg. 52: 1-11.

Snow, J. 1858. On chloroform and other anesthetics. London, John Churchill.

Steward, A., P.R. Allot, A.L. Cowles and W.W. Mapleson. 1973. Solubility coefficients for inhaled anaesthetics for water, oil, and biological media. Brit. Jour. Anaesth. 45: 282-293.

Stewart, R.D. 1974. The use of breath analysis in clinical toxicology. In: Essays in Toxicology. New York, Academic Press. Chapter 5.

Stewart, R.D., H.C. Dodd, D.S. Erly and B.B. Holder. 1965. Diagnosis of solvent poisoning. Jour. Amer. Med. Assoc. 193: 1097-1100.

Storms, W.W. 1973. Chloroform parties. Jour. Amer. Med. Assoc. 225: 160.

Symons J.M., T.A. Bellar, J.K. Carswell, J. DeMarco, K.L. Kropp, G.G. Robeck, D.R. Seeger, C.J. Slocum, B.L. Smith and A.A. Stevens. 1975. National Organics Reconnaissance Survey for Halogenated Organics. Amer. Water Works Assoc. Jour. 67: 634-647.

Taylor, D.C., D.M. Brown, R. Kuble and P.F. Langley. 1974. Metabolism of chloroform. II. A sex difference in the metabolism of ^{14}C-chloroform in mice. Xenobiotica. 4: 165-174.

Thompson, D.J., S.D. Warnet and V.V. Robinson. 1974. Teratology studies on orally administered chloroform in the rat and rabbit. Toxicol. Appl. Pharmacol. 29: 348-357.

Torkelson, T.R., F. Oyen and V.K. Rowe. 1976. The toxicity of chloroform as determined by single and repeated exposure of laboratory animals. Amer. Ind. Hyg. Assoc. Jour. 37: 697-704.

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

Tsurata, H. 1977. Percutaneous absorption of organic solvents. 2. A method for measuring the penetration rate of chlorinated solvents through excised rat skin. Ind. Health. 15: 131-140.

Uehleke, H. and T. Werner. 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: 289-303.

Uehleke, H., K.H. Hellmer and S. Tarabelli. 1973. Binding of ^{14}C-carbon tetrachloride to microsomal proteins in vitro and formation of $CHCl_3$ by reduced liver microsomes. Xenobiotic. 3: 1-11.

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

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

Villarruel, M.C. and J.A. Castro. 1975. Irreversible binding of carbon tetrachloride to microsomal phospholipids. Free radical nature of the reactive species and alterations in the physico-chemical properties of the target fatty acids. Res. Commun. Chem. Pathol. Pharmacol. 10: 105-115.

Villarruel, M.C., M.I. Diaz-Gomes and J.A. Castro. 1975. The nature of the irreversible binding of carbon-tetrachloride to microsomal lipids. Toxicol. Appl. Pharmacol. 33: 106-116.

von Oettingen, W.F. 1964. The Halogenated Hydrocarbons of Industrial and Toxicological Importance. Amsterdam, Elsevier Publ. Co. pp. 77-108.

Waters, R.M. 1951. Chloroform. A Study After 100 Years. Madison, University of Wisconsin Press.

Withey, J.R., B.T. Collins and P.G. Collins. 1982. Effect of vehicle on the pharmacokinetics and uptake of four halogenated hydrocarbons from the gastrointestinal tract of the rat. Pre-print paper submitted to J. Appl. Toxicol., December, 1982.

Withey, J.R. and B.T. Collins. 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. Path. Toxicol. 3: 313-332.

Witte, C. 1874. XXXIV. Experiments concerning the effects of chloroform on blood circulation. Dtsch. Z Chir. 4: 548-566.

Wolf, C.R., D. Mansuy, W. Nastainczyk, G. Deutschmann and V. Ullrich. 1977. The reduction of polyhalogenated methanes by liver microsomal cytochrome P-450. Molec. Pharmacol. 13: 698-705.

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

5. TOXICITY

5.1 EFFECTS OF ACUTE EXPOSURE TO CHLOROFORM

In both humans and experimental animals, characteristic effects of acute exposure to chloroform are depression of the central nervous system and hepatic damage. Renal and cardiac effects also occur. The systemic toxic effects of chloroform appear to be similar regardless of whether exposure or administration occurred by inhalation, oral, or parenteral routes. The only systemic effect documented for dermal administration, however, is renal damage.

5.1.1 Humans

5.1.1.1 ACUTE INHALATION EXPOSURE IN HUMANS -- Information on the effects of acute inhalation exposure of chloroform on humans has been obtained primarily during its use as an inhalation anesthetic. The relationship of the concentration of chloroform in inspired air and blood to anesthesia is described in Table 5-1 (Goodman and Gilman, 1980). Concentrations of chloroform used for the induction of anesthesia were in the range of 2-3 volumes % (20,000-40,000 ppm), followed by lower maintenance levels (NIOSH, 1974; Adriani, 1970).

Chloroform inhalation has a depressive effect on the central nervous system. Excitement due to release of inhibitions is followed by progressive depression of the cortex, higher centers, medulla and spinal cord (Wood-Smith and Stewart, 1964). Centers controlling temperature regulation, respiration, vomiting, vasomotor, and vagal activity are all depressed (Adriani, 1970).

The cardiovascular system is also affected by anesthetic use of chloroform. The myocardium is directly depressed in deeper planes of anesthesia. A blood level sufficient to cause respiratory failure may also cause cardiac arrest. In addition, chloroform sensitizes the autonomic tissues of the heart to epinephrine, causing arrhythmias. It has been found that under chloroform

TABLE 5-1

Relationship of Chloroform Concentration in Inspired Air and Blood to Anesthesia*

	In Inhaled Air Volumes %	In Blood mg%
Not sufficient for anesthesia	<0.15	<2
Light anesthesia (after induction)	0.15 to 0.20	2 to 10
Deep anesthesia	0.20 to 1.50	10 to 20
Respiratory failure	2.0	20 to 25

*Source: Goodman and Gilman, 1980

anesthesia regarded as normal, the heart is subject to arrythmias and extrasystoles (Kurtz et al., 1936; Orth et al., 1951). Orth et al. (1951) found a high incidence of ventricular arrhythmias, 20 of 52 cases investigated, and four cases of temporary cardiac arrest. Blood pressure is lowered by chloroform as a result of a 3-fold action: cardiac slowing due to vagal stimulation, depression of the vasomotor center, and dilation of splanchnic blood vessels (Krantz and Carr, 1965).

Respiratory effects of chloroform inhalation include increased rate and depth of respiration during induction and in light anesthesia, and decreased minute volume exchange in deeper planes of anesthesia. The Hering-Breuer reflex remains active. Bronchial smooth muscle is relaxed and secretions are increased. Laryngeal spasms are caused by high concentrations (Adriani, 1970).

In the gastrointestinal tract, chloroform markedly stimulates the flow of saliva during induction and recovery, but salivation is inhibited in deeper planes of anesthesia (Goodman and Gilman, 1980). The pharyngeal or gag reflex is depressed. Under anoxic conditions, pharyngeal muscle spasms result in stertorous respiration and thick mucus is excreted (Adriani, 1970). Stomach movements are decreased or abolished as tone is reduced. Gastric secretory activity is inhibited or abolished. Post-anesthetic dilation of the stomach occurs in nearly all cases. Nausea and vomiting often occur during recovery from anesthesia. The mechanism is central rather than local, but may be due in part to irritation of the stomach by swallowed vapor (Goodman and Gilman, 1980). Intestinal tone, motility, and secretory activity are inhibited or abolished (Adriani, 1970).

In the urinary tract, chloroform anesthesia results in a decrease in urine flow, possibly due to the release of antidiuretic hormone and renal vasoconstriction, leading to a decrease in renal blood flow and glomerular filtration.

Polyuria occurs after recovery (Goodman and Gilman, 1980). Chloroform anesthesia may be followed by albuminuria and glycosuria. Post-operative urine retention occurs frequently. Renal tubular necrosis has been found in cases of severe poisoning (Wood-Smith and Stewart, 1964).

During obstetric use of chloroform, uterine contractions are only slightly decreased by light anesthesia, but are markedly inhibited in deeper planes. Chloroform rapidly crosses the placental barrier, and respiratory depression in the infant is likely to occur (Wood-Smith and Stewart, 1964).

Chloroform anesthesia also has metabolic effects in humans. A rise in blood glucose accompanies anesthesia. Levels may rise $\geq$2-fold and remain elevated for several hours. The liver glycogen falls coincident with the rise in blood sugar. This, in turn, is a result of the release of epinephrine from the adrenal medulla during the period of excitation. There is also a decrease in glucose utilization in the periphery (Goodman and Gilman, 1980; Krantz and Carr, 1965). Acidosis occurs, characterized by a fall in plasma biocarbonate and phosphate.

Chloroform is acutely toxic to the liver, although in so-called delayed chloroform poisoning, the full effects of damage done during and shortly after administration are not seen for 24-48 hours. The glycogen content of the liver is rapidly depleted; three-fourths in the first half-hour and less rapidly thereafter. There is centrilobular and, in severe cases, mid-zonal and massive necrosis. Cells which survive show fatty degeneration. Symptoms include progressive weakness, prolonged vomiting, delirium, coma, and death. They develop from the first to the third day after exposure. Jaundice, increased serum bilirubin, bile in the urine, reduction in liver function, increased nitrogen excretion, lowered blood prothrombin and fibrinogen, and the appearance of leucine, tryosine, acetone, and diacetic acid in the urine are some of the more prominent findings. The hemorrhagic tendency is due to reduced prothrombin

formation by the injured liver. Death usually occurs on the fourth or fifth day, and autopsy reveals degeneration and necrosis of liver tissue, most marked around the central veins (Goodman and Gilman, 1980; Wood-Smith and Stewart, 1964).

Hematologic effects due to acute chloroform inhalation are seen during anesthesia. Erythrocytes are increased in number as the spleen is constricted and red blood cells are extruded into the circulation. Leukocytes are increased in number during the post-anesthetic period, reaching a maximum within 24 hours and returning to normal in 48 hours. There is an increase in polymorphonuclear cells. Platelets remain unchanged. One half-hour after exposure, there is a decrease in clotting time. Prothrombin time is increased. Prothrombin synthesis is impaired by liver toxicity as previously noted (Adriani, 1970).

The effects of chloroform on the eye include dilation of the pupils, with reduced reaction to light as well as reduced intraocular pressure (Sax, 1979; Winslow and Gerstner, 1978).

Signs of chloroform poisoning include a characteristic sweetish odor on the breath, cold and clammy skin, and dilated pupils (Winslow and Gerstner, 1978). Nausea and vomiting commonly occur. Ketosis, due to incomplete oxidation of fats, as well as a rise in blood sugar, accompanies chloroform intoxication. Initial excitation alternating with apathy is followed by prostration, unconsciousness, and possible death due to cardiac and central nervous system depression (Winslow and Gerstner, 1978).

The above discussion presents observations made on the effects of chloroform inhalation during general anesthesia. Information on the effects of experimental acute inhalation exposure of chloroform in humans is limited to the work of Lehman and Hasegawa (1910) and Lehman and Schmidt-Kehl (1936) as reviewed by NIOSH (1974). The duration of exposure was $\leq$30 minutes and only the subjective

responses of the subjects were measured. The dose-response relationships as tabulated by NIOSH (1974) are presented in Table 5-2.

5.1.1.2 ACUTE ORAL EXPOSURE IN HUMANS -- Case reports of suicides (Piersol et al., 1933; Schroeder, 1965) and of recreational abuse (Storms, 1973) of chloroform present some information on the effects of acute imbibition. A fatal dose of ingested chloroform may be as little as one-third of an ounce (10 mℓ) (Schroeder, 1965). The initial effect is usually unconsciousness and possibly death (within 12 hours without treatment) due to respiratory or cardiac arrest. If the patient survives, delayed effects are observed within 48 hours after regained consciousness. These symptoms include vomiting, anorexia, jaundice, liver enlargement, albuminuria, ketosis, ketonuria and glucosuria, hemorrhage due to lowered blood fibrinogen and prothrombin, reduced serum bicarbonate, increased blood sugar, coma and possible death. Upon autopsy, extensive hepatic centrilobular necrosis is evident.

5.1.1.3 ACUTE DERMAL AND OCULAR EXPOSURE IN HUMANS -- Chloroform is absorbed through the intact skin (von Oettingen, 1964). Application of chloroform to the skin is followed after 3 minutes by a pungent and burning pain reaching its maximum after 5 minutes, associated with erythema, hyperemia, and finally vesication (Oettel, 1936). Exposure of the eye to concentrated chloroform vapors causes a stinging sensation. Splashing the substance into the eyes evokes burning, pain, and redness of the conjunctival tissue. The corneal epithelium is sometimes impaired; however, regeneration starts rapidly and leads to full recovery within 1-3 days (Winslow and Gerstner, 1978).

5.1.2 Experimental Animals

5.1.2.1 ACUTE INHALATION EXPOSURE IN ANIMALS -- Tolerance of animals to chloroform has been summarized by Lehmann and Flury (1943) and by Sax (1979). Similar central nervous system effects are seen in animals at approximately the

TABLE 5-2

Dose-Response Relationships*

160 ppm (0.8 mg/ℓ) for unspecified time - no odor

205 ppm (1.0 mg/ℓ) for unspecified time - light transient odor

390 ppm (1.9 mg/ℓ) for 30 minutes -light transient odor

920 ppm (4.5 mg/ℓ) for 7 minutes - stronger, lasting odor; dizziness, vertigo after 3 minutes

680 ppm (3.3 mg/ℓ) to 1000 ppm (5.0 mg/ℓ) for 30 minutes - moderately strong odor; taste

1100 ppm (5.4 mg/ℓ) for 5 minutes - still stronger, permanent odor; dizziness, vertigo after 2 minutes

1400 ppm (6.6 mg/ℓ) to 1800 ppm (8.57 mg/ℓ) for 30 minutes - stronger odor, tiredness, salivation, giddiness, vertigo, headache, taste

3000 ppm (14.46 mg/ℓ) for 30 minutes - all above plus pounding heart, gagging

4300 ppm (20.8 mg/ℓ) to 5000 ppm (25 mg/ℓ) for 20 minutes - dizziness and light intoxication

5100 ppm (25 mg/ℓ) for 20 minutes - dizziness and light intoxication

7200 ppm (35.3 mg/ℓ) for 15 minutes - dizziness and light intoxication as above but more pronounced

*Source: Lehman and Hasegawa (1910) and Lehman and Schmidt-Kehl (1936).

same magnitude of exposure that produced these effects in humans. In mice, exposure to 2500 ppm for 2 hours produced no obvious effects, 3100 ppm for 1 hour produced slight narcosis, while 4000 ppm induced deep narcosis within one-half hour. Only slight symptoms are seen at 2000-6000 ppm for longer exposures. Fatal exposures were 4100-8200 ppm for mice, 12,300 ppm for rabbits, and 16,300-20,500 ppm for guinea pigs (duration of fatal exposures not specified). In cats, exposure to 7200 ppm resulted in disturbance of the equilibrium after 5 minutes, light narcosis after 60 minutes, and deep narcosis after 93 minutes of exposure. Exposure to 21,500 ppm produced disturbances in equilibrium after 5 minutes, light narcosis after 10 minutes, and deep narcosis in cats after 13 minutes of exposure.

Kylin et al. (1963) described the effects of a single exposure of mice to 100, 200, 400, or 800 ppm of chloroform for 4 hours. The mice exposed to 100 ppm did not develop demonstrable liver necroses, although moderate fatty infiltration of the liver was noted. In mice exposed to 200 ppm, some necrotic areas appeared in the liver and there was an increase in serum ornithine-carbamyl transferase. Exposure to chloroform at 400 and 800 ppm resulted in increased hepatic necrosis and serum enzyme activity.

More recent data regarding toxic effects of acute inhalation exposure to chloroform were presented by Wood et al. (1982), although the study was designed primarily to investigate the role of hydrogen bonding in the anesthetic mechanism. Groups of mice in a rotating cage were given a single exposure of upto 3 hours of varying concentrations of chloroform or deuterated chloroform, each concentration being held constant for about 20 minutes and then being raised until the mice had lost their righting reflex. The concentration was then lowered to 1/2 the ED_{50} ($\simeq$1500 ppm) where it remained until the mice had regained their righting reflex. The duration of these manipulated exposures never

exceeded 3 hours. Only 4 of 47 mice given chloroform gained the righting reflex; indeed, some mice died or were comatose. Upon histological examination of animals sacrificed 3-6 hours after exposure, mild hepatic centrilobular necrosis and very mild renal tubular necrosis was observed. The animals receiving deuterated chloroform survived for 24 hours, after which they were sacrificed. The liver and kidney lesions in these mice were more severe, perhaps owing to the longer survival time, which may have allowed these lesions to develop.

5.1.2.2 ACUTE ORAL EXPOSURE IN ANIMALS

Kimura et al. (1971) performed acute oral toxicity studies in newborn (5-8 g), 14-day-old (16-50 g), young adult (80-160 g), and older adult (360-470 g) rats. The chloroform was given in undiluted form to unfasted rats. LD_{50} values in mℓ/kg (95% confidence limits) were reported as follows: 14-day-old, 0.3 (0.2-0.5); young adult, 0.9 (0.8-1.1), and older adult, 0.8 (0.7-0.9). The young and older adult rats were males; the other two groups contained rats of both sexes. When compared with 15 other solvents included in this study, the LD_{50} values for chloroform were the lowest in the two adult groups and next to lowest in the 14-day-old rats. Only a rough approximation of the LD_{50} could be obtained for the newborn rats; volumes of 0.01 mℓ/10 kg body weight were generally fatal. Lower volumes could not be measured with any degree of accuracy and were not attempted.

Torkelson et al. (1976) reported an oral LD_{50} of 2.0 g/kg (1.05-3.80) in male rats. Animals receiving as little as 0.25 g/kg showed adverse effects. Other recent studies of acute oral toxicity have reported LD_{50} values (with 95% confidence limits) of 1120 mg/kg (789-1590) in ICR male mice and 1400 mg/kg (1120-1680) in females (Bowman et al., 1978), and 908 mg/kg (750-1082) and 1117 mg/kg (843-1514), respectively, in male and female rats (Chu et al., 1980).

In a study that compared the toxicities of halogenated hydrocarbons, a single oral dose of 60 mg/kg chloroform to mice had no toxic effect (Hjelle et al., 1982).

Hill (1978) performed experiments in mice designed to study variability in susceptibility to chloroform toxicity from single oral doses based upon genetic sex differences. For three strains of mice, the LD_{50} values (mℓ/kg) were: DBA/2J, 0.08; B6D2F1/J, 0.20; and C57BL/6J, 0.33. The animals more sensitive to chloroform-induced death were also found to be more susceptible to renal toxicity. Males were found to be more sensitive to renal damage and death than were females. This difference was related to testosterone and it was further noted that C57BL/10J males are relatively testosterone deficient in comparison to DBA males. The C57BL/6J strain used in the Hill (1978) study is closely related to the C57BL/10J strain and may, therefore, also be testosterone deficient.

In male B6C3F1 mice, severe diffuse renal necrosis occurred after a single oral dose of 240 mg/kg and focal tubular regeneration occurred after a single dose of 60 or 240 mg/kg. These effects were not seen after 15 mg/kg (Reitz et al., 1980). Liver damage (hepatocellular necrosis and swelling with inflammatory cell infiltration) occurred only at the highest doses.

Chu et al. (1982a) studied the effects of acute oral exposure of chloroform on Sprague-Dawley rats. Groups consisted of 10 male and 10 female animals given a single oral dose of 0, 546, 765, 1071, 1500, or 2100 mg/kg of chloroform in a volume of 5 mℓ/kg corn oil. Clinical signs of toxicity included depression and coma, but the authors did not specify whether these signs occurred at all dose levels. Treated rats surviving for 14 days consumed less food and had depressed growth rates. Gross examination revealed increased liver and kidney weights at 1071 mg/kg. Upon comprehensive histological examination, only mild to moderate

lesions, even at high doses, were observed in these organs. No changes were noted in other organs, including brain and heart. The hepatic and renal lesions were characterized by hepatocyte variations and occasional vesiculation of biliary epithelial nuclei in the liver, and by bilateral focal interstitial nephritis and fibrosis in the kidney. Changes in hematological and biochemical parameters were also observed in the 1071 and 1500 mg/kg treated groups. Cholesterol levels increased while lactate dehydrogenase activity and liver protein levels decreased. In female rats, the activity of microsomal aniline hydroxylase was induced by chloroform exposure. The numbers of lymphocytes were reduced in both males and females, as were hemoglobin and hematocrit values. Upon longer exposures of 5, 50, or 500 ppm chloroform in drinking water for 28 days, the only toxic effect observed was a decreased number of neutrophils in the highest dose-exposed rats. Examinations were performed as in the single-dose experiment.

5.1.2.3 ACUTE DERMAL AND OCULAR EXPOSURE IN ANIMALS -- Torkelson et al. (1976) found that chloroform, when applied to the skin of rabbits, produced slight to moderate irritation and delayed healing of abraded skin. When applied to the uncovered ear of rabbits, slight hyperemia and exfoliation occurred after one to four treatments. No greater injury was noted after 10 applications. One to two 24-hour applications, on a cotton pad bandaged on the shaven belly of the same rabbits, produced a slight hyperemia with moderate necrosis and a resulting eschar formation. Healing appeared to be delayed on the site as well as on abraded areas which were also covered for 24 hours with a cotton pad soaked in chloroform.

Single application of either 1.0, 2.0, or 3.98 g/kg for 24 hours under an impermeable plastic cuff held tightly around the clipped bellies of each of two rabbits did not result in any deaths. However, extensive necrosis of the skin

and considerable weight loss occurred at all levels. All animals were sacrificed for study 2 weeks after exposure. All treated rabbits exhibited degenerative changes in the kidney tubules graded in intensity with dosage levels. The livers were not grossly affected.

In the same study (Torkelson et al., 1976), liquid chloroform, dropped into the eyes of three rabbits, caused slight irritation of the conjunctiva which was barely detectable 1 week after treatment. In addition, slight but definite corneal injury occurred, as evidenced by staining with fluorescein. A purulent exudate occurred for $\geq$2 days after treatment. Although started 30 seconds after instilling the chloroform, thorough washing of one eye of each rabbit with a stream of running water for 2 minutes did not significantly alter the response in the washed eyes from that of the unwashed eyes.

5.1.2.4 INTRAPERITONEAL AND SUBCUTANEOUS ADMINISTRATION IN ANIMALS -- The toxicity of chloroform in mice after subcutaneous administration (Kutob and Plaa, 1962b) and intraperitoneal administration (Klaassen and Plaa, 1966) has been compared with that of other halogenated hydrocarbons (Pohl, 1979). In these studies, the LD_{50} values for carbon tetrachloride, chloroform, and dichloromethane were 200, 27.5, and 76 mmol/kg after subcutaneous administration and 20, 14, and 23 mmol/kg when given intraperitoneally. When the relative hepatotoxicity of these compounds was compared, a subcutaneous dose of 0.5 mmol/kg of carbon tetrachloride produced approximately the same degree of liver damage as 6.2 mmol/kg of chloroform. After intraperitoneal administration, these values dropped to 0.01 mmol/kg for carbon tetrachloride and 2.3 mmol/kg for chloroform. Dichloromethane did not cause significant histological changes in the liver by either route of administration. At doses that produced liver toxicity, chloroform caused kidney lesions which ranged from the presence of hyaline droplets,

nuclear pycnosis, hydropic degeneration, and increased eosinophilia, to necrosis with karyolysis and loss of epithelium of the convoluted tubules.

Ilett et al. (1973) found that intraperitoneal administration of chloroform caused centrilobular hepatic necrosis in mice of both sexes, whereas renal necrosis was observed only in male mice.

5.2. EFFECTS OF CHRONIC EXPOSURE TO CHLOROFORM

A characteristic effect of chronic exposure to chloroform is hepatic damage; this effect has been documented primarily in studies with experimental animals. As was the case for acute exposure to chloroform, hepatic damage in chronic studies results from either inhalation or oral administration of this chemical. Effects on the kidneys and thyroids have also been observed in some experiments. This section will discuss both subchronic (≈90 days) and chronic exposure studies, because many of the subchronic studies were preliminary range-finding tests for the chronic studies.

5.2.1. Humans

5.2.1.1 CHRONIC INHALATION EXPOSURE IN HUMANS -- Only two chronic inhalation studies that reported measurements of exposure concentrations, as well as effects on human health, were found. Neither study (Challen et al., 1958; Bomski et al., 1967) is particularly adequate or recent.

Challen et al. (1958) investigated complaints of workers (mainly women) in a plant manufacturing lozenges that contained chloroform as a principle ingredient. Before exhaust ventilation was installed, 9 of the 10 exposed workers had complained of symptoms of tiredness, dull-wittedness, depression, gastrointestinal distress, and frequent and scalding urination. Breathing-zone monitoring during simulation of "pre-ventilation" working conditions suggested that the emplyees had been exposed to ≈77-237 ppm. Discussions with management revealed that some of these workers had occasionally been observed to behave in a

silly manner or to stagger about during the workday. Another group of workers (N = 10) had been exposed primarily to concentrations of 22-71 ppm. Eight of these workers complained of less severe symptoms. Apparently, both groups of workers had been exposed to occasional peak concentrations of ≈1163 ppm lasting 1.5-2 minutes. At least four workers in each group worked half-time. None of the five controls reported symptoms similar to those reported by the exposed workers. Eight of the higher exposure employees (77-237 ppm for 3-10 years, followed by ≈2 years without exposure), nine of the lower exposure employees (22-77 ppm for 10-24 months), and five unexposed employees submitted to physical examinations, including liver function tests (thymol turbidity, serum bilirubin, and urine urobilinogen tests). These examinations and tests revealed no evidence of any organic lesion, including liver damage, attributable to exposure to chloroform.

In humans, hepatic damage is the most common toxic effect of acute exposure to chloroform, as noted previously. According to Pohl (1979), only one report of liver abnormalities in humans after chronic exposure to chloroform has been found in the literature and no additional reports were found in the more recent literature. In this study (Bomski et al., 1967), 17 cases of hepatomegaly were found in a group of 68 industrial workers who were exposed to chloroform in concentrations ranging from 2-205 ppm for 1-4 years. These were unknown rather than breathing zone concentrations. Three of the 17 workers with hepatomegaly were judged to have toxic hepatitis on the basis of elevated serum enzymes. The frequency of viral hepatitis among the 68 chloroform-exposed workers was higher (4.4% versus 0.38%) than the frequency among a group of inhabitants of the city, ≥18 years of age. This phenomenon also occurred in the 2 previous years. Ten cases of splenomegaly were also diagnosed among the 68 workers. There appears to be no comparison with incidences of these conditions in nonexposed workers.

5.2.1.2 CHRONIC ORAL EXPOSURE IN HUMANS -- There are relatively few reports of toxic effects following chronic ingestion of chloroform (Pohl, 1979), and more recent reports were not located in the literature. In one case, it was estimated that a male patient ingested 1.6-2.6 g of chloroform in a cough medicine daily for ≈10 years (Wallace, 1950). Blood and urine analyses, as well as liver function tests, indicated the individual suffered from hepatitis and nephrosis. Another report described three patients addicted to chlorodyne, a tincture containing chloroform and morphine (Conlon, 1963). Liver biopsy showed severe cellular damage in one of these individuals who had ingested 21 mℓ of chloroform daily for an undetermined period of time. All three displayed evidence of serious mental and physical deterioration, including peripheral neuropathy. It is not possible to determine if the adverse effects were due to chloroform, morphine, or ethanol.

More recently, the safety of a dentifrice containing 3.4% chloroform and a mouthwash containing 0.43% was assessed in studies lasting ≥1 year (DeSalva et al., 1975). The subjects using the dentifrice were exposed to ≈70 mg (0.047 mℓ) of chloroform each day, whereas the groups using the mouthwash were exposed to ≈178 mg (0.12 mℓ). The results of liver function tests and blood urea nitrogen determinations showed no statistical differences between control and experimental subjects.

Epidemiologic studies of humans exposed to chloroform in their drinking water have focused on carcinogenic endpoints, and, hence, are discussed in the chapter on carcinogenicity.

5.2.2 Experimental Animals

5.2.2.1 CHRONIC INHALATION EXPOSURE IN ANIMALS -- Experiments with several species of animals (Torkelson et al., 1976) give some information regarding potential effects of long-term inhalation exposure to chloroform. The

animals were exposed to chloroform 5 days/week for 6 months. Exposure to 25 ppm of chloroform for up to 4 hours/day had no adverse effects in male rats as judged by organ and body weights, and by gross and histological examination of livers and kidneys. Exposure to 25 ppm for 7 hours/day, however, produced histopathological changes in the livers and kidneys of male but not female rats. These changes were characterized as lobular granular degeneration and focal necrosis throughout the liver and cloudy swelling of the kidneys. The hepatic and renal effects appeared to be reversible because rats exposed according to the same protocol, but given a 6 week recovery period following exposure, appeared normal by the criteria tested. Increasingly pronounced changes were observed in the livers and kidneys of both sexes of rats exposed to 50 or 85 ppm for 7 hours/day. Hematologic indices, clinical chemistry, and urinalysis values, tested at higher levels of exposure, were within normal limits. Each exposure group and control group had 10-12 rats/sex except for the 25 ppm, 4 hour/day group, which had 10 male rats and no females.

Similar experiments with guinea pigs (N = 8-12 sex/group) and rabbits (N = 2-3/sex/group) gave somewhat inconsistent results. Histopathological changes were observed in livers and kidneys of both species at 25 ppm but not at 50 ppm in either species, nor even at 85 ppm in guinea pigs. The results of these studies are summarized in Table 5-3.

Other reports of effects of chronic inhalation exposure to chloroform in experimental animals were not found in the more recent literature.

5.2.2.2 CHRONIC ORAL EXPOSURE IN ANIMALS -- The data from several studies on the effects of chronic and subchronic oral exposure to chloroform are summarized in Table 5-4. Low levels of exposure (15-64 mg/kg/day, 6 days/week) have been reported to increase survival in mice and rats (Roe et al., 1979; Palmer et al., 1979) and to be associated with possible transient CNS depression

TABLE 5-3

Effects of Inhalation Exposure of Animals to Chloroform, 5 Days/Week for 6 Months*

Species	Sex	ppm	Exposure hours/day	Number in Group Started	Survived	Effects
rats	M	85	7	10	6	Excess mortality attributed to pneumonia on basis of gross and microscopic appearance of lungs; slight depression of final body weight; increase ($p<0.05$) in relative but not absolute weights of liver, kidneys, and testes; no effect on spleen weight; histological findings included marked centrilobular granular degeneration of the livers and cloudy swelling of the kidneys but no histopathological changes in testes; hematologic values (including differential count), urinalysis values and SGPT, SUN, and SAP values all "within normal limits"
	F	85	7	10	10	No evidence of pneumonia; final body weights and weights of liver and spleen unaffected, relative and absolute kidney weights increased; histological findings included marked centrilobular granular degeneration of the livers and cloudy swelling of the kidneys; hematologic, urinalysis, and SGPT, SUN, and SAT values all "within normal limits"
	M	50	7	10	9	Depression of final body weights ($p<0.05$); increases in relative ($p<0.05$) but not absolute weights of kidneys, spleen, and testes; histopathological changes in livers and kidneys similar to those seen at 85 ppm; hematologic values, urinalysis values, and SGPT, SUN, and SAP values all "within normal limits"
	F	50	7	10	10	Final body weights and weights of liver and spleen unaffected; increase in relative kidney weight ($p<0.05$); histopathological changes in livers and kidneys similar to those seen at 85 ppm but somewhat less marked; hematologic values, urinalysis values, and SGPT, SUN, and SAP values all "within normal limits"
	M	25	7	12	9	No effect on final body weights or weight of liver, spleen, or testes; increased relative kidney weight ($p<0.05$); lobular granular degeneration with focal areas of necrosis throughout the liver; cloudy swelling of renal tubular epithelium
	F	25	7	12	12	No statistically significant effect on body weight; increased relative but not absolute kidney and spleen weights; no histopathologic changes in kidneys and spleen; microscopic appearance of livers not specified

TABLE 5-3

Effects of Inhalation Exposure of Animals to Chloroform, 5 Days/Week for 6 Months* (cont.)

Species	Sex	ppm	Exposure hours/day	Number in Group Started	Survived	Effects
rats	M,F	25 plus 0 ppm for 6 weeks (recovery period)	7	12/sex	8 M, 10 F	"Normal" by the criteria tested at this dosage level (see 25 ppm above)
	M	25	1,2, or 4	10, 10, 10	7,8,4, respectively	No evidence of adverse effects by the criteria tested (i.e., final body weight; weights of livers, kidneys, spleen, testes; and probably gross and microscopic appearance of at least the liver and kidneys)
guinea pigs	M,F	85,50, or 25	7	8 to 12/sex/ exposure level	50 to 92% (mortality not related to exposure)	No adverse effects at 50 or 85 ppm other than marked pneumonitis in F at 85 ppm; some histopathological changes in livers of both sexes and kidneys of M at 25 ppm (criteria tested were body weights, organ weights, and gross and microscopic appearance of organs)
rabbits	M,F	85, 50, or 25	7	2 to 3/sex/ exposure level	0 to 1 death/ group, not related to exposure	No adverse effects at 50 ppm; some histopathologic changes in kidneys and liver and pneumonitis in lungs at 25 and 85 ppm. Hematologic and clinical chemistry values within normal limits at 85 ppm (criteria tested were same as for rats)
dogs	M,F	25	7	1/sex	1/sex	No adverse effects in M; marked cloudy swelling of renal tubular epithelium and increase in capsular space in glomeruli of kidneys in F (criteria tested were same as for rats and included clinical chemistry and hematological studies)

*Source: Torkelson et al., 1976

M = male; F = female; SGPT = serum glutamic pyruvic transaminase; SUN = serum urea nitrogen; SAP = serum alkaline phosphatase

Controls for each species and sex included at least one unexposed and one air-exposed group, each comparable in number of animals to the exposed groups. In statistical comparisons of organ and body weights, values for the control group (unexposed or air-exposed) closer in body weight to the test group were used. Mortality in control groups was similar to mortality in treated groups, with the exception of excess mortality in male rats exposed to 85 ppm for 7 hours/day or to 25 ppm for 4 hours/day. No explanation was given by Torkelson et al. (1976) for the high mortality in the 4 hours/day group. Strains of animals and age or weight at the start of the experiment were not specified. Purity of the chloroform used was 99.3% (0.4% ethyl alcohol and <0.3% of an unknown).

TABLE 5-4

Effects of Subchronic or Chronic Oral Administration of Chloroform to Animals

Species, Strain Age/Weight at Start	Sex	No. at Start	Vehicle	Dosage	Duration	Response	Reference
Rats, Sprague-Dawley weanling, 101 g M, 94 g F	M,F	20/sex/dose level	drinking water	0, 5, 50, 500, or 2500 ppm in drinking water ad lib. corresponding to intakes[c] of 0, 0.11-0.71, 1.2-1.5, 8.9-14, or 29-55 mg/rat/day The highest dose[c] corresponds to ≈291 mg/kg/day F, 310 mg/kg/day M	90 days, after which 10 rats/group were killed and 10 rats/group observed for additional 90 days	Increased mortality, decreased growth rate, and decreased food intake at highest dose; increased frequency of mild to moderate liver and thyroid lesions at highest dose, including increases in cytoplasmic homogeneity, hepatocyte density, and cytoplasmic volume, vacuolization due to fatty infiltration, some vesiculation of biliary epithelial nuclei, and hyperplasia in livers; and reduced follicle and colloid density, increased epithelial height, some focal collapse of follicles in thyroid; no histopathological effects in kidney, brain, and heart; after the 90-day recovery, the lesions were very mild and similar to those seen in controls	Chu et al., 1982b

TABLE 5-4

Effects of Subchronic or Chronic Oral Administration of Chloroform to Animals (cont.)

Species, Strain Age/Weight at Start	Sex	No. at Start	Vehicle	Dosage	Duration	Response	Reference
Rats, Osborne-Mendel 6 weeks/190 g	M	30/group except 40 ad lib. controls	drinking water	0, 200, 400, 600 900, or 1800 ppm in drinking water ad lib. corresponding to intakes[a] of 0, 20, 38, 57, 81, or 160 mg/kg/day, plus 0 ppm group matched with 1800 ppm group for water consumption	10 rats/group killed at 30, 60, and 90 days of exposure	Dose-related signs of depression during 1st week only; dose-related reduction in water consumption; decreased weight gain in 160 mg/kg group; increased incidence of "hepatosis" in livers of treated rats at 30 and 60 days but not 90 days (not dose-related); no other treatment-related effects on serum clinical chemistry values or urinalysis values, or gross and microscopic appearance of tissues, including kidney.	Jorgenson and Rushbrook, 1980

TABLE 5-4

Effects of Subchronic or Chronic Oral Administration of Chloroform to Animals (cont.)

Species, Strain Age/Weight at Start	Sex	No. at Start	Vehicle	Dosage	Duration	Response	Reference
rats, Osborne-Mendel 52 days/240 g M 175 g F	M,F	50/sex/dose level; 20/sex matched controls; ≈99/sex colony controls	corn oil, gavage	M: 0, 90, or 180 mg/kg/day; F: 0, 100, or 200 mg/kg/day (TWA); 5 days/week	78 weeks treatment plus 33 weeks observation	Treated animals had dose-related decrease in survival and weight gain, slight decrease in food consumption, increased severity and incidence of pulmonary lesions characteristic of pneumonia; necrosis of hepatic parenchyma NS in controls, 3/50 low dose M, 4/50 high dose M, 3/49 low dose F, 11/48 high dose F; hyperplasia of urinary bladder epithelium 1/18 control M, 7/45 low dose M, 1/45 high dose M, NS for control F, 6/43 low dose F, 2/40 high dose F; increased splenic hematopoiesis in 1/18 control M, 3/45 low dose M, 6/45 high dose M; both control and treated animals had chronic nephritis; low but statistically significant increased incidence of renal epithelial tumors in treated M (see Carcinogenicity section)	NCI, 1976

TABLE 5-4

Effects of Subchronic or Chronic Oral Administration of Chloroform to Animals (cont.)

Species, Strain Age/Weight at Start	Sex	No. at Start	Vehicle	Dosage	Duration	Response	Reference
rats, Sprague-Dawley NS	M,F	10/sex/dose level	toothpaste, gavage	0, 15, 30, 150, or 410 mg/kg/day 6 days/week	13 weeks	At 410 mg/kg/day, increased liver weight with fatty change and necrosis, gonadal atrophy, increased cellular proliferation in bone marrow; at 150 mg/kg/day, changes less pronounced but effect (NS) on relative liver and kidney weights; presumably no effects at lower dosage levels	Palmer et al., 1979
rats, Sprague-Dawley SPF. 180 to 240 g M 130 to 175 g F	M,F	50/sex/group	toothpaste, gavage	0 or 60 mg/kg/day 6 days/week	80 weeks exposure plus 15 weeks observation	Survival of treated animals slightly better than that of controls (32% treated M, 22% control M, 26% treated F, 14% control females survived to 95 weeks); body weights of treated rats slightly and progressively depressed; intercurrent respiratory and renal disease in all groups; minor histological changes in livers but no evidence of "treatment-related toxic effect" in livers; decrease ($p<0.01$) in relative liver weight in treated females; no gross or histologic treatment-related changes in brain; possible effect (NS) on incidence of severe glomerulonephritis; decrease in plasma cholinesterase in treated females	Palmer et al., 1979

TABLE 5-4 (cont.)

Effects of Subchronic or Chronic Oral Administration of Chloroform to Animals (cont.)

Species, Strain Age/Weight at Start	Sex	No. at Start	Vehicle	Dosage	Duration	Response	Reference
mice, B6C3F1, 6 weeks/19 g	F	30/group except 40 ad lib. controls	drinking water	0, 200, 400, 600, 900, 1800, or 2700 ppm in drinking water ad lib. corresponding to intakes[b] of ≈0, 32, 64, 97, 145, or 290 mg/kg/day; additional 0 ppm group matched with 2700 ppm group for water consumption	10 mice/group killed at 30, 60, and 90 days of exposure	Dose-related signs of depression during first week only; marked reduction in water consumption in higher-dose groups during first 2 weeks; body weight losses ($p<0.05$) in 97, 145, and 290 mg/kg groups and in matched controls during first week; mild hepatic centrilobular fatty change in 64, 97, 145, and 290 mg/kg groups at 30 days, but only in 2 highest dosage groups at 60 and 90 days; increase in liver fat/liver weight ($p<0.05$) for 290 mg/kg group at all 3 periods; no other treatment-related changes in serum enzyme levels, urinalysis values, or gross or microscopic appearance of tissues including kidney	Jorgenson and Rushbrook, 1980
mice, B6C3F1 35 days/18g M, 17 g F	M,F	50/sex/dose level; 20/sex matched controls	corn oil, gavage	M: 0, 138, or 227 mg/kg/day; F: 0, 238, or 447 mg/kg/day; 5 days/week	78 weeks treatment plus 14 to 15 weeks observation	Survival decreased in high dose F, unaffected in other treated groups; high incidences of hepatocellular carcinoma in treated mice (see Carcinogenicity section); renal inflammation in 10/18 control M, 2/50 low dose M, 1/50 high dose M	NCI, 1976

TABLE 5-4

Effects of Subchronic or Chronic Oral Administration of Chloroform to Animals (cont.)

Species, Strain Age/Weight at Start	Sex	No. at Start	Vehicle	Dosage	Duration	Response	Reference
mice, Schofield	M,F	10/sex per dosage	toothpaste, gavage	0, 60, 150, or 425 mg/kg/ day; 6 days/ week	6 weeks	At 425 mg/kg, 100% mortality; at 150 mg/kg, 8/10 M died and weight gain of F markedly retarded; at 60 mg/kg, weight gain of both sexes moderately retarded; no other observations mentioned	Roe et al., 1979
mice, ICI (expt. 1) ICI (SPF) (expt. 2) ICI, CBA, C57BL, CF/1 (expt. 3) ≤10 weeks old	expt. 1: M,F expt. 2 and 3: M treated and control, plus some F control	treated: 52/sex/dose level; control: 52 to 206/sex/ strain/ vehicle plus untreated	toothpaste in all 3 expt. for all strains and sexes plus arachis oil in expt. 3 for ICI, gavage	0, 17 (expt.1), or 60 mg/kg/day, 6 days/week	80 weeks treatment; 16 to 24 weeks observation according to numbers of survivors	Survival generally better in 60 mg/kg groups than in controls except for CF/1 animals or when chloroform given in arachis oil; slight retardation of weight gain in 60 mg/kg groups; no effect on hematologic values (tested in expt. 2 only); no treatment-related adverse effect on liver or other tissues except in kidneys as follows: 60 mg/kg in toothpaste - increased incidence of moderate to severe renal changes ($p < 0.001$) in CBA and CF/1 M, 60 mg/kg in oil- increased incidence of moderate to severe kidney disease ($p < 0.05$) in 1C1 M; increased incidence of benign and malignant kidney tumors in ICI M treated with 60 mg/kg in toothpaste or oil (see Carcinogenicity section)	Roe et al., 1979

TABLE 5-4

Effects of Subchronic or Chronic Oral Administration of Chloroform to Animals (cont.)

Species, Strain Age/Weight at Start	Sex	No. at Start	Vehicle	Dosage	Duration	Response	Reference
dogs, beagle 18 to 24 weeks 7 to 8 g	M,F	1/sex/dose level for 90 and 120 mg/kg; 2/sex/dose level for lower dosages	toothpaste in gelatin capsule, orally	30, 45, 60, 90, or 120 mg/kg/day, 7 days/week	13 weeks for 30 and 45 mg/kg, 18 weeks for 60 mg/kg, 12 weeks for 90 and 120 mg/kg	No deaths; occasional vomiting; marked weight loss in all dogs and poor general condition in some at 60 mg/kg or higher; apparent suppression of appetite initially at all dosages and throughout at 60 mg/kg and higher; jaundice and increased SAP, SGOT, SGPT, bilirubin, and ICD values in male at 120 mg/kg; increased SGPT values in 4/4 and increased SAP and SGOT values in 2/4 at 60 mg/kg; hepatocyte enlargement and vacuolation with fat deposition at 60 mg/kg and higher; discoloration of liver, increased liver weight, and slight fat deposition in hepatocytes at 45 mg/kg; no effect on any of these clinical chemistry or histological parameters at 30 mg/kg	Heywood et al., 1979

TABLE 5-4

Effects of Subchronic or Chronic Oral Administration of Chloroform to Animals (cont.)

Species, Strain Age/Weight at Start	Sex	No. at Start	Vehicle	Dosage	Duration	Response	Reference
dogs, beagle, 8 to 24 weeks, 7 to 8 kg	M,F	8/sex/dose level; 16/sex vehicle controls; plus other controls	toothpaste in gelatin capsule, orally	0, 15, or 30 mg/kg/day; 6 days/week	7.5 years treatment plus 20 to 24 weeks observation	No effect on survival, growth, organ weights, hematologic or urinalysis values (checked at intervals throughout); moderate dose-related elevation of SGPT reaching peak in sixth year of study, reverting to normal levels after treatment discontinued; other serum enzyme indicators of hepatic damage (checked during the latter portion of the study) followed pattern similar to SGPT, but BSP retention and ICD values were unaffected; aggregation of vacuolated histiocytes ("fatty cysts") in livers of all groups but cysts were larger and more numerous in treated dogs and persisted after treatment ended; fat deposition affected more renal glomeruli in 30 mg/kg group than in other groups	Heywood et al., 1979

[a]Calculated by Jorgenson and Rushbrook (1980) from measured average body weights and water consumption.

[b]Calculated from Jorgenson and Rushbrook's statement that the mice had actual intake levels of from 148 to 175% of the intended levels of 20, 40, 60, 90, 180, and 270 mg/kg/day.

[c]Calculated by Chu et al. (1982b) by multiplying the fluid intake volume by the concentration of chloroform

[d]Growth rate data were given only for the highest dose and mg/kg/day were calculated from this information by taking average weights over the 90-day period of exposure.

SPF = specific pathogen-free; SGPT = serum glutamic-pyruvic transaminase; SAP = serum alkaline phosphatase; BSP = bromsulphthalein; ICD = isocitric dehydrogenase (serum)

Purity of the chloroform samples used in all these studies was generally high and is discussed in the section on Carcinogenicity.

M = male; F = female; TWA = time-weighted average dose for days on which chemical was administered; NS = Not specified;

and mild hepatic changes in mice and rats (Jorgenson and Rushbrook, 1980; Palmer et al., 1979), hepatic damage in dogs (Heywood et al., 1979), and renal damage in male mice of some sensitive strains (Roe et al., 1979), and in dogs (Heywood et al., 1979). In addition to hepatic damage, ingestion of ≈300 mg/kg/day of chloroform produced thyroid lesions in rats (Chu et al., 1982b). In one study, a decreased incidence of renal inflammation occurred in male mice treated with chloroform at 138 or 227 mg/kg/day, 5 days/week (NCI, 1976). A similar effect may have occurred in rats (Palmer et al., 1979), but the authors did not specify whether the effect of chloroform was to increase or decrease the incidence of intercurrent renal disease. The NCI (1976) report stated that hepatic necrosis, hyperplasia of the urinary bladder epithelium, and increased splenic hematopoiesis in rats may have been related to chloroform treatment, but incidences in controls for some of these effects were not reported and the data, shown in Table 5-4, are difficult to interpret. Many of the studies summarized in Table 5-4 were at least partially designed as investigations of carcinogenicity and, hence, are also discussed in the carcinogenicity section of this document; some experimental details are discussed more fully in that section.

From the data presented in Table 5-4, it appears that rats and mice can tolerate higher daily intakes of chloroform when it is given in their drinking water or in a toothpaste base (by gavage) than they can when the chemical is administered in corn or arachis oil (by gavage). In the subchronic study of Jorgenson and Rushbrook (1980), rats and mice appeared to adapt to low levels of chloroform intake (up to ≈100 mg/kg/day); signs of depression and mild hepatic damage that occurred initially had disappeared by 90 days of treatment. Elevated indices of liver damage (e.g., SGPT levels) in dogs chronically exposed to chloroform reverted to "normal" after treatment was discontinued, although histological changes persisted. Similarly, the mild liver and thyroid lesions

seen in rats exposed to high doses of chloroform via their drinking water for 90 days were no longer apparent in rats allowed to recover for an additional 90 days (Chu et al., 1982b).

5.3 INVESTIGATION OF TARGET ORGAN TOXICITY IN EXPERIMENTAL ANIMALS

5.3.1 Hepatotoxicity. An extensive review of the early literature dealing with chloroform-induced liver damage by von Oettigen (1964) notes studies beginning in 1891. More recently, Groger and Grey (1979) summarized reports describing chloroform-induced liver hepatotoxicity as follows: typical effects of chloroform on liver cells are extensive vacuolization, disappearance of glycogen, fatty degeneration, swelling, and necrosis, all starting in the centrilobular areas. There is also often hemorrhaging into the parenchyma and infiltration of polymorphonuclear cells and monocytes. Electron-microscopic observations of liver parenchymal cells from chloroform-intoxicated rats as carried out by Scholler (1966, 1967) revealed deposition of lipid droplets in the cytoplasm, partial destruction of the mitochondrial matrix, proliferation of smooth endoplasmic reticulum, and swelling of the rough endoplasmic reticulum with detachment of ribosomes.

Kylin et al. (1963) conducted a study of the hepatotoxic effects of inhaled trichloroethylene, tetrachloroethylene, and chloroform in mice with the objective of finding the lowest concentration of the substances producing signs of liver damage after a single 4-hour exposure period. Histological examination showed that a concentration of 100 ppm caused moderate fatty infiltration in mice killed 1 day after exposure. At $\geq$200 ppm, the extent of the alteration increased with concentration and was more pronounced after 1 day than 3. Thus, judging from the histological picture, the smallest concentrations (ppm) of the different agents to produce more severe alterations in the exposed group than in the controls were as follows:

	Trichloro-ethylene (ppm)	Tetrachloro-ethylene (ppm)	Chloroform (ppm)
1 day after exposure	1600-3200	<200	<100
3 days after exposure	>3200	200 to 400	100 to 200

On this basis, the hepatotoxic effects of trichloroethylene, tetrachloroethylene, and chloroform are in the approximate ratios 1:10:20. The amount of liver fat was raised at 400 ppm of chloroform. A third indicator of liver toxicity was an increase in serum ornithine carbamyl transferase (S-OCT) activity at 24 hours in animals exposed to 200, 400, and 800 ppm of chloroform.

A study of the effect of oral doses of chloroform on the extent of liver damage in white mice ("of a Swiss strain") was conducted by Jones et al. (1958). Minimal changes characterized by midzonal fatty infiltration were observed 72 hours after the administration of 30 mg (0.02 mℓ)/kg. When the dose was increased to 133 mg (0.09 mℓ)/kg, a massive fatty infiltration of the total liver lobule was found. At a level of 355 mg (0.24 mℓ)/kg, massive fatty infiltraton occurred along with severe central lobular necrosis. Information on the hepatotoxicity of long-term chloroform administration has been presented in the sections on Effects of Chronic Exposure to Chloroform. Inhalation exposure of rats to 25, 50, or 85 ppm chloroform for 7 hours/day, 5 days/week for 6 months produced centrilobular granular degeneration and focal necrosis in their livers. In subchronic studies, ingestion of up to ≈100 mg/kg/day of chloroform produced mild, transient histological changes in the livers of rats and mice (Jorgenson and Rushbrook, 1980), ingestion of 145 or 190 mg/kg/day produced fatty change in the livers of mice (Jorgenson and Rushbrook, 1980), and administration of 410 mg/kg/day by gavage produced fatty change and necrosis in the livers of rats. Dogs treated subchronically with 45 mg/kg/day by the oral route had histological

evidence of slight hepatic fatty change, with increasingly severe changes noted at dosages of 60 and 120 mg/kg/day.

In chronic oral studies, rats had minor histological change in their livers and a decrease in relative liver weights when given 60 mg/kg/day of chloroform, 6 days/week, while the livers of mice were unaffected at this dosage. Dogs had some evidence of liver damage (clinical chemistry parameters) and an increase in the number and size of fatty cysts in their lifetime when administered 15 or 30 mg/kg/day orally for 6 days/week. The mechanism by which chloroform exerts its hepatotoxic effects has been widely investigated and efforts have been made to identify the responsible metabolite(s).

As long ago as 1928, it was suspected that the liver damage induced by chloroform may be due not only to the chemical itself, but might be caused by a degradation product (Lucas, 1928). The concept that chloroform is excreted unchanged was disproved since a large number of studies (Butler, 1961; Paul and Rubenstein, 1963; Van Dyke et al., 1964; and Reid and Krishna, 1973) indicated that the tissue necrosis induced by chloroform is associated with the covalent binding of toxic metabolites and alkylation of tissue proteins. Autoradiograms have revealed that this binding occurs predominantly in the necrotic areas (Illet et al., 1973). It was also shown by McLean (1970) that pretreatment of rats with phenobarbital (a microsomal enzyme inducing agent) greatly enhances the lethality of chloroform.

Brown et al. (1974) proposed a mechanism of chloroform hepatotoxicity implicating a free radical metabolite which can react with glutathione (GSH) (a tripeptide which protects against hepatotoxicity), diminishing GSH levels in the liver. According to this hypothesis, once GSH levels are depleted, further metabolism would result in the reaction of the metabolite with microsomal protein, and hence, necrosis. This proposal was based on observations in pheno-

barbital pre-treated rats anesthetized with chloroform, that hepatotoxicity was enhanced and GSH levels were decreased by the induction of microsomal enzymes. Covalent binding of chloroform metabolites to microsomal proteins in vitro was also enhanced by enzyme induction, an effect prevented by GSH. Similar findings were reported in mice by Ilett et al. (1973), who found severe chloroform-induced centrilobular necrosis in phenobarbital pretreated mice, but only slight centrilobular damage in mice exposed only to chloroform.

Thus, the hepatotoxicity of chloroform appears to depend on 1) the rate of its biotransformation to produce reactive metabolite(s), and 2) by the amount of GSH available to conjugate with and thus inactivate the metabolite(s).

The role of GSH in chloroform-induced hepatotoxicity was further studied by Docks and Krishna (1976), who found that only thoses doses of chloroform that decreased liver GSH caused liver necrosis when administered to phenobarbital pretreated rats.

More recently, Ekström et al. (1982) studied the mechanism of GSH depletion by chloroform in rats pretreated with phenobarbital. The synthesis of GSH proceeds via two enzymatic steps, the first of which is rate limiting:

$$\text{glutamate} + \text{cysteine} \xrightarrow[\text{synthetase}]{\gamma\text{-glutamyl-cysteine}} \text{dipeptide}$$

In the presence of glycine, the reaction continues via GSH synthetase to produce GSH. When the soluble fraction from livers of rats sacrificed at various times after chloroform exposure was incubated in the presence of these amino acids, it was found that GSH synthesis was inhibited within 4-6 hours, while liver necrosis was evident only after 6 hours. When glycine was eliminated from the initial part of the incubation, the dipeptide accumulated, but at a lower rate in the presence of chloroform than in its absence. Later addition of glycine resulted in GSH synthesis at a rate similar to control values. Thus, it appears that chloroform, or rather a reactive metabolite, inhibited GSH synthesis at the rate

limiting step (i.e., the formation of dipeptide by γ-glutamyl-cysteine synthetase).

The biotransformation of chloroform (as discussed in Chapter 4) depends on the activity of the microsomal drug metabolizing enzymes. Substances that induce these enzymes were shown to, indeed, enhance the hepatotoxicity of chloroform as evidenced by increased serum glutamic-pyruvic transaminase (SGPT) levels and decreased hepatic glucose-6-phosphatase activity (Lavigne and Marchand, 1974). An inhibitor of the drug metabolizing enzymes SKF-525A, however, while increasing the excretion of ^{14}C-carbon monoxide in rats administered ^{14}C-labelled chloroform, failed to diminish the hepatotoxicity of chloroform, leading these authors to conclude that factors other than metabolism may be involved.

McMartin et al. (1981) demonstrated that altering the cytochrome P-450 concentrations in the livers of chloroform-exposed rats also altered the hepatotoxicity, as measured by the incidence of hepatic lesions and by serum alanine aminotransferase activities. Both fasting and phenobarbital pretreatment increased the cytochrome P-450 content and liver damage, while cadmium produced the opposite effect.

Theories of chloroform hepatotoxicity involve the formation of reactive intermediates by liver enzymes. How these intermediates exert their hepatotoxic effect has been the subject of several studies. It has been suggested by Masuda et al. (1980) that, based on the chloroform-induced indices of hepatotoxicity of decreased microsomal glucose-6-phosphatase activity and cytochrome P-450 content with increased hepatic malondialdehyde levels, the lipid peroxidation hypothesis proposed for carbon tetrachloride may also apply to the case of chloroform. Qualitative and mechanistic differences of hepatotoxicity between the two chemicals were noted, however.

The interactive hepatotoxicity of chloroform and carbon tetrachloride was studied by Harris et al. (1982) who found that, while neither chemical alone given at subthreshold dose altered SGPT activity, hepatic triglyceride content, or hepatic calcium content, when given together, these chemicals increased the toxic response in rats. Administration of either or both chemicals had no effect on GSH levels or conjugated diene formation, but ethane expiration was increased in rats given both chemicals. Diene conjugation and ethane expiration are indices of lipid peroxidation. Histopathological changes were more severe from the combination than from either chemical alone. Although the mechanism of the hepatotoxic interaction between chloroform and carbon tetrachloride is unclear, the authors suggested that there might be a combined effect of phosgene formation and lipid peroxidation initiation.

It should be noted that the prevailing theories implicate phosgene as the major metabolite responsible for chloroform hepatotoxicity (Reynolds and Yee, 1967; Sipes et al., 1977; Mansuy et al., 1977; Pohl et al., 1977). Other potential toxic metabolites discussed by Pohl (1979) in a review of this subject are a trichloromethyl radical and dichlorocarbene; however, they are considered less important than phosgene in this regard.

A study by Stevens and Anders (1981) supports the phosgene-mediated mechanism. The time course of changes in SGPT levels and covalent binding of ^{14}C to proteins was examined in microsomal and soluble fractions from phenobarbital-pretreated rats sacrificed at various times after chloroform or ^{14}C-chloroform administration. It was found that ^{14}C binding was maximal at 6 hours while indices of liver damage peaked at 18 hours after chloroform exposure. Further experiments were performed in which diethyl maleate (a GSH depletor) treatment caused increased ^{14}C-binding to soluble and microsomal fractions and increased SGPT levels, perhaps by inhibiting the metabolism of phosgene to carbon

monoxide or stable conjugates. Cysteine, which reacts with phosgene to produce 2-oxothiazolidine-4-carboxylic acid, had a protective effect. Diethyl maleate also diminished, but did not eliminate the deuterium isotope effect on the GSH dependent chloroform metabolism to carbon monoxide, which would be expected if carbon monoxide formation occurred subsequent to phosgene production. Thus, the hepatotoxicity of chloroform can be altered by altering the various reaction pathways of phosgene, strongly indicating that phosgene is the toxic intermediate.

From the above discussion, it appears that, to be hepatotoxic, chloroform must first be metabolized by microsomal drug metabolizing enzymes to an active intermediate, probably phosgene, which in turn can react by various pathways, depending on GSH levels, one of which is the covalent binding to liver proteins resulting in necrotic lesions.

5.3.2 Nephrotoxicity

As noted by Watrous and Plaa (1972), the extensive body of research on the hepatotoxicity of halogenated hydrocarbons has tended to overshadow the fact that some of these agents are also nephrotoxic. Earlier reports of chloroform nephrotoxicity include those of Heller and Smirk (1932), who found that rats anesthetized with chloroform showed a diminished ability to excrete a water load given prior to anesthesia, and Knocher and Mandelstam (1944), who noted that chloroform injection produced a fatty infiltration of the kidney.

Renal necrosis produced by the oral administration of chloroform was described by Eschenbrenner and Miller (1945a). The necrosis, observed only in male mice, involved portions of both proximal and distal convoluted tubules. The nuclei of the epithelial cells were often absent or fragmented and the cytoplasm was coarsely granular and deeply eosinophilic. The glomeruli and collecting tubules appeared normal.

Sex and strain differences in the sensitivity of mice to chloroform nephrotoxicity were further studied by Deringer et al. (1953). Exposure of strain C3H mice to air containing ≈5 mg/ℓ of chloroform for 1, 2, or 3 hours resulted in lesions of the kidneys of all of the males but in none of the females. In animals dying within 1 day after exposure, epithelium of the proximal tubules and portions of the distal tubules were generally necrotic. The lumens of those segments of tubules were dilated. The glomeruli were relatively unaffected. The mice dying or sacrificed at later time intervals exhibited calcification in the necrotic area.

Similar lesions were found in males of strains C3H, C3Hf, A, and HR. However, strains C57BL, C57BR/cd, C57L, and ST were resistant to chloroform-induced nephrotoxicity.

Comparable results were reported by Krus and Zaleska-Rutczynski (1970). Subcutaneous administration of chloroform to C3H/He male mice resulted in renal tubular necrosis, with death ensuing 4-9 days later. The lesions were calcified with no evidence of regeneration. Female mice of this strain, males and females of the C57BL/6JN and BN strains, and F_1 generation males of the cross of female C3H/He with male C57BL/6JN mice survived the administration of chloroform (0.1 mℓ of 0.05 g chloroform in 1 mℓ ethyl laureate). Additional studies were performed with males and females of this F_1 generation and the resistant BN strain, in which animals were sacrificed at various time after chloroform administration. All mice survived, while all female mice were resistant, showing no kidney lesions at any time in the experiment. Renal damage was morphologically apparent in all male mice by 12 hours, but regeneration developed by day 4 and continued until the end of the experiment. It was concluded that although all male mice had tubular lesions, the ones surviving had tubules that did not calcify and a large degree of renal regeneration.

Several investigators have studied the influence of testosterone on chloroform-induced renal damage. Eschenbrenner and Miller (1945b) performed experiments in which they saw extensive necrosis of portions of the proximal and distal renal tubules in normal male and in testosterone-treated castrated male mice following the acute oral administration of chloroform. However, no necrosis was found after chloroform was administered to female mice or castrated male mice. Continuing this line of investigation, Culliford and Hewitt (1957) reported the following results:

1) Adult male mice of two strains (CBA and WH) developed extensive necrosis of the renal tubules after exposure to low concentrations of chloroform vapor (7-10 mg/ℓ for 2 hours). Adult females showed no renal damage after equivalent exposure.

2) Adult females became fully susceptible to necrosis after treatment with androgens. The susceptibility of males was greatly reduced by treatment with estrogens.

3) Castration removed the susceptibility of the males of one strain, but did not completely remove it in another. The residual susceptibility of castrates was abolished by adrenalectomy.

4) Male mice under 11 days old were not susceptible to necrosis even after massive doses of androgen. Between 11 and 30 days, they were susceptible if given androgen. Thereafter, they became spontaneously susceptible.

5) Liver damage occurred in nearly all exposed mice and was not correlated with sex hormone status.

6) Susceptibility could be induced in gonadectomized mice by methyl testosterone, testosterone propionate, dehydroepiandrosterone, progesterone, and large doses of cortisone acetate.

Hill (1978) also performed experiments demonstrating similar strain and sex differences in chloroform-induced renal toxicity. The renal toxicity of a fixed oral dose of chloroform to castrated male mice was increased with increasing doses of administered testosterone. Plasma levels of testosterone in resistant strains tended to be lower than levels in susceptible strains. Hill (1978) conjectured that a testosterone may act by sensitizing the renal proximal convoluted tubules to chloroform through a testosterone receptor mechanism.

Eschenbrenner and Miller (1945b), however, linked susceptibility to the nephrotoxic action of chloroform to differences in kidney morphology and physiology induced by testosterone.

Information on the nephrotoxicity of long-term chloroform administration has been presented in the section on Effects of Chronic Exposure to Chloroform. Inhalation of 25, 50, or 85 ppm of chloroform 7 hours/day, 5 days/week for 6 months produced cloudy swelling of the renal tubular epithelium in rats. Male mice of certain sensitive strains had increased incidences of moderate to severe renal disease when treated orally with chloroform at a dosage of 60 mg/kg/day, 6 days/week in a chronic study (Roe et al., 1979). Chronic oral administration of 30 mg/kg/day of chloroform, 6 days/week, to dogs produced an increase in the numbers of renal glomeruli affected by fat deposition (Heywood et al., 1979).

The mechanism of the nephrotoxicity of chloroform has been less extensively studied than has that of hepatotoxicity. Ilett et al. (1973) suggested that the hepatotoxic metabolite produced in the liver is transported via the circulation to the kidney where it exerts its nephrotoxic effects. More recent studies (McMartin et al., 1981; Kluwe and Hook, 1981) suggest that chloroform may also be metabolized in the kidney, but by a different mechanism.

McMartin et al. (1981) altered the concentrations of cytochrome P-450 by fasting, by phenobarbital pretreatment, or by administration of cadmium to rats given chloroform as a challenge. Fasting increased cytochrome P-450 concentrations in both liver and kidney, and chloroform-induced damage was enhanced in both organs of fasted animals. Pretreatment with cadmium decreased cytochrome P-450 in livers but not kidneys and significantly diminished liver damage due to chloroform while having no effect on kidney damage due to chloroform exposure. Phenobarbital pretreatment resulted in increased liver but not kidney cytochrome P-450 and, likewise, chloroform-induced damage was enhanced in livers but not

kidneys. Thus, pretreatments that altered hepatic cytochrome P-450 levels had no effect on chloroform-produced renal effects, suggesting that a metabolite of chloroform, which is responsible for kidney damage, is produced in the kidney.

The mechanism of chloroform nephrotoxicity was also investigated by Kluwe and Hook (1981), who found no difference between nephrotoxicity and hepatotoxicity with respect to the effects of microsomal enzyme inhibitors and diethyl maleate. Mice were injected intraperitoneally with chloroform either before piperonyl butoxide or SKF-525-A administration or after diethyl maleate, piperonyl butoxide or SKF-525A exposure. Although it inhibits microsomal enzymes, SKF-525-A, administered either before or after chloroform administration, did not reduce the hepatotoxicity or the nephrotoxicity of chloroform. This is consistent with a similar finding by Lavigne and Marchand (1974). Piperonyl butoxide, when given before chloroform, protected against toxicity in both organs, but when given after chloroform, did not. This finding indicated that an enzymatic step in the metabolism of chloroform by both organs was inhibited. The effect of diethyl maleate was to enhance the toxicity of chloroform in both organs. Thus, the mechanism of chloroform nephrotoxicity appears to be similar to that of hepatotoxicity with respect to these substances.

5.4 FACTORS MODIFYING THE TOXICITY OF CHLOROFORM

From the preceding discussion, it is evident that the alterations of microsomal enzyme activity or hepatic GSH levels influence the severity of toxic effects induced by a given amount of chloroform. It follows then that many factors could alter chloroform toxicity by affecting these parameters or acting through other mechanisms. These substances are of interest because they fall into categories of accidental or intentional exposure to humans. Alcohol, dietary components, pesticide, and steroids are some of the substances which are discussed below.

5.4.1 Factors that Increase the Toxicity. The effect of ethanol pretreatment on chloroform-induced hepatotoxicity in mice was studied by Kutob and Plaa (1962a). An intoxicating dose (5 g/kg) of ethanol was administered orally to mice daily for 15 days initially, with a systematic shortening of the duration to a single exposure. A challenging dose of chloroform (0.08 mℓ/kg) was administered subcutaneously either 12, 15, or 24 hours after ethanol treatment. Liver dysfunction was measured by prolongation of phenobarbital sleeping time, bromsulphalein (BSP) retention, liver succinic dehydrogenase activity, and histological examination. Regardless of the ethanol treatment period, phenobarbital sleeping time was significantly increased in mice receiving ethanol followed by chloroform when compared with mice receiving either substance alone. Similar findings were found for BSP retention. The in vitro succinic dehydrogenase activity was significantly reduced by ethanol pretreatment followed by chloroform administration 12 or 24 hours, but not 48 hours, later, when compared with activities from mice receiving only chloroform. Histological changes were seen in the livers of mice given ethanol 15 hours to 4 days prior to chloroform challenge, while mice receiving either chemical alone had morphologically normal livers. It was also determined that the ethanol treatment increased liver triglyceride content, with a maximum at 15 hours, and that ethanol pretreatment significantly increased the concentration of chloroform in the livers with a maximum at 12 hours after chloroform challenge. From these results, it was noted that a single dose of ethanol was just as effective as multiple doses. A mechanism was proposed for the ethanol enhanced chloroform-induced hepatotoxicity in which ethanol increases liver lipid content (as evidenced by increased triglycerides) resulting in increased concentrations of chloroform to be metabolized in the liver.

In support of this mechanism is the observation that oral isopropanol pretreatment for 5 days (0.3 mℓ/100 g for 2 days and 0.15 mℓ/100 g for 3 days) followed 12 hours later by 5 daily inhalations of chloroform (5000 ppm first day, 2500 ppm on the next four days), 2 hours/day led to severe fatty infiltration of the liver. Chloroform alone increased the pool of triglycerides (Danni et al., 1981).

In contrast, Sato et al. (1980) studied the mechanism by which ethanol enhances hydrocarbon metabolism, including that of chloroform. Rats ingesting ethanol in their drinking water for 3 weeks were sacrificed 10 hours after the final exposure. Control rats were given isocaloric glucose solutions. Liver microsomal enzyme systems were prepared and liver protein and cytochrome P-450 contents analyzed, the increased contents being indicative of microsomal enzyme synthesis in response to alcohol. When chloroform was added as a substrate, its metabolism was enhanced by 6 times, much more than could be accounted for by enzyme induction alone. Microsomes prepared from rats that were withdrawn from ethanol 24 hours prior to sacrifice did not show enhanced activity. In a subsequent study (Sato et al., 1981), rats receiving a single gavage dose of 0, 2, 3, 4, or 5 g/kg ethanol were sacrificed 18 hours later. The in vitro metabolism of chloroform by microsomes prepared from these rats was enhanced very little at 2 g/kg, slightly more at 3 g/kg, and dramatically at 4 g/kg. At 5 g/kg, however, enhanced enzyme activity was no greater than at 3 g/kg ethanol. When ethanol was added directly to the incubation system, the metabolism of chloroform was inhibited. Rats receiving 5 g/kg ethanol retained relatively large amounts in the blood and liver, while those receiving 4 g/kg retained almost none. If the ethanol remaining in the rats exerted an inhibitory effect on enzyme activity, then microsomal enzymes prepared from 5 g/kg ethanol-treated rats, mixed with the soluble fraction from control rats, should show increased activity

when compared with both microsomal and soluble fractions from ethanol-treated rats. This was found to be the case.

Based on these results and studies on metabolism of other hydrocarbons *in vitro* and *in vivo*, Sato et al. (1981) suggested that ethanol is both a stimulator and an inhibitor of drug metabolizing enzymes, depending on how much ethanol remains in the body and thus how much time has elapsed since ethanol ingestion. Thus, when ethanol is first ingested, it acts as a competitive inhibitor of microsomal enzyme activity, but as it disappears from the body, an optimum for stimulation may be reached and metabolism enhanced. It was postulated that since the metabolism of chloroform was enhanced to a much greater extent than can be explained from enzyme induction alone, perhaps ethanol modifies the enzyme activities by other mechanisms such as modification of membrane properties, allosteric effects, or by displacement of substrate already bound.

Polybrominated biphenyls (PBBs) have also been found to potentiate the toxicity of chloroform (Kluwe and Hook, 1978). Mice were fed diets containing 0, 1, 25, or 100 ppm PBB for 14 days. One day before sacrifice, the mice were given a single intraperitoneal injection of 0, 0.5, 2.5, 5.0, or 50 μℓ/kg chloroform. PBB enhanced the toxicity of chloroform in both the liver and the kidney as evidenced by results of blood urea nitrogen (BUN) and serum glutamic oxaloacetic transaminase (SGOT) determinations and by inhibition of p-aminohippuric acid (PAH) uptake by renal slices. PBB also reduced the LD_{50} of chloroform in these mice and the deaths were attributed to hepatic necrosis. Since PBBs were known to induce the drug metabolizing enzymes, their effects on chloroform were assumed to be due to enhanced chloroform metabolism.

Steroids appear to play a role in the potentiation of chloroform toxicity, especially in the kidney as seen from the sex-related differences in the response of mice (Eschenbrenner and Miller, 1945a; Deringer et al., 1953) and by experi-

ments involving testosterone administration to castrated male mice (Eschenbrenner and Miller, 1945b; Culliford and Hewitt, 1957; Hill, 1978) discussed previously. Clemens et al. (1979) further studied this phenomenon in castrated male and intact female mice. Dose-dependent testosterone sensitization of renal tubules to a fixed dose of chloroform was observed in castrated males with the response ranging from kidney dysfunction to death at high doses. The androgenic progestin, medroxyprogesterone acetate, enhanced chloroform-induced kidney damage in both castrated males and intact females. Progesterone or hydrocortisone potentiated chloroform toxicity in DBA/2J castrated male mice, but not in the C57BL/J6 strain males nor in any of the females. The mechanism by which the androgens exerted their potentiation may have been mediated through strain specific androgen receptors of the proximal convoluted tubular cells. The mechanism for the potentiating action of the other steriods was less clear.

The potentiation of chloroform toxicity by ketones and ketogenic substances has been studied extensively in recent years. Hewitt et al. (1979) and Cianflone et al. (1980) found that while pretreatment of mice with the insecticide, kepone (a ketone), enhanced the liver damage caused by chloroform exposure, the structurally related mirex (a non-ketone) did not. Other ketones were compared for their ability to enhance the hepatotoxic and nephrotoxic action of chloroform in rats with the following results: methyl n-butyl ketone and 2,5-hexanedione were the most potent enhancers, followed by acetone, followed by n-hexane (a ketogenic chemical) (Hewitt et al., 1980). Jernigan and Harbison (1982) studied the potentiation by 2,5-hexanedione of chloroform hepatotoxicity in mice with specific reference to sex differences. Female mice were more susceptible to the dose-dependent enhancement of chloroform hepatotoxicity as well as to the dose-related increase in hepatic microsomal enzymes. Pretreatment of male mice with 2,5-hexanedione potentiated the toxicity of deuterated chloroform but to a

lesser extent than chloroform; however, this deuterium isotope effect did not occur in female mice in vivo. Phenobarbital pretreatment did elicit a deuterium isotope effect in female mice in vivo, suggesting that 2,5-hexanedione pretreatment altered chloroform metabolism by a different mechanism than did phenobarbital. Jernigan and Harbison (1982) speculated that perhaps female mice have greater microsomal enzyme activities, different membrane properties, or perhaps produce a different reactive metabolite of chloroform than do males.

The mechanism of ketone potentiation of chloroform-induced hepato- and nephrotoxicity was also investigated by Branchflower and Pohl (1981) using methyl n-butyl ketone (MBK). Male rats were pretreated with MBK followed by chloroform administration. The metabolism of chloroform by liver and kidney microsomal enzymes and the toxicity to these organs were examined. Control experiments were conducted in which rats were either not pretreated, not given chloroform, or given deuterated chloroform ($CDCl_3$) instead of chloroform. MBK increased cytochrome P-450 levels and NADPH-dependent cytochrome reductase activity in liver microsomes, while having no effect on renal levels of these microsomal components. MBK pretreatment doubled the rate of metabolism of chloroform to diglutathionyl dithiocarbonate (GSCOSG) in microsomal preparations, and more GSCOSG was excreted into the bile of the pretreated animals when compared with rats receiving only chloroform. The amount of GSCOSG in bile was less in MBK-$CDCl_3$ treated animals. GSH levels were significantly decreased by MBK treatment and this decrease was enhanced following chloroform exposure, and to a lesser extent, following $CDCl_3$ exposure. Rats pretreated with MBK followed by chloroform had greatly elevated levels of SGPT associated with liver necrosis and significantly greater BUN levels associated with renal cortical tubule lesions over the control groups. A mechanism was proposed whereby MBK, by increasing cytochrome P-450 levels, enhanced the metabolism of chloroform to

phosgene. Furthermore, according to the hypothesis, the phosgene was converted to GSCOSG through GSH, levels of which were diminished by MBK, because the more phosgene formed, the more GSH was depleted in the reaction. The results with $CDCl_3$ indicated that C-H bond was involved in the mechanism. Although MBK also potentiated chloroform toxicity to the kidney, a different mechanism may have been involved since renal cytochrome P-450 and renal GSH levels were not affected.

5.4.2 Factors that Decrease the Toxicity. As discussed above, the experiments of Sato et al. (1980, 1981) indicate that ethanol is both a stimulator and an inhibitor of microsomal enzymes, and hence, of chloroform metabolism and toxicity, depending upon the length of time after ingestion.

Disulfiram and its metabolites have also been studied with respect to their protective effects of chloroform-induced hepatotoxicity (Scholler, 1970; Masuda and Nakayama, 1982). Disulfiram is used in treating chronic alcoholism and is metabolized to carbon disulfide and diethyldithiocarbamate (a herbicide) (IARC, 1976). Disulfiram, a known inhibitor of the microsomal drug metabolizing enzymes, given to rats prior to chloroform anesthesia completely prevented the elevated SGPT activity and liver necrosis observed in rats administered chloroform alone (Scholler et al., 1970). More recently, Masuda and Nakayama (1982) studied the effects of diethyldithiocarbamate and carbon disulfide pretreatment in mice challenged with chloroform. Both substances had a protective effect as measured by SGPT activity, liver calcium content, and centrilobular necrosis. Diethyldithiocarbamate and carbon disulfide decreased the activity of the drug metabolizing enzymes *in vivo* and *in vitro*, but only in the presence of NADPH, indicating that these substances must first be metabolized before exerting their inhibitory effect on chloroform metabolism. Gopinath and Ford (1975) also found that dithiocarbamate and carbon disulfide protected against chloroform hepato-

toxicity in rats, and the effect was presumed to be due to suppression of the drug metabolizing enzymes.

Dietary components can also alter the toxicity of chloroform. It is a widely held opinion that low protein content of the diet decreases microsomal enzyme activities, while a high protein diet increases the activities (McLean and McLean, 1969; Nakajima et al., 1982). If this is the case, a low protein diet should protect against chloroform hepatotoxicity by inhibiting the enzymes responsible for chloroform metabolism. It was found, however, that protein depletion did not alter the toxicity of chloroform in rats given a single oral dose (McLean and McLean, 1969; McLean, 1970). If pretreated with phenobarbital or NDDT to induce microsomal enzymes, rats maintained on a standard diet were no more susceptible to chloroform-induced liver damage that were pretreated, protein-depleted rats (McLean, 1970).

More recently, Nakajima et al. (1982) studied the individual effects of protein, fat, and carbohydrate on the metabolism of chloroform in relation to its toxicity in male rats. Test diets were varied with respect to carbohydrate, protein, or fat while maintaining isocaloric contents. Microsomal enzymes were prepared and chloroform was added as a substrate. The following results were obtained: decreased food intake increased liver microsomal enzyme activities; decreased sucrose content in the diet increased the metabolic rate; varying the protein and fat content, while holding the sucrose content constant, had no effect on the metabolic rate; a carbohydrate-free diet, which contained high protein and high fat, accelerated the rate of chloroform metabolism almost as much as 1 day of food deprivation. The authors concluded that it is a high carbohydrate content, rather than a low protein content, which is responsible for the decreased microsomal enzyme metabolism of chloroform and, hence, its toxicity.

5.5 SUMMARY; CORRELATION OF EXPOSURE AND EFFECT

The purpose of this section is to delineate dose-response relationships for the systemic toxicity of chloroform.

5.5.1 Effect of Acute Inhalation Exposure. The adverse effects on humans of inhaling high concentrations of chloroform have been well documented in the course of its use as an anesthetic. Studies that define the threshold region of exposure for such effects in humans are, however, sparse at best. Experiments involving subchronic exposure of several species of animals give some information on toxicity thresholds for renal and hepatic effects, but little for CNS and none for cardiovascular effects.

The only experimental studies conducted with humans (Lehmann and Hasegawa, 1910; Lehmann and Schmidt-Kehl, 1936) involved relatively short exposures and subjective responses. The results of these studies indicate that the odor of chloroform can be perceived at about 200 ppm. Subjective CNS effects (dizziness, vertigo) apparently did not occur at 390 ppm during a 30-minute exposure but were perceived at about 900 ppm after 2-3 minutes of exposure. Subjects exposed to 1400 ppm for 30 minutes experienced tiredness and headache in addition to the above CNS symptoms. The threshold for "light intoxication" was about 4300 ppm (20 minutes). An exposure duration of 30 minutes or less is insufficient to achieve pulmonary steady state (or total body equilibrium, Section 4.2.3). Hence, longer exposures at these concentrations would be expected to cause more severe effects.

Chloroform concentrations used for the induction of anesthesia ranged from about 20,000-40,000 ppm (NIOSH, 1974; Adriani, 1970) and for the maintenance of anesthesia ranged from 1500 ppm (light anesthesia) to 15,000 ppm (deep anesthesia) (Goodman and Gilman, 1980). Continued exposure to 20,000 ppm could result in respiratory failure, direct depression of the myocardium, and death

(Section 5.1.1). Levels of exposure sufficient to produce anesthesia have also caused cardiac arrythmias and extrasystoles (Kurtz et al., 1936; Orth et al., 1951) and hepatic necrosis and fatty degeneration (Goodman and Gilman, 1980; Wood-Smith and Stewart, 1964).

Data from acute animal exposures tend to show similar CNS effects at roughly the same levels of exposure that produced these effects in humans (Lehmann and Flury, 1943). In addition, some data on the threshold for hepatic effects has been obtained for mice. Kylin et al. (1963), in experiments with female mice of an unspecified strain, found that single, 4-hour exposures to chloroform produced mild hepatic effects (increased incidence of moderate fatty infiltration) at 100 ppm. At 200 ppm, in addition to fatty infiltration, hepatic necrosis and increased serum ornithine carbamyl transferase activity occurred. (An elevation in serum levels of this enzyme indicates liver damage according to Divincenzo and Krasavage, 1974.) Further increases in fatty infiltration, necrosis, and serum enzyme activity were observed at 400 and 800 ppm. These effects appeared to be reversible because the extent of change was less severe 3 days after exposure than it was 1 day after exposure.

Damage to the kidneys of male mice of sensitive strains (e.g., C3H) has occurred at exposure levels as low as 5 mg/ℓ (1025 ppm) for 1 hour (Deringer et al., 1953). The damage consisted of necrosis of the epithelium of the proximal tubules.

5.5.2 Effects of Acute Oral Exposure. Dose-response data for acute oral exposure of humans to chloroform is limited to case reports. A fatal dose of as little as 1/3 ounce (10 mℓ) was reported (Schroeder, 1965).

A variety of dose-response data is available for acute oral administration of chloroform to animals. Single doses that were sufficient to adversely affect kidney function (measured as excessive loss of glucose and/or protein in the

urine in male mice) ranged from 89-149 mg/kg in sensitive and relatively insensitive strains (Hill, 1978). At single oral doses of 1071 mg/kg, but not 756 or 546 mg/kg chloroform, increases in organ weights and mild to moderate lesions were observed in the livers and kidneys of Sprague-Dawley rats (Chu et al., 1982a). In male B6C3F1 mice, renal necrosis occurred after 20 mg/kg and focal tubular regeneration occurred after 60 or 240 mg/kg but not after 15 mg/kg (Reitz et al., 1980). A low observed adverse-effect level (LOAEL) for hepatic effects in mice can be identified from the study of Jones et al., 1958, in which 30 mg/kg caused midzonal fatty infiltration. Doses in the range of 133-355 mg/kg (Jones et al., 1958; Reitz et al., 1980) represent a FEL (Frank-Effect-Level) for hepatic damage (including centrilobular necrosis) in mice. According to Torkelson et al. (1976), rats given "as little as" 250 mg/kg chloroform "showed adverse effects" on liver and kidney as determined by gross pathological examination. Reported oral LD_{50} values for mice ranged from 119-1400 mg/kg, depending on sex, strain, and age (Kimura et al., 1971; Hill, 1978; Bowman et al., 1978). For rats, LD_{50} values of 908-2000 mg/kg have been reported (Chu et al., 1980; Torkelson et al., 1976). The lethal dose studies included both 24-hour and delayed deaths.

5.5.3 Effects of Dermal Exposure. Chloroform is irritating to the skin. It has been reported to cause degenerative changes in the renal tubules of rabbits exposed dermally to high doses under extreme conditions (1-3.98 g/kg body weight for 24 hours under an impermeable plastic cuff) (Torkelson et al., 1976). In humans, toxicity from dermal exposure is probably not important in comparison with other routes.

5.5.4 Effects of Chronic Inhalation Exposure. Limited information on the effects of long-term intermittent exposure of humans or animals to chloroform is available. A study involving a small number of workers (Challen et al., 1958) indicates that long-term exposure to 20-71 ppm (98-346 mg/m^3) for a 4-8 hour

workday, with occasional brief excursions to ≈1163 ppm, may represent a LOAEL for symptoms of CNS toxicity. No evidence of liver damage or other organic lesion was detected by physical examination and clinical chemistry tests. A single report linking liver enlargement and viral hepatitis to occupational exposure to 10-200 ppm chloroform (Bomski et al., 1967) is flawed by the apparent lack of suitable controls. The available data do not define a NOAEL (no-observed-adverse-effect-level) or NOEL (no-observed-effect- level) for humans.

Experiments with several species of animals (Torkelson et al., 1976) give some information regarding the threshold region for hepatic and renal effects of inhalation exposure to chloroform (Table 5-3). The animals were exposed to chloroform 5 days/week for 6 months. Exposure to 25 ppm of chloroform for up to 4 hours/day had no adverse effects in male rats as judged by organ and body weights and probably the gross and microscopic appearance of at least the liver and kidneys, although the authors were not explicit about the latter. Exposure to 25 ppm for 7 hours/day, however, produced histopathologic changes in the livers and kidneys of male but not female rats. These changes were characterized as lobular granular degeneration and focal necrosis throughout the liver and cloudy swelling of the kidneys. The hepatic and renal effects appeared to be reversible because rats exposed in the same way, but given a 6-week "recovery period" after the exposure period, appeared normal by the criteria tested. Increasingly pronounced changes were observed in the livers and kidneys of both sexes of rats exposed to 50 or 85 ppm. Hematologic, clinical chemistry, and urinalysis values, tested at the two higher levels of exposure, were "within normal limits."

Similar experiments with guinea pigs and rabbits gave somewhat inconsistent results. Histopathological lesions were observed in liver and kidneys of both species at 25 ppm but not at 50 ppm in either species and not in guinea pigs even at 85 ppm (Torkelson et al., 1976).

The experiments of Torkelson et al. (1976) indicate that subchronic exposure to 25 ppm (123 mg/m^3), 4 hours/day, 5 days/week represents a NOAEL and exposure to 25 ppm, 7 hours/day, 5 days/week respresents a LOAEL for rats. Guinea pigs and rabbits may be slightly less sensitive.

5.5.5 Effects of Chronic Oral Exposure. Little dose-response data for oral exposure of humans to chloroform appear to be available (Chapter 5). A single controlled study has been performed. In this study, subjects were exposed to 70 or 178 mg of chloroform/day (≈1 or 2.5 mg/kg/day assuming 70 kg body weight) for at least 1 year (DeSalva et al., 1975). Neither liver function tests nor blood urea nitrogen determinations (a measure of kidney function) revealed statistically significant differences between exposed and control subjects. Case reports involving abuse of medicines containing chloroform (Wallace, 1950; Conlon, 1963) are not adequate for risk assessment because of the small numbers of patients, exposure to other agents, and imprecise estimates of intake.

Subchronic and chronic toxicity experiments with rats, mice, and dogs, when considered together (Table 5-4), do not clearly establish a NOAEL or NOEL. Although no adverse effects were observed in four strains of mice given 17 mg/kg/day of chloroform, 6 days/week for 2 years (Roe et al., 1979), at the lowest dose level tested (i.e., 15 mg/kg/day, 6 days/week for 7.5 years) in dogs, chloroform treatment was associated with an elevation in SGPT in some but not all of the other tested clinical chemistry indicies of hepatic damage (Heywood et al., 1979). The livers of dogs treated with chloroform at this dosage level had larger and more numberous "fatty cysts" than were found in controls. These fatty cysts consisted of aggregations of vacuolated histiocytes. No effect on survival, growth, organ weights, gross and histological appearance of other organs, or hematologic or urinalysis values was observed at this dosage level. Hence 15 mg/kg/day (6 days/week) represents a LOAEL for dogs for effects on the

liver. Chronic oral administration of 60 mg/kg/day of chloroform (6 days/week) was associated with slight hepatic changes in rats (Palmer et al., 1979) and with increased incidences of moderate to severe renal disease in male mice of sensitive strains (Roe et al., 1979).

None of the three species tested in long-term experiments appeared to be markedly more sensitive to the toxicity of chloroform than any other; dogs may have been slightly more sensitive. There were considerable differences among strains of mice in the sensitivity of the males to chloroform nephrotoxicity, as had also been observed in acute toxicity experiments.

As would be expected, dosages that produced little or no histologic or clinical chemistry evidence of toxicity when given subchronically (15 and 30 mg/kg/day; rats, dogs) resulted in greater evidence of toxicity when given for longer periods of time (Palmer et al., 1979; Heywood et al., 1979). The response to chloroform in the long-term studies may have been modified by the presence or absence of intercurrent respiratory and renal disease, but no consistent pattern is obvious from an inspection of the data in Table 5-4.

5.5.6 Target Organ Toxicity. Target organs characteristic of the acute toxicity of chloroform are the central nervous system, liver, kidney, and heart. For chronic exposure to chloroform, characteristic target organs are the liver and kidney, and possibly the central nervous system. Some dose-response data are available for the toxicity of chloroform to the liver, kidney, and central nervous system; these data are summarized in Table 5-5 by target organ. The studies from which these data are drawn are discussed more fully elsewhere in Chapter 5, but a comparison on the basis of endpoint (target organ) was also considered to be useful.

Manifestations of liver damage include centrilobular necrosis, vacuolization, disappearance of glycogen, fatty degeneration and swelling (Groger and

Table 5-5

Target Organ Toxicity of Chloroform

Target Organ	Route and Type of Exposure	Species	Dose or Exposure	Effect on Target Organ	Reference
liver	inhalation, acute (surgical anesthesia)	human	induction = 20,000-40,000 ppm x a few minutes, plus maintenance = 1500-15,000 ppm x variable duration	necrosis, fatty degeneration in some patients	NIOSH, 1974; Goodman and Gilman, 1980; Wood-Smith and Stewart, 1964
liver	inhalation, acute	mice	100 ppm x 4 hours, single exposure	fatty infiltration	Kylin et al., 1963
liver	inhalation, acute	mice	200 ppm x 4 hours, single exposure	necrosis, fatty infiltration, increase in SOCT	Kylin et al., 1963
liver	inhalation, chronic	rats	25 ppm, 4 hours/day, 5 days/week x 6 months	no effect	Torkelson et al., 1976
liver	inhalation, chronic	rats	25 ppm, 7 hours/day, 5 days/week x 6 months	lobular granular degeneration, focal necrosis	Torkelson et al., 1976
liver	inhalation, chronic	rats	50 or 85 ppm, 7 hours/day, 5 days/week x 6 months	marked centrilobular granular degeneration	Torkelson et al., 1976

Table 5-5

Target Organ Toxicity of Chloroform (cont.)

Target Organ	Route and Type of Exposure	Species	Dose or Exposure	Effect on Target Organ	Reference
liver	oral, acute	mice	30 mg/kg bw; single dose	fatty infiltration	Jones et al., 1958
liver	oral, acute	mice	133 mg/kg, single dose	massive fatty infiltration and severe necrosis	Jones et al., 1958
liver	oral, acute	mice	355 mg/kg, single dose	massive fatty infiltration and severe necrosis	Jones et al., 1958
liver	oral, acute	mice	60 mg/kg, single dose	no effect	Reitz et al., 1980
liver	oral, acute	mice	240 mg/kg, single dose	hepatocellular necrosis and swelling; inflammation	Reitz et al., 1980
liver	oral (drinking water) subchronic	rats	20, 38, 57, 81, or 160 mg/kg/day x 90 days	transient hepatosis (at 30 and 60, but not at 90 days)	Jorgenson and Rushbrook, 1980
liver	oral (drinking water) subchronic	mice	64 or 97 mg/kg/day x 90 days	transient centrilobular fatty change (at 30 and 60 but not at 90 days)	Jorgenson and Rushbrook, 1980

Table 5-5

Target Organ Toxicity of Chloroform (cont.)

Target Organ	Route and Type of Exposure	Species	Dose or Exposure	Effect on Target Organ	Reference
liver	oral (drinking water) subchronic	mice	145 or 190 mg/kg/day x 90 days	fatty change	Jorgenson and Rushbrook, 1980
liver	oral (gavage) subchronic	rats	410 mg/kg/day, 6 days/ week x 13 weeks	fatty change and necrosis	Palmer et al., 1979
liver	oral (capsule), subchronic	dogs	30 mg/kg/day, 7 days/ week x 13 weeks	no effect	Heywood et al., 1979
liver	oral (capsule), subchronic	dogs	45 mg/kg/day, 7 days/ week x 13 weeks	slight fatty change	Heywood et al., 1979
liver	oral (capsule), subchronic	dogs	60 mg/kg/day, 7 days/ week x 18 weeks	fatty degeneration, increase in SGOT and SGPT	Heywood et al., 1979
liver	oral (capsule), subchronic	dogs	120 mg/kg/day, 7 days/ week x 12 weeks	fatty degeneration, jaundice, increase in SGOT, SGPT, bilirubin	Heywood et al., 1979
liver	oral (gavage), chronic	rats	60 mg/kg/day, 6 days/ week x 80 weeks	minor histological changes and decrease in relative liver weight	Palmer et al., 1977
liver	oral (gavage), chronic	mice	60 mg/kg/day, 6 days/ week x 80 weeks	no effect	Roe et al., 1979

Table 5-5

Target Organ Toxicity of Chloroform (cont.)

Target Organ	Route and Type of Exposure	Species	Dose or Exposure	Effect on Target Organ	Reference
liver	oral (capsule), chronic	dogs	15 or 30 mg/kg/day, 6 days/week x 7.5 years	increases in SGPT and other serum indicators of hepatic damage, increase in size and number of fatty cysts (vacuolated histiocytes)	Heywood et al., 1979
liver	oral, chronic	humans	2.5 mg/kg/day for $\geq$1 year	no effect	De Salva et al., 1975
liver	dermal, acute	rabbits	3.98 g/kg x 24 hours under plastic cuff, single exposure	no macroscopic pathologic changes	Torkelson et al., 1976
kidney	inhalation, acute	mice, males of sensitive strains	5000 ppm, 1 hour, single exposure	necrosis and calcification of tubular epithelium	Deringer et al., 1953
kidney	inhalation, chronic	rats	25, 50, or 85 ppm, 7 hours/day, 5 days/week x 6 months	cloudy swelling of tubular epithelium	Torkelson et al., 1976
kidney	oral, acute	mice, males of sensitive strains	89 mg/kg, single dose	loss of glucose or protein in urine	Hill, 1978

Table 5-5

Target Organ Toxicity of Chloroform (cont.)

Target Organ	Route and Type of Exposure	Species	Dose or Exposure	Effect on Target Organ	Reference
kidney	oral, acute	mice, males of sensitive strains	149 mg/kg, single dose	loss of glucose or protein in urine	Hill, 1978
kidney	oral, acute	mice, male	15 mg/kg, single dose	no effect	Reitz et al., 1980
kidney	oral, acute	mice, male	60 mg/kg, single dose	focal tubular epithelial regeneration	Reitz et al., 1980
kidney	oral, acute	mice, male	240 mg/kg, single dose	severe diffuse cortical necrosis, focal tubular epithelial regeneration	Reitz et al., 1980
kidney	oral (drinking water) subchronic	rats	160 mg/kg/day x 90 days	no effect	Jorgenson and Rushbrook, 1980
kidney	oral (drinking water) subchronic	rats	≈300 mg/kg/day x 90 days	no effect	Chu et al., 1980b
kidney	oral (drinking water) subchronic	mice	290 mg/kg/day x 90 days	no effect	Jorgenson and Rushbrook, 1980

Table 5-5

Target Organ Toxicity of Chloroform (cont.)

Target Organ	Route and Type of Exposure	Species	Dose or Exposure	Effect on Target Organ	Reference
kidney	oral (capsule), subchronic	dogs	120 mg/kg/day, 7 days/week x 12 weeks	no effect	Jorgenson and Rushbrook, 1980
kidney	oral, chronic	humans	2.5 mg/kg/day, 7 days/week, for $\geq$1 year	no effect on BUN	De Salva et al., 1975
kidney	oral (gavage), chronic	rats	200 mg/kg/day, 5 days/week x 78 weeks	no effect	NCI, 1976
kidney	oral (gavage), chronic	mice	138 or 227 mg/kg/day, 5 days/week x 78 weeks	decreased incidence of renal disease	NCI, 1976
kidney	oral (gavage), chronic	mice, males of sensitive strains	17 mg/kg/day, 6 days/week x 80 weeks	no effect	Roe et al., 1979
kidney	oral (gavage) chronic,	mice, males of sensitive strains	60 mg/kg/day, 6 days/week x 80 weeks	increased incidence of moderate to severe renal disease	Roe et al., 1979

Table 5-5

Target Organ Toxicity of Chloroform (cont.)

Target Organ	Route and Type of Exposure	Species	Dose or Exposure	Effect on Target Organ	Reference
kidney	oral (gavage) chronic	mice, males of insensitive strains, females	60 mg/kg/day, 6 days/week x 80 weeks	no effect	Roe et al., 1979
kidney	oral (capsule), chronic	dog	15 mg/kg/day 6 days/week x 7.5 years	no effect	Heywood et al., 1979
kidney	oral (capsule), chronic	dog	30 mg/kg/day, 6 days/week x 7.5 years	increase in fat deposition in glomeruli	Heywood et al., 1979
kidney	dermal, acute	rabbits	1.0, 2.0, and 3.98 g/kg x 24 hours under plastic cuff, single exposure	degenerative changes in tubules	Torkelson et al., 1976
central nervous system (CNS)	inhalation, acute	humans	900-1400 ppm for $\geq$30 minutes, single exposure	dizziness, tiredness, headache	Lehmann and Hasegawa, 1910; Lehmann and Schmidt-Kehl, 1936
CNS	inhalation, acute	humans	4300-5100 ppm x 20 minutes, single exposure	dizziness, light intoxication	Lehmann and Hasegawa, 1910; Lehmann and Schmidt-Kehl, 1936

Table 5-5

Target Organ Toxicity of Chloroform (cont.)

Target Organ	Route and Type of Exposure	Species	Dose or Exposure	Effect on Target Organ	Reference
CNS	inhalation, acute	humans	1500-2000 ppm, single exposure	maintenance of light anesthesia (after induction)	Goodman and Gillman, 1980
CNS	inhalation, acute	humans	15,000 ppm, single exposure	maintenance of heavy anesthesia (after induction)	Goodman and Gillman, 1980
CNS	inhalation, acute	humans	20,000-40,000 ppm x a few minutes, single exposure	induction of anesthesia	NIOSH, 1974 Adriani, 1970
CNS	inhalation, acute	mice	2500 ppm x 12 hours, single exposure	no obvious effects	Lehmann and Flury, 1943
CNS	inhalation, acute	mice	3100 ppm x 1 hour, single exposure	light narcosis	Lehmann and Flury, 1943
CNS	inhalation, acute	mice	4100 ppm x 0.5 hours, single exposure	deep narcosis	Lehmann and Flury, 1943
CNS	inhalation, acute	cats	7200 or 21,500 ppm x 5 minutes, single exposure	disturbance of equilibrium	Lehmann and Flury, 1943
CNS	inhalation, acute	cats	7200 ppm x 60 minutes single exposure	light narcosis	Lehmann and Flury, 1943

Table 5-5

Target Organ Toxicity of Chloroform (cont.)

Target Organ	Route and Type of Exposure	Species	Dose or Exposure	Effect on Target Organ	Reference
CNS	inhalation, acute	cats	7200 ppm x 93 minutes single exposure	deep narcosis	Lehmann and Flury, 1943
CNS	inhalation, acute	cats	21,500 ppm x 10 minutes single exposure	light narcosis	Lehmann and Flury, 1943
CNS	inhalation, acute	cats	21,500 ppm x 13 minutes single exposure	deep narcosis	Lehmann and Flury, 1943
CNS	inhalation, chronic	humans	20-71 ppm (with excursions to 1163 ppm lasting 1.5-2 minutes) for 4-8 hours/day, 5 days/week	tiredness	Challen et al., 1958
CNS	inhalation, chronic	humans	77 to 237 ppm (with excursions to ≈1163 ppm lasting 1.5-2 minutes) for 4-8 hours/day, 5 days/week	tiredness, depression, occasional silliness or staggering during the workday	Challen et al., 1958

Table 5-5

Target Organ Toxicity of Chloroform (cont.)

Target Organ	Route and Type of Exposure	Species	Dose or Exposure	Effect on Target Organ	Reference
CNS	oral, acute	rats	350 mg/kg single dose	minimum narcotic dose (MND_{50})	Jones et al., 1958
CNS	oral (drinking water), subchronic	rats	20-160 mg/kg/day x 90 days	dose-related signs of depression during 1st week only	Jorgenson and Rushbrook, 1980
CNS	oral (drinking water), subchronic	mice	32-290 mg/kg/day x 90 days	dose-related signs of depression during 1st week only	Jorgenson and Rushbrook, 1980
CNS	oral (drinking water) subchronic	rats	≈300 mg/kg/day x 90 days	no histopathologic changes in brain	Chu et al., 1982b
CNS	oral (gavage), subchronic	rats	60 mg/kg/day 6 days/week x 80 weeks	no effect on gross or histologic appearance of brain	Palmer et al., 1977

Grey, 1979). In the kidney, chloroform exposure produces necrosis of the proximal and distal convoluted tubules (Eschenbrenner and Miller, 1945a). The mechanism by which chloroform produces these effects has been extensively studied in experimental animals. From the studies summarized in Section 5.3.1 (Brown et al., 1974; Ilett et al., 1973; Docks and Krishna, 1976; Ekström et al., 1981; Lavigne and Marchand, 1974; McMartin et al., 1981; Masuda et al., 1980; Harris et al., 1982; Stevens and Anders, 1981), it appears that chloroform is first metabolized in the target organ by microsomal drug metabolizing enzymes to a reactive intermediate, probably phosgene, which in turn can react by various pathways, depending on glutathione levels, one of which is the covalent binding to liver proteins resulting in necrotic lesions. A similar mechanism may or may not occur in the kidney (Kluwe and Hook, 1981).

5.5.7 Factors that Modify the Toxicity of Chloroform. Several substances alter the toxicity of chloroform, most probably by modifying the metabolism of chloroform to a reactive intermediate (see Section 5.4). These substances are of interest because humans may be accidentally or intentionally exposed to them. Factors that potentiate the toxic effects induced by exposure to chloroform include ethanol (Kutob and Plaa, 1962; Sato et al., 1980, 1981), polybrominated biphenyls (Kluwe and Hook, 1978), steroids (Clemens et al., 1979), and ketones (Hewitt et al., 1979; Jernigan and Harbison, 1982; Branchflower and Pohl, 1981). Disulfiram and its metabolites (Scholler et al., 1970; Masuda and Nakayama, 1982; Gopinath and Ford, 1975) and high carbohydrate diets (Nakajima et al., 1982), appear to protect against chloroform toxicity.

5. REFERENCES

Adriani, J. 1970. The Pharmacology of Anesthetic Drugs. Springfield, Illinois, Charles C. Thomas. pp. 57-60.

Bomski, H., A. Sobolweska and A. Strakowski. 1967. [Toxic damage of the liver by chloroform in chemical industry workers.] Arch. Gewerbepathol. Gewerbehy. 24: 127-134. (Ger.) (Cited in Utidjian, 1976; NIOSH, 1974.)

Bowman, F.J., J. Borzelleca and A.E. Munson. 1978. The toxicity of some halomethanes in mice. Toxicol. Appl. Pharmacol. 44: 213-215.

Branchflower, R.V. and L.R. Pohl. 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.

Brown, B.R., Jr., I.G. Sipes and A.M. Sagalyn. 1974. Mechanisms of acute hepatic toxicity: Chloroform, halothane and glutathione. Anesthesiology. 41: 554-561.

Butler, T.C. 1961. Reduction of carbon tetrachloride *in vivo* and reduction of carbon tetrachloride and chloroform *in vitro* by tissues and tissue constituents. Jour. Pharmacol. Exp. Ther. 134: 311-319.

Challen, P.J.R., D.E. Hickish and J. Bedford. 1958. Chronic chloroform intoxication. Brit. Jour. Ind. Med. 15: 243-249.

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

Chu, I., D.C. Villenuve, V.E. Secours and G.C. Becking. 1982a. Toxicity of trihalomethanes: I. The acute and subacute toxicity of chloroform, bromodichloromethane, chlorodibromomethane and bromoform in rats. J. Environ. Sci. Health. B17: 205-224.

Chu, I., D.C. Villenuve, V.E. Secours, G.G. Becking and V.E. Valli. 1982b. Toxicity of trihalomethanes: II. Reversibility of toxicological changes produced by chloroform, bromodichloromethane, chlorodibromomethane and bromoform in rats. J. Environ. Sci. Health. B17: 225-240.

Cianflone, D.J., W.R. Hewitt, D.C. Villenueve and G.L. Plaa. 1980. Role of biotransformation in the alterations of chloroform hepatotoxicity produced by Kepone and Mirex. Toxicol. Appl. Pharmacol. 53: 140-149.

Clemens, T.L., R.N. Hill, L.P. Bullock, W.D. Johnson, L.G. Sulatos and E.S. Vesell. 1979. Chloroform toxicity in the mouse: Role of genetic factors and steroids. Toxicol. Appl. Pharmacol. 48: 117-130.

Conlon, M.F. 1963. Addiction to chlorodyne. Brit. Med. Jour. 2: 1177-1178.

Culliford, D. and H.B. Hewitt. 1957. The influence of sex hormone status on the susceptibility of mice to chloroform-induced necrosis of the renal tubules. Jour. Endocrinol. 14: 381-393.

Danni, O., O. Brossa, E. Burdino, P. Milillo and G. Ugazio. 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: 105-112.

Deringer, M.K., T.B. Dunn and W.E. Heston. 1953. Results of exposure of strain C3H mice to chloroform. Proc. Soc. Exp. Biol. Med. 83: 474-479.

DeSalva, S., A. Volpe, G. Leigh and T. Regan. 1975. Long-term safety studies of a chloroform-containing dentifrice and mouth-rinse in man. Food Cosmet. Toxicol. 13: 529.

Divincenzo, G.D. and W.J. Krasavage. 1974. Serum ornithine carbamyl transferase as liver response test for exposure to organic solvents. Amer. Indust. Hyg. Assoc. J. 35: 21-29.

Docks, E.L. and G. Krishna. 1976. The role of glutathione in chloroform-induced hepatotoxicity. Exp. Molec. Pathol. 24: 13-22.

Ekström, T., J. Hoegbers and B. Jernstroem. 1982. Inhibition of hepatic, gamma-glutamyl-cysteine synthetase by chloroform. Biochem. Pharmacol. 31: 3510-3513.

Eschenbrenner, A.B. and E. Miller. 1945a. Induction of hepatomas in mice by repeated oral administration of chloroform, with observations on sex differences. Jour. Natl. Canc. Instit. 5: 251-255.

Eschenbrenner, A.B. and E. Miller. 1945b. Sex differences in kidney morphology and chloroform necrosis. Science. 102: 302-303.

Goodman, L.S. and A. Gilman. 1980. The Pharmacological Basis of Therapeutics. 6th ed. New York, MacMillan and Co.

Gopinath, C. and E.J.H. Ford. 1975. The role of microsomal hydroxylases in the modification of chloroform hepatotoxicity in rats. Brit. Jour. Exp. Pathol. 56: 412-422.

Groger, W.K.L. and T.F. Grey. 1979. Effect of chloroform on the activities of liver enzymes in rats. Toxicology. 14: 23-28.

Harris, R.N., J.H. Ratnayake, V.F. Garry and M.W. Anders. 1982. Interaction hepatotoxicity of chloroform and carbon tetrachloride. Toxicol. Appl. Pharmacol. 63: 281-291.

Heller, H. and F.H. Smirk. 1932. The influence of anaesthetics and hypnotics on the absorption and excretion of water. Jour. Physiol. (London). 76: 292-302.

Hewitt, W.R., H. Mujajima, M.G. Côté and G.L. Plaa. 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, W.R., H. Miyajima, M.G. Côté and G.C. Plaa. 1979. Acute alteration of chloroform-induced hepato- and nephrotoxicity by Mirex and Kepone. Toxicol. Appl. Pharmacol. 48: 509-527.

Heywood, R., R.J. Sortwell, P.R.B. Noel, A.E. Street, D.E. Prentice, F.J.C. Roe, P.F. Wardsworth, A.N. Worden and N.J. Van Abbe´. 1979. Safety evaluation of toothpaste containing chloroform. III. Long-term study in beagle dogs. Jour. Environm. Pathol. Toxicol. 2: 835-851.

Hill, R.N. 1978. Differential toxicity of chloroform in the mouse. Ann. New York Acad. Sci. 298: 170-175.

Hjelle, J.J., A.S. Gordon and D.R. Peterson. 1982. Studies on carbon tetrachloride-ethanol interactions in mice. Toxicol. Lett. 10: 17-24.

IARC (International Agency for Research on Cancer). 1976. Disulfirams. In: Some Carbamates, Thiocarbamates and Carbazides. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man. WHO: Lyon, France. IARC, p. 85-96.

Ilett, K.F., W.D. Reid, I.G. Sipes and G. Krishna. 1973. Chloroform toxicity in mice: Correlation of renal and hepatic necrosis with covalent binding of metabolites to tissue macromolecules. Exp. Molec. Pathol. 19: 215-229.

Jernigan, J.D. and R.D. Harbison. 1982. Role of biotransformation in the potentiation of halocarbon hepatotoxicity by 2,5-hexanedione. J. Toxicol. Environ. Health. 9: 761-781.

Jones, W.M., G. Margolis and C.R. Stephen. 1958. Hepatotoxicity of inhalation anesthetic drugs. Anesthesiology. 19: 715.

Jorgenson, T.A. and C.J. Rushbrook. 1980. Effects of chloroform in the drinking water of rats and mice: Ninety-day subacute toxicity study. SRI International, Menlo Park, California. Publication No. EPA-600/1-80-030. Available from National Technical Information Service, Springfield, Virginia (NTIS PB80-219108).

Kimura, E.T., D.M. Ebert and P.W. Dodge. 1971. Acute toxicity and limits of solvent residue for sixteen organic solvents. Toxicol. Appl. Pharmacol. 19: 699-704.

Klaassen, C.D. and G.L. Plaa. 1966. Relative effect of various chlorinated hydrocarbons on liver and kidney function in mice. Toxicol. Appl. Pharmacol. 9: 139-151.

Kluwe, W.M. and J.B. Hook. 1978. Polybrominated biphenyl-induced potentiation of chloroform toxicity. Toxicol. Appl. Pharmacol. 45: 861-869.

Kluwe, W.M. and J.B. Hook. 1981. Potentiation of acute chloroform nephrotoxicity by the glutathione depletion diethyl maleate and protection by the microsomal enzyme inhibitor piperonyl butoxide. Toxicol. Appl. Pharmacol. 59: 457-466.

Knocker, P. and J. Mandelstam. 1944. Rhythmic changes in the rat produced by a single injection of chloroform. Nature (London). 154: 148-149.

Krantz, J.C., Jr. and C.J. Carr. 1965. Pharmacologic Principles of Medical Practice. 6th ed. Baltimore, Williams and Wilkins.

Krus, S. and Z. Zaleska-Rutczynski. 1970. Morphological counterparts of the genetically determined resistance of mice to chloroform. Experientia. 26: 101-102.

Kurtz, C.M., J.H. Bennett and H.H. Shapiro. 1936. Electrocardiographic studies during surgical anesthesia. J. Amer. Med. Assoc. 106: 434-441.

Kutob, S.D. and G.L. Plaa. 1962a. The effect of acute ethanol intoxication on chloroform-induced liver damage. Jour. Pharmacol. Exp. Teratol. 135: 245-251.

Kutob, S.D. and G.L. Plaa. 1962b. A procedure of estimating the hepatotoxicity potential of certain industrial solvents. Toxicol. Appl. Pharmacol. 4: 354.

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

Lavigne, J.G. and C. Marchand. 1974. The role of metabolism in chloroform hepatotoxicity. Toxicol. Appl. Pharmacol. 29: 312-326.

Lehmann, K.B. and F.F. Flury. 1943. Chlorinated hydrocarbons. In: Toxicology and Hygiene of Industrial Solvents, 1943. K.B. Lehmann and F.F. Flury, Eds. Williams and Wilkins Co., Baltimore. p. 138-145, 191-196.

Lehmann, K.B. and Hasewaga. 1910. [Studies of the absorption of chlorinated hydrocarbons in animals and humans.] Arch. Hyg. 72: 327-342. (Ger.) (Cited in NIOSH, 1974.)

Lehmann, K.B. and L. Schmidt-Kenl. 1936. [The thirteen most important chlorinated aliphatic hydrocarbons from the standpoint of industrial hygiene.] Arch. Hyg. 116: 131-200. (Ger.) (Cited in NIOSH, 1974.)

Lucas, G.H.W. 1928. A study of the fate and toxicity of bromine and chlorine containing anesthetics. Jour. Pharmacol. Exp. Ther. 34: 223.

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

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

Masuda, Y., I. Yano and T. Murano. 1980. Comparative studies on the hepatotoxic actions of chloroform and related halomethanes in normal and phenobarbital-pretreated animals. Jour. Pharmacobio. Dyn. 3: 53-64.

McLean, A.E.M. and E.K. McLean. 1969. Diet and toxicity. Brit. Med. Bull. 25: 278-281.

McLean, A.E.M. 1970. The effect of protein deficiency and microsomal enzyme induction by DDT and phenobarbitone on the acute toxicity of chloroform and a pyrrolizidiue alkaloid, retrorsine. Brit. Jour. Exp. Pathol. 51: 317-321.

McMartin, N.D., J.A. O'Connor, Jr., L.S. Kaminsky. 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: 99-110.

Nakajima, T., Y. Koyama and A. Sato. 1982. Dietary modification of metabolism and toxicity of chemical substances with specific reference to carbohydrate. Biochem. Pharmacol. 31: 1005-1011.

NCI (National Cancer Institute). 1976. Report on Carcinogenesis Bioassay of Chloroform. Available from National Technical Information Service, Springfield, Virginia. (NTIS PB-264-018).

NIOSH (National Institute for Occupational Safety and Health). 1974. Criteria for a Recommended Standard-Occupational Exposure to Chloroform. Available from National Technical Information Service, Springfield, Virginia. (NTIS PB-246-695).

Oettel, H. 1936. Arch. Exp. Pathol. Pharmacol. 183: 655.

Orth, O.S., R.R. Liebenow and R.T. Capps. 1951. III-The effect of chloroform on the cardiovascular system. In: R.M. Waters (Ed). Chloroform - A Study After 100 Years. University of Wisconsin Press, Madison, 1951. p. 39-75.

Palmer, A.K., A.E. Street, F.J.C. Roe, A.N. Worden and N.J. Van Abbé. 1979. Safety evaluation of toothpaste containing chloroform. II. Long-term studies in rats. Jour. Environm. Pathol. Toxicol. 2: 821-833.

Paul, B.B. and D. Rubinstein. 1963. Metabolism of carbon tetrachloride and chloroform by the rat. Jour. Pharmacol. Exp. Ther. 141: 141-148.

Piersol, G.M., A.J. Tumen and L.S. Kau. 1933. Fatal poisoning following the ingestion of chloroform. Med. Clin. North Am. 17: 587-601.

Pohl, L.R., B. Bhooshan, N.F. Whittaker and G. Krishna. 1977. Phosgene: A metabolite of chloroform. Biochem. Biophys. Res. Commun. 79: 684-691.

Pohl, L.R. 1979. Biochemical toxicology of chloroform. Rev. Biochem. Toxicol. 1: 79-107.

Reid, W.D. and G. Krishna. 1973. Centrilobular hepatic necrosis related to covalent binding of metabolites of halogenated aromatic hydrocarbons. Exp. Molec. Pathol. 18: 80-95.

Reitz, R.H., J.F. Quast, W.T. Stott, P.G. Watanabe and P.J. Gehring. 1980. Pharmacokinetics and macromolecular effects of chloroform in rats and mice: Implications for carcinogenic risk estimation. Water Chlorinat. Environm. Impact Health Eff. 3: 983-993.

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

Roe, F.J.C., A.K. Palmer, A.N. Worden and N.J. Van Abbe´. 1979. Safety evaluation of toothpaste containing chloroform. I. Long-term studies in mice. Jour. Environm. Toxicol. 2: 799-819.

Sato, A., T. Nakajima and Y. Koyama. 1980. Effects of chronic ethanol consumption on hepatic metabolism of aromatic and chlorinated hydrocarbons in rats. Br. J. Indust. Med. 37: 382-386.

Sato, A., T. Nakajima and Y. Koyama. 1981. Dose-related effect of a single dose of ethanol on the metabolism in rat liver of some aromatic and chlorinated hydrocarbons. Toxicol. Appl. Pharmacol. 60: 8-15.

Sax, N.I. 1979. Dangerous Properties of Industrial Materials, Fifth edition. New York, Van Nostrand Reinhold Company, Inc. p. 193.

Scholler, K.L. 1966. Elektronmikroskopische untersuchungen an leberzellen der Ratte nach narkosen mit verschiedenen inhlalationsnarkotica. Der Anaesthetist. 15: 145-148. (Ger).

Scholler, K.L. 1967. Effect of haloethane and chloroform on the fine structure and protein synthesis of rat liver. Experimentia. 23: 652-655. (In German).

Scholler, K.L. 1970. Modification of the effects of chloroform on the rat liver. Brit. Jour. Anaesth. 42: 603-605.

Schroeder, H.G. 1965. Acute and delayed chloroform poisoning. Brit. J. Anaesth. 37: 972-975.

Sipes, I.G., G. Krishna and J.R. Gillette. 1977. Bioactivation of carbon tetrachloride, chloroform, and bromotrichloromethane: Role of cytochrome P-450. Life Sci. 20: 1541-1548.

Stevens, J.L. and M.W. Anders. 1981. Effect of cysteine, diethyl maleate, and phenobarbital treatment on the hepatoxicity of (1H)- and (2H)-chloroform. Chem. Biol. Interact. 37: 207-217.

Storms, W.W. 1973. Chloroform parties. J. Amer. Med. Assoc. 225: 160.

Torkelson, T.R., F. Oyen and V.K. Rowe. 1976. The toxicity of chloroform as determined by single and repeated exposure of laboratory animals. Amer. Ind. Hyg. Assoc. Jour. 37: 697-705.

Utidjian, H.M.D. 1976. Recommendations for a chloroform standard. Jour. Occupat. Med. 18: 253.

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

von Oettigen, W.F. 1964. The halogenated hydrocarbons of industrial and toxicological importance. Amsterdam, Elsevier.

Wallace, C.J. 1950. Hepatitis and nephrosis due to cough syrup containing chloroform. Calif. Med. 73: 442.

Watrous, W.M. and G.L. Plaa. 1972. Effect of halogenated hydrocarbons on organic ion accumulation by renal cortical slices of rats and mice. Toxicol. Appl. Pharmacol. 22: 528-543.

Winslow, S.G. and H.B. Gerstner. 1978. Health aspects of chloroform - A review. Drug Chem. Toxicol. 1: 259-275.

Wood, S., B. Wardley-Smith, M. J. Halsey and C.J. Green. 1982. Hydrogen bonding in mechanisms of anesthetic tested with chloroform and deuterated chloroform. Br. J. Anaesth. 54: 387-391.

Wood-Smith, F.G. and H.C. Stewart. 1964. Drugs in Anaesthetic Practice. Washington, D.C. Butterworth, Inc. pp. 131-135.

United States
Environmental Protection
Agency

Office of Health and
Environmental Assessment
Washington DC 20460

EPA-600/8-84-004A
March 1984
External Review Draft

Research and Development

EPA

Health Assessment Document for Chloroform Review Draft

(Do Not Cite or Quote)

Part 2 of 2

NOTICE

This document is a preliminary draft. It has not been formally released by EPA and should not at this stage be construed to represent Agency policy. It is being circulated for comment on its technical accuracy and policy implications.

EPA-600/8-84-004A
March 1984
External Review Draft

Review
Draft

(Do Not
Cite or Quote)

Health Assessment Document for Chloroform

Part 2 of 2
External Review Draft

NOTICE

This document is a preliminary draft. It has not been formally released by the U.S. Environmental Protection Agency and should not at this stage be construed to represent Agency policy. It is being circulated for comment on its technical accuracy and policy implications.

U.S. ENVIRONMENTAL PROTECTION AGENCY
Office of Research and Development
Office of Health and Environmental Assessment
Environmental Criteria and Assessment Office
Research Triangle Park, NC 27711

March 1984

PREFACE

The Office of Health and Environmental Assessment has prepared this health assessment to serve as a "source document" for EPA use. This health assessment document was developed for use by the Office of Air Quality Planning and Standards to support decision-making regarding possible regulation of chloroform as a hazardous air pollutant.

In the development of the assessment document, the scientific literature has been inventoried, key studies have been evaluated and summary/conclusions have been prepared so that chemical's toxicity and related characteristics are qualitatively identified. Observed effect levels and other measures of dose-response relationships are discussed, where appropriate, so that the nature of the adverse health response are placed in perspective with observed environmental levels.

This document will be subjected to a thorough copy editing and proofing following the revision based on the EPA's Scientific Advisory Board review comments.

TABLE OF CONTENTS

TABLE OF CONTENTS (cont.)

TABLE OF CONTENTS (cont.)

TABLE OF CONTENTS (cont.)

LIST OF TABLES

LIST OF TABLES (cont.)

LIST OF TABLES (cont.)

LIST OF TABLES (cont.)

LIST OF FIGURES

LIST OF FIGURES (cont.)

The Office of Health and Environmental Assessment (OHEA), U.S. EPA, is responsible for the preparation of this health assessment document. The Environmental Criteria and Assessment Office (ECAO/RTP), OHEA, had the overall responsibility for coordination and the document production effort.

Project Manager

Si Duk Lee, Ph.D.
Environmental Criteria and Assessment Office
U.S. EPA, Research Triangle Park, N.C. 27711
(919) 541-4159

Authors and Reviewers

The principal authors of this document are:

Larry Anderson Ph.D.
Carcinogen Assessment Group
U.S. EPA, Washington, D.C.

David Baylis, M.S.
Carcinogen Assessment Group
U.S., EPA, Washington, D.C.

Chao W. Chen, Ph.D.
Carcinogen Assessment Group
U.S., EPA, Washington, D.C.

Carol Sakai, Ph.D.
Reproductive Effects Assessment Group
U.S., EPA, Washington, D.C.

Sheila Rosenthal, Ph.D.
Reproductive Effects Assessment Group
U.S., EPA, Washington, D.C.

I.W.F. Davidson, Ph.D.
Bowman Gray School of Medicine
Winston Salem, N.C.

D. Anthony Gray, Ph.D.
Syracuse Research Corp.
Syracuse, N.Y.

Sharon B. Wilbur, M.A.
Syracuse Research Corp.
Syracuse, N.Y.

Joan P. Coleman, Ph.D.
Syracuse Research Corp.
Syracuse, N.Y.

The following individuals provided peer-review of this draft or earlier drafts of this document:

U.S. Environmental Protection Agency

Joseph Padgett
Office of Air Quality Planning and Standards
U.S. EPA

Karen Blanchard
Office of Air Quality Planning and Standards
U.S. EPA

Jerry F. Stara, D.V.M.
Office of Health and Environmental Assessment
Environmental Criteria and Assessment Office
U.S. EPA

Lester D. Grant, Ph.D.
Office of Health and Environmental Assessment
Environmental Criteria and Asssessment Office
U.S. EPA

Participating Members of the Carcinogen Assessment Group

Roy E. Albert, M.D. (Chairman)
Elizabeth L. Anderson, Ph.D.
Larry D. Anderson, Ph.D.
Steven Bayard, Ph.D.
David L. Bayliss, M.S.
Chao W. Chen, Ph.D.
Margaret M. L. Chu, Ph.D.
Bernard H. Haberman, D.V.M., M.S.
Charalingayya B. Hiremath, Ph.D.
Robert E. McGaughy, Ph.D.
Dharm W. Singh, D.V.M., Ph.D.
Todd W. Thorslund, Sc.D.

Participating Members of the Reproductive Effects Assessment Group

Peter E. Voytek, Ph.D. (Director)
John R. Fowle, III, Ph.D.
Carol Sakai, Ph.D.
Ernest Jackson, M.D.
K.S. Lavappa, Ph.D.
Sheila Rosenthal, Ph.D.
Vicki Vaughn-Dellarco, Ph.D.

External Peer Reviewers

Dr. Karim Ahmed
Natural Resources Defense Fund
122 E. 42nd Street
New York, N.Y. 10168

Dr. Eula Bingham
Graduate Studies and Research
University of Cincinnati (ML-627)
Cincinnati, Ohio 45221
(513) 475-4532

Dr. James Buss
Chemical Institute of Industrial Toxicology
Research Triangle Park, N.C. 27709

Dr. I.W.F. Davidson
Wake Forest University
Bowman Gray Medical School
Winston Salem, N.C.

Dr. Larry Fishbein
National Center for Toxicological Research
Jefferson, Arkansas 72079
(501) 542-4390

Dr. Derek Hodgson
Department of Chemistry
University of North Carolina
Chapel Hill, N.C. 27514

Dr. Marshall Johnson
Thomas Jefferson Medical College
Department of Anatomy
1020 Locust Street
Philadelphia, Pennsylvania 19107

Dr. Trent Lewis
National Institute of Occupational Safety and Health
26 Columbia Parkway
Cincinnati, Ohio 45226
(513) 684-8394

Dr. Richard Reitz
Dow Chemical, USA
Toxicology Research Laboratory
1803 Building
Midland, Michigan 48640

Dr. Marvin A. Schneiderman
Clement Associates, Incorporated
Arlington, Virginia 22209
(703) 276-7700

Dr. Bernard Schwetz
National Institute of Environmental Health
Research Triangle Park, NC 27709
(919) 541-7992

Dr. James Selkirk
Oak Ridge National Laboratory
Oakridge, Tennessee 37820
(615) 624-0831

Dr. Samuel Shibko
Food and Drug Administration
Division of Toxicology
200 C Street, S.W.
Washington, D.C. 20204
Telephone:

Dr. Robert Tardiff
1423 Trapline Court
Vienna, Virginia 22180
(703) 276-7700

Dr. Norman M. Trieff
University of Texas Medical Branch
Department of Pathology, UTMB
Galveston, Texas 77550
(409) 761-1895

Dr. Benjamin Van Duuren
Institute of Environmental Medicine
New York University Medical Center
New York, New York 10016
(212) 340-5629

Dr. James Withey
Health and Protection Branch
Department of National Health & Welfare
Tunney's Pasture
Ottawa, Ontario
CANADA, KIA 01Z

6. TERATOGENICITY AND REPRODUCTIVE EFFECTS

Schwetz et al. (1974) evaluated the effects of reagent grade chloroform (lot no. 9649, Burdick and Jackson Laboratories, purity not reported) on the maternal and fetal well-being of Sprague-Dawley rats. Twenty female rats were exposed, by inhalation (7 hr/day), to 30, 100, and 300 ppm chloroform on days 6 through 15 of gestation. The authors analyzed the results statistically using Fisher's Exact Probability test, analysis of variance, Dunnett's test, or Tukey's test to compare the frequency of anomalies, resorptions, maternal and fetal weights, body lengths, liver weights, or serum glutamic pyruvic transaminase (SGPT) activity in the exposed versus the control groups. The level of significance was chosen at $p < 0.05$, and the litter was used as the experimental unit.

When the animals were exposed to the highest dose of chloroform (300 ppm), there was a significant increase in the number of resorptions and a decrease in the conception rate (Schwartz et al., 1974). At the lower doses (30 and 100 ppm), no alterations in resorption rate, fetal body weight, conception rate, number of implantations, or average litter size were observed. Fetal crown-rump length was significantly decreased at 30 and 300 ppm, but not at the 100 ppm level. At 100 ppm, an increase in the incidence of acaudia (absence of tail), short tail, imperforate anus, subcutaneous edema, missing ribs, and delayed ossification of sternebrae were observed. At 300 ppm, subcutaneous edema and abnormalities of the skull and sternum were observed, but the incidence of these was not statistically significant. The authors pointed out that the small numbers of survivors in the 300 ppm group (4 ± 7 versus the control 10 ± 4 live fetuses/litter) may have prevented adequate statistical evaluation of this effect.

In this study (Schwetz et al., 1974), chloroform also produced maternal toxicity, such as a decrease in the rate of maternal weight gain at all dose levels and a decrease in food consumption during pregnancy at the 100 and 300 ppm level. Other maternal effects observed were changes in liver weight gain during pregnancy (no change in absolute liver weight gain at 30 ppm, but an increase at 100 ppm, possibly due to the concomitant anorexia at this dose). No significant changes in SGPT activity were observed in groups exposed to chloroform at the 100 and 300 ppm levels (the only two doses evaluated). Since the developmental effects observed in the 300 ppm group were associated with anorexic effects in the mother, a starvation control group was included in this study. This starvation control group was restricted to food consumption comparable to the 300 ppm chloroform group. Animals on a starvation diet (allowed 3.7 g/day of food on days 6 through 15 compared with control animals whose food consumption average 19-25 g/day on days 6 through 15) had a significant decrease in the absolute weight of the liver and an increase in the relative weight of the liver. The effects of 300 ppm chloroform on the increase in the relative weight of the liver were much greater than starvation alone. Additionally, and perhaps most importantly, exposure to 300 ppm chloroform resulted in a dramatic decrease in the number of animals pregnant at sacrifice (15% pregnant versus 88% in air control), a decrease in the number of live fetuses per litter (4 versus 10 live fetuses/litter), and an increased percentage of resorptions (100% vs. 57%). Examination of the uteri indicated that the conceptus had been completely resorbed very soon after implantation. These effects appeared to result primarily from chloroform exposure, and not maternal toxic influence, since anorexia and liver weight changes associated with starvation were not accompanied by embryotoxic and teratogenic effects.

Murray et al. (1979) evaluated the effects of chloroform (spectral grade, Mallinckrodt, lot CSZ, code 4434, purity not reported) administered by inhalation (7 hr/day) to CF-1 mice. Thirty-five animals per group were exposed on gestation days 1 through 7 and 6 through 15; forty animals per group were exposed on gestation days 8 through 15. Only one dose, 100 ppm, was tested during three different time periods (days 1 through 7, 6 through 15, 8 through 15 of gestation) in CF-1 mice. The varying exposure periods were designed to evaluate the effects of chloroform in very early pregnancy, organogenesis, and somewhat later in pregnancy. Special sodium sulfide staining of the uteri was used to detect very early pregnancies.

The authors (Murray et al., 1979) analyzed the results statistically using the Fisher's Exact Probability test to evaluate pregnancy incidence; the modified Willcoxan test for fetal outcomes; the Mann-Whitney signed rank test for SGPT activity; and one-way analysis of variance for fetal body weights and body measurements, maternal body weights, liver weights, food consumption, and number of implantations and resorptions. The level of significance was chosen at $p < 0.05$.

Murray et al. (1979) reported that 100 ppm chloroform resulted in a decrease in the total number of pregnancies when the animals were exposed on days 1-7 or 6-15 of gestation but not on days 8-15 (see Table 6-1 for summary of data). In the pregnant animals, however, there was no significant effect on the average number of implantation sites. In animals exposed on days 1-7 of gestation, but not in those exposed on days 6-15 or 8-15, there were significant increases in resorptions per litter. This effect was accounted for by the loss of two entire litters. Mean fetal body weight and crown-rump length were significantly decreased in the groups exposed on days 1-7 and 8-15, but not in those exposed on days 6-15. Maternal toxicity (slight decrease in body weight gain during

TABLE 6-1. SUMMARY OF EFFECTS
(Murray et al., 1979)

Chemical: Chloroform, spectral grade, Lot CSZ, Code 4434, Mallinckrodt, Inc.
Animal: CF-1 mice, 35 animals in groups exposed on days 1-7 and 6-15;
40 animals in groups exposed on days 8-15
Route of exposure: Inhalation, 100 ppm (one dose only)
Duration of exposure: 7 hr/day, days 6-15 of gestation

Days	Pregnant (Implantation sites)		Additional pregnancies (special stain) (number of animals)		Total pregnancies (implantation sites and special stain)	
	Exposed	Control	Exposed	Control	Exposed	Control
1-7	11/34 (32%)	22/35 (63%)	4	4	15/34 (44%)	26/35 (74%)
6-15	13/35 (37%)	29/34 (85%)	2	2	15/35 (43%)	31/34 (91%)
8-15	18/40 (45%)	25/40 (62%)	6	1	24/40 (60%)	26/40 (65%)

(continued on the following page)

TABLE 6-1. (continued)

Days	1-7	6-15	8-15
Fetal effects	resorption	---	---
	fetal body weight and crown-rump length	---	fetal body weight and crown-rump length
	---*	---	cleft palate
	delayed skeletal ossification	---	delayed skeletal ossification
Maternal effects	body weight gain	body weight gain	body weight gain
	---	liver weight	liver weight
	---	SGPT (only one dose)	---

*Not significantly different.

pregnancy) was seen in groups exposed on days 6-15, with a more severe decrease in groups exposed on days 1-7 and 8-15. Less food and water were consumed in all experimental groups as compared to controls. Absolute and relative weight of the liver were increased in groups exposed on days 6-15 and 8-15, but not in those exposed on days 1-7. Serum glutamic pyruvic transaminase (SGPT) activity was increased in mice exposed on days 6-15, which was the only time period evaluated for this measurement.

A summary of this study (Murray et al., 1979) is presented in Table 6-1. One major result was that chloroform, under the conditions of this experiment, caused a decrease in pregnancy. The authors concluded that chloroform affected the stages either prior to, or in, early implantation. However, because of the small numbers of animals and the lack of dose-response evaluation (only one dose was tested, 100 ppm), this conclusion must be considered tentative until future studies confirm this observation. Other results of this study (Murray et al., 1979) indicated that the incidence of cleft palates increased in pups exposed in utero on days 8-15 of gestation, but not on days 1-7 or 6-15. The authors (Murray et al., 1979) suggested three possibilities to explain this last result. The first was that earlier exposure on days 6-15 prevented susceptible concepti from implanting. The second possibility was that the number of litters available (11 in the group exposed on days 6-15) was insufficient to detect this effect. The third was that the teratogenic effect (cleft palate) did not occur in concepti exposed on days 1-7 since they were exposed before organogenesis. The number of offspring coming to term was consistently less in all exposure groups than in the controls (days 1-7, 9 litters versus 22 in control; days 6-15, 11 litters versus 29 in control; days 8-15, 18 litters versus 24 in control). Therefore it was not clear whether chloroform produced teratogenicity separate from embryotoxicity. Since the pups with cleft palate in groups

were also retarded in growth, it was suggested that the ability of chloroform to cause malformations was indirect and not direct. However, this was not experimentally determined, and it is not known if the tendency for lowered fetal weight and/or delayed skeletal growth is correlated with a higher incidence of malformations.

In conclusion, this study (Murray et al., 1979) indicated that chloroform administration (100 ppm) by inhalation (7 hr/day) produced teratogenic and embryotoxic effects and interfered with pregnancy in addition to causing maternally toxic effects (changes in liver weight and decreases in weight gain during pregnancy). Exposure in the early stages of pregnancy appeared to produce a decreased incidence of conception, but the results of this study did not conclusively determine which days of pregnancy were most susceptible to the effects of chloroform. To answer this question, it would be necessary to use a greater number of doses and larger numbers of animals.

Thompson, Warner, and Robinson (1974) investigated the effect of chloroform administered orally, using Sprague-Dawley rats and Dutch-Belted rabbits. The rats were intubated with chloroform (Mallinckrodt, Batch ZJL dissolved in corn oil, purity not reported) twice a day in divided doses of 20 to 501 mg/kg/day. The rabbits were intubated once a day in doses of 20 to 398 mg/kg/day. Each study was divided into two parts, a range-finding portion designed to establish the proper dose range (six rats were administered 79, 126, 300, 316, and 501 mg/kg/day of chloroform; five rabbits were administered 63, 100, 159, 251, and 398 mg/kg/day), and a teratology study, using greater numbers of animals and three doses (25 rats were administered 20, 50, and 126 mg/kg/day). The rats were exposed to chloroform on days 6 through 15 of gestation, while the rabbits were exposed on days 6 through 18 of gestation.

Statistical evaluations of maternal body weight gains, food consumption, implantations, corpus luteum, resorptions, litter size, and fetal weights were made by an analysis of variance and Dunnett's Two-Tailed Multiple Range test. Sex ratios and frequency of anomalies among the fetal population and among litters were analyzed by the chi-square test. In all analyses, the level of significance chosen was $p < 0.05$.

The data for the range-finding portion of this study (Thompson et al., 1974) was not presented; however, the authors reported that rats treated orally with greater than 126 mg/kg/day chloroform had signs of maternal toxicity, such as a decrease in food consumption, acute toxic nephrosis, hepatitis, and gastric erosion. Fetal development was adversely affected in rats receiving 316 and 501 mg/kg/day with a decrease in fetal viability, litter size, and fetal weight and an increase in the number of resorptions. Only two rats survived when given 501/mg/kg/day; one was not pregnant, the other had complete early resorptions. In the rat teratology study, animals receiving 50 and 126 mg/kg/day displayed signs of maternal toxicity (lowered body weight gain, lowered food consumption, fatty changes in the liver). No overt toxic effects were observed in animals given 20 mg/kg/day, and no malformation was noted at any dose level.

In the range-finding portion of the study by Thompson et al. (1974) using rabbits, severe acute hepatitis and nephrosis were observed in animals given 63 mg/kg/day and higher. No overt signs of toxicity were observed at the 25 mg/kg/day level. In the two surviving dams given 100 mg/kg/day, one had four resorption sites with no viable concepti, while the other was not pregnant. No other embryotoxic or teratogenic effect was observed. In the teratology study of rabbits, maternal toxicity (depressed weight gain) was observed at the 50 mg/kg/day level. In the fetus, mean body weight was depressed at the 20 and 50 mg/kg/day levels, but no abnormalities were observed. Incomplete skeletal

ossification at the 25 or 35 mg/kg/day level was observed amongst fetuses, but not amongst litters when analyzed statistically.

In this study (Thompson et al., 1974), adverse effects resulting from chloroform exposure, such as skeletal deformities and deficiencies in pregnancy maintenance that were observed by Schwetz et al. (1974) and Murray et al. (1979), were not observed except at doses acutely toxic to the dam. The authors suggested that the greater maternal toxicity observed in this study compared to that of others (Schwetz et al., 1974; Murray et al., 1979) could be explained by the route or duration of exposure. In the study by Thompson et al. (1974), rats and rabbits were exposed orally to chloroform or twice a day, while Schwetz et al. (1974) and Murray et al. (1979) administered chloroform by inhalation 7 hr/day. The lack of information on the pharmacokinetic interaction of chloroform, however, prevents an evaluation of the role of exposure in producing adverse reproductive outcome.

Burkhalter and Balster (1979) evaluated the potential of chloroform to adversely affect behavior in developing ICR mice. This study was designed as a preliminary screening study, with the parental generation of male and female mice exposed 21 days prior to mating, during mating, and for an additional 21 days. The offspring were exposed starting on day seven until day 21 after birth. Only one oral dose of chloroform, 31.1 mg/kg/day, was administered to five control and five experimental animals. Each litter was reduced to eight pups, and three pups were randomly selected for behavorial teratogenic testing. The chloroform (Mallinckrodt, nanograde purity) was administered by gavage and delivered in a solution of 1 part polyoxyethylated vegetable oil, Emulphor (EL-620, GAF Corp., New York), and 8 parts saline. A variety of behavorial responses were evaluated which included: righting reflex, forelimb placing response, forepaw grasp, rooting reflex, cliff drop aversion, auditory startled

response, bar-holding ability, eye opening, motor performance, and learning ability. The scoring for these responses was based upon predesignated criteria. These criteria established behavioral ability by measuring both objective standards (time to complete test) and subjective standards ("weak" or "complete" grasp of paws).

Analysis of variance was used to statistically evaluate tests of passive avoidance. Screen test latencies were analyzed by t-test. The data from the neurobehavioral developmental scale were analyzed using a Mann-Whitney U test. The level of statistical significance was chosen at $p < 0.05$.

Burkhalter and Balster (1979) reported that the sizes of litters were similar for both the control and experimental groups; however, fetal body weight gain of pups during the 14 days of exposure (days 7-21 following birth) was decreased. Forelimb placement response was reduced in the exposed group on day 5 and 7 of birth, but not on day 9. The significance of this reduction is not known, although the recovery on day 9 suggested that the effect was reversible. The other behavorial responses were not significantly different in the exposed groups. Burkhalter and Balster (1979) concluded that 31.1 mg/kg/day of chloroform produced no significant adverse behavorial effects in pups exposed both in utero and after birth (days 7-21). However, since this study was designed as a preliminary screening study, using one dose and small numbers of animals, there was no attempt to evaluate the full range of dose-related effects. In future studies, it would be desirable to evaluate at least three doses, including doses high enough to produce some maternal toxicity, in addition to using a larger sample size.

In an abstract, Dilley et al. (1977) reported the effects of exposure to chloroform on pregnant rats (strain not reported, number of animals not reported). The animals were exposed by inhalation (20 ± 1.2 gm3/day) on days 7-14 of

gestation. Two lower concentrations were administered in the study, but the doses were not reported in the abstract. Dilly reported that chloroform increased fetal mortality and decreased fetal weight gain; however, there were no malformations.

In another abstract, Ruddick et al. (1980) exposed 15 Sprague-Dawley rats to 100, 200, and 400 mg/kg/day of chloroform by gavage on days 6-15 of gestation. Chloroform (purity not reported) was reported to cause maternal toxicity (changes in weight gain, biochemical and hematological parameters, and liver or kidney changes). Chloroform also produced adverse effects in fetal development (type not specified), but the authors attributed these effects primarily to maternal toxicity and not directly to chloroform exposure. Without the presentation of this data, however it is not possible to fully evaluate these results.

6.1 SUMMARY

In summary, the results of four articles and two abstracts indicated that under the conditions of the experiments, chloroform has the potential for causing adverse effects in pregnancy maintenance, delays in fetal development and production of terata in laboratory animals. The adverse effects on the conceptus were observed in association with maternal toxicity. The type and severity of effects appeared to be specific to the conceptus, affecting the fetus to a much greater degree than the mother. Therefore, it was concluded that chloroform has the potential for causing embryotoxicity and teratogenicity (Schwetz et al., 1974; Murray et al., 1979). The results of other studies indicated that chloroform has no significant effect on neonatal behavior (Burkhalter and Balster, 1979) and does not cause adverse fetal effects except at maternally toxic levels (Thompson et al., 1974). The two abstracts did not contain enough detail for critical scientific review (Dilley et al., 1977;

Ruddick et al., 1980).

Studies administering chloroform by inhalation for 7 hr/day (Schwetz et al., 1974; Murray et al., 1974) reported more severe outcomes than other studies which administered chloroform by intubation once or twice a day (Thompson et al., 1974; Burkhalter and Balster, 1979). However, since the pharmocokinetic relationship associated with route or duration of exposure have not been studied, it is not possible to evaluate the importance of the route of exposure in causing adverse reproductive outcome. To evaluate more fully the influence of these factors, additional investigations would have to be conducted.

6.2 REFERENCES

Burkhalter, J., and R.L. Balster. 1979. Behavioral teratology evaluation of chloroform in mice. Neurobehavioral Toxicol. 1:199-205.

Dilley, J.V., N. Chernoff, D. Kay, N. Winslow, and E.W. Newell. 1977. Inhalation teratology studies of five chemicals in rats. Toxicol. Appl. Pharmacol. 41:196.

Murray, F.A., B.A. Schwetz, J.G. McBride, and R.E. Staples. 1979. Toxicity of inhaled chloroform in pregnant mice and their offspring. Toxicol. Appl. Pharmacol. 50:151-522.

Ruddick, J.A., D.C. Villenouve, I. Chu, and V.E. Balli. 1980. Teratogenicity assessment of four halomethanes. Teratology 21:66A.

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

Thompson, D.J., S.D. Warner, and V.B. Robinson. 1974. Teratology studies on orally administered chloroform in the rat and rabbit. Toxicol. Appl. Pharmacol. 29: 348-357.

7. MUTAGENICITY

7.1 INTRODUCTION

The mutagenic potential of chloroform ($CHCl_3$) has been assessed by evaluation of the results from five *in vitro* bacterial studies, one host-mediated assay using *Salmonella* as the indicator organism, one yeast study, one *Drosophila* sex-linked recessive lethal test, one *in vitro* mammalian cell mutagenicity assay, two sperm head abnormality tests, three chromosome aberration studies, and six DNA damage studies (sister chromatid exchange and unscheduled DNA synthesis). These reports are discussed below. Also, several assays from a recently published screening study, in which 42 chemicals were tested in various short-term protocols, are briefly discussed. The majority of the above studies were negative. Information relating to the binding of metabolically activated $CHCl_3$ to cellular macromolecules is presented before the sections assessing the genetic damage caused by $CHCl_3$. This was done to set the stage for the discussion of the negative mutagenicity studies described below and to support the suggestion that $CHCl_3$ may be a weak mutagen. In addition, suggestions for further testing are presented.

7.2 COVALENT BINDING TO MACROMOLECULES

As mentioned in Chapter 4, the primary reactive metabolite of $CHCl_3$ is phosgene, $COCl_2$. Phosgene is a crosslinking agent and may covalently bind to and crosslink macromolecules. Thus, the toxicity and carcinogenicity of $CHCl_3$ may be related to its metabolism to phosgene. The DNA binding potential and carcinogenicity of phosgene are currently under investigation in Dr. B.L. Van Duuren's laboratory at New York University Medical Center. Preliminary evidence indicates that phosgene binds to DNA (Dr. Sipra Banerjee, New York University Medical Center, personal communication). The binding potential of metabolically activated $CHCl_3$ has been assessed in several

studies. Some of these studies have been described in Chapter 4, section 4.6. Additional studies and studies not adequately covered in Chapter 4 for the purposes of this chapter on mutagenicity are described below.

Diaz Gomez and Castro (1980a) assessed the $CHCl_3$ activation potential of purified rat liver nuclei by measuring covalent binding of nuclear-activated $CHCl_3$ to nuclear protein and lipid. The results were compared to results obtained from similar incubation mixtures containing microsomes instead of purified nuclei. The incubation mixtures containing either nuclei (1.3 mg protein/ml) or microsomes (1.56 mg protein/ml) were incubated for 30 min in 6.4 nM $^{14}CHCl_3$ (5.4 Ci/mol) and an NADPH generating system. The authors observed that the extent of binding to protein in the nuclear preparation was approximately 40% of that observed for microsomes (nuclei, 27 pmol/mg; microsomes, 68 pmol/mg). Binding to nuclear lipid was approximately 35% of that observed for microsomes (nuclei, 20 pmol/mg; microsomes, 57 pmol/mg). Thus, isolated nuclei were less efficient than microsomes in metabolizing $CHCl_3$, but the results were within the same order of magnitude.

This study suggests that metabolism of $CHCl_3$ to a reactive intermediate(s) can occur in nuclear membranes, as may be the case with other xenobiotics (Weisburger and Williams, 1982). It should be mentioned, however, that the nuclear preparations were contaminated with trace amounts of endoplasmic reticulum, which may have been sufficient to result in at least part of the nuclear activation observed.

In a subsequent study, Diaz Gomez and Castro (1980b) exposed rat or mouse liver DNA or RNA to $^{14}CHCl_3$ *in vivo* or *in vitro* without finding any significant binding of ^{14}C to the nucleic acids. However, the specific activity of the $^{14}CHCl_3$ was only 5.4 Ci/mol, which may have been too low to allow for observation of binding to DNA (Brookes and Lawley, 1971),

especially with a background count of 160 dpm.

Reitz et al. (1980) published results of DNA alkylation studies in livers and kidneys of male mice that were exposed orally to $CHCl_3$ at 240 mg/kg. A very small amount (1-3 x 10^{-6} alkylations/deoxynucleotide) of alkylation was observed. However, these results cannot be interpreted because the specific activity of the $^{14}CHCl_3$ used was not specified and because the experimental procedures used were not described.

In summary, binding of metabolically activated $CHCl_3$ to liver microsomal and nuclear protein and lipid has been observed. The only studies that attempted to measure binding to nucleic acids were inconclusive.

7.3 MUTAGENICITY STUDIES IN BACTERIAL TEST SYSTEMS

Uehleke et al. (1977) tested $CHCl_3$ for mutagenicity in suspension assays with S. typhimurium strains TA1535 and TA1538. No mutagenic activity was observed. About 6-9 x 10^8 bacteria were incubated for 60 min under N_2 in tightly closed test tubes with 5 mM $CHCl_3$ and microsomes (5 mg protein) plus an NADPH generating system. The mutation frequencies (his^+ colony forming units/10^8 his^- colony forming units) were less than 10 for both strains and the spontaneous mutation frequencies were 3.9 $\pm$ 3.7 for strain TA1535 and 4.4 $\pm$ 3.5 for strain TA1538. At this concentration of $CHCl_3$, survival of the bacteria was at least 90%. Additional higher concentrations should have been tested, because the mutagenic range can occur at higher toxicities. Dimethylnitrosamine (50mM), cyclophosphamide (0.5 mM), 3-methylcholanthrene (0.1 mM), and benzo[a]pyrene (0.1 mM) were the positive controls used in this study. Information was not provided on the survival of the bacteria at these concentrations of the positive control chemicals. Although these chemicals were mutagenic in the presence of the S9 activation system, they may not be appropriate as controls for $CHCl_3$ because they are not halogenated alkanes

and therefore are not metabolized like them.

Studies demonstrating that metabolically activated $CHCl_3$ binds to protein and lipid in the presence of rabbit microsomes were also described in the paper by Uehleke et al. (1977) and are mentioned in Chapter 4 of this document. However, it is not clear from the description provided in this report by Uehleke et al. (1977) whether rat, mouse, or rabbit microsomes were used in the mutagenicity studies. If mouse or rat microsomes rather than rabbit microsomes were used for the mutagenicity experiments, it cannot be assumed that $CHCl_3$ was sufficiently activated, since activation sufficient for binding of $^{14}CHCl_3$ to macromolecules was shown in this paper only with rabbit microsomes. Another deficiency in this study is that the Ames strains TA98 and TA100 were not used. These strains contain an R factor plasmid that increases the sensitivity of the tester strains to certain mutagens.

The mutagenicity of $CHCl_3$ was also tested in a study designed to evaluate the mutagenic potential of chemicals identified in drinking water (Simmon et al., 1977). No mutagenic activity was detected with $CHCl_3$. The authors tested 71 of the 300 chemicals that had been identified in public water supplies. $CHCl_3$ was tested at 10% by volume (1.24 M) in a suspension assay with Salmonella strains TA1535, TA1537, TA1538, TA98, and TA100 and S9 mix prepared from Aroclor 1254-treated rats. This concentration of $CHCl_3$ exceeds its solubility. Mutagenic activity was not observed. However, information on toxicity was not provided.

$CHCl_3$ was also tested in this study in a desiccator to assess mutagenicity due to vapor exposure (Simmon et al., 1977). Agar plates were placed uncovered in a desiccator above a glass petri dish containing the $CHCl_3$. The desiccator contained a magnetic stirrer which acted as a fan to aid in evaporation of the measured amount of $CHCl_3$ and to maintain an even

distribution of the vapors. Plates were exposed to the vapors for 7-10 hr and then removed from the desiccators, covered, and incubated approximately 40 hr before scoring. As in the suspension assay described above, mutagenic activity was not observed and no information on toxicity was provided.

This study by Simmon et al. (1977), although lacking some specific details of the $CHCl_3$ assay, clearly identifies other trihalomethanes ($CHBr_3$, $CHBr_2Cl$, $CHBrCl_2$) as mutagens in the vapor assay in desiccators. Methyl bromide, methyl chloride, methyl iodide, and methylene chloride were also found to be mutagenic in the desiccator assay. However, these seven halogenated compounds did not require metabolic activation to exhibit mutagenic activity. It may be that $CHCl_3$ itself is not mutagenic and the rat liver S9 was not sufficient to metabolize $CHCl_3$ to a potential mutagenic reactive intermediate (? phosgene), even though the demonstration of mutagenicity of three of the chemicals tested [bis(2-chloroisopropyl)ether, vinyl chloride, and vinylidene chloride] required or was enchanced by this S9 mix. Because $CHCl_3$ is a liver carcinogen in the mouse and not in the rat (NCI bioassay, 1976), mouse liver microsomes may be more appropriate than rat liver microsomes as a component of an activation system for $CHCl_3$ mutagenesis. It may also be that a reactive intermediate was formed, but it was too reactive or short-lived to be detected in a test system that uses exogenous metabolic activation.

Kirkland et al. (1981) studied the mutagenicity of $CHCl_3$ in Escherichia coli strains WP2p and WP2uvrA$^-$p, using reversion to tryptophan prototrophy as the endpoint. The bacteria were treated with $CHCl_3$ in plate incorporation and preincubation tests both with and without rat liver microsomes (plus cofactors) prepared from Aroclor 1254-induced rats. The concentration of protein in the microsomal suspension was not given. $CHCl_3$

was added at 10,000, 1,000, 100, 10, 1, or 0.1 ug/plate. Negative results were obtained in both tests. However, there was no indication that volatilization of $CHCl_3$ was prevented in the preincubation test. Also, it appears from the description of the protocol for the plate incorporation test that the procedure used to prevent loss of $CHCl_3$ was inadequate. $CHCl_3$ was added to suspensions of the bacteria in molten agar, and each mixture was rapidly mixed on a Whirlimixer and poured onto agar plates. The plates were then incubated in gas-tight containers. Excessive evaporation of $CHCl_3$ may have occurred during the mixing of the molten agar/bacteria/$CHCl_3$ suspension. 2-Aminoanthracene was used as a positive control requiring metabolic activation and N-methyl-N'-nitro-N-nitrosoguanidine was used as the positive control not requiring activation. These chemicals are not volatile and are therefore inadequate positive controls for $CHCl_3$. Also, it cannot be assumed that a microsomal activation system that metabolizes 2-aminoanthracene is sufficient to metabolize $CHCl_3$.

Gocke et al. (1981) assessed the mutagenicity of 31 chemicals (including $CHCl_3$) used as ingredients in European cosmetics. Three test systems were used: the Salmonella/microsome test, the sex-linked recessive lethal test in Drosophila, and the micronucleus test for chromosome aberrations in mice. The latter two tests will be discussed in the following sections.

For the Salmonella/microsome assays at least five doses of each compound were tested, usually up to 3.6 mg/plate for nontoxic and soluble compounds. Salmonella strains TA1535, TA100, TA1538, TA98, and TA1537 were used with and without activation by S9 mix prepared from Aroclor-pretreated rats. Because this was a screening study in which 31 chemicals were tested, details of the assay protocols were not given. Three halogenated aliphatic hydrocarbons were tested (1,1,1-trichloroethane, dichloromethane, and $CHCl_3$). Because of the

volatility of these compounds, the bacteria were exposed in airtight desiccators for 8 hr. The composition and purity of these chemicals were not specified.

The first two substances exhibited mutagenic activity with and without metabolic activation. $CHCl_3$ was inactive. However, as discussed above in the evaluation of the Simmon et al. (1977) paper, $CHCl_3$ may require metabolic activation to be mutagenic, and the rat liver S9 preparation may not have been sufficient. Also, if a reactive metabolite was formed, it may have been too reactive to be detected under conditions of exogenous activation. Phosgene, the primary reactive metabolite of $CHCl_3$, is very reactive and unstable (Kirk-Othmer, 1971).

In a recently published screening study of 42 chemicals (de Serres and Ashby, 1981), $CHCl_3$ was evaluated in 38 in vivo and in vitro short-term tests designed to assess potential genotoxicity. Results from bacterial assays carried out in 18 laboratories using Salmonella (Ames reversion test) or E. coli (forward mutation test) were essentially negative. However, these results are inconclusive, because nowhere was it mentioned in the protocols that excessive volatilization and escape of $CHCl_3$ from the culture dishes was prevented. In addition, the problems with external activation systems mentioned above also apply to the bacterial assays carried out in this screening study.

Agustin and Lim-Sylianco (1978) investigated the mutagenicity of $CHCl_3$ in a host-mediated assay using Salmonella strains TA1535 and TA1537 as the indicator organisms that were injected into male and female mice. The authors found that male mice metabolized the $CHCl_3$ to a mutagen active in strain TA1537. However, they reported only the ratios of mutation frequencies for treated vs. control animals and gave no indication of the actual colony counts

observed. The mutation frequency (tested/control) for strain TA1537 in male mice was 36.75 and that for female mice, 2.30. The mutation frequencies for strain TA1535 were 0.61 and 0.12, respectively. Details of the procedures used (i.e., doses of $CHCl_3$, numbers of bacteria injected and recovered, route of exposure, and time of exposure before the animals were killed) were not presented. Thus, although there is suggestive evidence of a positive response, definitive conclusions cannot be reached because of inadequacies in the way the data were reported and because details of the procedures used were not provided.

Agustin and Lim-Sylianco (1978) also studied the mutagenicity of ether-extracted urine concentrates from 10 male mice in a bacterial spot test, using strain TA1537. The mice were exposed to $CHCl_3$ at 700 mg/kg. Urine concentrates from $CHCl_3$-treated mice yielded 302 revertant colonies and a zone of inhibition of 29 mm, whereas urine from control animals yielded 10 revertant colonies with no zone of inhibition. Details of the ether extraction procedure were not provided, but the likelihood of a false positive result due to the presence of histidine in the extracted urine is unlikely, because the urine concentrate from the control animals yielded only 10 colonies and was presumably subjected to the same extraction procedure.

In summary, the results from the above bacterial studies are inconclusive, because false negative results could have been obtained due to a number of factors, including:

1. The activation systems used may have been inadequate for metabolism of $CHCl_3$.
2. Phosgene, the primary reactive metabolite of $CHCl_3$, is unstable and highly reactive. Because exogenous activation systems were used in many of these studies, any phosgene generated (assuming an adequate

activation system) may have been scavenged by microsomal protein or lipid before reaching the DNA.

3. Adequate exposure to $CHCl_3$ may not have occurred if appropriate precautions were not taken to prevent the evaporation of $CHCl_3$.

It is, of course, also conceivable that the negative results reflect the possibility that $CHCl_3$ is not a mutagen.

The positive result in the host-mediated study utilizing *in vivo* metabolism of $CHCl_3$ could not be evaluated because the details of the procedures used and the appropriate data were not reported. This result may be suggestive of a positive response and indicates the need for additional testing. The results from the urine spot test are suggestive of a positive response.

7.4 MUTAGENICITY STUDIES IN EUCARYOTIC TEST SYSTEMS

Callen *et al.* (1980) carried out a study on the mutagenicity of $CHCl_3$ in the D7 strain of *Saccharomyces cerevisiae*, which contains an endogenous cytochrome P-450 dependent monooxygenase metabolic activation system. By using this strain of yeast, Callen and his coworkers eliminated the need for the exogenous type of metabolic activation system used in the above bacterial studies. Three different genetic endpoints can be examined with this system: gene conversion at the *trp5* locus, mitotic crossing over at the *ade2* locus, and gene reversion at the *ilv1* locus. The effect of $CHCl_3$ on these endpoints was measured by exposing cells in suspension to 2.5, 5.0, and 6.3 g of $CHCl_3$ per liter of buffer (21 mM, 41 mM, and 54 mM, respectively). The purity of the $CHCl_3$ sample (from J.T. Baker) was not provided. Escape of volatilized $CHCl_3$ is not expected to have occurred to any significant extent, because the incubations were carried out in screw-capped glass tubes. Results of the Callen *et al.* study are presented in Table 7-1. A 1-hr

TABLE 7-1. GENETIC EFFECTS OF CHLOROFORM ON STRAIN D7 OF S. CEREVISIAE

	Concentration, mM			
	0	21	41	54
Survival				
Total colonies	1423	1302	982	84
% of control	100	91	69	6
trp5 locus (gene conversion)				
Total convertants	246	274	450	278
Convertants/10^5 survivors	1.7	2.1	4.6	33.1
ade2 locus (mitotic crossing over)				
Total twin spots	1	1	2	4
Mitotic cross-overs/10^4 survivors	1.6	1.7	4.1	44.8
Total genetically altered colonies	6	11	43	47
Total genetically altered colonies/ 10^3 survivors	1.0	1.9	8.9	52.7
ilv1 locus (gene reversion)				
Total revertants	61	46	81	50
Revertants/10^6 surviviors	4.3	3.5	8.2	60.0

treatment of cells with 54 mM $CHCl_3$ resulted in an increased convertant to survivor ratio with a marginal increase in the observed number of convertant colonies. Similar results were obtained for mitotic crossing over and gene reversion. Toxicity at this concentration was high (6% survival).

At the lower concentrations of $CHCl_3$ (21 mM and 41 mM), a small dose-related increase (1.2-fold and 2.7-fold, respectively) in gene convertants was observed. In addition, a 9-fold increase in the frequency of genetically altered colonies, which are due to gene conversion and mitotic crossing over, was observed at 41 mM $CHCl_3$. For gene reversion, a 2-fold increase was observed. Toxicity was low at these levels. These results are suggestive of a positive response, but additional studies are needed before it can be conclusively stated that CCl_4 causes genetic effects in yeast.

Sturrock (1977) tested the mutagenic effects of $CHCl_3$ at the 8-azaguanine locus in Chinese hamster lung fibroblast cells (V-79 cells) in culture. The cells were grown to a monolayer and exposed for 24 hr to an atmosphere containing 1 to 2.5% $CHCl_3$. Cells were then plated onto media with or without 8-azguanine. After incubation, the plates were examined for mutations and survival. No significant increase in the frequency of mutants was observed in treated cultures as compared with untreated controls. However, the xenobiotic biotransformation capability of the cells used in this study is unknown.

Gocke et al. (1981) evaluated the mutagenicity of $CHCl_3$ by carrying out tests in Drosophila to detect sex-linked recessive lethal mutations. The flies were exposed by the adult feeding method to 25 mM $CHCl_3$. Three successive broods (3-3-4 days) of flies were examined for sex-linked recessive lethal mutations. Over 4000 chromosomes per brood were tested. In two of the broods, small increases in mutations were observed. Results (sex-linked

recessive lethals/chromosomes) were as follows: Brood 1, 20/4616 (0.43%); Brood 2, 13/4349 (0.29%). Controls were 0.27% and 0.14%, respectively. These increases were not significant. To obtain significant increases more chromosomes would have to be tested.

Because sperm head abnormalities are thought by Wyrobek and Bruce (1978) to arise from mutations in the genes that code for spermatogenesis, it is possible that assays for sperm head abnormalities may be used as an indication of the mutagenic potential of chemicals. In a screening study in which 54 chemicals were tested for induction of sperm head abnormalities, Topham (1980) reported that $CHCl_3$ did not induce sperm head abnormalities when injected in mice. Groups of five male mice received five daily intraperitoneal injections of corn oil alone (5 ml/kg/day) or $CHCl_3$ in corn oil at 0.025, 0.05, 0.075, 0.1, and 0.25 mg/kg/day. Topam (1980) reported that the highest of these doses (0.25 mg/kg/day) was lethal. However, in Chapter 5 of this document, it is stated that the LD_{50} of $CHCl_3$ for ICR male mice is 789-1590 mg/kg. The reason for the discrepancy in reported topic response to $CHCl_3$ is not clear. Five weeks after the last dose, caudal sperm smears were examined for head abnormalities. The raw data for the experiments with $CHCl_3$ were not presented in the paper. Topham stated that $CHCl_3$ induced sporadic small increases in abnormal sperm heads at a high dose, but this result could not be repeated.

Another sperm head abnormality study was carried out by Land et al. (1981). This study was designed to determine whether certain anesthetics affect mouse sperm morphology. Groups of five male mice (11 weeks old) were exposed by inhalation to $CHCl_3$ at 0.04 and 0.08% (vol/vol) for 4 hr/day for 5 days in glass exposure chambers. Control mice (N = 15) were exposed to compressed air under similar conditions. Twenty-eight days after the first

exposure, the nine survivors from each exposure level were killed and the caudal sperm were examined for head abnormalities. The results were reported as % abnormal sperm (+ SEM) and were as follows: control, 1.42 (0.08); 0.08% $CHCl_3$, 3.48 (0.66); and 0.04% $CHCl_3$, 2.76 (0.31). The authors concluded that exposure of mice to $CHCl_3$ resulted in a significant increase in sperm head abnormalities compared to the control ($P < 0.01$). However, significance was calculated by the t-test and by the F test. Use of these tests may not be appropriate in this case because of the non-homogeneity of the variance in $CHCl_3$-treated and control groups (Dr. Chao Chen, Carcinogen Assessment Group, U.S. EPA, personal communication). This study is suggestive of a positive response, but a more appropriate statistical analysis of the data is needed. However, the data necessary to carry out such a statistical analysis were not provided.

Testing of the mutagenic potential of $CHCl_3$ in eucaryotic systems was carried out in the same screening study edited by de Serres and Ashby (1981) that was discussed in the previous section of this chapter for bacterial assays. Seven yeast assays, two in vitro mammalian DNA damage assays (unscheduled DNA synthesis and sister chromatid exchange), and three whole-animal tests (Drosophila sex-linked recessive lethal, mouse bone marrow micronucleus, and mouse sperm abnormality) were described for $CHCl_3$. The DNA damage studies will be discussed in the next section of this chapter.

The seven yeast assays involved both forward and reverse point mutations, mitotic crossing over, mitotic gene conversion, and induction of aneuploidy in mitotic cells. The latter three assays test for DNA damage. A positive result was obtained only in the forward mutation assay utilizing Schizosaccharomyces pombe as the test organism. In the reverse mutation assay, $CHCl_3$ was tested only with stationary cells in the presence of rat S9

mix. Exposure was for 24 hr. Growing cells are more sensitive to the mutagenic effects of several chemicals than are stationary cells, possibly because log-phase yeast cells contain an endogenous cytochrome P-450 metabolizing system (Callen et al., 1980). The DNA damage studies in yeast yielded negative results. The negative results in the mitotic gene conversion assay, which was carried out in strain D7, are in conflict with the weakly positive results reported for $CHCl_3$ by Callen et al. (1980) as described above.

The three whole-animal tests on $CHCl_3$ (Drosophila sex-linked recessive lethal, mouse bone marrow micronucleus, and mouse sperm abnormality) described in the de Serres and Ashby (1981) report yielded negative results. However, de Serres and Ashby, in their overview of the performance of the assay systems used in this study, state that the whole-animal tests had low sensitivity and that a negative result has "very little significance." It was recommended that $CHCl_3$ be tested further in in vivo short-term tests.

In summary, the results from the eucaryotic test systems suggest that $CHCl_3$ may be a weak mutagen. Results indicative of a positive response were obtained only in studies using test organisms possessing endogenous activation systems (i.e., yeast and mice). More studies, particularly with organisms possessing endogenous activation, are needed before a definite conclusion on the mutagenicity of $CHCl_3$ can be reached. Suggested studies are described later in this chapter.

7.5 OTHER STUDIES INDICATIVE OF DNA DAMAGE

Two types of DNA damage studies, sister chromatid exchange (SCE) and unscheduled DNA damage (UDS), are described in this section.

Sister chromatid exchange is thought to involve DNA breakage. For this reason, assays for SCE have been used as an indicator of primary DNA damage.

White et al. (1979) studied the induction of SCEs by $CHCl_3$ and other anesthetics. Information about the purity of these compounds was not given. Exponentially growing Chinese hamster ovary cells were exposed to the gases in vitro with and without S9 mix (10% by volume) prepared from Aroclor-induced rat livers. Exposure was at 0.71% (vol/vol) $CHCl_3$ (88 mM) for 1 hr in closed screw-capped culture flasks. The cells were then incubated for 24 hr in medium containing 10 uM 5-bromo-2-deoxyuridine. SCEs per chromosome were 0.544 $\pm$ 0.018 for $CHCl_3$-exposed cells and 0.536 $\pm$ 0.018 for controls. The short exposure time and low concentration raise serious questions concerning the conduct of this assay. Also, even though the rat liver S9 was sufficient for activation of vinyl-containing compounds to derivatives (presumably epoxides) that induced SCE, it may not have been adequate for activation of $CHCl_3$ and the other haloalkanes that tested negative.

Another SCE study was carried out by Kirkland et al. (1981) using human lymphocytes. The cells were treated with $CHCl_3$ at 25, 50, 75, 100, 200, and 400 ug/ml (0.2, 0.4, 0.6, 0.8, 1.6, and 3.3 mM, respectively) for 2 hr in the presence of S9 mix from Aroclor-induced rats. Metaphase spreads from approximately 100 cells per treatment were examined. Acetone was the negative control, and a positive control was not included in the assay because the same donor's lymphocytes had previously shown a dose-related increase in SCE after treatment with benzo[a]pyrene in the presence or absence of S9 mix. A small increase in SCE occurred at 50 ug of $CHCl_3$ per ml, but no dose-response trend was observed.

There are several problems with this study. First, since a positive dose-related increase in SCE after treatment of lymphocytes with benzo[a]pyrene was not dependent on S9 mix, this positive control is inadequate for substances that are likely to require metabolic activation. In

addition, this control was not concurrent and is therefore not an appropriate positive control. Second, there is no indication that volatilization and escape of $CHCl_3$ was prevented. Third, the maximal dose was only 3.3 mM. Fourth, information about the toxicity of $CHCl_3$ for the lymphocytes was not provided.

Two *in vitro* mammalian DNA damage studies on $CHCl_3$ (UDS and SCE) were described in the volume edited by de Serres and Ashby (1981). The SCE assay (Chapter 51) utilized an exogenous activation system and yielded negative results. The Chinese hamster ovary cells were exposed to $CHCl_3$ in the presence of rat liver S9 mix for only 1 hr. This length of time may be insufficient, particularly since a positive response for 2-acetylaminofluorene was obtained in the presence of S9 after 2 hr of exposure and not after a 1-hr exposure. Also, as mentioned above, exogenous activation may be inappropriate when testing $CHCl_3$ for genotoxic activity. Thus, the negative results obtained in this study are inconclusive. In addition, as in the bacterial tests described above, there was no indication that precautions were taken to prevent evaporation and loss of $CHCl_3$ from the culture flasks.

Unscheduled DNA synthesis (UDS), measured as repair of chemically induced DNA damage, is an additional method of testing for genetic damage. Mirsalis *et al.* (1982) measured UDS in primary rat hepatocyte cultures following *in vivo* treatment of adult male Fischer-344 rats (175-275 g) with $CHCl_3$ at 40 and 400 mg/kg by gavage. Control rats received corn oil (the vehicle for $CHCl_3$) by gavage. Several additional chemicals were also tested in this study. At 2 or 12 hr after treatment, the livers were perfused *in situ* and hepatocytes were isolated. Approximately 6×10^5 viable cells were seeded in 35-mm culture dishes containing coverslips and allowed to attach to the coverslips for about 90 min. After the coverslip cultures were washed, they

were incubated in a medium containing 10 uCi [^{3}H]thymidine (40-50 Ci/mmol) per ml for 4 hr. The cultures were washed again and incubated in medium containing 0.25 mM cold thymidine for 14-16 hr. The extent of UDS was assessed by autoradiography. Net grains/nucleus were calculated as the silver grains over the nucleus minus the highest grain count of three adjacent nuclear-sized areas over the cytoplasm.

Cells from negative control animals (given vehicle only) ranged from -3.0 to -5.1 net grains/nucleus. Several chemicals tested positive in this assay ($\leq$ 5 net grains/nucleus was considered positive), including methyl methanesulfonate, dimethylnitrosamine, 2-acetylaminofluorene, benzidine, and others. $CHCl_3$ at 40 and 400 mg/kg yielded a negative response (-2.7 to -4.4 net grains/nucleus). However, rats are not susceptible to $CHCl_3$-induced hepatocarcinogenesis (NCI bioassay, 1976). The negative response observed in this study is consistent with this fact. Benzo[a]pyrene and 7,12-dimethylbenz[a]anthracene, carcinogens that, like $CHCl_3$, are not rat liver carcinogens, also tested negative in this assay. These chemicals tested positive in the *in vitro* rat hepatocyte UDS assay (Williams *et al.*, 1981). This discrepancy suggests that the *in vitro* test may be more sensitive than the *in vivo* assay. $CHCl_3$ has not been tested in the *in vitro* rat hepatocyte UDS assay. The mouse is susceptible to $CHCl_3$-induced liver tumors (NCI bioassay, 1976) and therefore may be a more appropriate test animal for the *in vivo* UDS assay.

Also, it is uncertain whether the method of assaying for UDS used in this study (subtraction of cytoplasmic grain counts from nuclear grain counts) will allow for detection of a weak response. In a recent article discussing the validity of the autoradiographic procedure for detecting UDS in rat hepatocytes, Lonati-Galligani *et al.* (1983) describe some potential problems

with this method. First, they found that it is difficult to obtain hepatocyte preparations of reproducible quality. Preparations can differ in their metabolic capabilities. In order to avoid false negative results with potential weak UDS inducers due to poor hepatocyte preparations, they suggest that test chemicals should be studied in conjunction with a potent UDS-inducing analog and that negative results should be accepted only in tests in which the analog is strongly positive. No known positive analog of $CHCl_3$ was tested in the study of Mirsalis et al. (1982). Second, the cytoplasmic layer covering the nucleus is thinner than the cytoplasmic area next to the nucleus. Therefore, a variable overcorrection is probably applied, as witnessed by the usually higher cytoplasmic than nuclear counts observed in control cells (e.g., see above results for cells from control animals). This effect would tend to obscure a weakly positive UDS response. Lonati-Galligani et al. (1983) suggest that an alternative endpoint be determined. Instead of subtracting cytoplasmic grain counts from nuclear grain counts, the grains over the nucleus and over a cytoplasmic area should be scored and dose-response curves plotted separately. Both dose-response curves should be considered before a decision is reached on whether exposure to a certain chemical results in UDS.

Results of an additional DNA repair study were published by Reitz et al. (1980). Mice were exposed orally to $CHCl_3$ at 240 mg/kg and repair in the livers was assayed. Negative results were obtained. However, these results cannot be interpreted because no information on the methodology used to assay for DNA repair was given. In order to interpret these results one would have to know the length of time the mice were exposed to $CHCl_3$. Some compounds require a longer exposure than others (e.g., 2-acetylaminofluorine in Mirsalis et al., 1982). Also, the opportunity for false negative results, as discussed

above, exists in this study as well.

The UDS assay discussed in Chapter 48 of the de Serres and Ashby (1981) volume was carried out with Hela cells, which do not contain a P-450 activation system. An exogenous rat liver S9 activation system was employed. Although $CHCl_3$ tested positive in this assay in the absence of the activation system, the discussion of this assay in the de Serres and Ashby book suggests that this result is misleading because of an inadequacy in the statistical method employed. In the presence of rat liver S9, $CHCl_3$ tested negative. However, as already discussed, exogenous activation is probably inappropriate when testing compounds such as $CHCl_3$ for their ability to cause genetic damage.

In summary, because of various deficiencies in the above studies, the determination of the DNA-damaging potential of $CHCl_3$ requires additional studies. Indication of the DNA-damaging potential of $CHCl_3$ was suggested by the small increases in conversion and mitotic crossing over observed in yeast (Callen et al., 1980), as described in the previous section.

7.6 CHROMOSOME STUDIES

Kirkland et al. (1981) studied the ability of $CHCl_3$ to induce chromosome breakage in cultured human lymphocytes. The cells from one donor were treated with $CHCl_3$ at 50, 100, 200, and 400 ug/ml for 2 hr in the presence of an S9 activation system derived from Aroclor 1254-induced rats. The positive control compound, benzo[a]pyrene, in a separate experiment with the same donor's lymphocytes induced chromosome breakage with or without S9 treatment. The response of this donor's lymphocyte chromosomes to $CHCl_3$ was a random variation around the control value. The highest breakage level was at 200 ug/ml with 8 breaks/100 cells compared with 5.5 breaks/100 cells in the control. However, this difference was not significant according to the

chi-square test. The same four problems discussed in the previous section for the SCE study carried out by Kirkland et al. (1981) apply to their study on chromosome breakage.

According to Schmid (1976), the bone marrow micronucleus test can be used to detect clastogens and spindle poisons. Micronuclei are small elements that contain either pieces of chromosomal fragments originating from clastogenic events or whole chromosomes resulting from malfunction of the spindle aparatus. Gocke et al. (1981) used this test to study chromosome aberrations in mice exposed to $CHCl_3$. The animals were treated with $CHCl_3$ at 0 and 24 hr, and bone-marrow smears were prepared at 30 hr. The purity of the $CHCl_3$ (purchased from Merck) was not provided. Four mice (two males and two females) were used for each of three doses and one control. The animals were given two intraperitoneal injections of $CHCl_3$, each at 238, 476, and 952 mg/kg (2, 4, and 8 mmol/kg, respectively). The authors state that the assay was performed according to Schmid (1976); thus, it can be assumed that the doses chosen included the highest tolerable dose. Slides were coded, and 1000 polychromatic erythrocytes were scored per mouse.

The results were as follows (dose, % micronucleated polychromatic erythrocytes): 0 mg/kg, 1.2%; 2 x 238 mg/kg, 2.2%, 2 x 476 mg/kg, 2.6%; 2 x 952 mg/kg, 2.2%. Thus, a dose-related increase was not observed. Three halogenated alkanes were tested (dichloromethane, 1,1,1-trichloroethane, and $CHCl_3$) and all yielded negative results. Of 30 chemicals tested, only two (pyrogallol and hydroquinone) yielded positive results in the micronucleus test. Positive controls were not included in the assay, but the positive results for pyrogallol and hydroquinone indicate that the assay system was working.

The micronucleus test was also used by Agustin and Lim-Sylianco (1978) to study the clastogenic potential of $CHCl_3$. The authors tested seven concentrations of $CHCl_3$ up to 900 mg/kg in the mouse. The number of mice used and their sex was not specified. The $CHCl_3$ was purchased from Mallinckrodt and was redistilled before use. Information on purity of the $CHCl_3$ was not provided. For each slide, 1000 polychromatic erythrocytes were scored. The authors reported that $CHCl_3$ was clastogenic. Results were as follows (dose in mg/kg, number of micronucleated polychromatic erythrocytes per 1000 polychromatic erythrocytes $\pm$ SE): 0, 4 $\pm$ 1; 100, 3 $\pm$ 1; 200, 5 $\pm$ 1; 400, 5 $\pm$ 1; 600, 9 $\pm$ 2; 700, 17 $\pm$ 4; 800, 9 $\pm$ 2; 900, 10 $\pm$ 2. The authors stated that these data indicate that $CHCl_3$ must be metabolized to a clastogenic substance, because a straight-line dose-response relationship was not observed. However, the data provided by the authors are not sufficient to support this interpretation.

In the same paper, Agustin and Lim-Sylianco demonstrated that vitamin E administered 1 hr after $CHCl_3$ reduced the number of micronucleated cells observed at 700 mg of $CHCl_3$/kg (17 $\pm$ 4) to the control level (4 $\pm$ 1). The significance of this result is not clear.

This study by Agustin and Lim-Sylianco (1976) is difficult to interpret because details of the experimental procedures necessary to permit an evaluation of the results were not provided (e.g., number and sex of the animals and positive and negative controls). This study suggests that $CHCl_3$ may affect chromosomes. However, corroborative studies are needed to confirm or refute this suggested response.

In summary, based on the results of the three chromosome aberration studies described above, there are suggestive but not conclusive data that $CHCl_3$ is clastogenic. Negative results were reported by Kirkland *et al*.

(1981) and by Gocke et al. (1981), while Agustin and Lim-Sylianco (1976) reported a positive result. More studies are needed before it can be conclusively determined whether or not $CHCl_3$ is clastogenic.

7.7 SUGGESTED ADDITIONAL TESTING

More studies are needed on the covalent binding of $^{14}CHCl_3$ to DNA. Such studies should be done with $^{14}CHCl_3$ at a higher specific activity [about 40 Ci/mol (Brookes and Lawley, 1971)] than used by Diaz Gomez and Castro (1980b).

Additional studies on the ability of $CHCl_3$ to cause DNA damage are needed. Examples of such tests include measurement of unscheduled DNA synthesis in vivo in mice and in both rat and mouse hepatocytes after in vitro exposure (Williams, 1981), measuring endpoints suggested by Lonati-Galligani et al. (1983).

Additional studies are needed to corroborate and extend or refute the Callen et al. (1980) study in yeast, which possesses an endogenous activation system and is capable of assaying for point mutations, mitotic crossing over, and gene conversion.

Further testing for the ability of $CHCl_3$ to cause chromosome aberrations is needed, particularly in in vivo systems.

In addition to the study by Gocke et al. (1981), further testing of the ability of $CHCl_3$ to cause sex-linked recessive lethal mutations in Drosophila is needed. However, in order to detect a weak response, a larger number of chromosomes than analyzed by Gocke et al. (1981) should be scored.

7.8 SUMMARY AND CONCLUSIONS

It has been demonstrated that chloroform ($CHCl_3$) can be metabolized in vivo and in vitro to a substance(s) (presumably phosgene) that interacts with protein and lipid. However, the only experiment measuring interaction of

metabolically activated $CHCl_3$ with DNA, in which adequate information was given concerning the experimental procedures used, yielded a negative result (Diaz Gomez and Castro, 1980b). This result was judged as inconclusive because the specific activity of the $^{14}CHCl_3$ may have been too low.

The majority of the assays for mutagenicity and genotoxicity have also yielded negative results. However, many of these results are inconclusive because of various inadequacies in the experimental protocols used. The major problem is with those bacterial, sister chromatid exchange, and chromosome aberration studies that used reconstituted exogenous activation systems (i.e., S9 mix). In none of these studies was it shown that $CHCl_3$ was activated or metabolized by the activation system used. Metabolism of 2-aminoanthracene or vinyl compounds (used as positive controls) is probably an inadequate indication that the activation system can metabolize $CHCl_3$, because these substances are not halogenated alkanes and are therefore not metabolized like them. A better indication that the activation system is sufficient for metabolism of $CHCl_3$ may be to show that it metabolizes $^{14}CHCl_3$ to intermediates that bind to macromolecules. A second problem with experimental protocols utilizing exogenous activation systems relates to the possibility that any reactive metabolic intermediates formed may react with microsomal or membrane lipid or protein before reaching the DNA of the test organism. A third potential problem occurs in those *in vitro* protocols in which precautions were not taken to prevent escape of volatilized $CHCl_3$.

Studies in which endogenous or *in vivo* activation systems were used include those reported by Callen *et al.* (1980) in yeast, Gocke *et al.* (1981) in *Drosophila* (sex-linked recessive lethal test) and mice (bone-marrow micronucleus test), Topham (1980) and Land *et al.* (1981) in mice (sperm head abnormalities), and Agustin and Lim-Sylianco (1978) in mice (bone marrow

micronucleus test and host-mediated assay). The results from several of these studies suggest that $CHCl_3$ may be a weak mutagen.

In summary, with the present data, no definitive conclusions can be reached concerning the mutagenicity of $CHCl_3$. However, there is some indication (from the binding studies and from the mutagenicity tests that utilized endogenous or *in vivo* metabolism) that $CHCl_3$ may have the potential to be a weak mutagen. In order to substantiate this, only certain well-designed *in vivo* mutagenicity studies or studies with organisms possessing endogenous eucaryotic P-450 activation systems are recommended.

7.9 REFERENCES

Agustin, J.S., and C.Y. Lim-Sylianco. 1978. Mutagenic and clastogenic effects of chloroform. Bull. Phil. Biochem. Soc. 1:17-23.

Brookes, P., and P.D. Lawley. 1971. Effects on DNA: Chemical methods. In: A. Hollaender (ed.) Chemical Mutagens. Vol 1, pp. 121-144. New York: Plenum Press.

Callen, D.F. C.R. Wolf, and R.M. Philpot. 1980. Cytochrome P-450 mediated genetic activity and cytotoxicity of seven halogenated aliphatic hydrocarbons in Saccharomyces cerevisiae. Mutat. Res. 77:55-63.

de Serres, F.J., and J. Ashby (eds.) 1981. Evaluation of Short-Term Tests for Carcinogens. Progress in Mutation Research, Vol I. Elsevier/North Holland.

Diaz Gomez, M.I., and J.A. Castro. 1980a. Nuclear activation of carbon tetrachloride and chloroform. Res. Commun. Chem. Pathol. Pharmacol. 27:191-194.

Diaz Gomez, M.I. and J.A. Castro. 1980b. Covalent binding of chloroform metabolites to nuclear proteins-no evidence for binding to nucleic acids. Cancer Lett. 9:213-218.

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

Kirkland, D.J., K.L. Smith, and N.J. Van Abbe. 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.

Kirk-Othmer Encyclopedia of Chemical Technology, Second Edition, Supplement volume. 1971. pp. 674-683. Interscience Publishers.

Land, P.C., E.L. Owen, and H.W. Linde. 1981. Morphologic changes in mouse spermatozoa after exposure to inhalational anesthetics during early spermatogenesis. Anesthesiol. 54:53-56.

Lonati-Galligani, M., P.H.M Lohman, and F. Berends. 1983. The validity of the autoradiographic method for detecting DNA repair synthesis in rat hepatocytes in primary culture. Mutat. Res. 113:145-160.

Mirsalis, J.C., C.K. Tyson, and B.E. Butterworth. 1982. Detection of genotoxic carcinogens in the in vivo - in vitro hepatocyte DNA repair assay. Environ. Mutagen. 4:553-562.

National Cancer Institute (NCI). 1976. Report on Carcinogenesis Bioassays of Chloroform. National Technical Information Service, Springfield, Virginia (NTIS PB-264-018).

Schmid, W. 1976. The micronucleus test for cytogenetic analysis. In: A. Hollaender (ed.). Chemical Mutagens. Vol. 4, pp. 31-53. New York: Plenum Press.

Simmon, V.F., K. Kauhanen, and R.G. Tardiff. 1977. Mutagenic activity of chemicals identified in drinking water. In: D. Scott, B.A. Bridges, and F.H. Sobels (eds.), Progress in Genetic Toxicology, pp. 249-258. New York: Elsevier/North Holland Biomedical Press.

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.

Topham, J.C. 1980. Do induced sperm-head abnormalities in mice specifically identify mammalian mutagens rather than carcinogens? Mutat. Res. 74:379-387.

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

Weisburger, J.H. and G.M. Williams. 1982. Metabolism of chemical carcinogens. In: F.F. Becker (ed.). Cancer, a Comprehensive Treatise, 2nd Edition. vol. 1, pp. 241-333. New York: Plenum Press.

Reitz, R.H., J.F. Quast, W.T. Stott, P.G. Watanabe, and P.J. Gehring. 1980. Pharmacokinetics and macromolecular effects of chloroform in rats and mice: Implications for carcinogenic risk estimation. In: R.L. Jolley, W.A. Brungs, and R.B. Cumming (eds.). Water chlorination: Environmental Impact and Health Effects, Vol. 3, pp. 983-992.

White, A.E., S. Takehisa, E.I. Eger, S. Wolff, and W.C. Stevens. 1979. Sister chromatid exchanges induced by inhaled anesthetics. Anesthesiol. 50:426-430.

Williams, G.M. 1981. Liver culture indicators for the detection of chemical carcinogens. In: Short-Term Tests for Chemical Carcinogens, H.F. Stich and R.H.C. San (eds.), pp. 275-289. New York: Springer Verlag.

Wyrobek, A. and W.R. Bruce. 1978. The induction of sperm-shape abnormalities in mice and humans. In: A. Hollaender (ed.). Chemical Mutagens. Vol. 5, pp. 257-285. New York: Plenum Press.

8. CARCINOGENICITY

8.1. ANIMAL STUDIES

The carcinogenicity of chloroform has been evaluated in mice, rats, and dogs. Evidence for carcinogenic activity by chloroform includes induction of renal epithelial tumors in male Osborne-Mendel rats (National Cancer Institute [NCI] 1976), hepatocellular carcinomas in male and female B6C3F1 mice (NCI 1976), kidney tumors in male ICI mice (Roe et al. 1979), and hepatomas in female strain A mice (Eschenbrenner and Miller 1945) and NLC mice (Rudali 1967). Capel et al. (1979) demonstrated an ability of chloroform to promote growth and metastasis of murine tumors. Chloroform was not shown to be carcinogenic in (C57 x DBA2 F1) mice (Roe et al. 1968), female Osborne-Mendel rats (NCI 1976), female ICI mice, and male mice of the CBA, C57BL, and CF/1 strains (Roe et al. 1979), male and female Sprague-Dawley rats (Palmer et al. 1979), and male and female beagle dogs (Heywood et al. 1979). Chloroform was negative in a pulmonary tumor induction bioassay in male strain A/St mice (Theiss et al. 1977). Chloroform in liquid solution did not induce transformation of baby Syrian hamster kidney (BHK-21/C1 13) cells *in vitro*. Under the conditions of the carcinogenicity bioassays showing carcinogenic activity for chloroform specifically in kidney and liver of mice and rats, the conclusion can be made, by applying the IARC classification approach for carcinogens, that there is sufficient evidence for the carcinogenicity of chloroform in experimental animals.

8.1.1. Oral Administration (Gavage): Rat

8.1.1.1. NATIONAL CANCER INSTITUTE (1976) -- A carcinogenesis bioassay on chloroform in Osborne-Mendel rats was reported by the NCI (1976). The chloroform product (Aldrich Chemical Company, Milwaukee, Wisconsin) was shown to be 98% pure chloroform and 2% ethyl alcohol (stabilizer) by gas-liquid chromatography, flame ionization detection, and infrared spectrometry at the carcinogenesis bioassay laboratory. Chloroform solutions in corn oil were prepared fresh each week and stored under refrigeration.

Fifty animals of each sex were assigned to each of two dose groups. Treated animals were compared with matched vehicle-control groups (20 males and 20 females) and with vehicle colony control groups (99 males and 98 females) that included the matched control group and three other controls groups put on study within 3 months of the matched control group. Matched control and treated animals were housed in the same room, and colony controls were housed in two different rooms.

Doses selected for the main study in rats were estimated as those maximally and one-half maximally tolerated based on survival, body weights, clinical signs, and necropsy examinations in a preliminary toxicity test in which chloroform was given by gavage for 6 weeks with a subsequent observation period of 2 weeks without treatment. The chronic study began when rats were 52 days old and ended with sacrifice of survivors at 111 weeks. Chloroform was administered in corn oil by gavage 5 days each week during the initial 78 weeks. Doses of 90 and 180 mg/kg/day were administered to male rats throughout the chronic study; however, since initial doses of 125 and 250 mg/kg/day were reduced to 90 and 180 mg/kg/day at 22 weeks, doses given to female rats were expressed as time-weighted averages of 100 and 200 mg/kg/day.

Decedents and survivors were necropsied, and tissues and organs were examined microscopically. Body weights and food consumption were monitored weekly for the first 10 weeks and monthly thereafter. Animals were observed twice daily.

In matched control and both dose groups, at least 50% of the male and female rats survived as long as 85 and 75 weeks, respectively. Seven matched control, 24 low-dose, and 14 high-dose males and 15 matched control, 22 low-dose, and 14 high-dose females survived until the end of the study. Only one control male rat died before 90 weeks; the increase in death rate of control males after 90 weeks was, according to the NCI (1976) report, "probably due to respiratory and renal conditions." Overall survival was less in treated animals than in controls (Figure 8-1).

Decreased body weight gain was evident in both sexes of rats in both treatment groups. Initial mean body weights for all groups were about 175 g for females and 250 g for males. By 50 weeks, mean body weights were approximately 400 g in control, 350 g in low-dose, and 330 g in high-dose females; by 100 weeks, mean body weights were about 375 g in all groups of females. In males, mean body weights were about 640 g in the control group, 550 g in the low-dose group, and 500 g in the high-dose group by 50 weeks; by 100 weeks, mean body weights were approximately 500 g in all groups. Food consumption was reported as slightly lower in treated animals, but data were not provided. Appearance and behavior among groups were generally similar, but hunching, urine stains on the lower abdomen, redness of eyelids, and wheezing were noted in treated animals early in the study.

A statistically significant ($P < 0.05$) increase in renal epithelial tumors of tubular cell origin was found in treated male rats (Table 8-1). The epithelial tumors were described as follows: Of 13 tumors in high-dose males, 10 were carcinomas and three were adenomas; two carcinomas and two adenomas

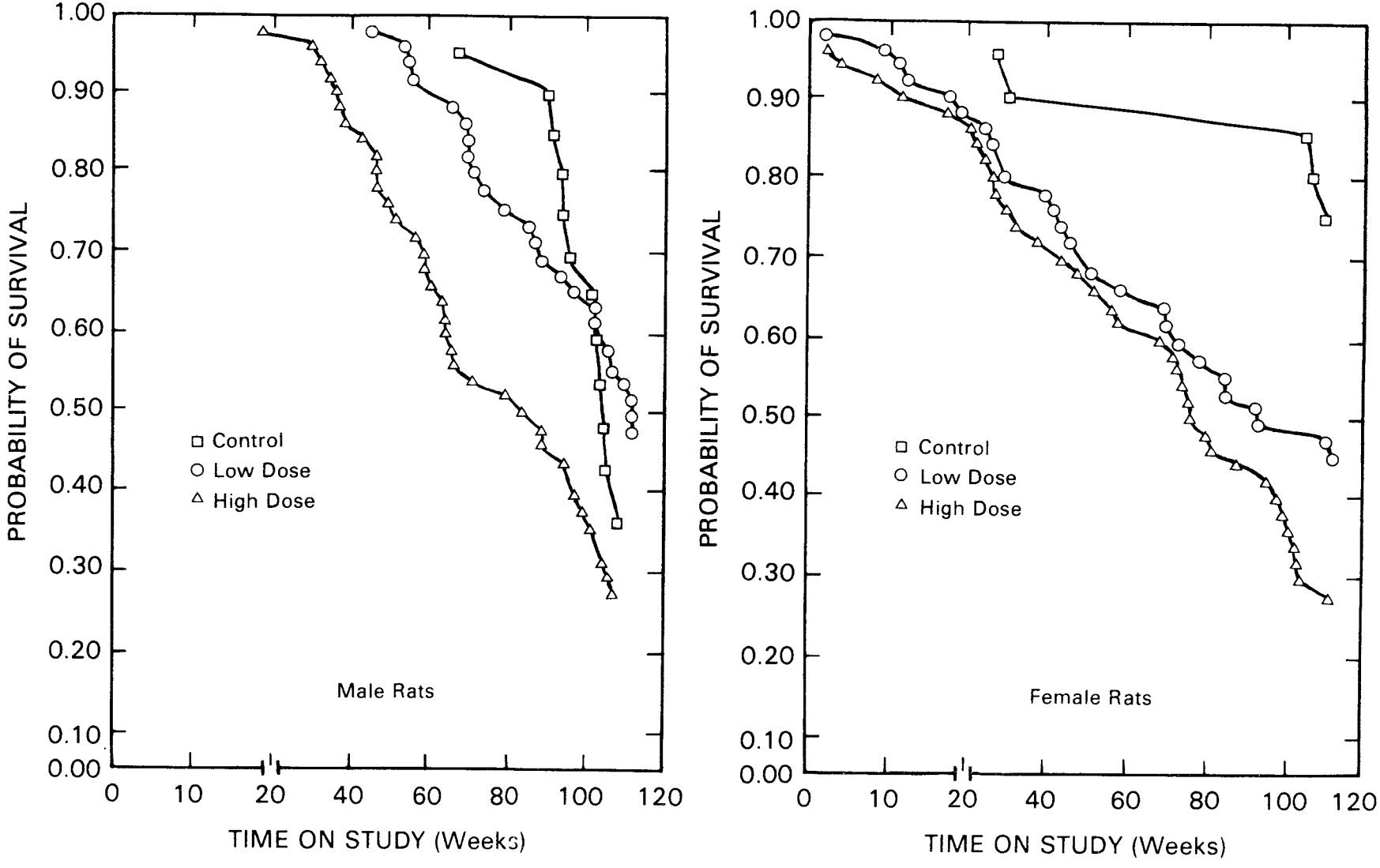

Figure 8-1. Survival curves for Fischer 344 rats in a carcinogenicity bioassay on chloroform. (NCI 1976)

TABLE 8-1. EFFECT OF CHLOROFORM ON KIDNEY EPITHELIAL TUMOR INCIDENCE IN OSBORNE-MENDEL RATS (NCI 1976)

	Male				Female			
	Controls[b]		Dose (mg/kg/day)[c]		Controls[b]		Dose (mg/kg/day)[c]	
Treatment[a]	Colony	Matched	90	180	Colony	Matched	100	200
Kidney tumor incidence[d]	0/99(0%)	0/19(0%)	4/50(8%)[e]	12/50(24%)[f]	0/98(0%)	0/20(0%)	0/49(0%)	2/48(4%)[g]
P value[h]			0.266	0.014[i]			---	0.495
Time to first tumor (weeks)	---	---	102	80	---	---	---	102
Survival at terminal sacrifice (111 weeks)	26%	37%	48%	28%	51%	75%	45%	29%

[a]Chloroform in corn oil administered by gavage 5 times per week for 78 weeks.
[b]Colony controls consist of four vehicle-control groups, including matched controls, given corn oil.
[c]Doses are time-weighted averages.
[d]Animals with tumor/animals examined.
[e]Two with tubular cell adenocarcinoma and two with tubular cell adenoma.
[f]Ten with tubular cell adenocarcinoma and two with tubular cell adenoma.
[g]One with tubular cell adenocarcinoma and one with squamous cell carcinoma in the renal pelvis.
[h]Fisher's Exact Test, compared with matched controls.
[i]For adenocarcinomas alone, P value is 0.03.

comprised the tumors found in four low-dose males; one renal epithelial carcinoma and one squamous cell carcinoma from renal pelvic transitional epithelium were noted in two high-dose females. One low-dose male had both a malignant mixed tumor and a tubular cell adenoma in the left kidney, and a high-dose male had a tubular cell carcinoma and a tubular cell adenoma in the right kidney. Renal epithelial carcinomas were large and poorly circumscribed, and they infiltrated surrounding normal tissue. Renal epithelial adenomas were circumscribed and well-differentiated. Additional kidney tumors included malignant mixed tumors in two low-dose and two colony control males and hamartomas in one low-dose male, one high-dose male, and one colony control male.

Although a statistically significant ($P < 0.05$) increase in thyroid tumors in both treatment groups of female rats as compared with colony controls was reported, the toxicologic significance of this finding appears questionable in that C-cell tumors and follicular cell tumors, which have different embryonic origins and different physiologic functions, are combined in the incidences described in Table 8-2; the majority of tumors were adenomas; the spontaneous incidence of thyroid tumors in Osborne-Mendel females is variable as stated, without presentation of historical data, in the NCI (1976) bioassay report; and the increased incidence of thyroid tumors in treated females is not significant ($P > 0.05$) when compared with data for matched controls.

No significant ($P < 0.05$) differences for other tumor types among groups were apparent. Four rats were lost (missing or autolyzed) for pathology.

Non-neoplastic lesions described as treatment-related include necrosis of liver parenchyma, epithelial hyperplasia in the urinary bladder, and hematopoiesis in spleen. Inflammatory pulmonary lesions characteristic of

TABLE 8-2. EFFECT OF CHLOROFORM ON THYROID TUMOR INCIDENCE IN FEMALE OSBORNE-MENDEL RATS (NCI 1976)

Dose[a,b] (mg/kg/day)	Follicular cell tumors[c]	C-cell tumors[d]	Total tumors[e]		
	Incidence[f]	Incidence	Incidence	P value[g]	Time to first tumors (weeks)
0 (matched)[h]	1/19(5%)	0/19(0%)	1/19(5%)		110
0 (colony)[h]	1/98(1%)	0/98(0%)	1/98(1%)		110
100	2/49(4%)	6/49(12%)	8/49(16%)	0.216	73
200	6/49(12%)	4/49(8%)	10/49(20%)	0.121	49

[a]Chloroform in corn oil administered by gavage 5 times per week.
[b]Time-weighted average doses.
[c]Adenomas except for carcinoma in one low-dose and two high-dose animals.
[d]Adenomas except for carcinoma in one high-dose animal.
[e]See text.
[f]Animals with tumors/animals examined.
[g]Fisher's Exact Test, compared with matched controls.
[h]Colony controls consist of four vehicle-control groups, including matched controls, given corn oil.

pneumonia were found in all groups, but the severity and incidence of these lesions were stated (data not reported) to have been greater in treatment groups.

Under the conditions of this bioassay, chloroform treatment significantly ($P < 0.05$) increased the incidence of renal epithelial tumors in male Osborne-Mendel rats. Although the number of matched vehicle controls was low, the use of pooled colony controls gives additional support for treatment-related effects. Moreover, historical control incidence of renal epithelial tumors in Osborne-Mendel rats was reported as rare.

Lower survival rates and body weights in rats than in matched controls provide evidence that the chloroform doses used were toxic to the rats used in this study, and a more precise estimate of dose-response perhaps could have been obtained if additional lower doses had been given, and if constant doses rather than time-weighted averages had been used. Treated animals were housed in the same room as rats treated with other volatile compounds (1,1,2,2-tetrachloroethane, 3-chloropropene, ethylene dibromide, carbon tetrachloride); however, since controls were in the same room as treated animals and oral chloroform doses probably would have been much higher than ambient levels of other volatiles, the likelihood that the other volatile compounds were responsible for the observed results is considered to be low. Additionally, these other volatile compounds did not induce kidney tumors in Osborne-Mendel rats (NCI 1976; Weisburger 1977). It should be noted that ambient levels of volatiles in the animal quarters were not measured.

8.1.1.2. PALMER ET AL. (1979) -- Palmer et al. (1979) reported carcinogenicity studies on chloroform in Sprague-Dawley rats. Chloroform was prepared in toothpaste, as described in Table 8-3 herein for the Roe et al. (1979) study, and administered by gavage. Dose levels of 15, 75, and 165 mg $CHCl_3$/kg/day were

selected for the carcinogenicity study based on results of a preliminary range-finding study showing the lowest toxic dose, indicated by liver and kidney changes, as 150 mg/kg/day.

TABLE 8-3. TOOTHPASTE FORMULATION FOR CHLOROFORM ADMINISTRATION (Roe et al. 1979)

Ingredient	Percentage w/w
Chloroform	3.51
Peppermint oil[a]	0.25
Eucalyptol[a]	0.50
Glycerol	39.35
Carragheen gum	0.45
Precipitated calcium carbonate	48.53
Sodium lauryl sulphate	1.16
Sodium saccharin	0.03
White mineral oil	1.10
Water	5.12
Total	100.00

[a]Essential oil flavor components.

An initial carcinogenicity study was done in which 25 rats of each sex per group received one of the selected doses in toothpaste containing essential oils (flavor components), indicated in Table 8-3, 6 days per week. A concurrent control group of 75 males and 75 females was administered toothpaste without chloroform and essential oils. A second carcinogenicity study was done in which

50 male and 50 female specific pathogen-free (SPF) Sprague-Dawley rats were dosed with 60 mg $CHCl_3$/kg/day in toothpaste with essential oils 6 days per week, and 50 control rats of each sex were given toothpaste without chloroform but with essential oils.

Body weights were measured weekly, and food consumption was recorded. Body weights were initially 180 to 240 g for males and 130 to 175 g for females. Blood and urine analysis were performed in the first study, and serum and erythrocyte cholinesterase activities were monitored in the second study. All animals were necropsied, and tissues and organs were examined histopathologically. Adrenals, kidneys, liver, lungs, and spleen were weighed.

Chloroform was not carcinogenic in these studies. Significant ($P < 0.05$) body weight loss in high-dose males in the first study (data not reported) and maximal body weight gain of approximately 370 g in control males, 330 g in treated males, 220 g in control females, and 180 g in treated females in the second study suggest an effect from chloroform treatment. Other than a 40% reduction of plasma cholinesterase levels and slight decreases in serum glutamic-pyruvic transaminase and serum alkaline phosphatase in treated females, additional toxic effects from chloroform treatment were not evident.

Low survival, attributed to respiratory disease, was apparent in both studies. The initial study was terminated at 52 weeks; 50% of the animals in all groups had died by 52 weeks in the second study, which was ended at 95 weeks. Except for 48 control females in the initial study, no more than 18 animals were alive in each group at the conclusion of either study. Although carcinogenic activity for chloroform was not observed, these studies on Sprague-Dawley rats are weakened by the high early mortality in control and treated animals.

8.1.2. Oral Administration (Gavage): Mouse

8.1.2.1. NATIONAL CANCER INSTITUTE (1976) -- The carcinogenicity of chloroform in B6C3F1 mice was evaluated by the NCI (1976). The chloroform product and chloroform solutions in corn oil were those used in the NCI (1976) carcinogenesis bioassay in rats discussed herein.

Each of two dose groups was composed of 50 males and 50 females. Treated mice were compared with matched vehicle control groups (20 males and 20 females) put on study 1 week earlier and with vehicle colony control groups (77 males and 80 females), which included the matched control group and three other control groups put on study within 3 months of the matched control group. All control mice were housed in the same room with treated mice.

Maximally and one-half maximally tolerated doses for the main study in mice were estimated from a preliminary toxicity test done as described for the NCI (1976) rat study. Mice were started in the chronic study at 35 days of age, and the study was concluded with sacrifice of survivors at 92-93 weeks. Chloroform in corn oil was administered by gavage 5 days per week during the first 78 weeks. Initial dose levels of 200 and 100 mg/kg/day for males and 400 and 200 mg/kg/day for females were raised to 300 and 150 mg/kg/day for males and 500 and 250 mg/kg/day for females at 18 weeks. Thus, doses expressed as time-weighted averages for the entire study were 138 and 277 mg/kg/day for males and 238 and 477 mg/kg/day for females.

Survival in mice was similar among groups except for high-dose females. At least 50% of the animals in each group survived as long as 85 weeks. Ten matched control, 33 low-dose, and 30 high-dose males, and 15 matched control, 34 low-dose, and 9 high-dose females survived for the duration of the study. All but two deaths in high-dose females occurred after 70 weeks.

Body weight gain among groups was comparable. Male and female mice initially weighed about 18 and 15 g, respectively. Mean body weights at 50 weeks were approximately 35 g in males and 28 g in females, and these levels were generally sustained throughout the remainder of the study. Food consumption was stated to have been equivalent among groups. Appearance and behavior among groups were similar except for bloating and abdominal distension noted in treated animals beginning after 42 weeks of treatment.

Statistically significant ($P < 0.05$) increases in hepatocellular carcinomas in both sexes in both treatment groups of mice were observed (Table 8-4). Various histopathologic types of hepatocellular carcinomas were observed. Hepatocellular carcinoma metastasized to the lung in two low-dose males and two high-dose females, and to the kidney in one high-dose male. Twenty animals were reported as missing or autolyzed, and therefore were not included in the pathology report.

Non-neoplastic lesions in mice attributed to treatment include nodular hyperplasia of the liver in 10 low-dose males, six low-dose females, and one high-dose female; and liver necrosis in one low-dose male, four low-dose females, and one high-dose female. Nine high-dose females with hepatocellular carcinoma had cardiac atrial thrombosis. Kidney inflammation was diagnosed in 10 matched control males, two low-dose males, and one high-dose male.

Under the conditions of this bioassay, chloroform treatment significantly ($P < 0.05$) increased the incidence of hepatocellular carcinoma in male and female B6C3F1 mice. Although the number of matched vehicle controls was low, the use of pooled colony controls gives additional support for treatment-related effects. Moreover, historical control incidence of hepatocellular carcinomas in B6C3F1 mice was reported as 5-10% in males and 1% in females.

TABLE 8-4. EFFECTS OF CHLOROFORM ON HEPATOCELLULAR CARCINOMA INCIDENCE IN B6C3F1 MICE (NCI 1976)

	Male				Female			
	Controls[b]		Dose (mg/kg/day)[c]		Controls[b]		Dose (mg/kg/day)[c]	
Treatment[a]	Colony	Matched	138	277	Colony	Matched	238	477
Hepatocellular carcinoma incidence[d]	5/77(8%)	1/18(6%)	18/50(36%)	44/45(98%)	1/80(1%)	0/20(0%)	36/45(80%)	39/41(95%)
P value[e]			0.011	3.13×10^{-13}			4×10^{-10}	3.7×10^{-14}
Time to first tumor (weeks)	72	72	80	54	90	---	66	67
Survival at terminal sacrifice (92 weeks)	48%	50%	65%	65%	81%	75%	75%	20%

[a]Chloroform in corn oil administered by gavage 5 times per week.
[b]Colony controls consist of four vehicle-control groups, including matched controls, given corn oil.
[c]Doses are time-weighted averages.
[d]Animals with tumor/animals examined.
[e]Fisher's Exact Test, compared with matched controls.

A more precise estimate of dose-response perhaps could have been obtained if additional lower doses had been used and if constant doses rather than time-weighted averages had been used. Treated animals were housed in the same room as animals treated with other volatile compounds*; however, since 1) controls were in the same room as treated animals, 2) oral chloroform doses probably would have been much higher than ambient levels of other volatiles, 3) the cages had filters to limit the amount of chemical released into the ambient air, 4) the total room air was exchanged 10 to 15 times per hour, and 5) dosing was done in another room under a large hood, the likelihood that the other volatile compounds were responsible for the observed results is considered to be low. It should be noted that ambient levels of volatiles in the animal quarters were not measured.

8.1.2.2. ROE ET AL. (1979) -- Roe et al. (1979) studied the carcinogenicity of chloroform in toothpaste in four strains of mice (C57BL, CBA, CF/1, and ICI). The toothpaste formulation used is presented in Table 8-3. The chloroform product was described as British Pharmacopoeia grade which was not contaminated with other haloalkanes or phosgene. Toothpaste was prepared fresh each month. Chloroform in arachis oil was also tested in ICI mice.

Dose levels for the carcinogenicity studies were selected based on results of a 6-week preliminary range-finding study in male and female Schofield mice which indicated moderate weight gain reduction at the lowest toxic dose of 60 mg $CHCl_3$/kg/day. Three different carcinogenicity studies were conducted in

*1,1,2,2-tetrachloroethane, 3-chloropropene, chloropicrin, 1,1-dichloroethane, trichloroethylene, sulfolene, iodoform, ethylene dichloride, methyl chloroform, 1,1,2-trichloroethane, tetrachloroethylene, hexachloroethane, carbon disulfide, trichlorofluoromethane, carbon tetrachloride, ethylene dibromide, dibromochloropropane.

which mice, initially no more than 10 weeks old, were given chloroform by gavage 6 days per week for 80 weeks followed by observation for 13 to 24 weeks. In one study, 52 male and 52 female ICI mice per dose group were given 17 or 60 mg/kg/day of chloroform in toothpaste and compared with 100 ICI mice of each sex concurrently given toothpaste without chloroform, peppermint oil, or eucalyptol. A second study, confined to male ICI mice, included 52 untreated mice, 260 mice given toothpaste alone without chloroform, eucalyptol, or peppermint oil, and groups of 52 mice each given, in toothpaste, 60 mg $CHCl_3$/kg/day, eucalyptol up to 32 mg/kg/day, or peppermint oil up to 16 mg/kg/day; treatment with chloroform, eucalyptol, or peppermint oil was done in the absence of the other two compounds. In the third study, groups of 52 male mice of each of the C57CL, CBA, CF/1, and ICI strains were given 60 mg $CHCl_3$/kg/day in toothpaste and compared with concurrent vehicle-control groups of 52 mice each, and with 100 untreated ICI mice. Fifty-two ICI male mice given 60 mg $CHCl_3$/kg/day in arachis oil, and concurrent control mice given arachis oil alone, were also evaluated in the third study.

Body weights were recorded in each study, and food consumption was estimated in the second and third studies. In each study, the animals were necropsied, and tumors and lesions as well as routine tissues and organs were examined histopathologically. Adrenals, kidneys, livers, lungs, and spleens were weighed.

Although the authors stated (data were not reported) that body weight gain was poorer in each treatment group than in controls in the third study on four mouse strains, differences in survival, body weights, and food consumption between control and treatment groups were not statistically ($P < 0.05$) significant, either as shown with data or as stated by the authors without data. Median survival was $\geq$ 73 weeks for all groups in the two studies on ICI mice

alone; by terminal sacrifice in the study on four strains (survival patterns were not reported), 52 to 79% of the C57BL and CBA mice and 12 to 31% of the CF/1 and ICI mice were alive. Liver and kidney weights were slightly lower (data not reported) in male ICI mice given chloroform in toothpaste. Incidences of tumors and lesions between control and chloroform-treated animals were not significantly ($P < 0.05$) different except for: 1) increased kidney tumor incidences in treated male ICI mice, as shown in Table 8-5, and 2) a significantly ($P < 0.001$, chi-square test) higher incidence of moderate to severe kidney "changes" in treated CBA and CF/1 males than in corresponding controls and of moderate to severe kidney disease ($P < 0.05$, chi-square test) in ICI males given $CHCl_3$ in arachis oil than in arachis oil controls, as described by the authors without presentation of data. Results in Table 8-5 indicate more effective induction of kidney tumors by chloroform in arachis oil than by chloroform in toothpaste. Kidney tumors were not found in C57BL, CBA, and female ICI mice, and malignant kidney tumors were diagnosed in two control and one treated CF/1 mice. Malignant kidney tumors were identified as hypernephromas, and benign kidney tumors were characterized as cortical adenomas. Eucalyptol and peppermint oil were not toxic in male ICI mice in these studies.

Results of the studies by Roe et al. (1979) show the ability of chloroform to induce kidney tumors in male ICI mice. The stronger induction of kidney tumors by chloroform in arachis oil compared with chloroform in toothpaste may reflect an effect of dosing vehicle on chloroform absorption, since Moore et al. (1982) demonstrated greater severity of acute toxicity and regenerative changes in kidneys of male CFLP mice given single gavage doses of 60 mg $CHCl_3$/kg when corn oil rather than toothpaste was the dosing vehicle. Kidney pathology was noted in treated animals in the study with four strains of mice; however, although poorer body weight gain reported for treated mice in each of the

TABLE 8-5. KIDNEY TUMOR INCIDENCE IN MALE ICI MICE TREATED WITH CHLOROFORM
(adapted from Roe et al. 1979)

Dose group	Numbers of mice examined histologically	Number of mice with kidney tumors		
		Benign	Malignant	Total
First study				
Vehicle-control[a]	72	0	0	0
17 mg $CHCl_3$/kg/day[b]	37	0	0	0
60 mg $CHCl_3$/kg/day[b]	38	5[h]	3[i]	8[j]
Second study				
Untreated control	45	1	0	1
Vehicle-control[a]	237	6	0	6
60 mg $CHCl_3$/kg/day[c]	49	7[h]	2[i]	9[j]
Third study				
Untreated control	83	0	0	0
Vehicle-control[d]	49	1	0	1
60 mg $CHCl_3$/kg/day[e]	47	2	3	5
Vehicle-control[f]	50	1	0	0
60 mg $CHCl_3$/kg/day[g]	48	3	9[h]	12[j]

[a]Toothpaste base vehicle without chloroform, eucalyptol, and peppermint oil.
[b]Chloroform given in toothpaste base with eucalyptol and peppermint oil.
[c]Chloroform given in toothpaste base without eucalyptol and peppermint oil.
[d]Toothpaste base vehicle without chloroform.
[e]Chloroform given in toothpaste base.
[f]Arachis oil.
[g]Chloroform given in arachis oil.
[h]Statistically significant versus vehicle-control ($P < 0.05$).
[i]Statistically significant versus vehicle-control ($P < 0.01$).
[j]Statistically significant versus vehicle-control ($P < 0.001$).

strains would suggest that a maximum tolerated dose was being approached, the observation that survival, body weights, and other pathology between control and treated mice in each of the four strains were not significantly ($P < 0.05$) different also suggests that higher doses could have been tested to more strongly challenge the mice for carcinogenicity. Since mice were as old as 10 weeks at the start of the studies, it is evident that treatment could have been started when the mice were younger to cover a greater portion of their lifespan during growth. A fuller evaluation of chloroform carcinogenicity could have been made if female mice had also been included in each study.

8.1.2.3. ESCHENBRENNER AND MILLER (1945) -- An early study on hepatoma induction by chloroform in mice was described by Eschenbrenner and Miller (1945). Strain A mice, initially 3 months old, with a historical spontaneous hepatoma rate of < 1% at 16 months of age were selected for treatment. "Chemically pure" chloroform was used, but chemical analysis was not indicated. Dose groups of five males and five females each were treated with doses of 2.4, 1.2, 0.6, 0.3, or 0.15 g/kg of chloroform in olive oil by gavage. Controls received olive oil alone.

In the study of hepatoma induction, mice were dosed every 4 days for a total of 30 doses. When 8 months old, mice were examined for hepatomas at one month after the last dose; however, these animals were given an additional dose of chloroform 24 hours before necropsy. Tissues and organs were examined histopathologically. Liver necrosis also was examined in mice given a single gavage treatment of one of the indicated doses of chloroform (one male and two females per group) 24 hours before removal of liver for microscopic evaluation.

Incidences of liver and kidney necrosis and hepatomas are shown in Table 8-6. Liver necrosis was noted in both sexes in the three highest-dose groups. Males in

all treatment groups developed kidney necrosis, whereas kidney necrosis was not apparent in females. No males in the three highest-dose groups and no females in the highest-dose group survived the study. All deaths occurred by 48 hours after the second administration of chloroform. All surviving females dosed with 0.6 or 1.2 g $CHCl_3$/kg had hepatomas.

TABLE 8-6. LIVER AND KIDNEY NECROSIS AND HEPATOMAS IN STRAIN A MICE FOLLOWING REPEATED ORAL ADMINISTRATION OF CHLOROFORM IN OLIVE OIL (adapted from Eschenbrenner and Miller 1945)

		Dose (g/kg)					
Observation	Sex	2.4	1.2	0.6	0.3	0.15	Control
Liver necrosis	F	+	+	+	0	0	0
	M	+	+	+	0	0	0
Kidney necrosis	F	0	0	0	0	0	0
	M	+	+	+	+	+	0
Deaths[a]	F	5/5	1/5	2/5	0/5	0/5	0/5
	M	5/5	5/5	5/5	2/5	0/5	0/5
Hepatomas in surviving animals receiving 30 doses[a]	F	--	4/4	3/3	0/5	0/5	0/5
	M	--	--	--	0/3	0/5	0/5

[a]Numerator is positive occurrences. Denominator is animals observed.

In the experiment on the ability of a single dose of chloroform to produce tissue necrosis, there was sharp distinction between normal and necrotic cells in liver. Doses of 2.4 and 1.2 g/kg produced extensive necrosis in all liver lobules, and the 0.6 g/kg dose produced necrosis in some lobes. Mice given 30 doses of chloroform in the hepatoma study had moderate liver cirrhosis and

necrosis, however, animals given 30 doses that did not result in necrosis had livers that appeared normal. Necrosis was not found in hepatoma cells, and hepatomas contained cords of enlarged liver-like cells which formed disorganized anastamosing columns. The hepatomas did not appear invasive, and metastasis was not found.

Renal necrosis in males was localized in the areas of the proximal and distal tubules. Glomeruli and collecting tubules appeared normal. The severity of renal necrosis was less with lower doses. The different kidney responses by males and females to chloroform treatment may be due to the unique lining of the Bowman's capsules with flat and cuboidal epithelium in females and males, respectively (an anatomic sexual dimorphism in mice). Although few animals were available for pathologic examination, the Eschenbrenner and Miller study (1945) indicates that hepatomas in female mice were induced at chloroform doses that also produced liver necrosis. Early mortality precluded all animals given chloroform doses that produced liver necrosis from developing hepatomas. Hepatomas were not induced by non-necrotizing doses of chloroform; however, a lifetime study perhaps could have given a stronger indication of the carcinogenic potential of chloroform at these lower doses. The observation of kidney necrosis in males without tumor formation and lack of necrosis in hepatomas suggests that liver in strain A mice was uniquely sensitive to tumor induction at necrotizing doses, or that there might have been additional factors in liver tumor formation besides necrosis. Furthermore, since a dose of chloroform was given 1 day before sacrifice--a factor which in itself could have been responsible for producing necrosis, as supported by liver necrosis found in mice which died after one or two treatments with chloroform--it is not clear what the extent of necrosis was during the last month of observation, when the animals were untreated.

8.1.2.4. RUDALI (1967) -- Rudali (1967) reported a carcinogenicity study on chloroform in NLC mice. Details such as age and sex of the mice were not given. The mice received twice-weekly doses of 0.1 ml of a 40% solution of chloroform in oil by force-feeding for an unspecified treatment period. Twenty-four animals were initially on study, but only five "sound mice" were evidently given a pathologic examination. An average survival period of 297 days was reported, but it is not clear if this period applied to the total group of 24 or to the smaller group of five. The observation period for the study was not mentioned. The use of a control group was not indicated, nor was a chemical analysis of the chloroform sample provided.

Three of the five mice examined in pathology were diagnosed with hepatomas and hepatic lesions; however, details of the pathologic observations were not reported. Although the study by Rudali (1967) gives evidence for carcinogenic activity by chloroform in NLC mice, it is weakened by a lack of experimental details, the absence of a control group, and the small number of animals examined in pathology.

8.1.3. Oral Administration (Capsules): Dog

8.1.3.1. HEYWOOD ET AL. (1979) -- The carcinogenicity of chloroform in toothpaste was evaluated in beagle dogs by Heywood et al. (1979). The toothpaste formulation used was that previously described in Table 8-3 herein except for reduced amounts of carragheen gum and glycerol. Chloroform in toothpaste was transferred from a syringe to gelatin capsules immediately before dosing.

Doses were selected from results of a preliminary range finding-study in which one or two dogs of each sex per group were given oral chloroform doses 7 days per week for 13 (30 and 45 mg/kg/day), 18 (60 mg/kg/day), or 12 (90 and

120 mg/kg/day) weeks. Because 45 mg/kg/day, which produced pathologic changes in the liver, was the lowest toxic dose, dose levels of 0, 15, and 30 mg $CHCl_3$/kg/day were chosen for the carcinogenicity study.

In the carcinogenicity study, chloroform was given orally in capsules 6 days per week for over 7 years. Eight males and eight females were assigned to each treatment group and to an untreated control group, and 16 dogs of each sex composed a vehicle-control group. The dogs were initially 18 to 24 weeks old. All of the dogs were clinically examined before treatment, and had been receiving medication annually for common diseases. Dogs were fed 200 g of diet twice daily until week 300, when obese dogs received reduced daily rations of 300 g. Body weights, food consumption, and water intake were estimated during the study. Hematology, serum biochemistry, and urinalysis were included in the evaluation of chloroform toxicity. Treatment was stopped at 376 weeks, and survivors were sacrificed for macroscopic examination at 395 to 399 weeks. Major organs were weighed. Tumors, lesions, and routine tissues and organs were evaluated microscopically. Liver and kidney specimens from control and high-dose dogs were also examined by electron microscopy.

Survival, body weights, food and water consumption, and appearance of the eyes were unaffected by chloroform treatment. Mean body weights increased from 7 to 8 kg initially to a maximum of 14 to 15 kg; however, reduction of diet portions for obese dogs complicated the body weight results. Results of blood and urine analyses were unremarkable except for dose-related increases in SGPT levels (Table 8-7), which could reflect liver pathology.

No treatment-related carcinogenic effects were found in necropsy and microscopic examination of tissues and organs. Non-neoplastic diagnoses showed that fatty cysts in the livers of all groups were larger and more numerous in treated dogs.

TABLE 8-7. SGPT[a] CHANGES IN BEAGLE DOGS TREATED WITH CHLOROFORM
(adapted from Heywood et al. 1979)

Treatment ($mg\ CHCl_3/kg/day$)	Group mean SGPT (MU/ml)											
	Pretreatment	Treatment stage (weeks)									Post-treatment (weeks)	
		6	26	52	104	156	208	260	312	372	14	19
30 mg	24	34[b]	58[c]	52[c]	64[c]	76[c]	91[c]	147[c]	128[c]	102[c]	105[d]	111
15 mg	22	29	33	32	45	46[d]	55[d]	95[c]	89[c]	66	53	48
Vehicle-control	22	29	30	29	40	30	40	33	47	51	56	128
Untreated	24	30	30	27	37	29	30	32	50	50	53	56

[a]Serum glutamic-pyruvic transaminase.
[b]Comparison with untreated group; $P < 0.05$.
[c]Comparison with untreated group; $P < 0.01$.
[d]Comparison with untreated group; $P < 0.001$.

The study by Heywood et al. (1979) did not show a carcinogenic effect of chloroform in toothpaste given to beagle dogs. Range-finding tests and SGPT and liver fatty cyst diagnoses in the carcinogenicity study suggest that a maximally tolerated dose was approached in the carcinogenicity study. It is not certain if 7 years was long enough for carcinogenicity testing with respect to the lifespan of the beagle dog (13 to 14 years), but by 7 years spontaneous tumor formation was becoming evident.

8.1.4. Intraperitoneal Administration: Mouse

8.1.4.1. ROE ET AL. (1968) -- Roe et al. (1968) investigated the carcinogenicity of chloroform in newborn (C57 X DBA_2 F1) mice. Chloroform was subcutaneously injected into one group of mice as a single dose of 200 ug when the animals were less than 24 hours old, and into another group of mice as eight daily doses of 200 ug, each beginning when the animals were 1 day old. Control groups were given the dosing vehicle, arachis oil, alone. Survivors were sacrificed for necropsy at 77 to 80 weeks.

No carcinogenic effect of chloroform was found. However, since the study was reported as an abstract, experimental details were not provided. Chloroform doses were rather low, and the use of newborn mice given one or a few doses of chloroform is not equivalent to lifetime treatment of animals given doses as high as those maximally tolerated. Additionally, there may be differences in chloroform metabolism between newborn and adult (C57 x DBA_2 F1) mice. Hence, it is concluded that the study by Roe et al. (1968) does not present sufficient evidence for an absence of carcinogenicity in chloroform.

8.1.4.2. THEISS ET AL. (1977) -- The carcinogenicity of chloroform was evaluated by Theiss et al. (1977) by means of the pulmonary tumor induction bioassay in strain A mice.

Test animals were male strain A/St mice initially 6 to 8 weeks old. Preliminary toxicity tests were performed for selection of maximum tolerated doses; in these tests, mice received six intraperitoneal injections of chloroform for 2 weeks and were observed for another 4 weeks. Results of the preliminary test were not reported. In the bioassay, chloroform doses in tricaprylin were 80 and 200 mg/kg, administered 3 times weekly for a total of 24 intraperitoneal injections; and 400 mg/kg, which was injected only twice. Fifty control mice were given tricaprylin alone. Each treatment group contained 20 animals. Mice were sacrificed 24 hours after the last dose, and lungs were removed for counting and examining surface adenomas microscopically. The chloroform product was reagent grade (Aldrich Chemical Company), but its chemical composition was not reported. A positive control group of 20 mice was given one injection of 1 g/kg of urethan in saline, and compared with 50 controls given saline alone.

Chloroform treatment did not produce a pulmonary adenoma response in this study. The average number of lung tumors per mouse was 0 to 0.39 in each group, except for the positive controls, which had an average of 19.6 lung tumors. At least 90% of the mice in each group survived, except for the mice given 400 mg $CHCl_3$/kg, where there was 45% survival. However, since this type of bioassay is basically a screen for carcinogens, a negative result does not necessarily indicate a lack of carcinogenic potential. Evidence for the carcinogenic activity of chloroform is available in other studies described in this document, and, according to the authors, there is evidence for carcinogenic activity in other compounds, e.g., 2-chloroethyl ether and hexachlorocyclohexane in liver, which also tested negative in the Theiss et al. (1977) study. Carcinogenic

effects of chloroform have been shown in the liver and kidney, whereas the lung was apparently not a target organ in the Theiss et al. (1977) study and in other studies.

8.1.5. Evaluation of Chloroform Carcinogenicity by Reuber (1979). Reuber (1979) evaluated the carcinogenicity of chloroform based on his review of slides in the NCI (1976) bioassay, and his review of data in other carcinogenicity studies described in this document. Reuber concurred with reported findings of rat kidney tumors and mouse hepatocellular carcinomas in the NCI (1976) study, mouse hepatomas in the Eschenbrenner and Miller (1945) and Rudali (1967) studies, and mouse kidney tumors in the Roe et al. (1979) study. However, Reuber also concluded that there were treatment-related neoplasms in the NCI study in addition to those reported. In rats, Reuber concluded that chloroform treatment induced liver tumors (hepatocellular carcinomas and neoplastic nodules) and cholangiofibromas and cholangiocarcinomas in addition to kidney tumors. Besides hepatocellular carcinomas, malignant lymphoma was also concluded by Reuber to have been induced by chloroform treatment in mice. Reuber also noted that treated rats and mice did not exhibit liver cirrhosis, that treated rats with thyroid tumors generally did not have liver or kidney tumors, and that liver necrosis was apparent only in high-dose female mice. The differences in histopathologic interpretation of tissue specimens in the NCI bioassay between the Reuber study and the NCI report, outside of a difference of opinion between pathologists, are not clear.

8.1.6. Oral Administration (Drinking Water): Mouse: Promotion of Experimental Tumors

8.1.6.1. CAPEL ET AL. (1979) -- The effect of chloroform on the growth of murine tumors was assessed by Capel et al. (1979). Redistilled analar chloroform was used, but chemical analysis of the product was not indicated. Test animals were male C57CL/105cSn/01a and male Theiller-Original (TO) mice, 20 to 22 g body weight. A cage of 20 mice drank 80 to 100 ml of water each day; hence, chloroform was added to yield doses of 0.15 or 15 mg $CHCl_3$/kg/day for two dose groups, with each mouse drinking 4 ml water per day. Fresh chloroform solution was given daily and was protected from light.

In one experiment, the authors stated that 100 TO mice in each dose group were divided into three "approximately equal" subgroups. One subgroup (pretreated) was treated with chloroform for 14 days before and after inoculation of Ehrlich ascites tumor cells. Another subgroup (post-treated) was given chloroform only after inoculation of tumor cells. The third subgroup, also inoculated with tumor cells, served as untreated controls. Tumor cells had been maintained in the peritoneal cavity of male TO mice by weekly passage of 10^6 cells. Peritoneal fluid was collected 7 days after inoculation of cells and diluted with buffered saline. All mice in the three subgroups were given intraperitoneal injections of 0.1 ml diluent (10^6 cells). At the end of exponential growth at 10 days following inoculation of cells, animals were sacrificed for removal of peritoneal fluid. The peritoneal cavity was washed with heparinized buffered saline. Fluid and washings were combined and diluted with buffered saline. Cells were disrupted by sonication for estimation of DNA levels per ml cell suspension as a measure of total cell content.

A second experiment was done in which 100 C57BL mice in each dose group were subdivided into three subgroups (pretreated, post-treated, control) each of which was treated with chloroform according to the protocol in the first

experiment. Each mouse received a subcutaneous injection of 10^6 B16 melanoma cells suspended in 0.1 ml buffered saline. Inoculum was obtained from a C57BL mouse which had received a transplant of syngeneic B16 melanoma cells maintained by intramuscular passage every 14 days. Animals were sacrificed at 21 days after inoculation, and spleen, mesenteric lymph nodes, and lungs were examined for metastases.

In the third experiment, Lewis lung tumor cells were maintained by serial intramuscular transplantation in C57BL mice. A group of 100 mice was divided into three approximately equal subgroups (pretreated, post-treated, control) to investigate the effect of 15 mg $CHCl_3$/kg/day on tumor growth and spread according to the protocol used in the first experiment. Each mouse received intramuscular injections of 2×10^6 cells suspended in 0.1 ml buffered saline into a thigh. Animals were killed 14 days after administration of tumor cells, and both the tumor-bearing and the normal thighs were skinned and severed at the knee and hip. Tumor weight was estimated as the difference between the weights of the thighs. Pulmonary tumor foci were also counted.

For estimation of the effect of 0.15 mg $CHCl_3$/kg/day in the third experiment, 100 mice were divided into subgroups of 20 animals each and were pretreated with chloroform before (for 8, 6, 4, or 2 weeks) and after injection of the Lewis cells. Mice were sacrificed at 16 days after inoculation of tumor cells, and tumor weights and numbers of lung foci were determined. In these animals, homogenates of primary tumors were prepared for B-glucuronidase estimation and protein content.

The results of these experiments are summarized in Tables 8-8, 8-9, and 8-10. Body weights and survival were not affected by chloroform treatment. Ehrlich ascites tumor cells, as equated with DNA content, were significantly ($P < 0.05$) increased in high-dose mice, and slightly though not significantly

TABLE 8-8. EFFECT OF ORAL CHLOROFORM INGESTION ON THE GROWTH OF EHRLICH ASCITES TUMORS
(Capel et al. 1979)

Dose	Treatment group	Number of animals per group	Average body weight (g)[a]	Tumor DNA (ug/ml)[a]	Significant
0.15 mg/kg/day	Control	33	38.3 ± 3.7	661 ± 222	
	Post-treated	33	39.4 ± 2.9	724 ± 254	NS
	Pretreated	33	37.9 ± 3.2	770 ± 283	NS
15 mg/kg/day	Control	43	39.4 ± 3.4	637 ± 221	
	Post-treated	37	37.5 ± 3.0	1143 ± 324	$P < 0.001$
	Pretreated	30	37.0 ± 3.9	827 ± 245	$P < 0.001$

[a]Results expressed are the mean ± S.D.
NS = Not significant; $P > 0.05$.

TABLE 8-9. EFFECT OF ORAL CHLOROFORM INGESTION ON METASTATIC "TUMOR TAKES" WITH B16 MELANOMA
(Capel et al. 1979)

Dose	Treatment	Number of animals per group	Animals with B16 melanoma invasion in organs (%)			
			Spleen	Mesenteric lymph nodes	Lung (a)[a]	Lung (b)[b]
0.15 mg/kg/day	Control	26	15	13	12	3
	Post-treated	31	35	10	10	5
	Pretreated	28	36	29	18	10
15 mg/kg/day	Control	30	15	12	6	4
	Post-treated	32	31	25	19	6
	Pre-treated	32	31	32	20	20

[a]Numbers in column (a) refer to the percentage of animals with tumor foci on the lungs.
[b]Numbers in column (b) refer to the average number of lung metastases.

TABLE 8-10. EFFECT OF ORAL CHLOROFORM INGESTION ON THE GROWTH AND SPREAD OF THE LEWIS LUNG TUMOR[a] (Capel et al. 1979)

Dose	Treatment	Number of animals per group	Average body weight (g)	Tumor weight (g)	Lung metastases	Significance	B-glucuronidase activity[b]	Protein content[c]
0.15 mg/kg/day	8[d]	20	30.6 ± 3.8	3.5 ± 0.81	165 ± 56	NS	0.33 ± 0.56	78.2 ± 4.2
	6	20	30.6 ± 3.8	3.3 ± 0.72	170 ± 41	NS	0.27 ± 0.79	66.8 ± 2.7
	4	20	29.0 ± 3.6	3.3 ± 0.54	154 ± 39	NS	0.38 ± 0.073	60.1 ± 5.1
	2	20	29.0 ± 3.5	3.2 ± 0.72	147 ± 44	NS	0.49 ± 0.070	60.3 ± 4.7
	0 (control)	20	29.3 ± 2.7	3.1 ± 0.11	142 ± 34		0.58 ± 0.094	50.8 ± 6.2
15 mg/kg/day	Control	33	23.6 ± 1.3	1.6 ± 0.31	44 ± 26			
	Post-treated	33	24.5 ± 2.3	1.7 ± 0.51	57 ± 19	$P < 0.05$		
	Pretreated	33	24.4 ± 2.2	1.8 ± 0.12	61 ± 19	$P < 0.01$		

[a]Results expressed are the means ± S.D.
[b]Expressed as mole product/mg protein/min.
[c]Milligrams of protein after extraction mg/g wet weight.
[d]Duration of treatment (weeks).
NS = not significant, $P > 0.05$.

increased in low-dose animals. Invasion by B16 melanoma cells, especially in the spleen, was augmented by both doses, and the numbers of lung foci were also greater in both treatment groups. Metastasis of Lewis cells was increased only by treatment with 15 mg $CHCl_3$/kg. There was no change in B-glucuronidase levels based on tumor protein content in the low-dose group. The increased tumor protein levels appear to reflect tumor growth which was not evident by weighing.

The study by Capel et al. (1979) shows an ability of chloroform to enhance the growth of three types of murine tumors in mice. A dose of 15 mg $CHCl_3$/kg was effective in each experiment, whereas a dose of 0.15 mg $CHCl_3$/kg was effective only in the test with B16 melanoma cells. Although this study does not evaluate the ability of chloroform to induce primary tumors, it does give evidence for a promoting effect of chloroform on the growth and spread of experimental tumors at low doses. However, the mechanism by which chloroform enhanced tumor growth in the study by Capel et al. (1979) is not certain, and the relevance of this study to the evaluation of the carcinogenic potential of chloroform is not clear.

8.2. CELL TRANSFORMATION ASSAY

8.2.1. Styles (1979). Styles (1979) reported an investigation on chloroform in a cell transformation system with BHK cells, using growth in semi-solid agar as an endpoint, as part of a larger study (Purchase et al. 1978) done to screen chemicals for carcinogenic potential. The BHK-agar transformation assay technique used has been described by Styles (1977) and Purchase et al. (1978). In the study reported by Styles (1979), baby Syrian hamster kidney (BHK-21/Cl 13) cells were exposed to five different doses of test substance in vitro in serum-

free liquid tissue culture medium in the presence of rat liver microsomal fraction and cofactors (S-9 mix; Ames et al. 1975). The liver microsomal fraction was obtained from Sprague-Dawley rats induced with Arochlor 1254.

Cells were grown and maintained in Dulbecco's modification of Eagle's medium in an atmosphere of 20% CO_2 in air. Cells were maintained at 37°C until confluent, and then were trypsinized and resuspended in fresh growth medium. Resuspended cells were grown until 90% confluent for transformation assays or 100% confluent for stock. Only cells with normal morphology were used for assays. To minimize spontaneous transformation frequency, cells were obtained at low passage, grown to 90% confluency, and frozen in liquid nitrogen. Cells were thawed at 37°C in growth medium for further use.

Test compounds were dissolved in DMSO or water as appropriate. Each dose was tested in replicate assays. Cells incubated until 90% confluency were trypsinized and resuspended in Medium 199 at a concentration of 10^6 cells/ml. Resuspended cells (10^6) were incubated with test chemical and S-9 mix at 37°C for 4 hours. After treatment, cells were centrifuged and resuspended in growth medium containing 0.3% agar. Survival after treatment was estimated by incubating 1,000 cells at 37°C for 6 to 8 days before counting colonies. Transformation was evaluated by counting colonies after cells were plated and incubated for 21 days at 37°C. The dose-response for transformation was compared with that for survival. Styles (1977) accepted a fivefold increase in transformation frequency above control values at the LC_{50} as a positive result. The spontaneous transformation frequency of BHK cells (72 experiments) in this study was 50 ± 16 per 10^6 survivors. The suitability of the soft agar medium for colony growth was checked by assays with polyoma-transformed BHK-21/C1 13 cells or Hela cells.

Cell transformation results were negative with exposure to chloroform solution in DMSO added to culture medium in a dose range that included levels at

which toxicity was observed (Figure 8-2). Although chloroform doses high enough to produce toxicity did not induce transformation, exposure of cells to chloroform as a vapor could have provided a comparison of the transformation potential of chloroform as a vapor and chloroform in liquid solution.

The study by Purchase et al. (1978), which was done on 120 chemicals of various classes, showed that the BHK-agar transformation assay system was about 90% accurate in discriminating between compounds with demonstrated carcinogenic or noncarcinogenic activity, and was in approximately 83% agreement with the results of assays done by the authors with S. typhimurium (TA 1535, TA 1538, TA 98, TA 100). Styles (1979) indicated, without presenting numerical data, that the results obtained in Salmonella assays on chloroform in liquid solution were similar to the findings of the transformation assays. Purchase et al. (1978) also observed that metabolically activated agents transformed BHK cells more strongly in the presence of S-9 mix, thus suggesting that BHK cells have limited intrinsic metabolic capability.

8.3 EPIDEMIOLOGIC STUDIES

In the last decade there has appeared in the literature a host of epidemiologic and statistical studies of cancer and exposure to the constituents of drinking water, of which chloroform is one (Harris 1974, Page et al. 1976, Tarone and Gart 1975, Buncher 1975, Vasilenko and Magno 1975, De Rouen and Diem 1975, McCabe 1975, Kruse 1977, Alavanja et al. 1978, Rafferty 1979, Kuzma et al. 1977, Harris et al. 1977, Salg 1977, Mah et al. 1977, Brenniman et al. 1978, Tuthill et al. 1979, Wilkins 1978). These studies have been subjected to several critical reviews (Wilkins et al. 1979, U.S. Environmental Protection

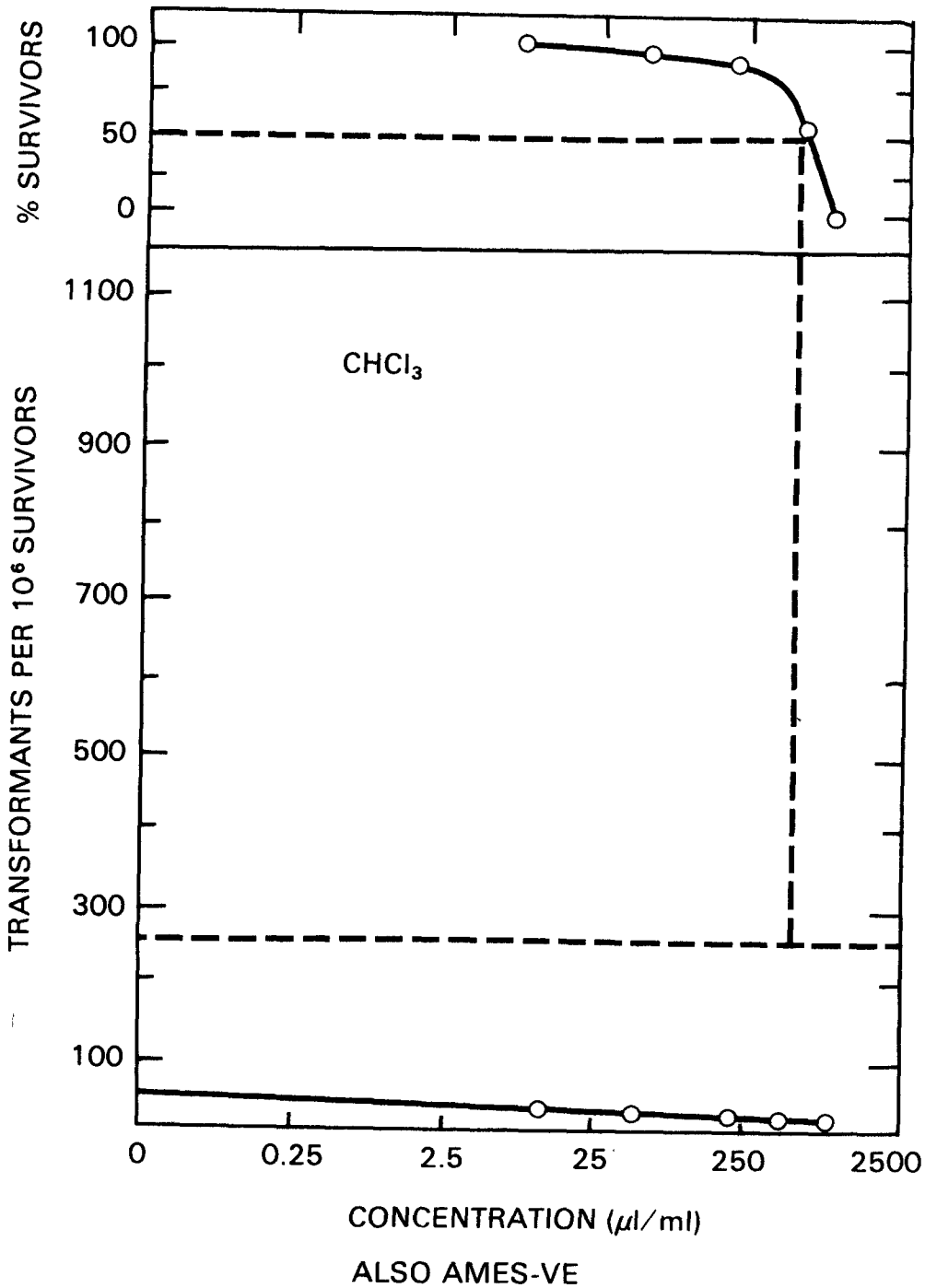

Figure 8-2. Negative result in transformation assay of chloroform which was also negative in the Ames assay. (Styles 1979)

Agency 1979, National Academy of Sciences 1978) and have been discussed in some detail. Some very general relationships have been noted by the reviewers. Of particular importance is the appearance of some consistency in the finding of cancer of the large intestine, rectum, and bladder associated with the constituents of drinking water.

It must be emphasized that none of the studies discussed in this section implicates chloroform directly as the sole or dominant constituent of drinking water responsible for the excess of cancer at these sites. Over 300 volatile organic contaminants have been identified in drinking water, and many of these have been identified as carcinogens (Wilkins et al. 1979).

However, chloroform at a peak concentration of 266 ug/l has been shown to exceed peak concentrations of other detected carcinogens by levels 37 times higher than those of the next highest carcinogen, vinyl chloride (Wilkins et al. 1979).

Chloroform measurements appear to range largely between 1 and 112 ug/l, according to a survey of 76 drinking water supplies (Cantor et al. 1978).

Although a direct association cannot be made, the possibility still exists that since chloroform is apparently the predominant component in chlorinated drinking water, it could be a contributing factor in the etiology of the cancer associated with the consumption of drinking water.

Almost all of the above-referenced studies were ecological correlation investigations, and only a few utilized case-control methods. The studies varied by sample size, cancer sites considered, control variables, and the types of endpoints used as indicators. Among the problems posed by the data in these studies are the following: 1) a lack of data measuring the quantity of chlorine and chloroform in drinking water; 2) the limited nature of recently acquired data on the quality and quantity of organics in drinking water; 3) the limited

amount of information given regarding personal consumption of drinking water; 4) the long latency periods associated with most cancers (current cancer rates reflect exposures received decades earlier); and 5) the demographic effects of migration, which adds another dimension of difficulty to the quantification of personal consumption of drinking water over time.

Since publication of the three reviews referred to above, several additional studies of cancer and exposure to trihalomethanes have been published. The following pages discuss each of these studies in detail.

8.3.1. Young et al. (1981). Young et al. (1981) conducted a case-control study in which cancer deaths in 8,029 white females were matched with non-cancer deaths in some 8,029 white females for county of residence, year of death, and age recorded on death certificates in 28 counties in the State of Wisconsin from 1972 through 1977. Information about the chlorine content of the drinking water of the 16,058 cases and controls was derived from mail-back questionnaires recently submitted to the superintendents of 202 waterworks encompassing the counties sampled. The questions pertained to prechlorination and postchlorination dosages used over the past 20 years (average daily dose in ppm). For 14% of the sample who were not served by a waterworks, decedents were assigned chlorine dosages of zero. The assignment was on the basis of water supplied to decedent's usual place of residence.

Odds ratios were calculated from a logistic regression model. This model provided estimates of the relative risk of site-specific cancer deaths for exposure of the previous 20 years to high, medium, and low chlorine doses, as compared with no chlorination. Urbanicity, marital status, and site-specific high-risk occupation were controlled in the model. Only colon cancer showed a significant ($P < 0.05$) association with chlorine intake in all three dosage

categories. However, no gradient of increasing risk with increasing dosage was apparent. For the high, medium, and low dosage categories, the odds ratios were 1.51, 1.53, and 1.53, respectively. All were significant at $P < 0.05$. In those counties where the drinking water supplies were exposed to rural runoff, the odds ratios for colon cancer increased to 3.43, 3.68, and 2.94 for high, medium, and low average daily chlorine doses when controlled for water source depth and purification. These were statistically significant at the $P = 0.025$ level. Colon cancer mortality was not related to chlorination in counties not exposed to rural runoff. This finding is consistent with the hypothesis that trihalomethanes are formed through the action of chlorine on organic substances in drinking water.

Nonsignificant risks were evident at the remaining sites, i.e., esophagus, stomach, rectum, liver, pancreas, kidney, bladder, lung, brain, and breast. The average daily chlorine dose categories were designated by the authors as follows: none (less than 0.01 ppm), low (0.01-0.99 ppm), medium (1.00-1.70 ppm), and high (1.71-7.00 ppm).

The authors made a number of assumptions regarding exposure of subjects and controls to chloroform. They assumed that chlorine in drinking water would represent a good surrogate for exposure of cases and controls to chloroform, reasoning that trihalomethanes such as chloroform are believed to result from the reaction of chlorine with naturally occurring organics in water. Although drinking water at the tap was not analyzed for chloroform or other trihalomethanes, the authors assumed that the measured levels of chlorine at the respective waterworks would correlate well with presumed exposure to chloroform in drinking water.

Such implicit assumptions appear questionable for several reasons. First, the latent period for the development of several, if not most, of the cancer sites

is most probably greater than 20 years. This is longer than the period covered by the exposure data on chlorination of water supplies used by the authors.

Second, migration within and around the 28-county area could have masked any real risk that was related to exposure. A diagnosis of colon cancer, which has a 5-year survival rate of better than 46%, could have induced victims to migrate to urban areas (where chlorine levels were higher) in order to obtain better medical care, thus leading to a false positive association.

Third, the amount of chloroform that is formed from the addition of chlorine is a function of several important variables: the quantity of organics in the water supply, treatment practices, and chlorine dosages. The quantity of organics in the water supply is, in turn, determined by the nature of the water supply source. Surface water (rivers and streams) receives large quantities of organics from land runoff, whereas groundwater contains little or no organic material; hence, the likelihood of chloroform formation from the addition of chlorine to a groundwater supply is minimal.

Fourth, liquids intake rates and amounts vary considerably from person to person. It is clear that most people satisfy their liquid requirements through a variety of drinks besides tap water, (e.g., milk, orange juice, coffee, soda). It is conceivable that many may drink little water because of these competing sources of liquid refreshment. Therefore, it is probable that many persons who were ranked as having been exposed to chloroform may in fact have had little exposure to it. The resulting misclassification of cases and controls by exposure category would tend to mask any gradient of increasing risk with exposure if one existed.

Another possibly confounding variable not controlled for in this study is the dietary intake of meat and foods low in fiber content (Reddy et al. 1980), both of which have been hypothesized as being related to colon or rectal cancer.

The dietary intake of such foods, however, is not known to be correlated with the quantity of chlorine in drinking water, although the possiblity of a spurious correlation cannot be ruled out. In more urbanized counties where chlorine levels are higher, residents may consume a diet of more meat and less fiber.

In summary, a definite association of chlorine or chloroform in drinking water with an increased risk of colon cancer should not be made for the reasons stated.

8.3.2. Hogan et al. (1979). Hogan et al. (1979) conducted an ecological study of site-specific cancer rates based on National Cancer Institute (NCI) cancer mortality data by county for the years between 1950 and 1969 (Mason and McKay 1974) and on chloroform levels in finished drinking water, as determined by the U.S. Environmental Protection Agency (EPA) in two separate surveys (U.S. EPA 1975). The first survey, known as the National Organics Reconnaissance Survey (NORS), consisted of samples from 80 water treatment facilities across the country. The second survey covered 83 utilities in the states of Illinois, Indiana, Michigan, Minnesota, Ohio, and Wisconsin. Linear multiple regression analyses were done for each set of data separately. The dependent variable was county site-specific cancer mortality. Weighted and unweighted regression coefficients were determined for a number of independent variables selected by the author based on a study by Hoover et al. (1976). A variety of demographic characteristics related to cancer mortality were used in addition to the variable "chloroform levels" as determined from the NORS and regional surveys to explain cancer mortality. These characteristics were: county population density, percent of urbanization per county, percent of nonwhite people, percent of foreign-born, county median family income, educational level, percent of workforce employed in manufacturing, chloroform level in drinking water samples, and

county population. According to the authors, the weighting was based on the inverse of the square root of the population of the race-sex county stratum, and was done chiefly to improve the precision of the regression estimates.

Significant positive statistical correlations were found between chloroform levels in treated drinking water and cancer mortality specific for bladder, rectum, and large intestine in the "weighted" regression for white females. On the other hand, only stomach cancer appeared to be positively correlated significantly with chloroform levels in white males. Without weighting, cancers of the bladder, rectum, thyroid, and breasts were significantly correlated with chloroform levels in white females. In white males, cancers of the pancreas and rectum were significantly correlated with chloroform without weighting. Only estimated regression coefficients were provided with their corresponding P values. The study contained no information on actual levels of chloroform observed in drinking water. Nonwhites were not considered because of the small sizes of the populations from which rates were derived.

Ecological studies such as this one are necessarily weak because their information is based on aggregate rather than individual data. The evidence for an association is indirect and definite conclusions cannot be drawn, although hypotheses may be formulated. It is not certain whether a multiple linear regression technique is the proper method for analyzing such data, since the assumption of linearity implied in its selection may not be warranted. Also, since the model contains no interaction terms, it is implicit that the chosen control variables are independent of each other, and such an assumption may also be unwarranted. Furthermore, as was mentioned in the Young et al. (1981) study, these data are weakened because it was assumed that the subjects were actually exposed to the levels of chlorine (or chloroform) indicated. Another limitation is that since the chloroform data were collected in 1975, the more

relevant exposure data (assuming a general cancer latency of 10 to 30 years) should be those of 1920 to 1959, given that the site-specific cancer mortality data covered the period 1950-1969.

8.3.3. Cantor et al. (1978). Cantor et al. (1978), in an ecological study of cancer mortality and halomethanes in drinking water, used age-standardized cancer mortality rates by site and sex in whites for the years 1968-1971, but only in the 923 U.S. counties that were more than 50% urban in 1970. This study was similar to the Hogan et al. (1979) study with respect to its design; i.e., a weighted linear regression model was used with sex- and site-specific cancer rates as the dependent variable. The weight was directly proportional to the square root of the counties' person-years at risk and thus inversely proportional to the standard deviation of the estimated mortality rate. Chloroform ($CHCl_3$), bromochloromethane (BTHM), and total trihalomethane (THM) levels were obtained from the two EPA surveys (U.S. EPA 1975) used in the Hogan et al. study. Demographic variables used in the regression model on a county-wide basis were: percent of urbanization (1970); median school years completed by persons over age 25; population size (ratio of 1970 to 1950 population); percentage of the work force in manufacturing; and percentage of foreign-born. Although a predicted, age-adjusted, site-specific cancer rate was calculated for each county based on this regression technique, only the data for 76 counties, where more than half of the population of the counties was served by a sampled water supply, were actually used in this correlation analysis of THM levels with residual mortality rates. Figure 8-3 gives a frequency distribution of the chloroform levels in these 76 U.S. drinking water supplies. The three indicators, chloroform, bromochloromethane, and total trihalomethane, were highly correlated with one another.

Positive nonsignificant correlations with THM levels were evident with

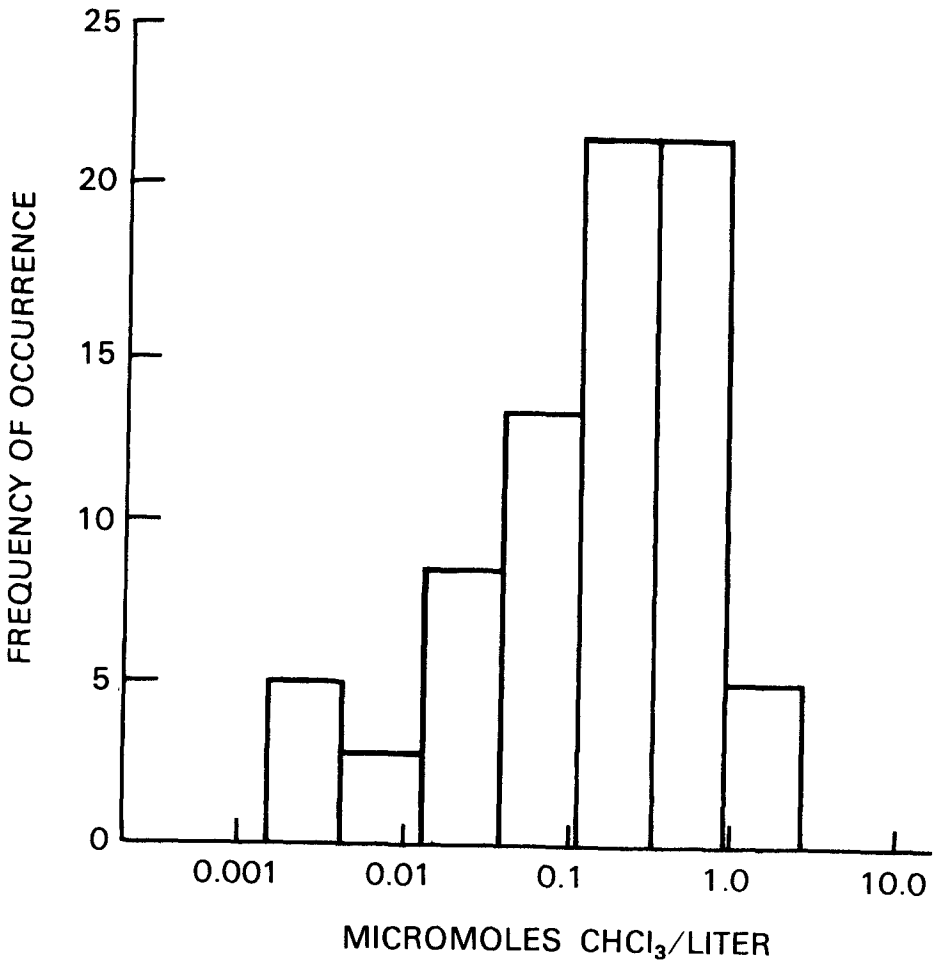

Figure 8-3. Frequency distribution of $CHCl_3$ levels in 76 U.S. drinking water supplies. The abscissa is linear in the logarithm of the level. (Cantor et al. 1978)

respect to several forms of cancer, including lymphoma and kidney cancer in males (Table 8-11). But according to the authors, bladder cancer mortality rates gave the strongest and most consistent association with THM exposure after controlling for differences in social class, ethnic group, urbanicity, region, and extent of county industrialization (Table 8-12). However, the association appeared to be greatest with respect to BTHM and not chloroform. The corresponding correlations for chloroform were positive but nonsignificant. The authors report that although other sites appeared to be positively correlated with THM levels, the inconsistencies "outweigh the consistencies," thus casting doubt on the reliability of these correlation coefficents; i.e., the direction and strength of the correlations bear little relationship to the percent of population served by treated drinking water and/or by region.

TABLE 8-11. CORRELATION COEFFICIENTS BETWEEN RESIDUAL MORTALITY RATES IN WHITE MALES AND THM LEVELS IN DRINKING WATER BY REGION AND BY PERCENT OF THE COUNTY POPULATION SERVED IN THE UNITED STATES (Cantor et al. 1978)

Site of cancer	THM Indicator	Correlation coefficients for regions of the U.S. North	South	Mountain	Pacific	All regions
Kidney	$CHCl_3$	0.11 (0.54)[a]	-0.11 (0.73)	0.66 (0.11)		0.14 (0.33)
Lymphoma (non-Hodgkins)	BTHM	0.06 (0.74)	0.08 (0.79)	0.05 (0.92)		0.06 (0.70)
		Correlation coefficients for counties in which the percent of the population served was:				
		50-64%	65-84%	85-100%		50-100%
Kidney	$CHCl_3$	-0.16 (0.44)	-0.11 (0.60)	0.42 (0.04)		0.07 (0.55)
Lymphoma (non-Hodgkins)	BTHM	-0.33 (0.11)	-0.19 (0.36)	0.36 (0.08)		-0.08 (0.81)

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

TABLE 8-12. CORRELATION COEFFICIENTS BETWEEN BLADDER CANCER MORTALITY RATES BY SEX AND BTHM LEVELS IN DRINKING WATER BY REGION OF THE UNITED STATES (Cantor et al. 1978)

Bladder cancer	Correlation coefficients by region			
	North	South	Mountain	Total
Number	31	13	7	51
Male white	0.52[a] (0.002)	0.04 (0.90)	-0.02 (0.96)	0.30 (0.03)
Female white	0.30 (0.11)	0.20 (0.51)	0.63 (0.13)	0.33 (0.02)

[a]P value for two-tailed t-test is shown in parentheses. Counties with at least 65% of their populations served by one water supply were included in this analysis.

The authors noted an association of kidney cancer with chloroform exposure that was restricted to males, but was significant only in counties where at least 85% of the public was served by treated drinking water. In counties where less than 85% was served by treated drinking water, the correlation coefficients were actually negative. Combining all counties with greater than 50% served by treated drinking water, the correlation coefficent was nonsignificant and close to zero. One interesting observation was that without controlling for ethnicity, the authors found a "fairly strong" association of THM levels with colon cancer and lung cancer rates in both sexes, and even a dose-response relationship between these tumor sites and the proportion of the population exposed. However, when ethnicity was added to the regression model, these relationships disappeared.

Again, this is a descriptive study from which hypotheses can be formulated only for future in-depth study. It cannot be concluded that even the significant positive correlations in the study indicate any evidence of real associations. As the authors point out, potential sources of error (i.e.,

control of confounders such as cigarette smoking and diet) are particularly difficult since no direct information is available on the individuals studied. The main problem with such studies, as mentioned earlier, is that the data are aggregate rather than individual. Such data frequently include large numbers of individuals who never received the exposure in question. Associations derived from such data may be misleading and are often unreliable.

8.3.4. Gottlieb et al. (1981). Gottlieb et al. (1981) completed a case-control study of the relationship between Mississippi River drinking water and the risk of rectum and colon cancer. The study was based on mortality data gathered from 20 parishes in southern Louisiana. Rectal and colon cancer deaths (692 and 1,167, respectively) from 1969 to 1975 were matched one-to-one to non-cancer deaths by age at death, year of death, sex, and race, within the same parish group. A parish group consisted of similar parishes with respect to industrial and urban-rural characteristics and were defined so that each parish included nearly equal populations using groundwater and surface water supply sources, based on information from the 1970 census.

Three different estimators of exposure were used. The first, "sourcelife," is defined as follows: "mostly surface" (birth and death in a surface-water-using parish); "some surface" (some known surface water use at birth or death); "possible surface" (death in a groundwater parish but had either unknown or out-of-state birthplace); and "least surface" (birth and death in a groundwater-using parish). Length of residence was also considered, if known and for more than 10 years. The second index used was chlorine level (none, low [less than 1.09 ppm], or high [greater than 1.08 ppm]). The third index was the level of organics in the drinking water (low [less than 68 ppm] and high [greater than or equal to 68 ppm]). Sourcelife could be determined for 99.2% of the entire

group of 3,718 cases and controls, but 51% had no data for length of residence or had lengths of residence of under 10 years. For those with lengths of residence of less than 10 years, water sources during the possible carcinogenic period were unknown. Chlorine values were available for some 78.9% of the 3,718 sources, while organics levels were available for only 50.1% of the sources. The analyses using the latter two variables were equivocal, possibly due to the lack of information on these parameters.

Colon cancer was found not to be related significantly to any water variable, although the number of colon cancer cases in this study (1,167) was greater than the number of rectum cancer cases (692). The authors hint that the earlier correlation found in ecological studies could have resulted from confounding with urban lifestyles. Rectal cancer, on the other hand, was found to be significantly elevated with respect to surface or Mississippi River water consumption. Based on sourcelife, the odds ratio for rectal cancer for those who were born and died using groundwater sources was 2.07 (95% confidence interval [C.I.] 1.49-2.88) based on a multidimensional contingency table analysis. Chlorination was significantly associated with rectal cancer, and for those who used river water, the risk decreased as the distance from the mouth of the river increased. The odds ratio for cancer of the rectum at a location below New Orleans versus one above the city was 1.82 (95% C.I. 1.01-3.26). The authors noted that both sexes were at increased risk. With respect to controlling for the effect of chlorination where adequate numbers existed, the surface water versus groundwater effect on rectal cancer was of only borderline significance ($P = 0.05$), implying a chlorine effect.

With respect to levels of organics, information was available for over 48% of the rectal cancer group and their controls. The odds ratio calculated based on these data was nonsignificant (Table 8-13), but was probably subject to some

TABLE 8-13. RISK OF MORTALITY FROM CANCER OF THE RECTUM ASSOCIATED WITH LEVELS OF ORGANICS IN DRINKING WATER (Gottlieb et al. 1981)

	Cases	Controls
High ($\geq$ 68 ppm)	110	97
Low (< 68 ppm)	232	220
Total	342	317
Odds ratio	1.08	

bias with respect to availability of exposure data as a function of date of death.

With respect to colon cancer, the authors felt that since they had grouped the parishes according to industry and urban characteristics (matching was done within the parish group), they successfully eliminated urban lifestyle as a confounder in their evaluation of colon cancer and drinking water.

The results of this study suggest that cancer of the rectum is linked to the consumption of surface water, and since chlorination appears to be an effect modifier altering the risk ratio to only borderline significance, it would seem that chlorination does contribute to the risk of rectal cancer.

8.3.5. Alavanja et al. (1978). Alavanja et al. (1978) reported on a case-control study of 3,446 gastrointestinal and urinary tract cancer deaths (1,595 females and 1,851 males) occurring during a 3-year period from 1/1/68 to 12/31/70 in seven counties of New York State. Some 3,444 individually matched noncancer deaths were also selected. Independent variables were: residence in an urban or rural area, residence in an area served by chlorinated or nonchlorinated water, residence in an area served by surface water or ground-

water, and occupation. Cases were taken from computer tapes of New York State death certificates, and were individually matched with an equal number of non-cancer deaths for the same year. Matching variables were age, race, sex, foreign- versus United States-born, and county of usual residence. If potentially confounding variables could not be controlled via the matching process, the cases and controls were stratified by these confounding variables. The data were analyzed by the chi-square test. A statistically significant excess of gastrointestinal and urinary tract cancer mortality occurred among women in the urban county of Erie (odds ratio [OR] = 2.08), with nonsignificant excesses in Schenectady County (OR = 2.98) and Allegany County (OR = 4.13). Likewise, among men a statistically significant excess of gastrointestinal and urinary tract cancer mortality occurred in Erie County (OR = 2.15) and Rensselaer County (OR = 1.98), and a nonsignificant excess occurred in Schenectady County (OR = 1.96) and Allegany County (OR = 2.85). Although the study encompassed a seven-county area, almost two-thirds of the deaths occurred in Erie County. The combined overall odds of dying from gastrointestinal and urinary tract cancer for all seven counties combined (including Erie), were only 1.79 based on 3,446 cases, whereas in Erie County alone they were 3.15 based on 2,177 cases. The authors concluded that males and females residing in the chlorinated water areas of the counties noted above were at a greater risk of gastrointestinal and urinary tract cancer mortality not due to age, race, ethnic distribution, urbanicity, occupation, inorganic carcinogens (Cd, As, Be, Pb, Ni, NO_3), or surface/groundwater difference. No environmental data are provided, however, to characterize quantities of chlorine (or chloroform) exposure. "Inadequate water quality data" prevented the authors from making a "definitive claim that the process of chlorination is directly or indirectly responsible for the

greater risk of gastrointestinal and urinary tract cancer mortality" in chlorinated cancer areas. No description is given of how residence was classified into chlorinated versus nonchlorinated water areas or surface water versus groundwater areas through the use of water distribution maps, a practice which can result in misclassification on the basis of exposure. Again, because of the lack of individual dosage data on chloroform exposure and the low significance of the risks described, this study can only be regarded as suggestive for gastrointestinal and urinary tract cancer mortality.

8.3.6. Brenniman et al. (1978). Brenniman et al. (1978) attempted to confirm the findings of Alavanja et al. (1978) in a case-control study of gastrointestinal and urinary tract cancer mortality among whites in 70 Illinois communities using both chlorinated and nonchlorinated groundwater. The authors limited the study to groundwater because of the possible introduction of confounding effects due to agricultural runoff and industrial sewage in surface water. The 3,208 cases and 43,666 controls used were those of Illinois deaths occurring between 1973 and 1976. Controls were selected from a pool of non-cancer deaths after the elimination of certain special types of deaths, such as perinatal deaths.

Chlorinated groundwater communities were matched with nonchlorinated groundwater communities that were similar with respect to urbanicity and Standard Metropolitan Statistical Area (SMSA) description. To ensure a minimum follow-up period, water supplies were categorized as chlorinated or nonchlorinated according to a "1963 inventory of municipal water facilities." Additionally, questionnaires were sent to water treatment plants in the communities to verify the 1963 data. The beginning dates for chlorination were obtained for many of the plants. Based on an EPA survey, it was found that 14 chlorinated groundwater

supply sources in Illinois had chloroform concentrations ranging from less than 1 ug/l to 50 ug/l, with a mean concentration of 10.8 ug/l.

In females, statistically significant increased relative risks of cancer of the large intestine and rectum (OR = 1.19, $P \leq 0.05$), as well as total digestive tract cancer (excluding liver) (OR = 1.15, $P \leq 0.05$), were found for chlorinated versus nonchlorinated Illinois groundwater supplies. With respect to total gastrointestinal and urinary tract cancer, the risk was significantly increased in females living within standard metropolitan statistical areas (OR = 1.28, $P \leq 0.025$) and within urban areas (OR = 1.24, $P \leq 0.025$) between chlorinated and nonchlorinated groundwater communities.

Where evidence was available concerning a history of chlorination, the authors noted that the relative risk of total gastrointestinal and urinary tract cancer tended to increase with time from initial chlorination, although the change was small. The greatest increase occurred in urban nonstandard metropolitan areas (OR = 1.14 if chlorinated since 1963 and nonsignificant, but OR = 1.28 if chlorinated since 1953 and significant, $P \leq 0.025$). Although several significant findings were observed in this study, it is surprising that the authors so readily dismissed the results of their own study on the basis that confounding factors such as diet, smoking, and occupation were not controlled. These authors felt that the findings were tenuous and did not confirm the findings of Alavanja et al. (1978) either in strength or in consistency. They state that "chlorination of groundwater does not seem to be a major factor in the the etiology of site-specific gastrointestinal and urinary tract cancers."

8.3.7. Struba (1979). Struba (1979), as part of his Ph.D. thesis, completed a case-control study of mortality in North Carolina on individuals who died at age 45 or under during the period 1975-1978. The cancer sites studied were the

rectum, colon, and urinary bladder. Between 700 to 1,500 cases per site were matched with controls by age, race, sex, and geoeconomic region (coastal, piedmont, or mountain). Non-cancer deaths were excluded if cancer was listed as a contributory or underlying cause of death. For colon and rectal cancer, certain precancerous colonic disorders were excluded (ulcerative colitis, familial polyposis, and adenomatous polyposis). Water data were classified by source, treatment, and previous use. "Source" was defined as ground, surface uncontaminated, or uncontaminated by upstream pollution. "Treatment" was defined as none, prechlorinated, post-chlorinated, or both. "Previous use" included the following 15 categories of upstream pollution for contaminated water only:

(1) Tobacco manufacturing
(2) Textile manufacturing
(3) Textile bleaching and dyeing
(4) Furniture manufacturing
(5) Pulp and paper mills
(6) Chemical industries
(7) Petroleum refining
(8) Rubber and plastics manufacturing
(9) Leather tanning and finishing
(10) Abrasives, asbestos, minerals
(11) Primary metals industries
(12) Electroplating
(13) Electric power generation
(14) Urban areas > 50,000
(15) Out-of-state upstream discharges

The author found small but significant odds ratios (1.3 to 2.0) for all three sites in rural areas, as well as significant odds ratios for each of the water quality variables in many stratified or combined analyses. Odds ratios for urban areas (population over 10,000) were generally not significant. Urbanization was shown to be an effect modifier for colon cancer and a likely confounder for rectal and bladder cancers. The author considered socioeconomic status to be a likely confounder for cancer of the rectum and bladder. Multivariate analyses showed no evidence that occupation acted as a confounder for bladder cancer in

this study. To estimate migration effects, cases and controls were stratified by place of birth and death (birth and death in the same county; birth and death in North Carolina; and death in North Carolina, birth unspecified) and substratified by region, age, race, sex, and urbanization. Odds ratios for treatment (chlorinated and nonchlorinated) were computed for all of the strata. For all three cancer sites, the group with least migratory influence had the highest odds ratio, thus lending support to the author's supposition that an increasing migratory effect is associated with a decreasing risk of cancer of all three sites.

Additionally, Struba found an increasing gradient of risk from the coastal regions of North Carolina to the mountains, a finding that he maintains is consistent with a stronger contrast between surface water and water from deep wells than between surface water and water from shallow wells, which are known to be susceptible to contamination by surface water seepage into groundwater aquifers. However, the author notes that this difference could be due to differences in water treatment practices or confounding by uncontrolled factors such as dietary habits or lifestyles.

8.3.8. Discussion. These later ecological and case-control studies of chlorine exposure and cancer risk from water supplies consistently support the finding of an increased risk of bladder, colon, and rectal cancer from exposure to chlorinated water. This association is at best weak, although significant, as evidenced by odds risk ratios that range up to 3.6 in the Young et al. (1981) study, but generally fall between 1.1 and 2.0 (see Table 8-14) in the remaining case-control studies. The risk ratios derived in these studies could be explained by the confounding effects of uncontrolled influences such as smoking, diet, air pollution, occupation, and lifestyle. However, the consistency of

TABLE 8-14. CANCER RISK ODDS RATIOS AND 95% CONFIDENCE INTERVALS (CHLORINATED VERSUS UNCHLORINATED) (Crump and Guess 1982)

Site	Alavanja et al. 1978[a]	Brenniman et al. 1978[b]	Young et al. 1981[c]	Gottlieb et al. 1981[d,e]	Struba 1979[d,e]
Rectum	1.93 (1.32, 2.83)	1.26 (crude) (0.98, 1.61) 1.22 (adjusted)	1.39 high (0.67, 2.86) 1.16 medium (0.58, 2.32) 1.13 low (0.61, 2.08)	1.41 (1.07, 1.87)	1.53 (1.24, 1.89)
Colon	1.61 (1.28, 2.03)	1.08 (crude) (0.96, 1.22) 1.1 (adjusted	1.51 high (1.06, 2.14) 1.53 medium (1.08, 2.00) 1.53 low (1.11, 2.11)	1.05 (0.95, 1.18)	1.30 (1.13, 1.50)
Bladder	1.69 (1.11, 2.56)	1.04 (crude) (0.81, 1.33) 0.98 (adjusted)	1.04 high (0.43, 2.50) 1.03 medium (0.42, 2.54) 1.06 low (0.60, 3.09)	1.07 (0.84, 1.36)	1.54 (1.26, 12.88)

[a]Calculated for both sexes and all races combined. Confidence intervals were not stated in Alavanja et al. (1978). Crump (1979) calculated them by applying the method of Fleiss (1979) to data in Alavanja et al. (1978).

[b]Calculated for Caucasians of both sexes. Adjusted values were adjusted for age, sex, urban/rural, and SMSA/nonSMSA. Confidence intervals were not stated in the original report. Crump (1979) calculated them by applying the method of Fleiss (1979) to data on total cases and total controls supplied by Dr. Brenniman in personal communication.

[c]Calculated for white females and for high, medium, and low average daily chlorine doses compared with no chlorination. Odds ratios and confidence intervals computed by logistic regression, controlling for urbanization, marital status, and site-specific occupation.

[d]Calculated for both sexes and all races combined.

[e]Struba and Gottlieb et al. also computed odds ratios for surface water versus groundwater as follows. Struba: rectum 1.55 (1.26, 1.91); colon 1.27 (1.10, 1.46); bladder 1.48 (1.22, 1.80). Gottlieb et al.: rectum 1.51 (1.21, 1.90); colon 0.95 (0.88, 1.03).

the finding across several independent and diverse study groups supports the finding of a definite risk. Of course, all of the case-control studies use residence data and cause-of-death information from death certificates, and thus are not strictly incidence studies. Bias can creep in from several sources: differential survivorship rates due to proximity to better medical care and treatment facilities, higher socioeconomic status, and the possibility of migration of newly diagnosed cancer patients to major medical care centers where chlorination is used to a greater extent. Underestimates of risk can result from failure to control for migration before to diagnosis, misclassification of cause of death, and use of chlorination as a surrogate variable in place of more direct measurements of chloroform, especially if the chlorinated source contains few organic contaminants. Hence, the association is weak but significant with regard to the three cancer types and exposure to chlorinated drinking water. Since exposure to chlorine in water is not the same as exposure to chloroform, the most that can be said is that there is a suggestion of an increased risk of cancer of these three sites from exposure to chloroform. If this risk truly exists, it may be due to an intermediate in the natural synthesis of chloroform (communication with Dr. Kenneth P. Cantor, NCI).

In summary, it appears that there may be a suggestion of an increased risk of certain forms of cancer (bladder, large intestine, and rectum) due to the presence of trihalomethanes in drinking water. Beyond this, little more can be said. The significant excess risk of colon cancer from chlorine in drinking water does not constitute evidence of an association of colon/rectal cancer with chloroform. The statistically significant positive correlation of bladder cancer and BTHM levels in drinking water is not readily attributable to chloroform. The evidence of a significant association of kidney cancer with chloroform exposure in drinking water is even more questionable, since it was based on

the findings of only one study, which was confined to males residing in counties where more than 85% of the population was served by treated drinking water. A statistically positive correlation was seen only in males residing in counties with over 85% treated drinking water. No association was observed in females in these same counties, and the correlations were actually negative for both males and females in counties with less than 85% treated drinking water.

It appears that these case-control and ecological studies in humans suggest a weak association of certain forms of cancer with trihalomethanes and chloroform in drinking water, but further epidemiologic research should be performed to confirm these findings.

8.4. QUANTITATIVE ESTIMATION

This section evaluates the unit risk for chloroform in air and water and the potency of chloroform relative to other carcinogens that the Carcinogen Assessment Group (CAG) has evaluated. The unit risk is defined as the lifetime cancer risk to humans from daily exposure to a concentration of 1 ug/l in water by ingestion or daily exposure to 1 ug/m^3 in air by inhalation. If the unit risk is calculated from a model that is linear at low doses, then the unit risk could be used as the slope for calculating risk at low doses.

The unit risk estimate for chloroform represents an extrapolation below the dose range of experimental data. There is currently no solid scientific basis for any mathematical extrapolation model that relates exposure to cancer risk at extremely low concentrations, including the unit concentration given above. For practical reasons, such low levels of risk cannot be measured directly, either by animal experiments or by epidemiologic studies. Low-dose extrapolation must therefore be based on current understanding of the mechanisms of carcinogenesis.

At the present time, the dominant view of the carcinogenic process involves the concept that most cancer-causing agents also cause irreversible damage to DNA. This position is based in part on the fact that a very large proportion of agents that cause cancer are also mutagenic. There is reason to expect that the quantal response, which is characteristic of mutagenesis, is associated with a linear non-threshold dose-response relationship. Indeed, there is substantial evidence from mutagenicity studies with both ionizing radiation and a wide variety of chemicals that this type of dose-response model is the appropriate one to use. This is particularly true at the lower end of the dose-response curve; at higher doses, there can be an upward curvature, probably reflecting the effects of multistage processes on the mutagenic response. The linear non-threshold dose-response relationship is also consistent with the relatively few epidemiologic studies of cancer responses to specific agents that contain enough information to make the evaluation possible (e.g., radiation-induced breast and thyroid cancer, skin cancer induced by arsenic in drinking water, liver cancer induced by aflatoxin in the diet). Some supporting evidence also exists from animal experiments (e.g., the initiation stage of the two-stage carcinogenesis model in rat liver and mouse skin). Linearity is also supported when the mode of action of the carcinogen in question is similar to that of the background cancer production in the exposed population.

Because its scientific basis, although limited, is the best of any of the current mathematical extrapolation models, the linear non-threshold model has been adopted as the primary basis for risk extrapolation to low levels of the dose-response relationship. The risk estimates made with such a model should be regarded as conservative, representing the plausible upper limits for the risk; i.e., the true risk is not likely to be higher than the estimate, but it

could be lower. For several reasons, the unit risk estimate based on animal bioassays is only an approximate indication of the risk in populations exposed to known carcinogen concentrations. First, there are important species differences in uptake, metabolism, and organ distribution of carcinogens, as well as species differences in target site susceptibility. Second, the concept of equivalent doses for humans compared with animals on a mg/surface area basis is virtually without experimental verification as to carcinogenic response. Finally, human populations are variable with respect to genetic constitution and diet, living environment, activity patterns, and other cultural factors.

The unit risk estimate can give a rough indication of the relative potency of a given agent compared with other carcinogens. The comparative potency of different agents is more reliable when the comparison is based on studies in the same test species, strain, and sex, and by the same route of exposure.

The quantitative aspect of the carcinogen risk assessment is included here because it may be of use in the regulatory decision-making process (setting regulatory priorities, evaluating the adequacy of technology-based controls, etc). However, it should be recognized that the estimation of cancer risks to humans at low levels of exposure is uncertain. At best, the linear extrapolation model used here provides a rough but plausible estimate of the upper limit of risk; i.e., it is not likely that the true risk would be much more than the estimated risk, but it could very well be considerably lower. The risk estimates presented in subsequent sections should not be regarded as an accurate representation of the true cancer risks even when the exposures are accurately defined. The estimates presented may be factored into regulatory decisions to the extent that the concept of upper risk limits is found to be useful.

8.4.1. Procedures for the Determination of Unit Risk.

8.4.1.1. LOW-DOSE EXTRAPOLATION MODEL -- The mathematical formulation chosen to describe the linear nonthreshold dose-response relationship at low doses is the linearized multistage model. This model employs enough arbitrary constants to be able to fit almost any monotonically increasing dose-response data, and it incorporates a procedure for estimating the largest possible linear slope (in the 95% confidence limit sense) at low extrapolated doses that is consistent with the data at all dose levels of the experiment.

Let P(d) represent the lifetime risk (probability) of cancer at dose d. The multistage model has the form

$$P(d) = 1 - \exp\left[-q_0 + q_1 d + q_1 d^2 + \ldots + q_k d^k)\right]$$

where

$$q_i \geq 0, \; i = 0, 1, 2, \ldots, k$$

Equivalently,

$$P_t(d) = 1 - \exp\left[q_1 d + q_2 d^2 + \ldots + q_k d^k)\right]$$

where

$$P_t(d) = \frac{P(d) - P(0)}{1 - P(0)}$$

is the extra risk over background rate at dose d.

The point estimate of the coefficients q_i, i = 0, 1, 2, ..., k, and consequently, the extra risk function, $P_t(d)$, at any given dose d, is calculated by maximizing the likelihood function of the data.

The point estimate and the 95% upper confidence limit of the extra risk, $P_t(d)$, are calculated by using the computer program GLOBAL79, developed by Crump and Watson (1979). At low doses, upper 95% confidence limits on the

extra risk and lower 95% confidence limits on the dose producing a given risk are determined from a 95% upper confidence limit, q_1^*, on parameter q_1. Whenever $q_1 > 0$, at low doses the extra risk $P_t(d)$ has approximately the form $P_t(d) = q_1^* \times d$. Therefore, $q_1^* \times d$ is a 95% upper confidence limit on the extra risk and R/q_1^* is a 95% lower confidence limit on the dose, producing an extra risk of R. Let L_0 be the maximum value of the log-likelihood function. The upper-limit q_1^* is calculated by increasing q_1 to a value q_1^* such that when the log-likelihood is remaximized subject to this fixed value q_1^* for the linear coefficient, the resulting maximum value of the log-likelihood L_1 satisfies the equation

$$2 (L_0 - L_1) = 2.70554$$

where 2.70554 is the cumulative 90% point of the chi-square distribution with one degree of freedom, which corresponds to a 95% upper limit (one-sided). This approach of computing the upper confidence limit for the extra risk $P_t(d)$ is an improvement on the Crump et al. (1977) model. The upper confidence limit for the extra risk calculated at low doses is always linear. This is conceptually consistent with the linear non-threshold concept discussed earlier. The slope, q_1^*, is taken as an upper bound of the potency of the chemical in inducing cancer at low doses. [In the section calculating the risk estimates, $P_t(d)$ will be abbreviated as P.]

In fitting the dose-response model, the number of terms in the polynomial is chosen equal to (h-1), where h is the number of dose groups in the experiment, including the control group.

Whenever the multistage model does not fit the data sufficiently well, data at the highest dose are deleted and the model is refit to the rest of the data. This is continued until an acceptable fit to the data is obtained. To determine

whether a fit is acceptable, the chi-square statistic

$$X^2 = \sum_{i=1}^{h} \frac{(X_i - N_i P_i)^2}{N_i P_i (1-P_i)}$$

is calculated where N_i is the number of animals in the i^{th} dose group, X_i is the number of animals in the i^{th} dose group with a tumor response, P_i is the probability of a response in the i^{th} dose group estimated by fitting the multistage model to the data, and h is the number of remaining groups. The fit is determined to be unacceptable whenever X^2 is larger than the cumulative 99% point of the chi-square distribution with f degrees of freedom, where f equals the number of dose groups minus the number of non-zero multistage coefficients.

8.4.1.2. SELECTION OF DATA -- For some chemicals, several studies in different animal species, strains, and sexes, each run at several doses and different routes of exposure, are available. A choice must be made as to which of the data sets from several studies to use in the model. It may also be appropriate to correct for metabolism differences between species and for absorption factors via different routes of administration. The procedures used in evaluating these data are consistent with the approach of making a maximum-likely risk estimate. They are as follows:

1. The tumor incidence data are separated according to organ sites or tumor types. The set of data (i.e., dose and tumor incidence) used in the model is the set where the incidence is statistically significantly higher than the control for at least one test dose level and/or where the tumor incidence rate shows a statistically significant trend with respect to dose level. The data set that gives the highest estimate of the lifetime carcinogenic risk, q_1^*, is selected in most cases. However, efforts are made to exclude data sets that produce spuriously

high risk estimates because of a small number of animals. That is, if two sets of data show a similar dose-response relationship and one has a very small sample size, the set of data having the larger sample size is selected for calculating the carcinogenic potency.

2. If there are two or more data sets of comparable size that are identical with respect to species, strain, sex, and tumor sites, the geometric mean of q_1^*, estimated from each of these data sets, is used for risk assessment. The geometric mean of numbers A_1, A_2, ..., A_m is defined as

$$(A_1 \times A_2 \times \ldots \times A_m)^{1/m}$$

3. If two or more significant tumor sites are observed in the same study, and if the data are available, the number of animals with at least one of the specific tumor sites under consideration is used as incidence data in the model.

8.4.1.3. CALCULATION OF HUMAN EQUIVALENT DOSAGES -- Following the suggestion of Mantel and Schneiderman (1975), it is assumed that mg/surface area/day is an equivalent dose between species. Since, to a close approximation, the surface area is proportional to the two-thirds power of the weight, as would be the case for a perfect sphere, the exposure in mg/day per two-thirds power of the weight is also considered to be equivalent exposure. In an animal experiment, this equivalent dose is computed in the following manner.

Let

L_e = duration of experiment

l_e = duration of exposure

m = average dose per day in mg during administration of the agent (i.e., during l_e), and

W = average weight of the experimental animal

Then, the lifetime exposure is

$$d = \frac{l_e \times m}{L_e \times W^{2/3}}$$

8.4.1.3.1. Oral -- Often exposures are not given in units of mg/day, and it becomes necessary to convert the given exposures into mg/day. Similarly, in drinking water studies, exposure is expressed as ppm in the water. For example, in most feeding studies exposure is given in terms of ppm in the diet. In these cases, the exposure in mg/day is

$$m = ppm \times F \times r$$

where ppm is parts per million of the carcinogenic agent in the diet or water, F is the weight of the food or water consumed per day in kg, and r is the absorption fraction. In the absence of any data to the contrary, r is assumed to be equal to one. For a uniform diet, the weight of the food consumed is proportional to the calories required, which in turn is proportional to the surface area, or two-thirds power of the weight. Water demands are also assumed to be proportional to the surface area, so that

$$m \propto ppm \times W^{2/3} \times r$$

or

$$\frac{m}{rW^{2/3}} \propto ppm.$$

As a result, ppm in the diet or water is often assumed to be an equivalent exposure between species. However, this is not justified for the present study, since the ratio of calories to food weight is very different in the diet of man

as compared with laboratory animals, primarily due to differences in the moisture content of the foods eaten. For the same reason, the amount of drinking water required by each species also differs. It is therefore necessary to use an empirically derived factor, f = F/W, which is the fraction of an organism's body weight that is consumed per day as food, expressed as follows:

Species	W	Fraction of body weight consumed as f_{food}	f_{water}
Man	70	0.028	0.029
Rats	0.35	0.05	0.078
Mice	0.03	0.13	0.17

Thus, when the exposure is given as a certain dietary or water concentration in ppm, the exposure in $mg/W^{2/3}$ is

$$\frac{m}{rW^{2/3}} = \frac{ppm \times F}{W^{2/3}} = \frac{ppm \times f \times W}{W^{2/3}} = ppm \times f \times W^{1/3}$$

When exposure is given in terms of mg/kg/day = m/Wr = s, the conversion is simply

$$\frac{m}{rW^{2/3}} = s \times W^{1/3}$$

8.4.1.3.2. Inhalation -- When exposure is via inhalation, the calculation of dose can be considered for two cases where 1) the carcinogenic agent is either a completely water-soluble gas or an aerosol and is absorbed proportionally to the amount of air breathed in, and 2) the carcinogen is a poorly water-soluble gas which reaches an equilibrium between the air breathed and the body

compartments. After equilibrium is reached, the rate of absorption of these agents is expected to be proportional to the metabolic rate, which in turn is proportional to the rate of oxygen consumption, which in turn is a function of surface area.

8.4.1.3.2.1. Case 1. Agents that are in the form of particulate matter or virtually completely absorbed gases, such as sulfur dioxide, can reasonably be expected to be absorbed proportionally to the breathing rate. In this case the exposure in mg/day may be expressed as

$$m = I \times v \times r$$

where I = inhalation rate per day in m^3, v = mg/m^3 of the agent in air, and r = the absorption fraction.

The inhalation rates, I, for various species can be calculated from the observations of the Federation of American Societies for Experimental Biology (FASEB 1974) that 25-g mice breathe 34.5 l/day and 113-g rats breathe 105 l/day. For mice and rats of other weights, W (in kilograms), the surface area proportionality can be used to find breathing rates in m^3/day as follows:

$$\text{For mice, } I = 0.0345\ (W/0.025)^{2/3}\ m^3/\text{day}$$

$$\text{For rats, } I = 0.105\ (W/0.113)^{2/3}\ m^3/\text{day}$$

For humans, the value of 30 m^3/day* is adopted as a standard breathing rate (International Commission on Radiological Protection 1977). The equivalent exposure in $mg/W^{2/3}$ for these agents can be derived from the air intake data in a

*From "Recommendation of the International Commission on Radiological Protection," page 9. The average breathing rate is 10^7 cm^3 per 8-hour workday and 2 x 10^7 cm^3 in 24 hours.

way analogous to the food intake data. The empirical factors for the air intake per kg per day, $i = I/W$, based on the previously stated relationships, are tabulated as follows:

Species	W	$i = I/W$
Man	70	0.29
Rats	0.35	0.64
Mice	0.03	1.3

Therefore, for particulates or completely absorbed gases, the equivalent exposure in $mg/W^{2/3}$ is

$$d = \frac{m}{W^{2/3}} = \frac{Ivr}{W^{2/3}} = \frac{iWvr}{W^{2/3}} = iW^{1/3}vr$$

In the absence of experimental information or a sound theoretical argument to the contrary, the fraction absorbed, r, is assumed to be the same for all species.

8.4.1.3.2.2. Case 2. The dose in mg/day of partially soluble vapors is proportional to the O_2 consumption, which in turn is proportional to $W^{2/3}$ and is also proportional to the solubility of the gas in body fluids, which can be expressed as an absorption coefficient, r, for the gas. Therefore, expressing the O_2 consumption as $O_2 = k\ W^{2/3}$, where k is a constant independent of species, it follows that

$$m = k\ W^{2/3} \times v \times r$$

or

$$d = \frac{m}{W^{2/3}} = kvr$$

As with Case 1, in the absence of experimental information or a sound theoretical argument to the contrary, the absorption fraction, r, is assumed to be the same for all species. Therefore, for these substances a certain concentration in ppm or ug/m^3 in experimental animals is equivalent to the same concentration in humans. This is supported by the observation that the minimum alveolar concentration necessary to produce a given "stage" of anesthesia is similar in man and animals (Dripps et al. 1977). When the animals are exposed via the oral route and human exposure is via inhalation or vice versa, the assumption is made, unless there is pharmacokinetic evidence to the contrary, that absorption is equal by either exposure route.

8.4.1.4. CALCULATION OF THE UNIT RISK FROM ANIMAL STUDIES -- The risk associated with d $mg/kg^{2/3}/day$ is obtained from GLOBAL79 and, for most cases of interest to risk assessment, can be adequately approximated by $P(d) = 1 - \exp(-q_1^* d)$. A "unit risk" in units X is simply the risk corresponding to an exposure of X = 1. This value is estimated by finding the number of $mg/kg^{2/3}/day$ that corresponds to one unit of X, and substituting this value into the above relationship. Thus, for example, if X is in units of ug/m^3 in the air, then for case (1), $d = 0.29 \times 70^{1/3} \times 10^{-3}$ $mg/kg^{2/3}/day$, and for case (2), d = 1, when ug/m^3 is the unit used to compute parameters in animal experiments.

If exposures are given in terms of ppm in air, the following calculation may be used:

$$1 \text{ ppm} = 1.2 \times \frac{\text{molecular weight (gas)}}{\text{molecular weight (air)}} \text{ mg/m}^3$$

Note that an equivalent method of calculating unit risk would be to use mg/kg for the animal exposures, and then to increase the j^{th} polynomial coefficient by

an amount

$$(W_h/W_a)^{j/3} \quad j = 1, 2, \ldots, k,$$

and to use mg/kg equivalents for the unit risk values.

8.4.1.4.1. Adjustments for Less Than Lifespan Duration of Experiment -- If the duration of experiment L_e is less than the natural lifespan of the test animal L, the slope q_1^*, or more generally the exponent g(d), is increased by multiplying a factor $(L/L_e)^3$. We assume that if the average dose d is continued, the age-specific rate of cancer will continue to increase as a constant function of the background rate. The age-specific rates for humans increase at least by the third power of the age and often by a considerably higher power, as demonstrated by Doll (1971). Thus, it is expected that the cumulative tumor rate would increase by at least the third power of age. Using this fact, it is assumed that the slope q_1^*, or more generally the exponent g(d), would also increase by at least the third power of age. As a result, if the slope q* [or g(d)] is calculated at age L_e, it is expected 1 that if the experiment had been continued for the full lifespan L at the given average exposure, the slope q_1^* [or g(d)] would have been increased by at least $(L/L_e)^3$.

This adjustment is conceptually consistent with the proportional hazard model proposed by Cox (1972) and the time-to-tumor model considered by Daffer et al. (1980), where the probability of cancer by age t and at dose d is given by

$$P(d,t) = 1 - \exp\,[-f(t) \times g(d)]$$

8.4.1.5. MODEL FOR ESTIMATION OF UNIT RISK BASED ON HUMAN DATA -- If human epidemiologic studies and sufficiently valid exposure information are available

for the compound, they are always used in some way. If they show a carcinogenic effect, the data are analyzed to give an estimate of the linear dependence of cancer rates on lifetime average dose, which is equivalent to the factor B_H. If they show no carcinogenic effect when positive animal evidence is available, then it is assumed that a risk does exist, but is smaller than could have been observed in the epidemiologic study, and an upper limit to the cancer incidence is calculated assuming hypothetically that the true incidence is below the level of detection in the cohort studied, which is determined largely by the cohort size. Whenever possible, human data are used in preference to animal bioassay data.

Very little information exists that can be used to extrapolate from high-exposure occupational studies to exposures at low environmental levels. However, if a number of simplifying assumptions are made, it is possible to construct a crude dose-response model whose parameters can be estimated using vital statistics, epidemiologic studies, and estimates of worker exposures.

In human studies, the response is measured in terms of the relative risk of the exposed cohort of individuals as compared with the control group. The mathematical model employed for the low-dose extrapolation assumes that for low exposures the lifetime probability of death from cancer, P_0, may be represented by the linear equation

$$P_0 = A + B_H x$$

where A is the lifetime probability in the absence of the agent, and x is the average lifetime exposure to environmental levels in units such as ppm. The factor B_H is the increased probability of cancer associated with each unit increase of x, the agent in air.

If it is assumed that R, the relative risk of cancer for exposed workers as compared with the general population, is independent of length of exposure or age at exposure and depends only on average lifetime exposure, it follows that

$$R = \frac{P}{P_0} = \frac{A + B_H (x_1 = x_2)}{A + B_H \times 1}$$

or

$$RP_0 = A + B_H (x_1 + x_2)$$

where x_1 = lifetime average daily exposure to the agent for the general population, x_2 = lifetime average daily exposure to the agent in the occupational setting, and P_0 = lifetime probability of dying of cancer with no or negligible exposure.

Substituting $P_0 = A + B_H x_1$ and rearranging gives

$$B_H = P_0 (R - 1)/x_2$$

To use this model, estimates of R and x_2 must be obtained from epidemiologic studies. The value P_0 is derived by means of the life table methodology from the age- and cause-specific death rates for the general population found in U.S. vital statistics tables.

8.4.2. Unit Risk Estimates.

8.4.2.1. DATA AVAILABLE FOR POTENCY CALCULATION -- Evidences of carcinogenic activity of chloroform from lifetime treatment studies in laboratory animals include: 1) significantly ($P < 0.05$) increased incidences of hepato-

cellular carcinomas in female and male B6C3F1 mice (Table 8-15) and kidney tumors in male Osborne-Mendel rats (Table 8-16) in an NCI (1976) bioassay, where animals were given chloroform in corn oil by gavage; and 2) kidney tumors in male ICI mice given chloroform in arachis oil by gavage (Roe et al. 1979, Table 8-17). These data sets are used to estimate the carcinogenic potency of chloroform using the linearized multistage model and the dose conversion procedure as described previously. For comparison, three other low-dose extrapolation models, the probit, Weibull, and one-hit, are also used to calculate the carcinogenic potency of chloroform.

TABLE 8-15. INCIDENCE OF HEPATOCELLULAR CARCINOMAS IN FEMALE AND MALE B6C3F1 MICE (NCI 1976)

	Human (animal) dose (mg/kg/day)[a]	Incidence rate
Female	0	0/20 (0%)
	11.59 (238)	36/45 (80%)
	23.23 (477)	39/41 (95%)
Male	0	1/18 (6%)
	6.72 (138)	18/50 (36%)
	13.49 (277)	44/45 (98%)

[a]Human equivalent dose is calculated by d x (5/7) x (78/90) x $(0.034/70)^{1/3}$ = 4.87 x 10^{-2} x d, where d is the animal dose given 5 days per week for 78 weeks (out of a lifespan of 90 weeks). The body weights are assumed to be 34 g for mice and 70 kg for humans.

TABLE 8-16. INCIDENCE OF TUBULAR-CELL ADENOCARCINOMAS IN MALE OSBORNE-MENDEL RATS (NCI 1976)

Human (animal) dose (mg/kg/day)[a]	Incidence rate
0	0/19
8.62 (90)	2/50
17.24 (180)	10/50

[a]Human equivalent dose is calculated by d x (78/104) x (5/7) x $(0.4/70)^{1/3}$ = 9.58 x 10^{-2} x d, where d is the animal dose given 5 days per week for 78 weeks (out of a lifespan of 104 weeks). The body weights are assumed to be 400 g for rats and 70 kg for humans.

TABLE 8-17. INCIDENCE OF MALIGNANT KIDNEY TUMORS IN MALE ICI MICE (Roe et al. 1979)

Human (animal) dose (mg/kg/day)[a]	Incidence rates
0	0/50
3.79 (60)	9/48

[a]Human equivalent dose is calculated by d x (6/7) x (80/90) x $(0.04/70)^{1/3}$ = 6.32 x 10^{-2} x d, where d is the animal dose given 6 days per week for 80 weeks (out of a lifespan of 90 weeks). The body weights are assumed to be 400 g for mice and 70 kg for humans.

8.4.2.2. CHOICE OF LOW-DOSE EXTRAPOLATION MODELS -- In addition to the multistage model currently used by the CAG for low-dose extrapolation, three more models, the probit, the Weibull, and the one-hit models, are used for comparison (Appendix A). These models cover almost the entire spectrum of risk estimates that could be generated from existing mathematical extrapolation models. Generally statistical in character, these models are not derived from biological arguments, except for the multistage model, which has been used to support the somatic mutation hypothesis of carcinogenesis (Armitage and Doll 1954, Whittemore 1978, Whittemore and Keller 1978). The main difference among these models is the rate at which the response function P(d) approaches zero or P(0) as dose d decreases. For instance, the probit model would usually predict a smaller risk at low doses than the multistage model because of the difference of the decreasing rate in the low-dose region. However, it should be noted that one could always artificially give the multistage model the same (or even greater) rate of decrease as the probit model by making some dose transformation or by assuming that some of the parameters in the multistage model are zero. This, of course, would not be reasonable if the carcinogenic process for the agent were not known a priori. Although the multistage model appears to be the most reasonable or at least the most general model for low-dose extrapolation, the point estimate generated from this model is of limited value because the shape of the dose-response curve beyond the experimental exposure levels remains in question. Furthermore, point estimates at low doses extrapolated beyond the experimental doses could be extremely unstable and could differ drastically, depending on the amount of lowest experimental dose. Since upper-bound estimates from the multistage model at low doses are relatively more stable than point estimates, it is suggested that the upper-bound estimate for the risk (or the lower-bound estimate for the dose) be used in evaluating the

carcinogenic potency of a suspect carcinogen. The upper-bound estimate can be taken as a plausible estimate if the true dose-response curve is actually linear at low doses. The upper-bound estimate means that the risks are not likely to be higher, but could be lower if the compound has a concave upward dose-response curve or a threshold at low doses. Another reason one can, at best, obtain an upper-bound estimate of the risk when animal data are used is that the estimated risk is only a probability conditional on the assumption that an animal carcinogen is also a human carcinogen. Therefore, in reality, the actual risk could range from a value near zero to an upper-bound estimate.

8.4.2.3. CALCULATION OF THE CARCINOGENIC POTENCY OF CHLOROFORM -- Using the incidence data in Tables 8-15 to 8-17 and the corresponding human equivalent doses, the maximum likelihood estimates of the parameters were calculated for each of the four models referred to above (see Table A-1 in Appendix A). These models can be used to calculate either point estimates of risk at a given dose, or the virtually safe dose for a given level of risk. The upper-bound estimates of the risk at 1 mg/kg/day, calculated from each of these models on the basis of different data sets, are presented in Table 8-18. From this table, it is observed that the multistage model predicts a comparable risk on the basis of four different data sets, while the probit and Weibull models are very unstable and predict a wide range of risk depending on which data base is used for the risk calculation. Figures 8-4 and 8-5 present the point and upper-bound estimates of risk over the low-dose range, calculated from the four models. The carcinogenic potency of chloroform is represented by the geometric mean of the risk estimates calculated from the linearized multistage model, on the basis of liver tumor data for female and male mice. Although the risk calculated from the data for female mice is greater than that calculated from the data for male mice, the

TABLE 8-18. UPPER-BOUND ESTIMATES OF CANCER RISK OF 1 mg/kg/day, CALCULATED BY DIFFERENT MODELS ON THE BASIS OF DIFFERENT DATA SETS[a]

Data base	Multistage	Probit	Weibull	One-hit
Liver tumors in female mice (NCI 1976)	1.6×10^{-1}	1.5×10^{-1}	4.2×10^{-1}	1.6×10^{-1}
Liver tumors in male mice (NCI 1976)	3.0×10^{-2}	7.3×10^{-12}	2.4×10^{-3}	1.5×10^{-1}
Kidney tumors in male rats (NCI 1976)	1.3×10^{-2}	3.0×10^{-5}	1.3×10^{-3}	1.6×10^{-2}
Kidney tumors in male mice (Roe et al. 1979)	9.0×10^{-2}	NA	NA	9.0×10^{-2}

[a]Upper-bound estimates are calculated by the one-sided 95% confidence limit.

NA = not applicable. Models are not applicable because there is only one dosed group.

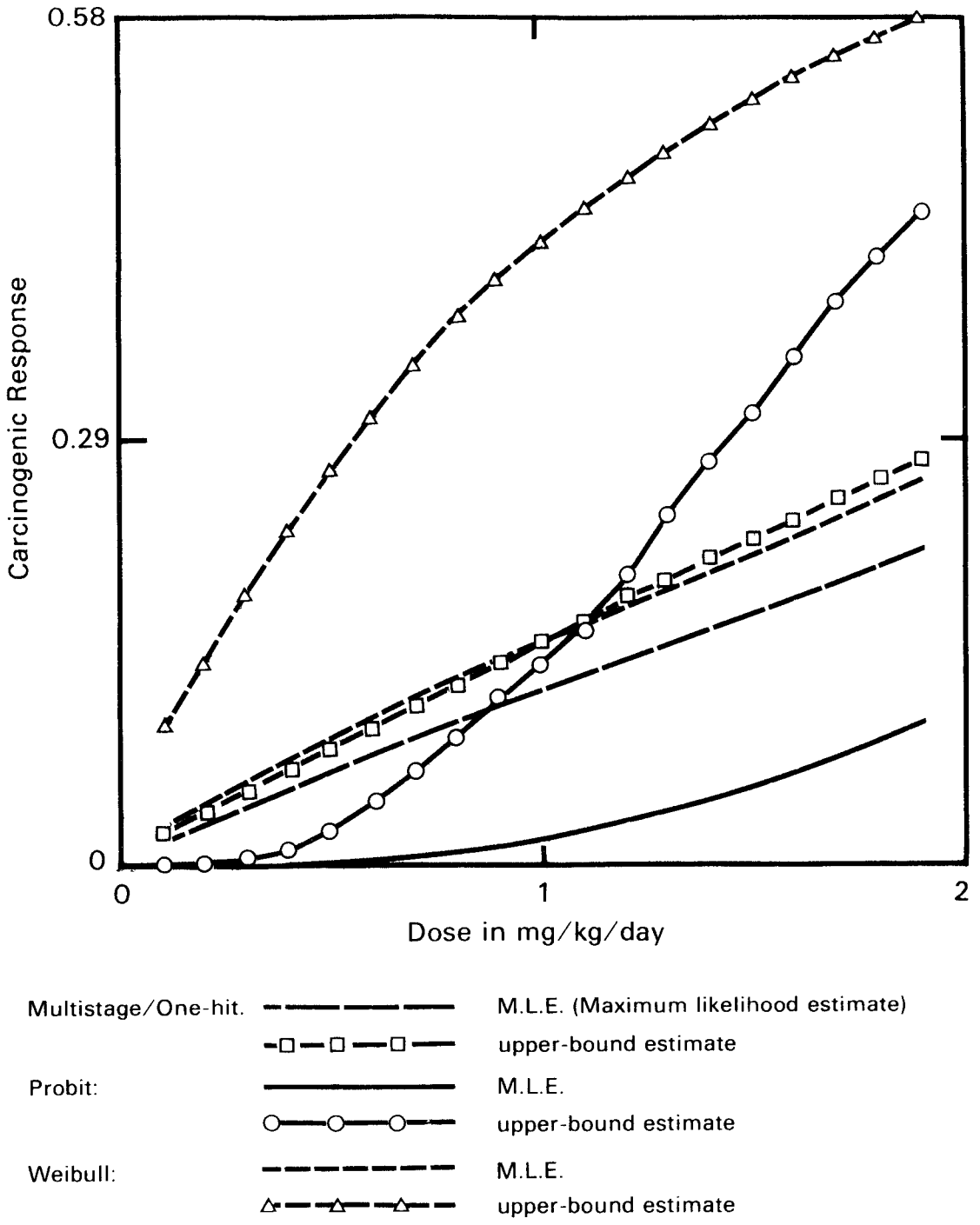

Figure 8-4. Point and upper-bound estimates of four dose-response models over low-dose region on the basis of liver tumor data for female mice. (NCI 1976)

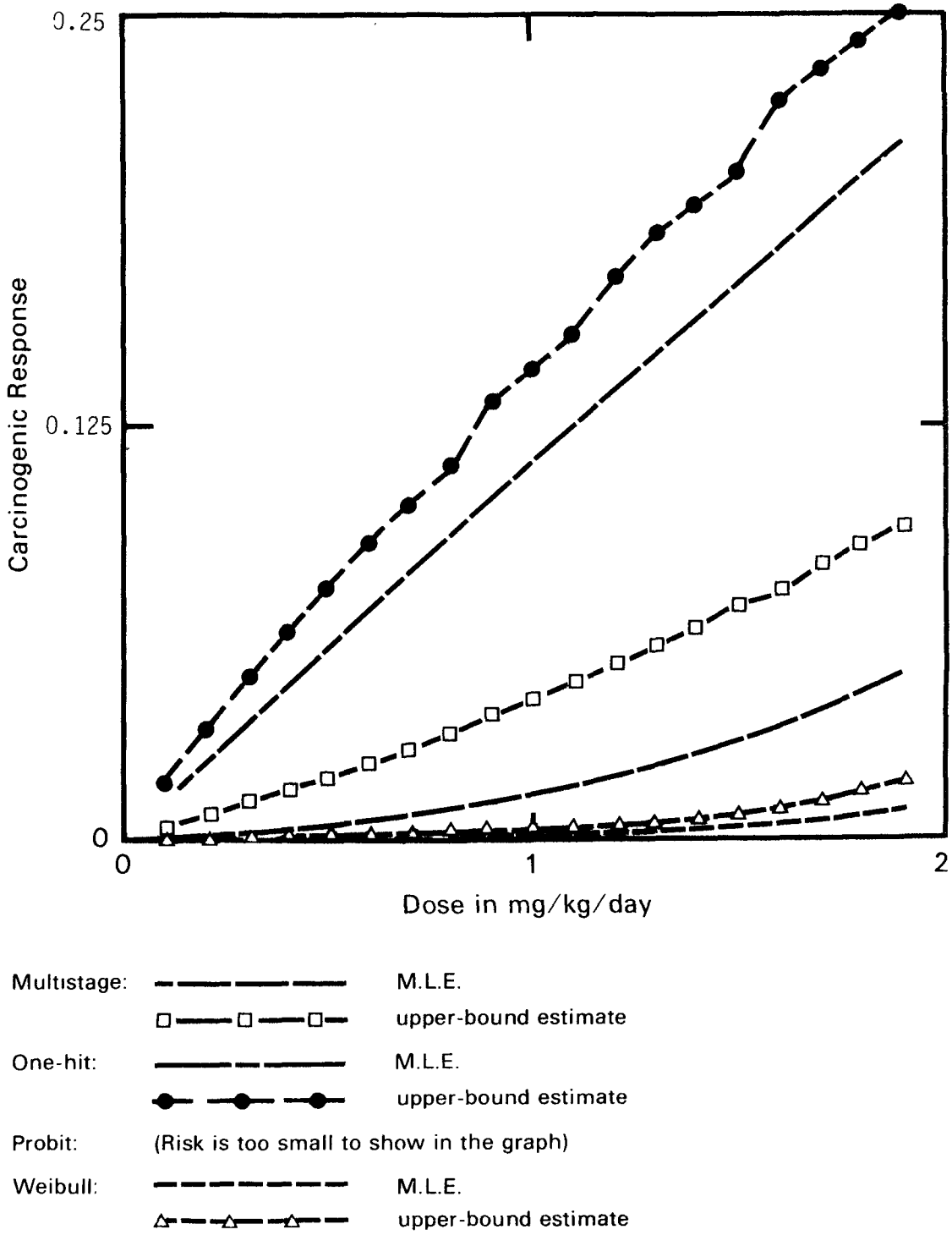

Figure 8-5. Point and upper-bound estimates of four dose-response models over low-dose region on the basis of liver tumor data for male mice. (NCI 1976)

estimates from both data sets are combined because the data for males includes an observation at a lower dose, and the response at this dose does not appear to be inconsistent with the female data, if the linear dose-response relationship is assumed.

Thus, the risk at 1 mg/kg/day is

$$P = (1.6 \times 10^{-1} \times 3.0 \times 10^{-2})^{1/2} = 7 \times 10^{-2}$$

This number differs little from the geometric mean of the q_1^* (upper-bound of the linear parameter) calculated from the two data sets, and thus is used herein as the slope for calculating risk at low doses.

8.4.2.4. RISK ASSOCIATED WITH 1 ug/m^3 OF CHLOROFORM IN AIR -- A paucity of information presently exists on the retention of inhaled chloroform. The only available estimate of pulmonary absorption of chloroform is a study by Lehmann and Hasegawa (1910), who estimated an approximate average of 64.1% (67.6%, 50.2%, 74.6%) retention of a mean chloroform exposure level of 4,592 ppm breathed by three humans for 20 minutes. The relationship is not certain between these early data on short exposures to high chloroform levels and data on pulmonary absorption of chloroform by humans for longer periods at lower exposure levels. In the absence of additional data, absorption rates of 65% by inhalation and 100% orally are assumed for the purpose of a unit risk estimate. Under this assumption, 1 ug/m^3 of chloroform in the air would result in an absorbed effective dose of 1.7×10^{-4} mg/kg/day or

$$d = 0.65 \times (10^{-3} \text{ mg/m}^3 \times 20 \text{ m}^3\text{/day})/70 \text{ kg} = 1.7 \times 10^{-4} \text{ mg/kg/day}$$

Therefore, the risk, P, associated with 1 ug/m^3 of chloroform in air is

$$P = 7 \times 10^{-2} \times 1.7 \times 10^{-4} = 1 \times 10^{-5}$$

8.4.2.5. RISK ASSOCIATED WITH 1 ug/LITER OF CHLOROFORM IN DRINKING WATER -- For drinking water exposure, it is assumed that 100% of the chloroform in drinking water can be absorbed, and that water intake is 2 l/day. Under these assumptions, the daily dose from consumption of water containing 1 ug/l (1 ppb) of chloroform is calculated as follows:

$$d = 1 \text{ ug/l} \times 2 \text{ l/day} \times 10^{-3} \text{ mg/ug} \times 1/70 \text{ kg} = 2.9 \times 10^{-5} \text{ mg/kg/day}$$

Therefore, the risk associated with 1 ug/l of chloroform in water is

$$P = 7 \times 10^{-2} \times 2.9 \times 10^{-5} = 2 \times 10^{-6}$$

This estimate appears consistent with available epidemiologic data such as the odd ratios for bladder cancer, which were estimated to range from 1.04 to 1.69 (Table 8-14). According to a survey of 76 water supply systems in the United States, the chloroform measurements ranged from 1 ug/l to 112 ug/l. A rough estimate of the cancer risk on the basis of these statistics ranges from:

$$B = (1.04 - 1) \times 7 \times 10^{-4}/112 = 3 \times 10^{-7}/(\text{ug/l})$$

to

$$B = (1.69 - 1) \times 7 \times 10^{-4}/1 = 5 \times 10^{-4}/(\text{ug/l})$$

where 7×10^{-4} is the estimated background bladder cancer mortality rate in the United States.

8.4.3. Comparison of Potency with Other Compounds. One of the uses of the quantitative potency estimate is to compare the relative potencies of carcinogens. Figure 8-6 is a histogram representing the frequency distribution of potency indices for 53 suspect carcinogens evaluated by the CAG. The actual data summarized by the histogram are presented in Table 8-19. The potency index is derived from q_1^*, the 95% upper bound of the linear component in the multistage model, and is expressed in terms of $(mMol/kg/day)^{-1}$. Where no human data were available, animal oral studies were used in preference to animal inhalation studies, since oral studies constitute the majority of animal studies.

Based on the available data concerning liver tumors in female and male mice (NCI 1976), the potency index for chloroform has been calculated as 8×10^0. This figure is derived by multiplying the slope $q_1^* = 7 \times 10^{-2}$ mg/kg/day and the molecular weight of chloroform, 119.4. This places the potency index for chloroform in the fourth quartile of the 53 suspect carcinogens evaluated by the CAG.

The ranking of relative potency indices is subject to the uncertainties involved in comparing estimates of potency for different chemicals based on varying routes of exposure in different species, by means of data from studies whose quality varies widely. All of the indices presented here are based on estimates of low-dose risk, using linear extrapolation from the observational range. These indices may not be appropriate for the comparison of potencies if linearity does not exist at the low-dose range, or if comparison is to be made at the high-dose range. If the latter is the case, then an index other than the one calculated above may be more appropriate.

8.4.4. Summary of Quantitative Assessment. Four data sets that contain sufficient information are used to estimate the carcinogenic potency of

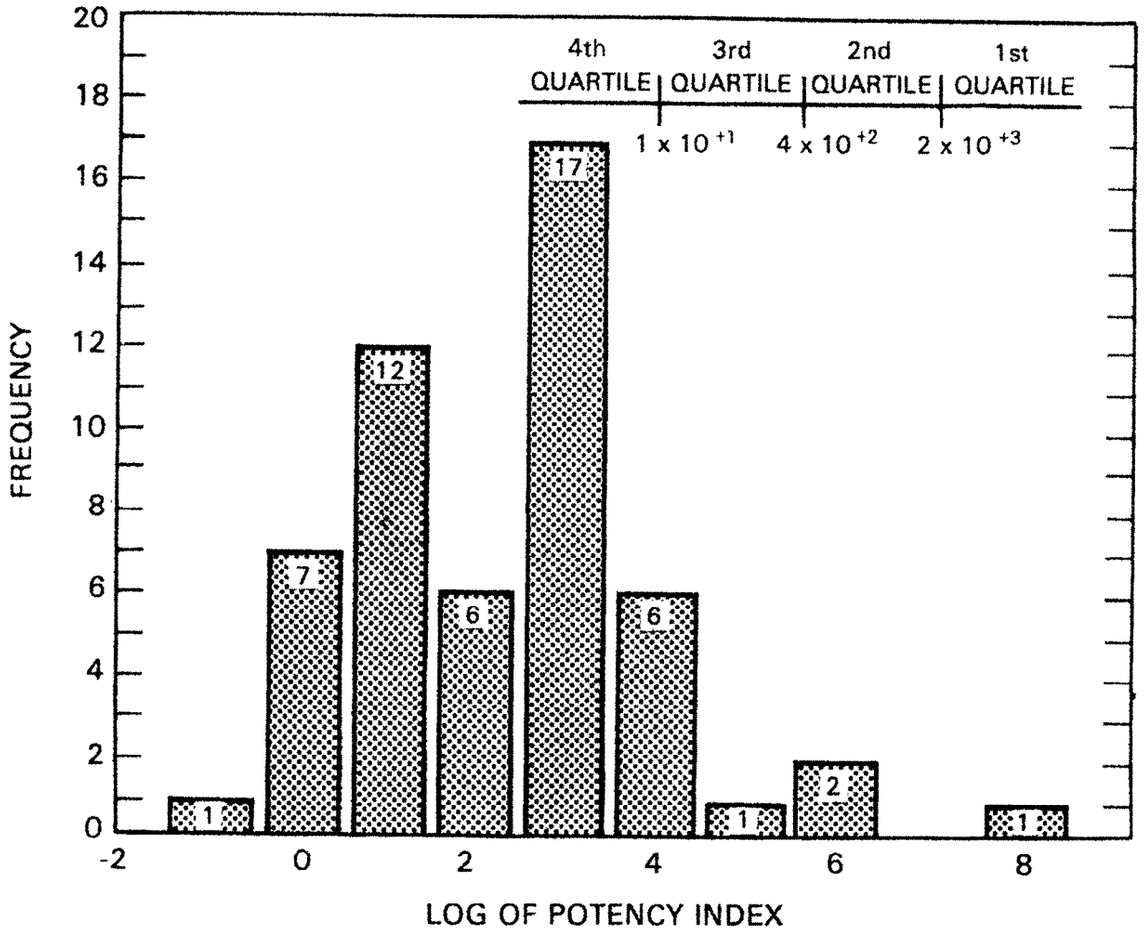

Figure 8-6. Histogram representing the frequency distribution of the potency indices of 53 suspect carcinogens evaluated by the Carcinogen Assessment Group.

TABLE 8-19. RELATIVE CARCINOGENIC POTENCIES AMONG 53 CHEMICALS EVALUATED BY THE CARCINOGEN ASSESSMENT GROUP AS SUSPECT HUMAN CARCINOGENS[1,2,3]

Compounds	Slope $(mg/kg/day)^{-1}$	Molecular weight	Potency index	Order of magnitude (log_{10} index)
Acrylonitrile	0.24(W)	53.1	$1x10^{+1}$	+1
Aflatoxin B_1	2924	312.3	$9x10^{+5}$	+6
Aldrin	11.4	369.4	$4x10^{+3}$	+4
Allyl Chloride	$1.19x10^{-2}$	76.5	$9x10^{-1}$	0
Arsenic	15(H)	149.8	$2x10^{+3}$	+3
B[a]P	11.5	252.3	$3x10^{+3}$	+3
Benzene	$5.2x10^{-2}$(W)	78	$4x10^{0}$	+1
Benzidine	234(W)	184.2	$4x10^{+4}$	+5
Beryllium	4.86	9	$4x10^{+1}$	+2
Cadmium	6.65(W)	112.4	$7x10^{+2}$	+3
Carbon tetrachloride	$1.30x10^{-1}$	153.8	$2x10^{+1}$	+1
Chlordane	1.61	409.8	$7x10^{+2}$	+3
Chlorinated ethanes				
1,2-dichloroethane	$6.9x10^{-2}$	98.9	$7x10^{0}$	+1
hexachloroethane	$1.42x10^{-2}$	236.7	$3x10^{0}$	0
1,1,2,2-tetrachloroethane	0.20	167.9	$3x10^{+1}$	+1
1,1,1-trichloroethane	1.6x10--3	133.4	$2x10^{-1}$	-1
1,1,2-trichloroethane	$5.73x10^{-2}$	133.4	$8x10^{0}$	+1
Chloroform	$7x10^{-2}$	119.4	$8x10^{0}$	+1
Chromium	41 (W)	100	$4x10^{+3}$	+4
DDT	8.42	354.5	$3x10^{+3}$	+3
Dichlorobenzidine	1.69	253.1	$4x10^{+2}$	+3
1,1-dichloroethylene	$1.47x10^{-1}$(I)	97	$1x10^{+1}$	+1
Dieldrin	30.4	380.9	$1x10^{+4}$	+4

(continued on the following page)

TABLE 8-19. (continued)

Compounds	Slope $(mg/kg/day)^{-1}$	Molecular weight	Potency index	Order of magnitude (log_{10} index)
Dinitrotoluene	0.31	182	$6x10^{+1}$	+2
Diphenylhydrazine	0.77	180	$1x10^{+2}$	+2
Epichlorohydrin	$9.9x10^{-3}$	92.5	$9x10^{-1}$	0
Bis(2-chloroethyl)ether	1.14	143	$2x10^{+2}$	+2
Bis(chloromethyl)ether	9300(I)	115	$1x10^{+6}$	+6
Ethylene dibromide (EDB)	8.51	187.9	$2x10^{+3}$	+3
Ethylene oxide	0.63(I)	44.0	$3x10^{+1}$	+1
Heptachlor	3.37	373.3	$1x10^{+3}$	+3
Hexachlorobenzene	1.67	284.4	$5x10^{+2}$	+3
Hexachlorobutadiene	$7.75x10^{-2}$	261	$2x10^{+1}$	+1
Hexachlorocyclohexane				
technical grade	4.75	290.9	$1x10^{+3}$	+3
alpha isomer	11.12	290.9	$3x10^{+3}$	+3
beta isomer	1.84	290.9	$5x10^{+2}$	+3
gamma isomer	1.33	290.9	$4x10^{+2}$	+3
Methylene chloride	$6.3x10^{-4}$	84.9	$5x10^{-2}$	-1
Nickel	1.15(W)	58.7	$7x10^{+1}$	+2
Nitrosamines				
Dimethylnitrosamine	25.9(not by q_1^*)	74.1	$2x10^{+3}$	+3
Diethylnitrosamine	43.5(not by q_1^*)	102.1	$4x10^{+3}$	+4
Dibutylnitrosamine	5.43	158.2	$9x10^{+2}$	+3
N-nitrosopyrrolidine	2.13	100.2	$2x10^{+2}$	+2
N-nitroso-N-ethylurea	32.9	117.1	$4x10^{+3}$	+4
N-nitroso-N-methylurea	302.6	103.1	$3x10^{+4}$	+4
N-nitroso-diphenylamine	$4.92x10^{-3}$	198	$1x10^{0}$	0
PCBs	4.34	324	$1x10^{+3}$	+3

(continued on the following page)

TABLE 8-19. (continued)

Compounds	Slope $(mg/kg/day)^{-1}$	Molecular weight	Potency index	Order of magnitude (log_{10} index)
Phenols				
2,4,6-trichlorophenol	$1.99x10^{-2}$	197.4	$4x10^{0}$	+1
Tetrachlorodioxin	$4.25x10^{5}$	322	$1x10^{+8}$	+8
Tetrachloroethylene	$3.5x10^{-2}$	165.8	$6x10^{0}$	+1
Toxaphene	1.13	414	$5x10^{+2}$	+3
Trichloroethylene	$1.9x10^{-2}$	131.4	$2.5x10^{0}$	0
Vinyl chloride	$1.75x10^{-2}$(I)	62.5	$1x10^{0}$	0

Remarks:

1. Animal slopes are 95% upper-limit slopes based on the linearized multistage model. They are calculated based on animal oral studies, except for those indicated by I (animal inhalation), W (human occupational exposure), and H (human drinking water exposure). Human slopes are point estimates based on the linear non-threshold model.

2. The potency index is a rounded-off slope in $(mMol/kg/day)^{-1}$ and is calculated by multiplying the slopes in $(mg/kg/day)^{-1}$ by the molecular weight of the compound.

3. Not all the carcinogenic potencies presented in this table represent the same degree of certainty. All are subject to change as new evidence becomes available.

chloroform. They are liver tumors in female mice (NCI 1976), liver tumors in male mice (NCI 1976), kidney tumors in male rats (NCI 1976), and kidney tumors in male mice (Roe et al. 1979). The unit risks at 1 mg/kg/day, calculated by the linearized multistage model on the basis of these four data sets, are comparable. The geometric mean, $q_1^* = 7 \times 10^{-2}/(mg/kg/day)$, of the potencies calculated from liver tumors in male and female mice, is taken to represent the carcinogenic potency of chloroform. The upper-bound estimate of the cancer risk due to 1 ug/m^3 of chloroform in air is $P = 1 \times 10^{-5}$. The upper-bound estimate of the cancer risk due to 1 ug/l in water is $P = 2 \times 10^{-6}$. The carcinogenic potency of chloroform is in the fourth quartile among the 53 suspect carcinogens evaluated by the CAG.

The unit risks given above are calculated under the assumption that mg per unit of body surface area is equivalent between mice and humans. If the dose in mg/kg/day is assumed to be equivalent, then these unit risks would be reduced approximately by a factor of 12.

8.5. SUMMARY

8.5.1. Qualitative. Chloroform in corn oil administered at estimated maximally and one-half maximally tolerated doses by gavage for 78 weeks produced a statistically significant increase in the incidence of hepatocellular carcinomas in male and female B6C3F1 mice and renal epithelial tumors (malignant and benign) in male Osborne-Mendel rats; a carcinogenic response of female Osborne-Mendel rats to chloroform was not apparent in this study. Use of more than two doses in these studies might have given a more precise estimate of dose-response.

A statistically significant increase in the incidence of renal tumors (benign and malignant) was found in another study in male ICI mice treated with chloroform in either toothpaste or arachis oil by gavage for 80 weeks; however, treatment with a gavage dose of chloroform in toothpaste for 80 weeks did not produce a carcinogenic response in female ICI mice and male mice of the CBA, C57BL, and CF/1 strains. Induction of malignant kidney tumors in male ICI mice was greater when chloroform was administered, at the same dose, in arachis oil instead of toothpaste. A carcinogenic response was not observed in male and female Sprague-Dawley rats given chloroform in toothpaste by gavage for 80 weeks, but early mortality was high in control and treatment groups. Gavage doses of chloroform in toothpaste did not show a carcinogenic effect in male and female beagle dogs treated for over 7 years. The results of preliminary toxicity tests and the carcinogenicity studies suggest that doses of chloroform in toothpaste given to mice, rats, and dogs in the carcinogenicity studies approached those maximally tolerated. However, chloroform doses given to mice and rats in toothpaste or arachis oil were lower than those above given in corn oil.

Hepatomas were found in NLC mice given chloroform in oil by force-feeding twice weekly for an unspecified period of time, and in female strain A mice given chloroform in olive oil by gavage once every 4 days for a total of 30 doses at a level which produced liver necrosis; however, small numbers of animals were examined for pathology, the duration of these studies was either uncertain or appeared to be below the lifetime of the animals, and no control group of NLC mice was apparent. Although a carcinogenic effect of chloroform was not evident in newborn (C57 x DBA2 - F1) mice given single or multiple subcutaneous doses during the initial 8 days of life and observed for their lifetimes, the dose levels used appeared well below a maximum tolerated dose and the period of

treatment after birth was quite short compared to lifetime treatment. Chloroform was ineffective at maximally tolerated and lower doses in a pulmonary adenoma bioassay in strain A mice; however, other chemicals that have shown carcinogenic activity in other tests were also ineffective in this pulmonary adenoma bioassay. Although an ability of chloroform to promote the growth and spread of Lewis lung carcinoma, Erlich ascites, and B16 melanoma cells in mice has been shown, the mechanism by which chloroform produced this effect is uncertain and the relevance of this study to the evaluation of the carcinogenic potential of chloroform is presently not clear. Chloroform in liquid solution did not induce transformation of baby Syrian hamster kidney (BHK - 21/C1 13) cells in vitro at doses high enough to produce toxicity; additional testing of chloroform as a vapor could have provided a comparison of cell transformation potential between chloroform as a vapor and chloroform in a liquid solution.

An additional carcinogenicity study on female B6C3F1 mice and male Osborne-Mendel rats given chloroform in drinking water over a wide range of dose levels is in progress at the Stanford Research Institute.

There are no epidemiologic cancer studies dealing with chloroform per se. Chlorinated drinking water can contain significant amounts of chloroform. There appears to be a weak but statistically significant risk of cancer of the bladder, large intestine, and rectum with the presence of chlorine in drinking water. The odd ratios calculated in the latter ecological and case-control studies range up to a high of 3.68 for cancer of the colon in the Young et al. study, but most fell between 1.1 and 2.0. The risk ratios derived in each study could be explained by the confounding effects of several factors; i.e., smoking, diet, air pollution, occupation, or lifestyle. However, the consistent finding of a statistically significant excess of cancer across several independent and diverse study populations supports the finding of a definite risk. Bias

can creep into these studies from differential surrounding rates due to proximity to better medical care and treatment facilities, higher socioeconomic status, and the possibility of migration of cancer patients to medical care facilities in areas where chlorination is used to a greater extent. Underestimates of risk may result from failure to control for migration effects prior to diagnosis, misclassification of cause of death, and use of chlorination as a surrogate variable for chloroform, especially if few organic contaminants are in the water. Exposure to chlorinated drinking water will not necessarily result in exposure to chloroform if organic contaminants are not present. Many contaminants found in drinking water other than chloroform are carcinogenic, but they generally are found in much smaller quantities as compared with chloroform levels found in water sources containing large quantities of organics. The presence of these other substances, some carcinogenic, makes it impossible to incriminate chloroform directly as the cause of the excess cancer at the three sites. Hence, there appears to be an increased risk of cancer of the bladder, rectum, and large intestine from chlorinated water and, by inference, from chloroform.

8.5.2. Quantitative. Four data sets that contain sufficient information are used to estimate the carcinogenic potency of chloroform. They are liver tumors in female mice (NCI 1976), liver tumors in male mice (NCI 1976), kidney tumors in male rats (NCI 1976), and kidney tumors in male mice (Roe et al. 1979). The unit risks at 1 mg/kg/day, calculated by the linearized multistage model on the basis of these four data sets, are comparable. The geometric mean, $q_1^* = 7 \times 10^{-2}/(mg/kg/day)$, of the potencies calculated from liver tumors in male and female mice, is taken to represent the carcinogenic potency of chloroform. The upper-bound estimate of the cancer risk due to 1 ug/m^3 of

chloroform in air is $P = 1 \times 10^{-5}$. The upper-bound estimate of the cancer risk due to 1 ug/liter in water is $P = 2 \times 10^{-6}$. This estimate appears consistent with the limited epidemiologic data available for humans.

8.6. CONCLUSIONS

Evidence that chloroform has carcinogenic activity is based on increased incidences of hepatocellular carcinomas in male and female B6C3F1 mice, renal epithelial tumors in male Osborne-Mendel rats, kidney tumors in male ICI mice, and hepatomas in NLC and female strain A mice. As concluded elsewhere in this document, no definitive conclusions can be reached concerning the mutagenicity of chloroform based on present evidence. The toxicity of chloroform in liver and kidney, as noted in this document, is considered to occur through covalent binding of a reactive metabolic intermediate, possibly phosgene, with cellular macromolecules; the evidence discussed in the metabolism section herein indicates that reactive metabolites of chloroform can react extensively with proteins and lipids, but minimally with nucleic acids. Applying the International Agency for Research on Cancer (IARC) criteria for animal studies, the level of evidence for carcinogenicity would be sufficient for concluding that chloroform is carcinogenic in experimental animals.

There are no epidemiologic studies of cancer and chloroform per se. There appears to be an increased risk of cancer of the bladder, rectum, and large intestine from chlorinated drinking water and, by inference, from chloroform. Applying the IARC criteria to assess the carcinogenicity of chloroform in humans, there is limited evidence for the carcinogenicity of chlorinated drinking water in humans, and inadequate evidence for the carcinogenicity of chloroform in humans.

The overall IARC classification for chloroform is 2B, which by definition designates chloroform as probably carcinogenic to humans. In the IARC scheme, Group 2 chemicals are divided into higher (Group A) and lower (Group B) degrees of evidence depending on whether evidence for their carcinogenicity in humans is concluded to be limited (Group A) or inadequate (Group B).

REFERENCES

Alavanja, M., I. Goldstein, and M. Susser. 1978. A case-control study of gastrointestinal and urinary tract cancer mortality and drinking water chlorination. Pages 395-409 in Water chlorination: Environmental impact and health effects, Vol. 2. R. Jolley, H. Gorchev, D.H. Hamilton, Jr., eds. Ann Arbor, Michigan: Ann Arbor Science Publishers.

Ames, B.N., J. McCann, and E. Yamasaki. 1975. Method for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. Mutat. Res. 31:347-364.

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

Brenniman, G.R., J. Vasilomanolakis-Lagos, J. Amsel, T. Namekata, and A.H. Wolff. 1978. Case-control study of cancer deaths in Illinois communities served by chlorinated or nonchlorinated water. Pages 1043-1057 in Water chlorination: Environmental impact and health effects. R. Jolley, H. Gorchen, and H. Hamilton, Sr., eds. Ann Arbor, Michigan: Ann Arbor Science Publishers.

Buncher, C.R. 1975. Cincinnati drinking water: An epidemiologic study of cancer rates. University of Cincinnati Medical Center, Cincinnati, Ohio.

Cantor, K.P., R. Hoover, T.J. Mason, and L.J. McCabe. 1978. Association of cancer mortality with halomethanes in drinking water. J. Natl. Cancer Inst. 61(4):979-985.

Capel, I.D., H.M. Dorrell, M. Jenner, M.H. Pinnock, and D.C. Williams. 1979. The effect of chloroform ingestion on the growth of some murine tumors. Eur. J. Cancer 15:1485-1490.

Cox, C.B. 1972. Regression model and life tables. J. Roy. Stat. Soc. B 34:187-220.

Crump, K.S. 1979. Dose-response problems in carcinogenesis. Biometrics 35:157-167.

Crump, K.S., and H.A. Guess. 1982. Drinking water and cancer: Review of recent epidemiological findings and assessment of risks. Ann. Rev. Public Health 3:339-357.

Crump, K.S., and W.W. Watson. 1979. GLOBAL79: A FORTRAN program to extra_ polate dichotomous animal carcinogenicity data to low dose. Natl. Inst. Environ. Health Science, Contract No. 1-ES-2123.

Crump, K.S., H.A. Guess, and L.L. Deal. 1977. Confidence intervals and test of hypotheses concerning dose-response relations inferred from animal carcinogencity data. Biometrics 33:437-451.

Daffer, P., K. Crump, and M. Masterman. 1980. Asymptotic theory for analyzing dose-response survival data with application to low-dose extrapolation problem. Mathematical Biosciences 50:207-280.

De Rouen, T.A., and J.E. Diem. 1975. The New Orleans drinking water controversy: A statistical perspective. Am. J. Public Health 65:1060-1062.

Doll, R. 1971, Weibull distribution of cancer: Implications for models of carcinogenesis. J. Roy Stat. Soc. A 13:133-166.

Dripps, R.D., J.E. Eckenhoff, and L.D. Vandam. 1977. Introduction to anesthesia: the principles of safe practice. 5th ed. Philadelphia, PA: W.B. Saunders Company, pp. 121-123.

Eschenbrenner, A.B., and E. Miller. 1945. Induction of hepatomas in mice by repeated oral administration of chloroform with observations on sex differences. J. Natl. Cancer Inst. 5:251-255.

Federation of American Societies for Experimental Biology (FASEB). 1974. 2nd ed. Vol. III. Philip L. Altman and Dorothy S. Dittmen, eds. Bethesda, Maryland. Library of Congress No 72-87738.

Fleiss, J.L. 1979. Confidence intervals for odds ratio in case-control studies: The state of the art. J. Chronic Dis. 32:69-77.

Gottlieb, M.S., J.K. Carr, and D.T. Morris. 1981. Cancer and drinking water in Louisiana: colon and rectum. Int. Epidemiol. 10(2):117-125.

Harris, R.H. 1974. Implications of cancer-causing substances in Mississippi River Water. Environmental Defense Fund, Washington, D.C.

Harris, R.H., T. Page, and N.A. Reiches. 1977. Carcinogenic hazards of organic chemicals in drinking water. Pages 309-330 in Origins of Human Cancer. H.H. Hiatt, J.D. Watson, and J.A. Winsten, eds. Cold Spring Harbor Laboratory.

Heywood, R., R.J. Sortwell, P.R.B. Noel, A.E. Street, D.E. Prentice, F.J.C. Roe, P.F. Wadsworth, A.N. Worden, and N.J. Van Abbe. 1979. Safety evaluation of toothpaste containing chloroform. III. Long-term study in beagle dogs. J. Environ. Pathol. Toxicol. 2:835-851.

Hogan, M.D., P. Chi, D.G. Hoel, and T.J. Mitchell. 1979. Association between chloroform levels in finished drinking water supplies and various site-specific cancer mortality rates. J. Environ. Pathol. Toxicol. 2:873-887.

Hoover, R.N., F.W. McKay, and J.F. Fraumeni. 1976. Fluoridated drinking water and the occurrence of cancer. J. Natl. Cancer Inst. 57:757-768.

Howe, R.B., and K.S. Crump. 1982. A computer program to extrapolate quantal animal toxicity data to low dose. OSHA Contract No. 41USC252C3.

International Commission on Radiological Protection (ICRP). 1977. Recommendation of the ICRP, Pub. No. 26, adopted Jan. 17, 1977. Oxford, England: Pergamon Press.

Kovar, J., and D. Krewski. 1981. Risk81: A computer program for low-dose extrapolation of quantal response toxicity data. Department of Health and Welfare, Canada.

Kruse, C.W. 1977. Chlorination of public water supplies and cancer - Washington County, Maryland experience. A final report, Johns Hopkins University, School of Hygiene and Public Health, to the Office of Research and Development, Health Effects Research Laboratory, Cincinnati, Ohio. Unpublished draft.

Kuzma, R.J., C.M. Kuzma, and C.R. Buncher. 1977. Ohio drinking water source and cancer rates. Am. J. Public Health 67:725-729.

Lehmann, K.B., and O. Hasegawa. 1910. Studies of the absorption of chlorinated hydrocarbons in animals and humans. Arch. Hyg. 72:327-342. (German).

Mah, R.A., G.H. Spivey, and E. Sloss. 1977. Cancer and chlorinated drinking water. Final report on EPA Contract No. CA-6-99-3349-J to Health Effects Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, Ohio.

Mantel, N., and M.A. Schneiderman. 1975. Estimating safe levels, a hazardous undertaking. Cancer Res. 35:1379-1386.

Mason, T.J., and F.W. McKay. 1974. U.S. cancer mortality by county: 1950-1969. Washington, D.C., U.S. Government Printing Office.

McCabe, L.J. 1975. Association between trihalomethanes in drinking water (NORS data) and mortality. Health Effects Research Laboratory, U.S. Environmental Protection Agency, Cincinnati, Ohio.

Moore, D.H., L.F. Chasseaud, S.K. Majeed, D.E. Prentice, and F.J.C. Roe. 1982. The effect of dose and vehicle on early tissue damage and regenerative activity after chloroform administation to mice. Fd. Chem. Toxicol. 20:951-954.

National Academy of Sciences. 1978. Epidemiological studies of cancer frequency and certain organic constituents of drinking water. A review of recent literature published and unpublished. Washington, D.C.

National Cancer Institute (NCI). 1976. Report on carcinogenesis bioassay of chloroform. Natl. Tech. Inf. Serv. PB-264018. Springfield, VA.

National Institute for Occupational Health and Safety (NIOSH). 1974. Criteria for a recommended standard: Occupational exposure to chloroform. DHEW Publication No. 75-114.

Page, T., R.H. Harris, and S.S. Epstein. 1976. Drinking water and cancer mortality in Louisana. Science 193:55-57.

Palmer, A.K., A.E. Street, F.J.C. Roe, A.N. Worden, and N.J. Van Abbe. 1979. Safety evaluation of toothpaste containing chloroform. II. Long-term studies in rats. J. Environ. Pathol. Toxicol. 2:821-833.

Purchase, I.F.H., E. Longstaff, J. Ashby, J.A. Styles, D. and P.A. Lefevre, and F.R. Westwood. 1978. An evaluation of 6 short-term tests for detecting organic chemical carcinogens and recommendations for their use. Br. J. Cancer 37:873-959.

Rafferty, P. 1979. Drinking water quality and cancer mortality patterns. Master's Thesis. University of North Carolina, Chapel Hill, North Carolina. Unpublished.

Reddy, B.S., L.A. Cohen, G.D. McCoy, P. Hill, J.H. Weisburger, and E.L. Wynder. 1980. Nutrition and its relationship to cancer. Naylor Dana Institute for Disease Prevention, American Health Foundation, Valhalla, New York. Advances in Cancer Research, Vol. 32.

Reuber, M.D. 1979. Carcinogenicity of chloroform. Environ. Health Perspect. 31:171-182.

Roe, F.J.C., R.L. Carter, and B.C.V. Mitchley. 1968. Test of chloroform and 8-hydroxyquinoline for carcinogenicity using newborn mice. Br. Emp. Cancer Campgn. 46:13.

Roe, F.J.C., A.K. Palmer, A.N. Worden, and N.J. Van Abbe. 1979. Safety evaluation of toothpaste containing chloroform. I. Long-term studies in mice. J. Environ. Pathol. Toxicol. 2:799-819.

Rudali, G. 1967. Oncogenic activity of some halogenated hydrocarbons used in therapeutics. UICC Monogr. Ser. 7:138-143.

Salg, J. 1977. Cancer mortality and drinking water in 346 counties of the Ohio River Valley Basin. Ph.D. Thesis. Department of Epidemiology, University of North Carolina. Also final report, EPA Contract No. 5-03-4528-J to Health Effects Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, Ohio.

Struba, R.J. 1979. Cancer and drinking water quality. Ph.D. Thesis. University of North Carolina, Chapel Hill, North Carolina. 156 pp. Available from: University Microfilms International, Ann Arbor, Michigan, Publication No. 8022514.

Styles, J.A. 1977. A method for detecting carcinogenic organic chemicals using mammalian cells in culture. Br. J. Cancer 36:558-563.

Styles, J.A. 1979. Cell transformation assays. Pages 147-164 in G.E. Pagett, ed. Topics in Toxicology: Mutagenesis in sub-mammalian systems: Status and significance. Baltimore, MD: University Park Press.

Tarone, R.E., and J.J. Gart. 1975. Review of "The implications of cancer-causing substances in Mississippi River water" by R.H. Harris. Bethesda, Maryland, Mathematical Statistics and Applied Mathematics Section, National Cancer Institute.

Theiss, J.C., G.D. Stoner, N.B. Shimkin, and E.K. Weisburger. 1977. Test for carcinogenicity of organic contaminants of United States drinking waters by pulmonary tumor response in strain A mice. Cancer Res. 37:2717-2720.

Tuthill, R.W., G.S. Moore, and D.W. Polakoff. 1979. A statistical model for predicting chloroform levels in chlorinated surface water supplies. J. Am. Water Works Assoc. 71:37-39.

U.S. Environmental Protection Agency. 1975. Preliminary assessment of suspected carcinogens in drinking water: Report to Congress, December 1975.

U.S. Environmental Protection Agency. 1979. National interim primary drinking water regulations; control of trihalomethanes in drinking water; final rule. Federal Register 44(231)29:68624-68707 (November 29). Part III.

Vasilenko, P., and L. Magno. 1975. Factors relating to the incidence of cancer mortality in New Jersey. Princeton University, Princeton, New Jersey. Unpublished.

Weisburger, E.K. 1977. CArcinogenicity studies on halogenated hydrocarbons. Environ. Health Perspect. 21:7-16.

Whittemore, A. 1978. Quantitative theories of carcinogenesis. In: Advances in cancer research, Vol. 27. New York, NY: Academic Press, Inc., pp. 55-58.

Whittemore, A., and B. Keller. 1978. Quantitative theory of carcinogenesis. Society for Industrial and Applied Mathematics Review 20:1-30.

Wilkins, J.R. 1978. Chlorination of public water supplies and cancer in Washington County, Maryland. Ph.D. Thesis. Department of Environmental Health Sciences, Johns Hopkins University School of Hygiene and Public Health. Also final report with C.W. Kruse on EPA Contract No. R805198-01-0 to Health Effects Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, Ohio.

Wilkins, J.R., N.A. Reiches, and C.W. Kruse. 1979. Organic chemical contaminants in drinking water and cancer. Am. J. Epidemiology 110:420-448.

Young, T.B., M.S. Kanarek, and A.A. Tslatis. 1981. Epidemiologic study of drinking water chlorination and Wisconsin female cancer mortality. J. Natl. Cancer Inst. 67(6):1191-1198.

APPENDIX A

COMPARISON AMONG DIFFERENT EXTRAPOLATION MODELS

Four models used for low-dose extrapolation, assuming the independent background, are:

Multistage: $$P(d) = 1 - \exp\left[-(q_i d + \ldots + q_k d^k)\right]$$

where q_i are non-negative parameters

Probit: $$P(d) = \int_{-\infty}^{A + B\ln(d)} f(x)\, dx$$

where f(.) is the standard normal probability density function

Weibull: $$P(d) = 1 - \exp\left[-bd^k\right]$$

where b and k are non-negative parameters

One-hit: $$P(d) = 1 - \exp\left[-bd\right]$$

where b is a non-negative parameter.

The maximum likelihood estimates (MLE) of the parameters in the multistage and one-hit models are calculated by means of the program GLOBAL82, which was developed by Howe and Crump (1982). The MLE estimates of the parameters in the probit and Weibull models are calculated by means of the program RISK81, which was developed by Kovar and Krewski (1981).

Table A-1 presents the MLE of parameters in each of the four models that are applicable to a data set.

TABLE A-1. MAXIMUM LIKELIHOOD ESTIMATE OF THE PARAMETERS FOR EACH OF THE FOUR EXTRAPOLATION MODELS, BASED ON DIFFERENT DATA BASES

Data base	Multistage	Probit	Weibull	One-hit
Liver tumors in female mice (NCI 1976)	$q_1 = 1.35 \times 10^{-1}$ $q_2 = 0$ $(q_1^* = 1.7 \times 10^{-1})$[a]	A = -2.03 B = 1.17	b = 1.68×10^{-1} k = 0.92	b = 1.35×10^{-1}
Liver tumors in male mice (NCI 1976)	$q_1 = 0$ $q_2 = 1.34 \times 10^{-2}$ $(q_1^* = 3.0 \times 10^{-2})$	A = -7.15 B = 3.51	b = 7.95×10^{-4} k = 3.25	b = 1.21×10^{-1}
Kidney tumors in male rats (NCI 1976)	$q_1 = 0$ $q_2 = 7.07 \times 10^{-4}$ $(q_1^* = 1.3 \times 10^{-2})$	A = -4.58 B = 1.31	b = 2.08×10^{-4} k = 2.45	1.56×10^{-2}
Kidney tumors in male mice (Roe et al. 1979)	$q_1 = 5.5 \times 10^{-2}$ $(q_1^* = 9.1 \times 10^{-2})$	NA	NA	5.5×10^{-2}

[a] q_1^* is the 95% upper-bound confidence limit of the linear parameter in the multistage model.

NA = not applicable. The models are not applicable since there is only one dose group.