Drinking Water and Health, Volume 5

Safe Drinking Water Committee, Board on Toxicology and Environmental Health Hazards, National Research Council

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Board on Toxicology and Environmental Health Hazards
Commission on Life Sciences
National Research Council

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Preface

The Safe Drinking Water Act of 1974 (PL 93-523) authorized the U.S. Environmental Protection Agency (EPA) to establish federal standards to protect humans from harmful contaminants in drinking water and to establish a joint federal-state system for assuring compliance with these standards and for protecting underground sources of drinking water. One section of the law [1412 (e)] and its amendments (42 USC Subpart 300f *et seq*., 1977) mandated that the National Academy of Sciences conduct studies on the health effects associated with contaminants found in drinking water. It stipulated that the study group should evaluate the available data for use in developing primary drinking water regulations, identify areas of insufficient knowledge, and make recommendations for future research. Amendments to the act in 1977 called for revisions of the Academy's studies to reflect "new information which has become available since the most recent previous report [and which] shall be reported to the Congress each two years thereafter."

The first study in this series was published in 1977 under the title *Drinking Water and Health*. That volume examines the health effects associated with microbiological, radioactive, particulate, inorganic, and organic chemical contaminants found in drinking water. It also gives numerical risk assessments, which are estimates of the probability that cancer will result from exposure to certain chemical contaminants in drinking water. Volumes 2 and 3 of *Drinking Water and Health* were published in 1980. Volume 2 compares the efficacy and practicability of chlorination and 11 alternative disinfection methods for inactivating microorganisms, identi

fies the by-products likely to be formed by the use of each major method, and evaluates the use of granular activated carbon for the reduction or removal of organic and other contaminants from drinking water. Volume 3 reviews 12 epidemiological studies concerning health effects associated with drinking water containing trihalomethanes. It also summarizes the current state of knowledge on the relationship between cardiovascular disease and water hardness. It adds to the 1977 publication's information on estimation of risk to human health by extrapolating carcinogenesis data from laboratory animals to humans and also evaluates six models for estimating the carcinogenic risk at low doses. Furthermore, it examines the acute and chronic health effects associated with the products of water disinfection and other selected contaminants. The volume also develops suggested no-adverse-response levels (SNARL's) for both acute and chronic exposure to the drinking water contaminants reviewed by the committee. One chapter reviews the contribution of selected inorganic elements in drinking water to the optimal nutrition of humans.

Volume 4 of *Drinking Water and Health* was published in 1982. This volume identifies chemical and biological contaminants associated with drinking water distribution systems and the health implications of deficiencies in those systems. It also contains an evaluation of information on the toxicity of selected inorganic and organic contaminants and provides SNARL's when appropriate. Some of the compounds were reviewed for the first time in this report; others had been reviewed in earlier volumes of this series. For the latter compounds, discussions were limited mainly to information that had become available after the earlier reports had been published, although the committee occasionally cited older references that were not previously assessed.

The study reported in this volume continues the assessment and evaluation procedures established in the earlier volumes. Twenty-one compounds are reviewed in Chapter 2. Chapter 3 contains an evaluation of epidemiological data on exposure to arsenic and asbestos in drinking water.

On behalf of the members of this committee, I would like to express our thanks and gratitude to Dr. Robert Golden, who served as the Project Officer for this study. Without his enthusiastic efforts and logistical support this endeavor would not have been possible. We also thank Ms. Frances Peter, who served as Editor of this report, and Ms. Virginia White, Ms. Edna Paulson, and Ms. Edna Millard, who assisted in an extensive search of the scientific literature and in reference verification. In addition, we acknowledge the assistance of members of the EPA staff, especially Drs. Krishan Khanna and Joseph Cotruvo. The committee is grateful to Ms. Agnes Gaskin for organizing meetings, preparing the manuscripts, and providing general secretarial support.

Special thanks are in order for Drs. David Hoel, Joseph Haseman, and Nathanial White from the National Institute of Environmental Health Sciences, who expended great effort in providing the committee with the carcinogenic risk estimates.

Finally, I thank the members of the committee, all of whom took time away from busy schedules to donate their expertise to this project. Their contributions will continue the level of excellence established by previous volumes in this series. It was a pleasure to have the opportunity to work with them.

DANIEL B. MENZEL, CHAIRMAN
SAFE DRINKING WATER COMMITTEE

Contents

I

Introduction

The 21 compounds reviewed in this volume were evaluated at the request of the EPA to assist that agency in the event that it becomes necessary to regulate these chemicals in the future. Since 13 of the 21 reviews are updates of evaluations published in earlier volumes of this series, the disscusions are limited to new data. Only 8 compounds were evaluated for the first time. Chapter 3 reviews epidemiological data concerning the diseases that may result from exposure to either arsenic or asbestos in drinking water. Both substances were reviewed comprehensively in the first and third volumes of *Drinking Water and Health* (National Research Council, 1977, 1980). The more limited review in this report puts into perspective the potential risk to human health posed by chronic exposure.

In general, the format for the toxicity evaluations in Chapter 2 is similar to that developed in the previous volumes. When the data were sufficient, the committee calculated a suggested no-adverse-response level (SNARL) for chronic toxicity. In most cases, whenever chronic SNARL's were estimated, data were available from studies lasting a major portion of the life-time of the test animal. For these SNARL's an arbitrary assumption (also used in previous volumes) was made that 20% of the intake of the chemical of concern was derived from drinking water. This was done to provide a basis for calculation and also because there is virtually no information on the relative contribution of other sources to intake of the compounds reviewed. In the event that this value becomes known for a particular compound, the SNARL value can be easily adjusted. Because of this assumption, it would be inappropriate to use these values as though they were

maximum contaminant intakes. These numbers are *not* a guarantee of absolute safety. Furthermore, SNARL's are based on exposure to a single agent and do not take into account possible interactions with other contaminants. In all cases, the safety (or uncertainty) factor used in the calculations of the SNARL's reflects the degree of confidence in the data as well as the combined judgment of the committee members. As in Volumes 1, 3, and 4 of *Drinking Water and Health* (National Research Council, 1977, 1980, 1982), the following assumptions were used to determine which uncertainty factor should be used in the calculations:

- A factor of 10 was used when there were good chronic exposure data on humans and supportive chronic data on other species.
- A factor of 100 was used when good chronic toxicity data were available for one or more species.
- A factor of 1,000 was used when the chronic toxicity data were limited or incomplete.

In previous volumes the committee calculated acute SNARL's in addition to chronic SNARL's when there were adequate data. This was not done in the present volume because it was anticipated that another National Research Council committee would do this as part of its ongoing activities.

Where there was evidence of carcinogenicity in one or more animal species, the committee calculated a numerical risk estimate using the methodology described in the first and third volumes of *Drinking Water and Health* (National Research Council, 1977, 1980). More details are provided under the "Carcinogenic Risk Estimate" section for each compound where this procedure was used. There is an expanded discussion of risk assessment in Chapter III of *Drinking Water and Health*, Volume 3 (National Research Council, 1980).

The committee that prepared Volume 4 (National Research Council, 1982) did not estimate risks for chemicals that were potentially carcinogenic to humans. The present committee decided that the inclusion of estimates would be useful, even if some people may disagree with the selection of a particular mathematical model. Presented with each cancer risk estimate are the tumor incidence data so that the reader may substitute any other mathematical model. In this volume (as in previous volumes) the risk estimates from data on different species and tumors that resulted from exposure to a particular compound were averaged to yield one composite estimate. This was done because when tumors develop in more than one species it is not possible to know which is more representative of humans and also to reduce the confusion that results when more than one risk es

timate number is associated with each compound. The individual risk estimates for each species and tumor site are presented in tabular format.

The committee discussed several different methods for interpreting the conflicting and ambiguous data on the carcinogenicity of several compounds. Many ambiguities could be resolved if better metabolic data in humans were available for classes of compounds, so that the metabolic fate of the compound in different animal species and strains could be compared to that in humans. Regrettably, little such information is now available. Since uncertainties in the interpretation of bioassay results may be caused by the use of high doses and by differences in response among the species, strain, and sex of the test animals, the committee agreed to adopt the approach of the International Agency for Research on Cancer (1980, pp. 18–19) for interpreting animal carcinogenicity tests:

> In general, the evidence that a chemical produces tumours in experimental animals is of two degrees: (a) *sufficient evidence* of carcinogenicity is provided by the production of malignant tumours; and (b) *limited evidence* of carcinogenicity reflects qualitative and/or quantitative limitations of the experimental results.
>
> *Sufficient evidence* of carcinogenicity is provided by experimental studies that show an increased incidence of malignant tumours: (i) in multiple species or strains, and/or (ii) in multiple experiments (routes and/or doses), and/or (iii) to an unusual degree (with regard to incidence, site, type and/or precocity of onset). Additional evidence may be provided by data concerning dose-response, mutagenicity or structure.
>
> In the present state of knowledge, it would be difficult to define a predictable relationship between the dose (mg/kg bw/day) of a particular chemical required to produce cancer in test animals and the dose which would produce a similar incidence of cancer in humans. The available data suggest, however, that such a relationship may exist at least for certain classes of carcinogenic chemicals. Data that provide *sufficient evidence* of carcinogenicity in test animals may therefore be used in an approximate quantitative evaluation of the human risk at some given exposure level, provided that the nature of the chemical concerned and the physiological, pharmacological and toxicological differences between the test animals and the humans are taken into account. However, no acceptable methods are currently available for quantifying the possible errors in such a procedure, whether it is used to generalize between species or to extrapolate from high to low doses. The methodology for such quantitative extrapolation to humans requires further development.
>
> Evidence for the carcinogenicity of some chemicals in experimental animals may be *limited* for two reasons. Firstly, experimental data may be restricted to such a point that it is not possible to determine a causal relationship between administration of a chemical and the development of a particular lesion in the animals. Secondly, there are certain neoplasms, including lung tumours and hepatomas in

> mice, which have been considered of lesser significance than neoplasms occurring at other sites for the purpose of evaluating the carcinogenicity of chemicals. Such tumours occur spontaneously in high incidence in these animals, and their malignancy is often difficult to establish. An evaluation of the significance of these tumours following administration of a chemical is the responsibility of particular Working Groups preparing individual monographs, and it has not been possible to set down rigid guidelines; the relevance of these tumours must be determined by considerations which include experimental design and completeness of reporting.
>
> Hence '*sufficient evidence*' of carcinogenicity and *'limited evidence*' of carcinogenicity do not indicate categories of chemicals: the inherent definitions of those terms indicate varying degrees of experimental evidence, which may change if and when new data on the chemical become available. The main drawback to any rigid classification of chemicals with regard to their carcinogenic capacity is the as yet incomplete knowledge of the mechanism(s) of carcinogenesis.

The committee agrees that rigid guidelines for establishing carcinogenicity are difficult to develop and that each compound must ultimately be evaluated on the basis of the available evidence. Occasionally a risk estimate was performed on data that, based on the above criteria, were judged to be *limited*. This was done so the relative risk of different chemicals could be compared, but the committee recommends that less weight be given to these estimates than to those based on *sufficient* evidence.

Although current risk assessment procedures are fraught with uncertainty, they are the only available means to obtain a comparable perspective on the potential for cancer development from chronic exposure to selected compounds. In this volume, the committee uses the process of quantitative risk assessment in estimating the probability of cancer after a lifetime daily consumption of 1 liter of water containing compounds in a concentration of 1 μg/liter. The committee recognizes that these risk estimates are indeed estimates and should not be considered in a sense of certitude. It should be realized that the risk estimation process involves a direct extrapolation of doses in animals to humans, based on body surface area. Implicit in this process are the assumptions that animal models and human beings respond in a quantitative manner and that it is appropriate to extrapolate to doses as much as six orders of magnitude lower than those given to animals.

Within the scientific community, there is considerable debate over the toxicological bases of the above assumptions, which are accepted here as workable hypotheses for the calculation of numerical risks to provide guidance for qualitative judgements. However, the committee is concerned that undue credence will be given to the quantitative aspects of such risk values, especially when quantitative risk estimates are fundamentally unverifi

able under commonly used research methodology and economic resources.

The committee recognizes that regulatory agencies must base their decisions on the most scientifically sound evaluation of the data. The deficiencies of such data must be recognized, but they should not preclude carefully reasoned, cautious judgments, To gain more confidence in current extrapolation models, it is essential that the level of uncertainty be reduced. This can be accomplished only by vigorous research efforts to overcome the many limitations of using animal models to predict potential responses in humans.

The biological differences in response to chemical exposure among species or strains should be regarded as important information in predicting the potential qualitative and quantitative responses of humans. When an adequate bioassay produces positive findings of carcinogenicity in one species and negative results in another, the differences in results should be explained in terms of the biological differences between the animals. These differences should then be considered in evaluating potential risk to humans. The committee recognizes that this usually cannot be done, but that there is a need to develop data that will make this possible. It strongly recommends that methods be developed to enhance the utility of laboratory animals to predict toxic effects in humans through an increased understanding of their biological similarities and differences.

Many short-term mutagenicity tests are currently used to aid in the evaluation of the carcinogenic potential of chemicals. Many of these tests are based on the use of microorganisms or cells from mammals or plants to detect agents that react with and modify deoxyribonucleic acid (DNA). This is an important step because ample evidence suggests that DNA damage leads to mutational events in all living organisms. A cellular genetic change is important to humans because it may result in a cellular transformation that, in turn, may produce neoplasia. This process is referred to as the somatic mutation theory of carcinogenesis. Since DNA is a target molecule for mutagenesis, a chemical was designated as a mutagen in this volume based on its ability to induce mutations in any one short-term test. However, it should be recognized that epigenetic mechanisms may also play a role in tumorigenicity in the absence of direct DNA change. Therefore, some carcinogens may not be detectable in mutagenicity screening tests.

There are now generally accepted guidelines for interpreting short-term test results as a basis for assessing risk to human health. A series of hierarchical tests may be used to evaluate the mutagenic effects of chemicals. From the findings of such tests, one can predict the qualitative, but not

quantitative, effects on human health. Evaluation of potential carcinogenicity is currently based on correlations between short-term mutagenicity test results and data obtained from studies of carcinogenicity in laboratory animals or epidemiological studies of humans. However, data on the limited number of chemicals known to be carcinogens in humans are not sufficient to provide an adequate basis for validation of the short-term tests. In addition, most validation studies have included chemicals that have been assayed in carcinogenicity studies in animals, but such studies do not always provide unequivocal answers as to the carcinogenic or noncarcinogenic nature of a chemical. Therefore, caution should be exercised in using mutagenicity test results to predict potential carcinogenicity, especially since not all mutagens are carcinogenic, nor are all carcinogens mutagenic.

The National Research Council Committee on Chemical Environmental Mutagens (National Research Council, 1983) made the following statement concerning the risk of mutation to future human populations:

> Efficient mutagenicity tests that use experimental organisms and *in vitro* systems are a product of great advances in basic knowledge and of a substantial research effort. The principal task of the Committee [on Chemical Environmental Mutagens] is to study how the results of such tests can be used to assess the risk to future human populations. There are no reliable data from direct human experience, so it is necessary to rely on experimental test systems. Some tests are exquisitely sensitive to chemical mutagens, but use microorganisms or mammalian-cell cultures of uncertain relevance to human germ cell damage. Although there has been great progress in the development of test systems, it is still not possible to predict the human impact of a mutagen with confidence. There are two reasons for this: first, in the absence of human data, it is not possible to validate the test systems, and second, one must assume that they predict human effects. Even if the damage to human germ cells could be measured precisely, we lack the knowledge to translate the measurements into a total impact on the health and welfare of future generations—this situation is not likely to change soon.

Assessment of teratogenicity is quite different from assessment of mutagenicity and carcinogenicity. A teratogen is defined as an agent that produces either a major or minor deviation from normal morphology or function during embryonic or fetal development. The molecular and cellular mechanisms that produce teratogenesis are multiple, which has made the search for a prescreen very difficult. There are at least 10 different *in vitro* systems under development and validation, but none of them have come into common usage. Another unique aspect of teratogenicity testing is that almost any agent administered at a high enough dose during a sensitive time in prenatal development can produce a teratogenic effect. Sucrose and sodium chloride are examples of such agents (Shepard, 1980). This

means that in humans, exposure level, time of exposure, individual sensitivity, and potency are probably the controlling factors for teratogenicity rather than solely the demonstration that an agent is teratogenic in a laboratory animal or in some *in vitro* test.

Another unique feature of teratology tests is that the exposure period in humans is generally limited to 9 months, and the end point is often measurable at birth. Examples of delayed teratogenic action have been identified but are not common. This relatively short exposure period and the end point measurement have enabled investigators to make some epidemiological analyses. To date, such studies have indicated that approximately 25 agents may be teratogenic in humans.

The data for most of the compounds reviewed in this report were not sufficient to judge with certainty that a particular compound was or was not teratogenic in animals. Therefore, many of the evaluations are based on what the committee considered to be limited evidence. Because of the limited nature of the data, no judgment can be made about teratogenic potential in humans. Postnatal studies were included in the reviews, but studies *in ovo* were not considered.

The reviews of arsenic and asbestos are limited to evaluations of epidemiological studies. Each of these substances has been reviewed and evaluated in previous volumes of *Drinking Water and Health*, but recent data make another evaluation timely.

In the case of arsenic, recent epidemiological studies conducted in the United States are at variance with the results of some older studies done in Taiwan. Furthermore, there is now some evidence that arsenic may be an essential nutrient. These issues are examined in detail in Chapter 3.

For asbestos there are now epidemiological data on populations exposed via drinking water in addition to the data on occupational exposures via inhalation. These oral exposure studies are examined in detail, and the risk of cancer is compared with the risk derived from the inhalation studies. Finally, the committee presents a model to estimate the risk of gastric cancer from ingested asbestos fibers.

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II

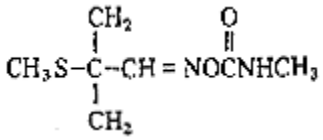
Toxicity of Selected Contaminants in Drinking Water

The health effects of 21 contaminants found in drinking water are evaluated in this chapter. The substances are discussed in alphabetical order: aldicarb, carbofuran, carbon tetrachloride, chlorobenzene, *o*-dichlorobenzene, *p*-dichlorobenzene, 1,2-dichloroethane, 1,1-dichloroethylene, *cis*-and *trans*-1,2-dichloroethylene, dichloromethane, dinoseb, hexachlorobenzene, methomyl, picloram, rotenone, tetrachloroethylene, 1,1,1-trichloroethane, trichloroethylene, vinyl chloride (monochloroethylene), and uranium. For each one, the committee reviewed data from studies of metabolism, health effects in humans and laboratory animals, mutagenicity, carcinogenicity, and teratogenicity. When there were sufficient data, it calculated numerical estimates of cancer risk. It also calculated suggested no-adverse-response levels (SNARL's) for chronic exposures to chemicals that were not known or suspected to be carcinogens.

The descriptions of some of the contaminants are limited to data generated since the last four volumes of *Drinking Water and Health* were published. Other contaminants and their health effects are evaluated for the first time in this report. This is one reason why no significance should be attached to the length of the discussion devoted to each contaminant.

ALDICARB

propanal, 2-methyl-2-(methylthio), *o*-[(methylamino)-carbonyl]oxime
CAS No. 116-06-3



Aldicarb was evaluated in the first volume of *Drinking Water and Health* (National Research Council, 1977, pp. 635-643). The following material, which became available after the 1977 report was prepared, updates and, in some instances, reevaluates the information contained in the previous review. Also included are some references that were not assessed in the earlier report.

Metabolism

Preliminary studies by Hurst and Dorough (1978, abstract) indicate that aldicarb, which is an inhibitor of acetylcholinesterase, inhibits certain carboxyesterases that play a role in the hydrolytic detoxification of this pesticide. Such anticarboxyesterase activity could enhance the toxicity of this agent. By administering radiocarbon-labeled aldicarb to bile-cannulated rats, Marshall and Dorough (1979) demonstrated that an oral dose is completely absorbed. In their experiment, approximately 28% of the dose appeared in the bile within 24 hours; 64% was excreted in the urine.

Reduced acetylcholinesterase activity was observed in the brain and liver of rat fetuses for as long as 24 hours after doses of 0.01 or 0.10 mg/kg body weight (bw) were administered orally to the dams (Cambon *et al*., 1979).

Health Aspects

Observations in Humans

Peoples *et al*. (1978) reviewed 38 reports of occupational illnesses that apparently resulted from exposure to aldicarb. These reports were obtained from the registry for the State of California, where the product is available only as a granular formulation. The symptoms described in the documents were consistent with those expected for a carbamate insecticide: dizziness, blurred vision, constricted pupils, nausea, and abdominal pain. Virtually all incidents involved employees who handled, loaded, or applied aldicarb.

Several of them were admitted to the hospital. Depressed plasma cholinesterase levels were observed in some patients. Although some of the subjects were reported as having been very ill, recovery, which was rapid and complete, was aided in some cases by the administration of atropine.

A committee of the National Research Council (1982) found no evidence that long-term health effects resulted from short-term exposure to a variety of anticholinesterase compounds. That committee did not specifically review aldicarb, which is a member of that broad class of chemicals. Nonetheless, this conclusion provides reassurance that short-term exposure to aldicarb is unlikely to produce long-term adverse effects on human health.

Observations in Other Species

The committee found no reports that extended or amplified previous discussions on the acute or chronic effects of aldicarb in animal species.

Mutagenicity Normal cultured skin cells from humans were treated with aldicarb. No breaks in DNA were detected (Blevins *et al*., 1977a,b).

A transplacental host-mediated hamster cell assay was used to study the ability of aldicarb to induce morphological transformation in fetal cell cultures. These cultures were also examined for growth in soft agar (0.3%) and for their ability to induce tumors in nude mice. Negative results were obtained with aldicarb (Quarles *et al*., 1979b). Negative results were also obtained in a test with bone marrow erythrocyte micronuclei from mice. However, because of the extreme toxicity of aldicarb (i.e., an LD_{50} of 1 mg/kg bw), it could not be administered at high doses (Seiler, 1977).

The evidence indicates that aldicarb is nonmutagenic in several assays that allow for the detection of genotoxicity. The results from the mouse micronucleus test remain inconclusive because of the toxicity of the compound.

Carcinogenicity Aldicarb was tested for carcinogenicity in Fischer 344 rats and $B6C3F_1$ mice (National Cancer Institute, 1979). The pesticide was administered to both the rats and the mice in their feed at doses of either 2 or 6 mg/kg. The animals were dosed for 103 weeks and sacrificed shortly thereafter. No evidence of carcinogenicity related to aldicarb was found in either sex of mice or rats. Because there was no indication of weight depression or early mortality, it is possible that a maximum tolerated dose was not used.

Under the conditions of this bioassay, technical-grade aldicarb was not carcinogenic to either Fischer 344 rats or $B6C3F_1$ mice of either sex.

Teratogenicity The committee found no data to evaluate.

Conclusions and Recommendations

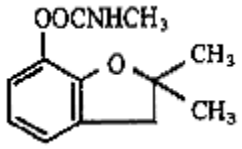
Suggested No-Adverse-Response Level (SNARL)

Chronic Exposure Long-term (2-year) feeding studies have been conducted in rats and dogs. The studies reviewed in the first volume of *Drinking Water and Health* (National Research Council, 1977, pp. 635-693) established 0.1 mg/kg/day no-adverse-effect levels in both animal species. This resulted in the calculation of an acceptable daily intake (ADI) for aldicarb of 0.007 mg/liter. This value continues to be the recommended SNARL for chronic exposure. Work cited in the first volume indicated that the acute effects of aldicarb toxicity are readily reversible. Unless exposure is sustained, therefore, cumulative effects are unlikely. None of three long-term rodent studies on aldicarb provided evidence of a carcinogenic effect. In fact, no chronic toxicity of any type was observed. This may be attributed to the limited maximum daily dose that could be given because of the potent anticholinesterase activity of aldicarb. Animal studies have provided no indication that aldicarb has any untoward toxicity other than that associated with anticholinesterase activity.

Since animal studies of sufficient duration have been conducted and since restrictions on dosage have been imposed by the intrinsic toxicity of the agent, it is unlikely that any further meaningful repeated exposure data will be forthcoming. The best judgment that can be made with the data in hand is that exposure of humans to doses of aldicarb that do not affect cholinesterase will not result in any adverse effects.

CARBOFURAN

7-benzofuranol, 2,3-dihydro-2,2-dimethyl-, methylcarbamate
CAS No. 1563-66-2



Carbofuran is a systemic insecticide, acaricide, and nematocide, which is applied directly to foliage and soil. The chemical was introduced in 1967 by the FMC Corporation under the trademark Furadan. It is a colorless, crystalline solid that is soluble in water at 700 mg/liter. Its half-life in soil ranges from 30 to 60 days. In alkaline media, the compound is unstable.

Carbofuran does not require activation, but is a direct inhibitor of acetylcholinesterase. For this reason, symptoms consistent with the toxicity of

insecticides of this class appear in mammals immediately after they have been exposed to the compound.

Metabolism

Carbofuran is readily and completely absorbed when administered orally to mice and rats (Ahdaya *et al*., 1981; Marshall and Dorough, 1979). In studies of bile-cannulated rats given an oral dose of ^{14}C-ring-labeled carbofuran, Marshall and Dorough (1979) accounted for 95% of the dose in the bile and urine within 48 hours. Biliary excretion was quite extensive and comprised about one-third of the dose given.

The products of the metabolism of carbofuran have been shown to be similar in plants and mammals (Metcalf *et al*., 1968). The most prominent of these products results from the hydroxylation of the dihydrobenzofuranyl ring to yield 3-hydroxycarbofuran (Dorough, 1968; Ivie and Dorough, 1968). In mice and rats, further oxidation to the 3-keto derivative occurs as well as hydrolysis of the carbamate. Studies with ring-labeled carbofuran in rats have shown that more than 90% of the dose is excreted in the urine and that the material is present primarily as water-soluble conjugates of the 3-hydroxy and 7-hydroxy metabolites, each of which appears to a limited extent in free form. Sulfate and glucuronide conjugates account for the majority of the urinary metabolites in the rat (Marshall and Dorough, 1979). The *N*-methylhydroxy derivative was also found in the urine in small quantities. As would be expected, the urinary product also included derivatives formed by metabolic alteration at more than one site (e.g., hydrolysis at position 7 plus 3-hydroxylation). In another study in rats, the major urinary metabolite was a water-soluble conjugate of 2,3-dihydro-2,2-dimethyl-3-keto-7-hydroxybenzofuran (Dorough, 1968). In the cow, the metabolic deposition of carbofuran was found to be very similar to that in the rat both in route of excretion and metabolites formed (Ivie and Dorough, 1968).

Health Aspects

Observations in Humans

Tobin (1970) related five case reports of individuals with apparent carbofuran intoxication. The symptoms that were rapid in onset were characteristic of compounds with anticholinesterase activity: nausea, blurred vision, muscular weakness, and perspiration. The effects subsided shortly after the onset, and all subjects had complete recovery, which was enhanced by the administration of atropine.

A committee of the National Research Council (1982) found no evidence

that long-term health effects resulted from short-term exposure to a variety of anticholinesterase compounds. That committee did not specifically review carbofuran, which is a member of that broad class of chemicals. Nonetheless, this conclusion provides reassurance that short-term exposure to carbofuran is unlikely to produce long-term adverse effects on human health.

Observations in Other Species

Acute Effects The acute oral LD_{50} of carbofuran has been reported to be less than 20 mg/kg bw for a number of mammalian species (Table II-1). The committee found no other reports describing the effects of acute treatment with carbofuran.

Chronic Effects Wolfe and Esher (1980) conducted an 8-month feeding study of carbofuran in two species of mice—the old-field mouse (*Peromyscus polionotus*) and the cotton mouse (*P. gossypinus*). They used one dietary concentration of 0.01%. The animals were housed in pairs, and reproductive performance was evaluated in addition to growth and behavior. This level of treatment produced no measurable effect on the food consumption or growth of the parents, or on reproductive capacity or growth and development of the young.

Tobin (1970) reported no-effect levels in the diet of 25 and 20 ppm for the rat and dog, respectively, after conducting 2-year feeding studies with carbofuran. This report contained no other data on the design or the evaluation of these studies.

Mutagenicity Quarles *et al.* (1979b) used a transplacental host-mediated hamster cell assay to study the ability of carbofuran to induce morphological transformation in fetal cell cultures. These cultures were also examined for growth in soft agar (0.3%) and for their ability to induce tumors in nude mice. Negative results were obtained with carbofuran.

Oral administration of sodium nitrite (50 mg/kg) and carbofuran

TABLE II-1 $LD_{50's}$ for Carbofuran in Mice, Rats, and Dogs

Animal	$LD_{50's}$, mg/kg	Reference
Mouse	2	Krieger *et al*., 1976
Rat	8-14	Tobin, 1970
Rat	4-11	Yu *et al*., 1972
Rat	11-15	Abdel-Aal and Helal, 1980
Dog	19	Tobin, 1970

(10 mg/kg) did not result in elevated micronuclei counts in the bone marrow cells of mice (Seiler, 1977). Negative results were obtained in the Ames *Salmonella* assay, yeast *Saccharomyces cerevisiae* D3 mitotic recombination assay, *E. coli* WP2 assay, and relative toxicity assay with *Escherichia coli* and *Bacillus subtilis* (Poole *et al*., 1977, abstract).

Germinating onion bulbs (*Allium cepa*) were treated with 0.1% carbofuran for 2 to 4 hours (Sathaiah *et al*., 1974). The investigators observed an irregular scattering of chromosomes during the metaphase stage as well as chromosome abnormalities such as fragments, clumping, scattered anaphases, and contractions.

In summary, carbofuran was found to be nonmutagenic in several short-term assays. Although chromosome abnormalities were observed in one plant assay, there is no evidence to suggest that DNA damage was directly involved in producing these effects.

Carcinogenicity No data were found by the committee.

Teratogenicity McCarthy *et al*. (1971, abstract) reported that although no increase in defects occurred in the offspring of treated rats, rabbits, and dogs, there was a reduced survival rate in rat offspring exposed to diets containing 100 ppm. Wolfe and Esher (1980) administered the same dietary dose level to mice and found a reduced survival rate, but the difference from controls was not significant, and fetal weights were not reduced.

After reviewing these limited data, the committee concluded that carbofuran does not appear to be teratogenic to mice, rats, rabbits, or dogs.

Conclusions and Recommendations

The published information is not sufficient to permit a calculation of a suggested no-adverse-response level (SNARL) or otherwise to assess the potential hazard of chronic exposure to carbofuran. There is a need for more data on the chronic toxicity, carcinogenicity, and teratogenicity of carbofuran.

CARBON TETRACHLORIDE

methane, tetrachloro-
CAS No. 56-23-5
CCl_4

Carbon tetrachloride was evaluated in the first and third volumes of *Drinking Water and Health* (National Research Council, 1977, pp. 703-706;

1980, pp. 96-98). The following material, which became available after the 1980 report was prepared, updates and, in some instances, reevaluates the information contained in the previous review. Also included are some references that were not assessed in the earlier reports.

Metabolism

Carbon tetrachloride is believed to be metabolized by cytochrome P450 enzymes contained in the membranes of the endoplasmic reticulum (microsomes) and in the nuclear membrane (Diaz Gomez and Castro, 1980; Recknagel and Glende, 1973). Low doses of the compound induce lipid peroxidation (LPO), whereas higher doses (> 0.5 ml/kg administered orally) inhibit the mixed-function oxidase system and further induce lipid peroxidation (Harris *et al*., 1982; Recknagel and Glende, 1973). The hypothesis that the metabolic pathway of carbon tetrachloride proceeds from the trichloromethyl radical ($\cdot CCl_3$) to phosgene ($COCl_2$) to carbon monoxide (CO) (Recknagel, 1967) has withstood the test of time. Recent evidence has suggested that chloroform ($CHCl_3$) may also form under anaerobic conditions in hepatic microsomes (Ahr *et al*., 1980). Inhibitors of cytochrome P450 decrease carbon tetrachloride toxicity, whereas Type I inducers of cytochrome P450 enhance the hepatotoxicity (National Research Council, 1980).

Health Aspects

Observations in Humans

No new data were found by the committee.

Observations in Other Species

Acute Effects Carbon tetrachloride toxicity is manifested by a rapid rise in LPO in the target organ (Recknagel, 1967). The reactive metabolite inducing LPO is believed to be the trichloromethyl radical, which alters membrane function by blocking ion pumps within the cell (Bernacchi *et al*., 1980; Lowrey *et al*., 1981; Moore, 1980). These effects have been observed in rats that were given acute oral doses ranging from 0.5 to 1 ml/kg.

Recently published evidence indicates that there is an age difference in susceptibility to carbon tetrachloride-induced hepatotoxicity. At intraperitoneal doses of 1 ml/kg, the increase in serum aspartate aminotransferase and triglyceride accumulation in the neonatal (1-to 14-day-old) rat was

equivalent to that observed in the adult rat; however, there was much less macromolecular binding and lipid peroxidation in the young rats, whereas blood acetoacetate levels were 3 to 5 times higher (Cagen and Klaassen, 1979). Ethanol toxicity in mice has been enhanced by oral pretreatment with 0.5 ml/kg doses of carbon tetrachloride, which resulted in a concomitant 3- to 5-fold increase in acetaldehyde production (Hjelle and Petersen, 1981). These authors concluded that carbon tetrachloride inhibits acetaldehyde oxidation and hepatic aldehyde dehydrogenase. In young rats, this inhibition may be the result of the exquisite sensitivity of these enzymes to carbon tetrachloride and may account for the enhanced toxicity of carbon tetrachloride in diabetic rats (Cagen and Klaassen, 1979; Hanasono *et al*., 1975).

Chronic Effects No new data were found by the committee.

Mutagenicity Callen *et al*. (1980) reported that carbon tetrachloride induced recombinations and mitotic gene conversions as well as point mutations when 21, 28, and 34 mM concentrations were incubated with *Saccharomyces cerevisiae* D7 cells growing in log phase for 1 hour at 37°C. No exogenous mammalian metabolic activation system was required to induce the genetic effects. The yeast D7 cytochrome P450 system apparently mediates the metabolic conversion.

In summary, carbon tetrachloride was mutagenic in one microbial mutagenicity assay.

Carcinogenicity The carcinogenicity of this compound was evaluated in the first volume of *Drinking Water and Health* (National Research Council, 1977, pp. 703-707) and more recently in a publication by the International Agency for Research on Cancer (1979a). In a study not reviewed in these two reports, Al'pert *et al*. (1972) gave 30 female rats subcutaneous injections of carbon tetrachloride twice weekly for 2 years. Eight of the dosed animals developed mammary adenocarcinomas. No mammary tumors developed in a control group of 15 female rats.

Teratogenicity Wilson (1954) gave oral (0.3 ml) or subcutaneous (0.8 ml) doses of carbon tetrachloride to rats on days 2 or 3 of gestation. No congenital defects were observed in the offspring. Adams *et al*. (1961) noted some degeneration of embryonic discs in the blastocysts of rabbits exposed *in vivo* to 1.0 ml/kg.

Schwetz *et al*. (1974) subjected pregnant rats to inhalation exposures of carbon tetrachloride in concentrations ranging from 300 to 1,000 ppm

(1,890 to 6,300 mg/m^3) for 7 hours/day on days 6 through 15 of gestation. Fetal size was reduced, but neither resorptions nor malformations occurred more frequently than in the controls.

Transplacental passage has been shown in pregnant mice (Roschlau and Rodenkirchen, 1969), rats (Tsirel'nikov and Dobrovol'skaya, 1973), and humans (Dowty *et al*., 1976). The amounts were not quantified. The carbon tetrachloride was identified by the use of gas chromatography and mass spectrometry.

The data indicate that carbon tetrachloride is not teratogenic to rats.

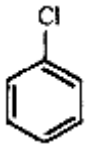
Conclusions and Recommendations

Carbon tetrachloride is apparently metabolized by mixed-function oxidases, which generate a reactive trichloromethyl radical. It is believed that the reactive intermediate induces lipid peroxidation, which leads to hepatotoxicity. The compound is mutagenic in yeast without metabolic activation, since yeast has an active mixed-function oxidase system.

Carbon tetrachloride is not teratogenic to rats exposed orally, subcutaneously, or via inhalation, but it has been shown to reduce fetal weight after maternally toxic inhalation exposures. The carcinogenicity of carbon tetrachloride was discussed in previous reviews.

CHLOROBENZENE

monochlorobenzene; benzene, chloro-
CAS No. 108-90-7



Chlorobenzene was reviewed in the first volume of *Drinking Water and Health* (National Research Council, 1977, pp. 709-710). The following material, which became available after the 1977 report was prepared, updates and, in some instances, reevaluates the information contained in the previous review. Also included are some references that were not assessed in the earlier report.

Metabolism

No significant data on the metabolism of chlorobenzene have been reported since the initial review. However, there have been reports providing

new information about chlorobenzene's effects on enzyme systems and on metabolism of other chemicals.

As noted in the 1977 review, the liver is the primary site of organ toxicity. The observed hepatotoxicity is probably caused by metabolic conversions of chlorobenzene to an epoxide and subsequent reaction with cellular macromolecules. Recent literature confirms these effects and also demonstrates additional effects on the hepatic metabolism of other chemicals with resulting hepatotoxicity.

Yang *et al*. (1979) reported that chlorobenzene produced a 10-fold increase in bile duct-pancreatic fluid (BDPF) flow along with a 70% decrease in protein content of the fluid; however, they observed no changes in the chloride concentration in the BDPF, in the bile flow, or in the levels of serum glutamic pyruvic transaminase (SGPT) (now known as plasma alanine aminotransferase, ALT). In this study, male rats (Holtzman Sprague-Dawley stock) received intraperitoneal injections of chlorobenzene (5 mmol/kg in sesame oil) 24 hours before measurements of hepatic function. Since serum SGPT levels were not increased, the authors concluded that stimulation of BDPF flow probably occurred independently of hepatotoxicity. In male mice, however, intraperitoneal injection of chlorobenzene produced dose-and time-dependent hepatotoxicity, as shown by increases in serum SGPT levels (Shelton and Weber, 1981). Results of these two studies suggest species differences in response to chlorobenzene since mice, but not rats, developed liver lesions. This observation may be important when results from any toxicity studies are evaluated.

Shelton and Weber (1981) also studied the combined metabolic effects of intraperitoneally administered chlorobenzene plus carbon tetrachloride. They reported additive effects with increased concentrations. The theoretical model used to analyze their results might provide a means to study the combined effects of organohalide chemicals found in drinking water. Before it can be used for this purpose, however, the model should first be verified in tests with oral exposures.

Health Aspects

Observations in Humans

No new data were found by the committee.

Observations in Other Species

Acute Effects The committee found no additional information on acute oral toxicity. In a study of inhalation exposures, Bonnet *et al*. (1979) observed an LC_{50} value of 1,886 ppm (range, 1,781-1,980 ppm, or 8,195-

9,110 mg/m^3) when female mice were exposed for 6 hours. The authors noted that this value is approximately 25 times higher than the time-weighted threshold limit value (TLV) of 75 ppm (350 mg/m^3), which is recommended in the United States by the American Conference of Governmental Industrial Hygienists (1981).

Feeding chlorobenzene to male albino rats at dietary levels that were acutely lethal to some of the animals produced a 6-fold increase in urinary excretion of coproporphyrin, followed by increased excretion of porphobilinogen and δ-aminolevulinic acid (Rimington and Ziegler, 1963); unfortunately, the number of animals in the study was small.

Chlorobenzene injected intraperitoneally into male Wistar rats produced dose-dependent increases in hepatic δ-aminolevulinic acid synthetase at doses of 100 and 200 mg/kg and in heme oxygenase at doses of 50 to 200 mg/kg and a significant decrease in cytochrome P450 at doses of 200 mg/kg (Ariyoshi *et al*., 1981). Maximum enzyme activities were noted 24 hours following injection, whereas the cytochrome P450 content was decreased 24 and 48 hours following injection. These authors suggested that chlorobenzene could induce a rapid degradation of heme or cytochrome P450 and that it also seemed to inhibit, in part, the heme biosynthesis pathway.

In adult male albino rats, chlorobenzene at oral doses of 200, 400, or 800 mg/kg/day for 14 days significantly decreased cytochrome P450 and glucose-6-phosphatase at the high dose and increased glucuronyl transferase at all doses but had no effect on isocitrate dehydrogenase, cytochrome-*c* reductase, benzo(*a*) pyrene hydroxylase, or detoxification of EPN (ethyl *p*-nitrophenyl phenylphosphonothionate) (Carlson and Tardiff, 1976). The lack of effect on isocitrate dehydrogenase led the authors to conclude that chlorobenzene did not produce frank liver damage, as measured by this test.

Chronic Effects No new data were found by the committee.

Mutagenicity No data were found by the committee.

Carcinogenicity Chlorobenzene was tested for carcinogenicity in both sexes of the B6C3F$_1$ mouse and the Fischer 344 rat (National Toxicology Program, 1982a). Doses of 60 or 120 mg/kg bw were administered by gavage to female mice and to male and female rats; lower doses of 30 or 60 mg/kg were administered to male mice because of their greater susceptibility to the toxicity of this compound. The doses were administered in corn oil to groups of 50 rats and 50 mice of each sex 5 days per week for

2 years. There were also corresponding untreated control groups of 50 rats and mice of each sex.

There was weak evidence of carcinogenicity in the male rats. The site and incidence rate are discussed in the following section.

Carcinogenic Risk Estimate In the above-cited study by the National Toxicology Program (1982a), there was a weak dose-related incidence of hepatic neoplastic nodules in the male rats. The NTP concluded that this provided "some, but not clear evidence of carcinogenic activity in male rats" (Table II-2).

The tumor incidence rates in Table II-2 were used to make statistical estimates of both the lifetime risk and an upper 95% confidence bound in the lifetime risk. The risk estimates are expressed as a probability of cancer after a lifetime daily consumption of 1 liter of water containing the compound in a concentration of 1 μg/liter. The estimates of risk are based on the multistage model for carcinogenesis, i.e.,

$$P(\text{tumor/dose "d"}) = 1 - \exp[-(q_0 + q_1 d + q_2 d^2 + \ldots + q_k d^k)],$$

where the q's, which are unknown nonnegative parameters, are estimated by maximum likelihood methods and k represents the number of transitional events in the carcinogenic process that are related to the carcinogen under test. The conversion of animal doses to human doses is based on body surface area, assuming the following weights: humans, 70 kg; rats, 400 g; and mice, 33 g. The conversion formula is: human consumption = animal consumption × (animal weight/human weight)$^{1/3}$. The human dose estimates were also reduced by a factor of 5/7 to take into account the fact that the test animals were only gavaged 5 days per week. Using the data from the National Toxicology Program (1982a) study, the committee estimated the lifetime risk and upper 95% confidence estimate of lifetime risk in humans after a daily consumption of 1 liter of water containing the compound in a concentration of 1 μg/liter (Table II-3).

TABLE II-2 Tumor Incidence in Rats Gavaged with Chlorobenzene[a]

Animal	Sex	Tumor Site	Dose, mg/kg/day	Tumor Rates
Fischer 344 rat	Male	Liver	0, 60, 120	2/50, 4/49, 8/49

[a] From National Toxicology Program, 1982a.

TABLE II-3 Carcinogenic Risk Estimates for Chlorobenzene[a]

Animal	Sex	Estimated Human Lifetime Risk at a Daily Dose of 1 μg/liter	Upper 95% Confidence Estimate of Lifetime Cancer Risk per μg/liter
Fischer 344 rat	Male	3.71×10^{-8}	2.13×10^{-7}

[a] Based on data from the National Toxicology Program, 1982a.

Using the criteria for interpreting animal carcinogenicity data as outlined in Chapter I, the committee based the above calculation on limited evidence.

Teratogenicity No data were fóund by the committee.

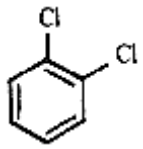
Conclusions and Recommendations

A recent report indicates that chlorobenzene was weakly carcinogenic at a dose of 120 mg/kg in male rats. Therefore, a chronic SNARL will not be calculated because chlorobenzene is considered to be potentially carcinogenic in humans.

The basic conclusions and recommendations contained in the 1977 *Drinking Water and Health* report remain valid, and mutagenicity and teratogenicity data are still needed. Bioassays for carcinogenesis fulfill the need for some data; however, well-designed studies of subchronic exposures should be conducted to identify species differences in potential hepatotoxicity and to learn which effects should be used as a basis for establishing limits in drinking water.

O-DICHLOROBENZENE

1,2-dichlorobenzene, benzene 1,2-dichloro-
CAS No. 95-50-1



o-Dichlorobenzene is used primarily as an intermediate in the synthesis of dyestuffs, herbicides, and degreasers. It is also used as a process solvent in the manufacture of toluene diisocyanate (Ware and West, 1977). It has a

melting point of – 17.6°C, a boiling point of 179°C, and a density of 1.3 g/ml at 20°C (Weast and Selby, 1975). Its solubility in water at 25°C is 145,000 μg/liter (Jacobs, 1957).

Most of the literature on *o*-dichlorobenzene does not distinguish this compound from other isomers or from dichlorobenzenes in general. Consequently, this review is limited to those data that clearly identify *o*-dichlorobenzene as the chemical under study.

Metabolism

Parke and Williams (1955) measured the excretion of *o*-dichlorobenzene by rabbits given a single oral dose of 500 mg/kg bw. Virtually all of the compound was excreted in 6 days, predominantly in urinary conjugates of glucuronide (48%), ethereal sulfate (21%), and mercapturic acid (5%). The remainder was excreted as monophenols.

Health Aspects

Observations in Humans

The committee found no information concerning the exposure of humans only to *o*-dichlorobenzene.

Observations in Other Species

Acute Effects The acute oral LD_{50} for guinea pigs was reported to range from 0.8 to 2.0 μg/kg (Hollingsworth *et al*., 1958). After 6-hour inhalation exposures the acute LC_{50} in mice was reported to be 1,236 ppm (6,825 mg/m^3); range, 1,201-1,279 ppm (Bonnet *et al*., 1979).

o-Dichlorobenzene produced increased urinary excretion of coproporphyrin, porphobilinogen, and δ-aminolevulinic acid, but the responses were less pronounced than those induced by the 1,4-isomer (Rimington and Ziegler, 1963). Female Wistar rats dosed orally with *o*-dichlorobenzene at a level of 250 mg/kg for 3 days had an increase in liver weights and microsomal protein content and enhanced aminopyrine demethylase activity in the liver (Ariyoshi *et al*., 1975).

o-Dichlorobenzene injected intraperitoneally into male Sprague-Dawley rats produced centrilobular necrosis that was enhanced by prior administration of phenobarbital (Reid *et al*., 1973), which was also found to enhance the excretion of metabolites of *o*-dichlorobenzene. However, the latter response was blocked by administration of SKF 525-A, confirming that

the hepatotoxicity of *o*-dichlorobenzene is probably mediated through an activated metabolite.

Ribonucleic acid (RNA) and protein synthesis was found to be strongly inhibited in HeLa cells exposed for 30 minutes to 350 μg/ml concentrations of *o*-dichlorobenzene. A possible mechanism leading to such inhibition is the uncoupling of oxidative phosphorylation (Myhr, 1973).

Chronic Effects Repeated oral doses as high as 376 mg/kg given to female rats for 192 days produced a moderate increase in liver weights and a slight increase in kidney weights. After 7-hour inhalation exposures administered 5 days/week for 6 to 7 months at levels of 49 or 93 ppm (271 or 513 mg/m^3), *o*-dichlorobenzene produced no adverse effects in rats, guinea pigs, rabbits, or monkeys (Hollingsworth *et al*., 1958). Because the number of animals used in this study was limited to 10 animals per dose level with up to 50% lethality, the data are of questionable value.

Mutagenicity *o*-Dichlorobenzene was found to be nonmutagenic in eight histidine-requiring *Salmonella* strains and in a bacteriophage T4 mutation assay. However, the results remain inconclusive because no metabolic activation system was included in the assay procedures (Andersen *et al*., 1972).

Carcinogenicity *o*-Dichlorobenzene was tested for carcinogenicity in both sexes of the B6C3F$_1$ mouse and the Fischer 344 rat (National Toxicology Program, 1982a). Dosages of 60 or 120 mg/kg bw were administered by gavage to both sexes of the mice and the rat. *o*-Dichlorobenzene was administered in corn oil to groups of 50 rats and 50 mice of each sex 5 days/week for 2 years. There were also corresponding vehicle and untreated control groups of 50 rats and 50 mice of each sex.

No compound-related carcinogenic effect was detected in either sex of the mice or rats; however, the maximum tolerated dose was probably not used in this study. No other effects were reported.

Teratogenicity No data were found by the committee.

Conclusions and Recommendations

Suggested No-Adverse-Response Level (SNARL)

Chronic Exposure A tentative chronic SNARL is calculated below. Using the lowest dose level from the carcinogenicity bioassay (60 mg/kg) and assuming that a 70-kg human consumes 2 liters of water daily, that 20% of

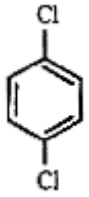
exposure is from water, an uncertainty factor of 1,000, and a factor of 5/7 to correct from a 5- to 7-day weekly exposure, one may calculate the chronic SNARL as:

$$\frac{60 \text{ mg/kg} \times 70 \text{ kg} \times 0.2}{1{,}000 \times 2 \text{ liters}} \times \frac{5}{7} = 0.3 \text{ mg/liter.}$$

The uncertainty factor of 1,000 is used because the bioassay from which this dose was selected has not been formally reviewed and published. This calculation must be reviewed when that occurs. Further studies of this chemical should be directed toward comparative pharmacokinetics, mutagenicity, and characterization of hepatotoxicity.

P-DICHLOROBENZENE

1,4-dichlorobenzene; benzene, 1,4-dichloro-
CAS No. 106-46-7



p-Dichlorobenzene was reviewed in the first volume of *Drinking Water and Health* (National Research Council, 1977, pp. 681-686). The following material, which became available after the 1977 report was prepared, updates and, in some instances, reevaluates the information contained in the previous review. Also included are some references that were not assessed in the earlier report. The literature contains no important new information that would alter the basic conclusions drawn in 1977.

Metabolism

The metabolism of *p*-dichlorobenzene was extensively studied in rats following repeated inhalation, oral, or subcutaneous doses (Hawkins *et al*., 1980). After these exposures, residues detected by ^{14}C content were observed in fat, kidneys, liver, and lungs, but they declined rapidly to levels below limits of detection within 5 days after exposure. From 91% to 97% of the dose was excreted in the urine. Biliary excretion ranged from 46% to 63%, but the excreted material apparently was reabsorbed from the intes

tinal tract since subsequent fecal excretion was negligible. The major urinary excretion products were the sulfate and glucuronide conjugates of 2,5-dichlorophenol. Two minor urinary components were identified as dihydroxydichlorobenzene and a mercapturic acid conjugate of *p*-dichlorobenzene. In contrast to their findings for *o*-dichlorobenzene, Reid *et al*. (1973) found that the metabolism (and excretion) of *p*-dichlorobenzene was not enhanced by pretreatment with phenobarbital, and *p*-dichlorobenzene was not hepatotoxic, even to the pretreated animals.

Health Effects

Observations in Humans

A single case of aplastic anemia allegedly occurred following occupational exposure to *p*-dichlorobenzene and naphthalene (Harden and Baetjer, 1978).

Observations in Other Species

Acute Effects The single dose LD_{50} in Wistar rats was reported to be 2.5 ± 0.01 g/kg (Zupko and Edwards, 1949). In another study, *p*-dichlorobenzene administered to rats in a single oral dose decreased hexobarbital-induced sleeping time (Carlson and Tardiff, 1976). In short-term studies with male rats given oral doses of *p*-dichlorobenzene generally ranging from 250 to 770 mg/kg/day, urinary excretion of coproporphyrin was increased severalfold, uroporphyrin and porphobilinogen slightly increased, and δ-aminolevulinic acid moderately increased (Rimington and Ziegler, 1963). In male Holtzman Sprague-Dawley rats, *p*-dichlorobenzene did not increase bile duct-pancreatic fluid flow following intraperitoneal injection at a dose of 1.4 mM bw (Yang *et al*., 1979).

Chronic Effects When *p*-dichlorobenzene was administered to male rats for 90 days at doses of 0, 10, 20, and 40 mg/kg bw, it significantly increased ethyl *p*-nitrophenyl phenylphosphonothionate (EPN) detoxification, benzo(a) pyrene hydroxylase, and azoreductase (Carlson and Tardiff, 1976); however, the chemical was not hepatotoxic. These results indicated that the chemical enhanced metabolism of foreign compounds. Oral doses of *p*-dichlorobenzene given to female rats at doses of 50, 100, or 200 mg/kg daily for as long as 120 days produced significant dose-dependent increases in liver weight and liver porphyrin content, but they did not affect urinary excretion of porphyrins (Carlson, 1977). Rimington and Ziegler

(1963) gave *p*-dichlorobenzene to male albino rats by gastric intubation for as long as 41 days. Urinary coproporphyrin, uroporphyrin, porphobilinogen, and δ-aminolevulic acid were all significantly increased over controls after only 5 days at a dose of 770 mg/kg. There was no change in hepatic glutathione content.

Mutagenicity No mutagenicity data were found by the committee.

Carcinogenicity No additional data on the carcinogenicity of this compound have become available since its last review in *Drinking Water and Health* (National Research Council, 1977). In June 1980, the National Cancer Institute/National Toxicology Program (1982a) initiated a carcinogenesis bioassay of *p*-dichlorobenzene in B6C3F_1 mice and Fischer 344 rats. Dosages of 200 or 600 mg/kg bw were administered by gavage to both sexes of the mice and to the female rats. Male rats were given 150 or 300 mg/kg. The *p*-dichlorobenzene was administered in corn oil to groups of 50 rats and 50 mice of each sex 5 days/week for 2 years. There were also corresponding vehicle and untreated control groups of 50 rats and 50 mice of each sex.

Histopathologic findings of the study were not available at the time of this review.

Teratogenicity No data were found by the committee.

Conclusions and Recommendations

In the 1977 review of this chemical, the Safe Drinking Water Committee calculated an acceptable daily intake (ADI) of 0.0134 mg/kg/day, based on the data available at that time. This resulted in the calculation of a suggested no-adverse-response level of 0.094 mg/liter. This value continues to be the recommended SNARL for chronic exposure. However, because this chemical is being tested as a possible carcinogen, the SNARL should be reviewed when the bioassay is complete. The other basic conclusions and recommendations contained in the earlier volume are still valid for this chemical.

Because large quantities of this chemical are used, studies should be conducted to determine its reproductive effects, teratogenicity, and mutagenicity, especially from oral exposures. Acute and subchronic toxicity tests emphasizing potential hepatic toxic responses would also be desirable. Any need for further chronic tests should be assessed after the results of the carcinogenesis assay have been published.

1,2-DICHLOROETHANE

ethylene dichloride; ethane, 1,2-dichloro-
CAS No. 107-06-2

$$ClH_2C–CH_2Cl$$

1,2-Dichloroethane was evaluated in the first and third volumes of *Drinking Water and Health* (National Research Council, 1977, pp. 723-725; 1980, p. 104). Because of its commercial availability as an intermediate in the production of vinyl chloride and 1,1,1-trichloroethane, it merits continued surveillance. In the United States approximately 10 billion pounds are consumed annually, and some 2 million workers are exposed to the chemical (Fishbein, 1979). Ten percent of those are exposed to high concentrations of 1,2-dichloroethane when used as a solvent in textile cleaning, metal degreasing, and adhesives. At least 45 fumigant formulations are now in use, and 36 U.S. gasoline formulations contain 1,2-dichloroethane as an additive (Fishbein, 1979). Consequently, it is likely to enter the water supply from a variety of activities.

Drury and Hammons (1979) surveyed a number of reports on the occurrence of 1,2-dichloroethane in water. They concluded that its presence in municipal drinking water is infrequent, probably because it is relatively volatile and unstable in the atmosphere through photooxidation (Drury and Hammons, 1979).

The following material, which became available after the 1980 report was prepared, updates and, in some instances, reevaluates the information in the previous reviews. Also included are some references not discussed in the earlier reports.

Metabolism

Significant progress has been made in our understanding of the metabolism of 1,2-dichloroethane (Anders and Livesey, 1980). The metabolic pathways that account for the known metabolites of this compound and other 1,2-dihaloethanes are shown in Figure II-1.

1,2-Dichloroethane is metabolized to ethylene by cytosolic hepatic enzymes dependent upon glutathione. Most likely the same enzymes involved in the metabolism of halogenated methanes also participate in the conversion of 1,2-dichloroethane to ethylene. The enzymatic formation of *S*-(2-chloroethyl) glutathione probably involves glutathione *S*-transferase (Anders and Livesey, 1980), based on similarities reported for this enzyme by Jakoby *et al.* (1976). Anders and Livesey (1980) theorized that 1,2-dichlo

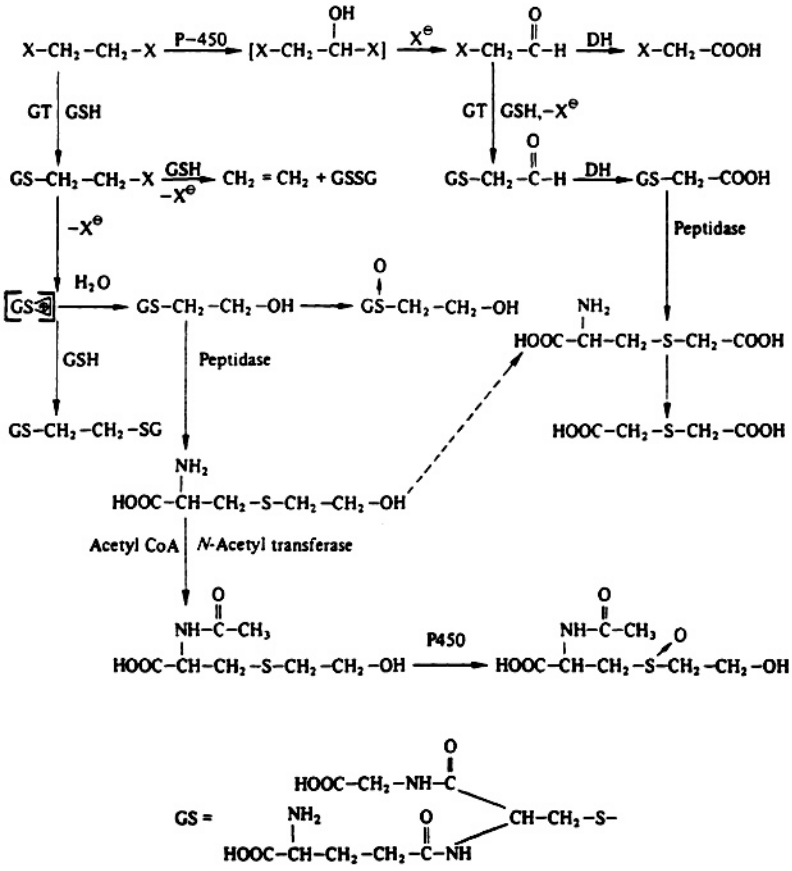


FIGURE II-1
Metabolic pathways of 1,2-dichloroethane and other 1,2-dihaloethanes.
GT = reduced glutathione (GSH) transferase; DH = dehydrogenase;
P450 = cytochrome P450; X = a halogen; and CoA = coenzyme A.
From Anders and Livesey, 1980, with permission.

roethane is metabolized through an initial enzyme-catalyzed nucleophilic (S_N2, i.e., bimolecular) attack of glutathione on the electron-deficient carbon of 1,2-dichloroethane to form *S*-(2-chloroethyl)-glutathione. A subsequent reaction of glutathione with the sulfur atom of *S*-(2-chloroethyl)-glutathione is followed by -elimination of halide to form ethylene. Loss of chloride ion from *S*-(2-chloroethyl)-GSH results in the formation of an episulfonium ion, which is highly reactive. This ion is believed to be the reac

tive intermediate that results in covalent reaction with biopolymers (Anders and Livesey, 1980).

Sipes and Gandolfi (1980) reported the covalent reaction of 1,2-dichloroethane with proteins, lipid, and DNA *in vitro* following activation by rat hepatic microsomes supplied with a system generating reduced nicotinamide adenine dinucleotide phosphate (NADPH). Oxygen was required for a covalent reaction with 1,2-dichloroethane, which was one of the least reactive aliphatic halogenated hydrocarbons tested. Since microsomes free of cytosolic enzymes were used in this study, the reaction measured by this assay was probably the formation of chloracetaldehyde, rather than the pathway leading to ethylene via the episulfonium ion. Glutathione greatly inhibited the covalent reaction.

We do not yet know which pathway is most important in determining the metabolic events that lead to the toxicity of 1,2-dichloroethane. The mutagenic activity of 1,2-dichloroethane is probably associated with the formation of the episulfonium ion (Anders and Livesey, 1980).

Observations in Humans

No new data were found by the committee.

Observations in Other Species

New data found by the committee are summarized below.

Mutagenicity 1,2-Dichloroethane was found to be nonmutagenic in the standard Ames *Salmonella* plate incorporation assay, but was reported to be mutagenic when the plates were placed in desiccators at doses of 3,6, and 9 mg/plate. Mutagenicity was detected with strains TA1535 (2-fold increase) and TA100 (20-fold increase), both in the presence and absence of an Aroclor 1254-induced rat liver S9 metabolic activation system (Nestmann *et al*., 1980; Stolzenberg and Hine, 1980). Isolated, perfused liver from male Wistar rats was used as a metabolizing system to study the mutagenicity of 1,2-dichloroethane in the *Salmonella* assay. At the beginning of the test and 90 minutes later, 360 μM of 1,2-dichloroethane was added to the perfused liver. Within 15 to 30 minutes, the extracted bile, which was diluted 10-fold, induced a strong mutagenic effect in strain TA1535 (800 and 600 colonies after 0 and 90 minutes, respectively). The perfusate produced only slight increases in the number of revertants in TA1535. A weak response was obtained with undiluted bile after the addition of only 24 μM of 1,2-dichloroethane to the perfusion system obtained from male Wistar and Sprague-Dawley rats (90 and 70 colonies, respectively). *In vivo*

experiments with CBA mice given a single intraperitoneal 80 mg/kg injection of 1,2-dichloroethane resulted in the production of bile that, after a 10-fold dilution, gave rise to approximately double the number of spontaneously produced revertant colonies in TA1535 (Rannug and Beije, 1979). Negative results were obtained in a micronucleus test in CBA male mice at a concentration of 100 mg/kg bw after 30 hours of exposure (Jenssen and Ramel, 1980).

In summary, the data suggest that 1,2-dichloroethane is mutagenic in microbial mutagenicity assays.

Carcinogenicity Van Duuren *et al*. (1979) examined the carcinogenicity of 1,2-dichloroethane by the mouse skin bioassay. When 126 mg of 1,2-dichloroethane was administered once dermally to 30 female Sch:HA(ICR) Swiss mice, followed by 5 μg of phorbol myristate acetate (PMA) 14 days later, all the mice had papillomas after 357 days. However, the authors attributed little significance to this result, which was supported by the failure of repeated applications of 1,2-dichloroethane to induce skin tumors. When 126 mg of 1,2-dichloroethane in 0.2 ml of acetone was administered topically for 440 to 594 days to 30 female mice, 26 mice developed lung tumors ($P < .0005$), and three had stomach tumors. The authors did not consider the papillomas significant in the two-stage skin bioassay, noting that no skin tumors appeared after repeated skin exposure. However, the lung tumors observed after repeated skin applications were regarded as significant.

Teratogenicity Rao *et al*. (1980) exposed rats to vapor at 100 and 300 ppm (409 and 1,227 mg/m^3) for 7 hours daily during days 6 to 15 of gestation. Ten of the 16 rats exposed to 300 ppm died, and only one rat with an implanted pregnancy had total resorption. At 100 ppm, there was neither increased resorption nor decreased fetal weight. Lane *et al*. (1982) administered 1,2-dichloroethane at concentrations up to 1,000 mg/kg/day in the drinking water of mice and found no adverse reproductive effects.

In studies of maternal exposures to 1,2-dichloroethane, Vozovaya (1976) and Vozovaya and Malyarova (1975) reported preimplantation reproductive failure and accumulation of the compound in fetal rat liver.

The limited studies available for review indicate that 1,2-dichloroethane is not teratogenic to mice or rats.

Conclusions and Recommendations

The information that has become available since the 1980 review of 1,2-dichloroethane in *Drinking Water and Health* does not permit any more realistic assessment of no-adverse-response levels than the data previously

assessed. The additional studies recommended in the last review are still needed.

Despite extensive knowledge concerning the metabolism of 1,2-dichloroethane, there is still a need to know the comparative pharmacokinetics in different species, including humans. The knowledge that 1,2-dichloroethane has been shown to have both mutagenic and carcinogenic properties in animals provides further reason to obtain these kinds of data following oral exposure in order to develop better estimates of the risk to humans exposed to low levels.

1,1-DICHLOROETHYLENE

ethene, 1,1-dichloro-
CAS No. 75-35-4
$H_2C = CCl_2$

1,1-Dichloroethylene is commonly called vinylidene chloride. This substance is used primarily as an intermediate in the synthesis of copolymers slated for food-packaging films and coatings. It is also used in the synthesis of 1,1,1-trichloroethane. This volatile liquid has a boiling point of 31.7°C, and it is practically insoluble in water, having a solubility of only 0.04 w/v (400 mg/liter) at 20°C (Hardie, 1964). It has been found in drinking water in concentrations as high as 0.1 μg/liter (U.S. Environmental Protection Agency, 1975b). In 1976, 120 million kg were produced in the United States; 50 million kg were used as an unisolated intermediate (International Agency for Research on Cancer, 1979a). The threshold limit value is 10 ppm (40 mg/m^3) (American Conference of Governmental Industrial Hygienists, 1981).

Metabolism

Orally administered 1,1-dichloroethylene is rapidly and completely absorbed in rats (Jones and Hathway, 1978a; Putcha *et al*., 1982; Reichart *et al*., 1979). Following distribution, the highest concentrations are found primarily in the liver and kidneys (Jones and Hathway, 1978a,b; McKenna *et al*., 1978). At low doses a major route of elimination is exhalation of the parent compound (Chieco *et al*., 1981; Jones and Hathway, 1978b; Reichert *et al*., 1979). The exhaled metabolic product is carbon dioxide. At higher doses, the parent compound predominates (Jones and Hathway, 1978a,b; McKenna *et al*., 1978).

The compound is metabolized to a number of metabolites, presumably

through the formation of an epoxide (Greim *et al*., 1975; Henschler, 1977; Henschler and Bonse, 1978; Leibman and Ortiz, 1977). Jones and Hathway (1978a) reported that 80% of an intragastrically administered 0.5 mg/kg dose appeared as urinary metabolites, whereas only 33% of a 350 mg/kg dose was metabolized. At the higher dose, 37% of the urinary metabolites were identified as thiodiglycolic acid and 48% as an *N*-acetyl-*S*-cysteinyl derivative.

McKenna *et al*. (1978) observed that 78% of a single oral 1 mg/kg dose was accounted for in metabolites found in the urine and feces, that 21% was exhaled as carbon dioxide, and that from 1% to 3% was exhaled as unchanged 1,1-dichloroethylene. After the administration of a 50 mg/kg dose, 19% was exhaled unchanged by fed rats and 29% by fasted rats. The two major urinary metabolites excreted within 25 hours following the administration of 1 mg/kg to fed rats were identified as thiodiglycolic acid (22% of the metabolites) and *S*-(2-hydroxyethyl)-*N*-acetylcysteine (13% of the metabolites). Reichert *et al*. (1979) observed dose-dependent metabolism: 1.3% was exhaled unchanged following an oral dose of 0.5 mg/kg, but 16.5% was exhaled after 50 mg/kg. Renal excretion was approximately 43% in both cases, but biliary excretion amounted to 16% after the low dose and 8% after the high dose. Thiodiglycolic acid was the main urinary metabolite, accounting for approximately 40%. Other metabolites, including chloroacetic acid, dithioglycolic acid, thioglycolic acid (Jones and Hathway, 1978a), *N*-acetyl-*S*-(2-carboxymethyl) cysteine, and methylthio-acetylaminoethanol (Reichert *et al*., 1979) have also been identified in rat urine. In *in vitro* studies with rat liver microsomes, monochloroacetate and dichloroacetaldehyde were identified as metabolites (Costa and Ivanetich, 1982a,b). Mice given an oral dose of 50 mg/kg metabolized the compound to a greater extent than did rats; they exhaled 6% unchanged in contrast to the 28% exhaled by rats (Jones and Hathway, 1978b). Dithioglycolic acid (23% of the dose) and the *N*-acetyl-*S*-cysteinyl acetyl derivative (50% of dose) were the primary metabolites.

Health Aspects

Observations in Humans

No definitive studies in humans have been reported. Early studies of workers exposed to traces of 1,1-dichloroethylene cannot readily be evaluated since the major compound to which the workers were exposed was vinyl chloride (Kramer and Mutchler, 1972). Ott *et al*. (1976) examined the health of 138 employees exposed to 1,1-dichloroethylene where vinyl chloride was not a copolymer. Time-weighted averages for exposure ranged

from less than 10 ppm to greater than 50 ppm (40-198 mg/m^3). The investigators observed no alterations in results of clinical laboratory tests, including hematological tests, urinalyses, and tests for respiratory functions, performed on these workers. There are no data on long-term exposures of large numbers of human beings.

Observations in Other Species

Acute Effects The most commonly reported toxic effect of 1,1-dichloroethylene is hepatotoxicity. Jenkins *et al.* (1972) reported that a single oral 100 mg/kg dose of 1,1-dichloroethylene in corn oil given to rats caused a decrease in liver glucose-6-phosphatase and an increase in alkaline phosphatase activity but did not cause elevations in plasma alkaline phosphatase and alanine aminotransferase. These plasma enzymes were elevated following doses of 300 mg/kg and 500 mg/kg.

Establishing a dose-response relationship for the oral lethality of 1,1-dichloroethylene in rats has presented some difficulties (Andersen *et al*., 1979). For large (> 200 g) male rats, the dose-response reached a plateau at about 400 mg/kg. Immature rats (73 g) were more sensitive: there was an almost 100% lethality at doses of 200 mg/kg, but there was an apparent decrease in lethality when dose levels greater than 200 mg/kg of the compound were administered. Smaller rats also responded with a greater increase in hepatotoxicity, as assessed by serum enzymes following a 50 mg/kg dose of 1,1-dichloroethylene in corn oil (Andersen and Jenkins, 1977). Plasma aspartate aminotransferase in these animals (100-110 g) was elevated at a dose of 25 mg/kg but not at 12.5 mg/kg. In one study, female rats were observed to be less sensitive to injury than were males (Andersen and Jenkins, 1977), but in another, female rats were reported to be more sensitive (Jenkins *et al*., 1972).

A confounding factor in the studies of 1,1-dichloroethylene has been the influence of fasting prior to administration of the compound. Jaeger *et al.* (1974) exposed rats by inhalation to various concentrations of 1,1-dichloroethylene and estimated that the minimum lethal concentration was 200 ppm (794 mg/m^3) for fasted rats and 10,000 ppm (39,700 mg/m ^{3}) for fed rats. Serum alanine aminotransferase levels were elevated at 150 ppm (595 mg/m^3) in fasted rats, but a level of at least 2,000 ppm (7,940 mg/m^3) was needed for the same effect in fed rats. This striking difference was related to the effect of fasting on hepatic glutathione concentrations. The importance of fasting was also observed histologically (Reynolds and Moslen, 1977; Reynolds *et al*., 1975). Hepatic lesions occurred earlier and were more extensive in fasted rats exposed to 200 ppm (794 mg/m^3) of 1,1-

dichloroethylene for 4 hours than were observed in fed rats subjected to the same exposures. These lesions included nuclear changes, swollen and ruptured mitochondria, and midzonal necrosis.

Other effects have been reported. Harms *et al*. (1976) observed in rats that a 0.5 ml/kg dose administered intraperitoneally caused an increase in bile-duct pancreatic fluid flow but had little or no influence on hepatic bile flow. Jenkins and Andersen (1978) reported the occurrence of nephrotoxicity in rats, based on elevations in blood urea nitrogen (BUN), which generally parallel hepatotoxic responses. The doses needed to elicit this response were higher than those needed to induce hepatotoxicity; a dose of 400 mg/kg caused elevations in BUN in fasted but not in fed rats.

Chronic Effects Prendergast *et al*. (1967) exposed rats, guinea pigs, dogs, rabbits, and monkeys by inhalation to 5, 15, 20, and 45 ppm (20, 60, 80, and 180 mg/m^3) 1,1-dichloroethylene for 90 days. At the highest level, 7 of 15 guinea pigs and 3 of 9 monkeys died. Dogs and monkeys lost weight, and rats gained less weight than did the controls. In the livers, there was fatty metamorphosis, focal necrosis, hemosiderin deposition, bile duct proliferation, and fibrosis. These signs were most severe in the dog. The morphologic changes were accompanied by elevations in liver alkaline phosphatase and serum aspartate aminotransferase, but serum urea nitrogen levels were not elevated. In the rat kidneys there was nuclear hypertrophy of the tubular epithelium. At 20 ppm, weight losses were observed in rabbits, dogs, and monkeys, and 3 of 15 guinea pigs and 2 of 3 monkeys died, but no defined histopathological changes were observed. At 15 ppm, 3 of 5 guinea pigs died, and the monkeys and rats had poor weight gain. At 5 ppm, 2 of 45 rats, 2 of 45 guinea pigs, and 1 of 21 monkeys died, but no signs of toxicity were observed. No histopathological lesions were found in the liver, and serum enzyme levels were normal. In the control group, 7 of 304 rats, 2 of 314 guinea pigs, none of 34 dogs, and 1 of 57 monkeys died. Quast *et al*. (1977) exposed male and female Sprague-Dawley rats to 60, 100, or 200 ppm (6, 10, or 19 mg/kg) daily for 90 days. No changes were observed in body weight gain, hematological indices, urine analysis, BUN, serum alkaline phosphatase, serum alanine aminotransferase, gross pathology, or organ weights. Hepatocellular vacuolization was seen in the animals receiving 200 ppm. Norris (1977) reported that no adverse effects were observed in dogs given 1,1-dichloroethylene in doses of 6.25, 12.5, or 25 mg/kg for 90 days. This assessment was based on clinical observation, body weights, and gross and histopathological examination.

Rampy *et al*. (1977) exposed male and female Sprague-Dawley rats to 1,1-dichloroethylene at concentrations of 68, 106, and 220 mg/liter in drinking water. They examined the groups at 6, 12, 18, and 24 months of

exposure. There were no consistent changes in body weight gain, hematological indices, urinalysis, and nonprotein sulfhydryl groups in the liver and kidneys. There was slightly higher mortality than in the controls for the males in all three treatment groups, but this was not related to dose and was not observed in the females. No differences were observed in organ weights except for a decreased kidney to body weight ratio in the males at the lowest dose levels at the 90-day sacrifice. The only histopathological change noted was a vacuolization of hepatocytes at the 220 mg/liter exposure.

1,1-Dichloroethylene was administered by gavage in corn oil to Fischer 344 rats and B6C3F_1 mice 5 times per week for 13 weeks at levels of 0, 5, 15, 40, 100, or 250 mg/kg bw (National Toxicology Program, 1982b). Centrilobular necrosis was observed in three rats, which died at the highest level of exposure. Cellular atypia, characterized as altered nuclear/cytoplasmic ratios, megalocytosis, basophilia, clear cells, and an increase in binucleated cells, was seen in the rats given the two highest dose levels. Foci of cellular alteration were observed in rats receiving the 5 and 15 mg/kg doses, but not in those at the higher doses. In the mice, there was considerable mortality at the higher doses, and a dose-related decrease in body weight was observed in the males. Dose-related hepatic lesions were observed but were minimal at 5 mg/kg.

In the same study, 1,1-dichloroethylene was administered for 104 weeks by gavage in corn oil at levels of 1 or 5 mg/kg bw (to rats) and 2 or 10 mg/kg bw (to mice) (National Toxicology Program, 1982b). There were no adverse effects on body weight or survival of the rats, and no compound-related clinical signs were observed. The only lesion of possible significance reported was a dose-dependent increase in chronic inflammation of the kidney, but this lesion was also present in the controls. In mice, the body weights of the treated males were lower than those of the controls, but in both males and females there were no clinical signs of toxicity or significant differences in survival.

Mutagenicity 1,1-Dichloroethylene was found to be mutagenic in the Ames *Salmonella* assay by the following investigators: Bartsch *et al*. (1975, 1979), Jones and Hathway (1978c), and Simmon *et al*. (1977). These assays were performed in desiccators. A variety of mammalian metabolic activation systems elicited or enhanced mutations in one or more of the *Salmonella* base-pair substitution mutants. Activating systems were: uninduced kidney and liver S9 fractions from male albino mice; Aroclor 1254-induced kidney and liver S9 fractions from male Sprague-Dawley rats; a presumed phenobarbital-induced S9 liver fraction from humans; phenobarbital-induced liver S9 fractions and phenobarbital-induced and uninduced kidney and lung S9 fractions from OF-1 mice; and uninduced S9 fractions from

the liver, kidney, and lung of female BD VI rats. Preparations that failed to induce mutations were uninduced liver S9 fractions from marmosets, humans, and male rats.

1,1-Dichloroethylene did not induce chromosome breakage in Chinese hamster DON-6 cells when tested at concentrations of 3×10^{-3} and 3×10^{-2} mM. It could not be determined from the data whether or not the compound induced toxic effects (Sasaki *et al*., 1980). No increase in 8-azaguanine-and ouabain-resistant colonies was observed when Chinese hamster V79 cells were exposed in desiccators to 2% and 10% 1,1-dichloroethylene vapor in air for 5 hours (Drevon and Kuroki, 1979).

Anderson *et al*. (1977) exposed male mice by inhalation to 50 ppm (198 mg/m^3) for 6 hours per day for 5 days prior to mating and found no evidence of dominant lethality. Short *et al*. (1977) reported similar findings in rats.

Mutagenic activity was observed in the multiple end point *E. coli* K12 strain when assayed in suspension at a concentration of 2.5 mM in the presence only of liver S9 fraction derived from phenobarbital-pretreated male mice (Greim *et al*., 1975). Both point mutation and mitotic gene conversion were observed in *Saccharomyces cerevisiae* D7 in the presence of liver S10 fraction obtained from male mice pretreated with Aroclor 1254 (Bronzetti *et al*., 1981). They also observed mutagenic activity in a mouse host-mediated assay with *S. cerevisiae* D7 after the animals were gavaged with 1,1-dichloroethylene in corn oil.

The data indicate that 1,1-dichloroethylene is mutagenic in several microbial mutagenicity assays and in a mouse host-mediated microbial assay. Negative results were obtained in an *in vitro* mammalian mutagenicity assay and in a mouse dominant lethal study. No chromosome breakage was observed in an *in vitro* study with Chinese hamster cells.

Carcinogenicity Evidence for a carcinogenic effect of 1,1-dichloroethylene was obtained in studies of mice (Maltoni, 1977). Of the 300 male and female Swiss mice exposed to 25 ppm (100 mg/m^3) of the compound 4 hours/day, 5 days/week for 52 weeks, 25 (8.3%) had developed kidney adenocarcinomas at the end of the experiment (98 weeks). No such tumors were found in controls. No tumors were found in rats or hamsters exposed to higher concentrations of 1,1-dichloroethylene for the same length of time.

In another study, mice and rats were exposed to 1,1-dichloroethylene at 55 ppm (220 mg/m^3), 6 hours/day, 5 days/week. The experiment was terminated after 12 months (Lee *et al*., 1978). Among the 70 male and female exposed mice, six bronchial lung tumors were found; the earliest of the tumors appeared between 4 and 6 months, and the latest after 12 months.

Three of the 70 mice had hepatic hemangiosarcomas. Because of the small number of tumors and the low incidence, it was questionable whether these findings were related to 1,1-dichloroethylene exposure. In 2 of 35 male rats, hemangiosarcomas were found in the mesenteric lymph nodes or in subcutaneous tissues. Despite the low incidence, the investigators believed that these tumors were related to the 1,1-dichlorethylene.

1,1-Dichloroethylene was found to produce skin tumors when applied once to mouse skin, followed by repeated treatments with phorbol myristate acetate. When given alone, it did not produce tumors when applied topically or by subcutaneous injection. Thus, 1,1-dichloroethylene can be regarded as an initiating agent for mouse skin (Van Duuren *et al*., 1979).

Several studies failed to demonstrate significant carcinogenic activity of 1,1-dichloroethylene. In a study by Viola and Caputo (1977), Wistar rats were exposed to 1,1-dichloroethylene by inhalation to 200 ppm (792 mg/m^3) for 5 months. The exposure was then reduced to 100 ppm (396 mg/m^3). Both exposures were administered 4 hours/day, 5 days/week. The second exposure was continued for the lifetime of the animals. There was no difference in the incidence of tumors between the exposed and control rats. The experiment was repeated with Sprague-Dawley rats, which were exposed to 1,1-dichloroethylene in concentrations of 100 or 75 ppm (396 or 297 mg/m^3). At the time of reporting, Viola and Caputo (1977) could not conclude that 1,1-dichloroethylene was carcinogenic. Male and female Sprague-Dawley rats were also exposed for their lifetime to concentrations of 1,1-dichloroethylene ranging from 60 to 200 mg/liter in drinking water. The authors reported no increase in tumor incidence, a conclusion based on gross tumor count only. The same laboratory confirmed that exposure to 1,1-dichloroethylene at 10 to 40 ppm (40-160 mg/m^3) in air (6 hours/day, 5 day/week for 18 months) was not carcinogenic to Sprague-Dawley rats (Rampy *et al*., 1977).

1,1-Dichloroethylene monomer was dissolved in olive oil and given orally to 24 pregnant female BD IV rats at a dose of 150 mg/kg on the 17th day of gestation. Their offspring (89 males and 90 females) then received weekly 50 mg/kg doses of 1,1-dichloroethylene, which was administered in olive oil by stomach tube. Animals were killed when moribund, and approximately one-half of the animals survived for 130 weeks. In the treated animals there was an increased incidence of liver tumors. In treated males there were more meningiomas than in the controls. However, the total number of tumor-bearing animals was the same for exposed animals and controls. There was some question whether the dose used in this experiment was the maximum tolerated dose (Ponomarkov and Tomatis, 1980).

A similar problem was encountered in a study commissioned by the National Toxicology Program (1982b). In a 104-week study, Fischer 344 rats were given 1 or 5 mg/kg doses of 1,1-dichloroethylene 5 times per week;

$B6C3F_1$ mice received 2 and 10 mg/kg 5 times per week. In this bioassay, there was no evidence that 1,1-dichloroethylene was carcinogenic for either the rats or the mice. However, there was some doubt as to whether a maximum tolerated dose had been used in this study.

To date, there has been no evidence that 1,1-dichloroethylene is carcinogenic in humans (Infante, 1977). However, the only epidemiological study found by the committee was based on a small population of 139 people exposed to measured levels of 1,1-dichloroethylene where vinyl chloride was not used as a copolymer (Ott *et al*., 1976). A more recent analysis of the data did not enable investigators to draw definite conclusions (Apfeldorf and Infante, 1981).

The committee's review indicated that the information on 1,1-dichloroethylene is not sufficient for a definite conclusion to be reached about its ability to induce cancer in laboratory animals or humans.

Teratogenicity Murray *et al*. (1979) subjected rats and rabbits to inhalation exposures of 1,1-dichloroethylene in concentrations as high as 160 ppm (635 mg/m^3) for 7-hour periods during days 6 through 18 and 6 through 15 of gestation, respectively. Maternal toxicity was observed in both species. No increased general malformation rate was found, but wavy ribs and delayed ossification were more frequent in rat fetuses exposed to 1,1-dichloroethylene at 80 and 160 ppm. Alumot *et al*. (1976) fed 1,1-dichloroethylene to pregnant rats at 200 ppm in their diets on days 6 through 15 of gestation. They observed no differences between the exposed fetuses and controls. The same investigators also fed rats doses as high as 500 ppm in feed over a 2-year period and found no alteration in fetal mortality or weight when litters were examined after birth. Approximately 60% to 70% of the substance was estimated to have been consumed.

1,1-Dichloroethylene was reported to be nonteratogenic when given to rats in drinking water at 200 mg/liter or when administered by inhalation to rats and rabbits at 0, 20, 80, or 160 ppm (0, 80, 240, or 580 mg/m^3) for 7 hours/day on days 6 to 15 of gestation in rats and days 6 to 18 of gestation in rabbits (Norris, 1977).

The data indicate that 1,1-dichlorethylene is not teratogenic to mice, rats, or rabbits. There are no data from which its effects in humans can be judged.

Conclusions and Recommendations

Suggested No-Adverse-Response Level (SNARL)

Chronic Exposure The following calculation for a chronic SNARL *is based on noncarcinogenic effects only*. Rampy *et al*. (1977) administered

1,1-dichloroethylene in the drinking water of male and female rats for as long as 2 years. At 220 mg/liter, but not at 106 ppm, vacuolization of the hepatocytes was observed. At 106 mg/liter, the mean daily consumption for males was 10.0 mg/kg and for females it was 12.6 mg/kg. However, a carcinogenesis bioassay of 1,1-dichloroethylene indicated that 10 mg/kg causes slight hepatotoxicity in mice (National Toxicology Program, 1982b). This was not observed at the 2 mg/kg dose. An uncertainty factor of 100 was assumed on the basis of the above study. Assuming that a 70-kg human consumes 2 liters of water daily, that 20% of the exposure of most individuals would be from drinking water, and a factor of 5/7 to correct from a 5- to 7-day weekly exposure, one may calculate the SNARL as:

$$\frac{2 \text{ mg/kg} \times 70 \text{ kg} \times 0.2}{100 \times 2 \text{ liters}} \times \frac{5}{7} = 0.10 \text{ mg/liter.}$$

If present in drinking water, 1,1-dichloroethylene would be rapidly and completely absorbed. At the low levels expected to be present, dose-dependent kinetics involving saturation of the metabolic pathways would not occur, so that nearly all of the compound would undergo metabolic conversion. Studies in animals suggest that the principal target organs in humans would be the liver and, to a lesser extent, the kidney.

1,2-DICHLOROETHYLENE

***cis* (ethene, 1,2-dichloro-(Z)-**
CAS No. 156-59-2
***trans* (ethene, 1,2-dichloro-(E)-**
CAS No. 156-60-5
ClHC = CHCl

1,2-Dichloroethylene exists in both the *cis* and *trans* forms. Both of these compounds are used, alone and in combination, as solvents and chemical intermediates. Their solubility in water is low. The solubility of *cis*-1,2-dichloroethylene is 0.35 g/100 g (3,500 mg/liter) and that of *trans*-1,2-dichloroethylene is 0.63 g/100 g (6,300 mg/liter) at 25°C (Hardie, 1964). Both isomers have been found in drinking water (U.S. Environmental Protection Agency, 1975b). The maximum concentrations found were 16 μg/liter for the *cis* isomer and 1.0 μg/liter for the isomer.

Metabolism

Using an isolated, perfused rat liver preparation, Bonse *et al*. (1975) found that both isomers were metabolized to the same metabolites—dichlo

roacetic acid and dichloroethanol. In this system, the *cis* isomer was metabolized to a greater extent than was the *trans* isomer. These metabolites are apparently formed via an epoxide intermediate. In studies of rats exposed to 1,2-dichloroethylene vapor, *trans*-1,2-dichloroethylene was found to be metabolized more slowly than was the *cis* isomer (Filser and Bolt, 1979). Studies with rat liver microsomes demonstrated a Type 1 difference spectra with these substances, implicating the involvement of cytochrome P450 (Costa and Ivanetich, 1982b).

Health Aspects

Observations in Humans

No data were found by the committee.

Observations in Other Species

Acute Effects The liver is the primary target for 1,2-dichloroethylene toxicity at doses that do not cause narcotic effects (i.e., between 1% to 2% in air). In male rats given single oral 400 mg/kg doses of either the *cis* or *trans* isomers, there were only slight changes in liver alkaline phosphatase and liver glucose-6-phosphatase, respectively (Jenkins *et al*., 1972). At 1,500 mg/kg, the *trans* isomer caused only a decrease in liver tyrosine transaminase, whereas the *cis* isomer caused decreases in liver glucose-6-phosphatase, liver tyrosine transaminase, and plasma glutamic-pyruvic transaminase (alanine aminotransferase), along with an increase in liver alkaline phosphatase. Using an isolated, perfused liver system, Bonse *et al*. (1975) found that both compounds, after a 2- to 3-hour perfusion period at a concentration of 55 μM, caused increases in glutamic-pyruvic transaminase, in glutamic-oxaloacetic transaminase (aspartate aminotransferase), and in the ratio of lactate to pyruvate in the perfusate.

An extensive study on *trans*-1,2-dichloroethylene indicated that the compound's LD_{50} when administered intraperitoneally was 3.2 ml/kg in the mouse and 6.0 ml/kg in the rat (Freundt *et al*., 1977). The *trans* isomer caused a greater lethality when given orally: the oral LD_{50} in the rat was 1.0 ml/kg. Inhalation exposure of rats for 8 hours to *trans*-1,2-dichloroethylene at 1,000 ppm (3,967 mg/m^3) resulted in reductions in serum albumin, urea nitrogen, and alkaline phosphatase, but these effects were not observed at 200 ppm (793 mg/m^3). Both the leukocyte count and erythrocyte count were lowered at the higher level, but only the leukocyte count was diminished at the lower exposure. Bromsulfophthalein clearance was not affected. After a single inhalation exposure to 200, 1,000, and 3,000

ppm (793, 3,967, and 11,901 mg/m^3) for 8 hours, there was slight fatty degeneration of the liver lobules in one of six rats exposed to 200 ppm (793 mg/m^3). Pulmonary capillary hyperemia and distention of the alveolar septa were increased. At 1,000 ppm (3,965 mg/m^3) two of six rats had fatty degeneration of the liver. The damage was slight in one animal and severe in the other. Changes in the lung were observed in five of the rats. The damage was slight in three rats and severe in two. There was no histopathological indication of damage to the kidneys, spleen, brain, striated muscle, or peripheral nerves. Cardiac muscle damage was observed at 3,000 ppm.

In an additional study, female rats exposed by inhalation to 200 ppm concentrations of both the *cis* and *trans* isomers for 8 hours exhibited increases in hexobarbital-induced sleeping times and zoxazolamine-induced paralysis times and decreased metabolism of aminopyrine (Freundt and Macholz, 1978). These effects were shown to be dose dependent and reversible. Those produced by the *cis* isomer were greater than those of the *trans* isomer. This mixed-function oxidase inhibition was shown by *in vitro* studies using various concentrations of the *trans* isomer to be competitive, as indicated by a Dixon plot.

Nephrotoxicity has been observed in Swiss mice following intraperitoneal exposures to high doses (2 to 4 ml/kg) of both isomers. This effect was demonstrated by an increase in urinary protein. Unlike observations made in studies of 1,1,2-trichloroethane (0.2 ml/kg) or 1,1,2,2-tetrachloroethane (1 ml/kg), the changes were not accompanied by an increase in urinary glucose or by histopathological evidence of damage to the proximal convoluted tubules (Plaa and Larson, 1965). In both the kidney and the liver, the *cis* isomer was more toxic than the *trans* isomer.

Chronic Effects In the study by Fruendt *et al*. (1977), minimal histopathological liver damage was observed after inhalation exposures to 200 ppm (793 mg/m^3) for 8 hours. However, after exposure to this level for 5 days/week for 16 weeks, fatty degeneration was observed in the livers of five of six rats, and slight pulmonary capillary hyperemia and alveolar septum distention were observed in all six animals.

Mutagenicity *cis*-1,2-Dichloroethylene at 2.9 mM and *trans*-1,2-dichloroethylene at 2.3 mM concentrations were found to be nonmutagenic when tested in a suspension assay with the multiple end point *E. coli* K12 strain, which detects reverse mutations at the *gal*, *nad*, and *arg* loci and forward mutation to resistance to 5-methyl-DL-tryptophan. The assay was performed both in the presence and absence of a phenobarbital-induced mouse liver activation system. An 88% survival occurred at the highest

concentration (2.9 mM) of *cis*-1,2-dichloroethylene; a 90% survival occurred at the highest concentration (2.9 mM) of *trans*-1,2-dichloroethylene used (Greim *et al*., 1975).

Under the conditions of these studies neither the *cis* nor *trans* isomer of 1,2-dichloroethylene was observed to be mutagenic.

Teratogenicity No data were found by the committee.

Carcinogenicity No data were found by the committee.

Conclusions and Recommendations

Both *cis*-and *trans*-1,2-dichloroethylene demonstrate a potential for liver and kidney damage. Little information is available on the effects resulting from chronic administration of the materials. Long-term studies, especially those involving oral administration, are needed before a chronic SNARL can be determined. In view of its structural similarity to vinyl chloride, a carcinogenesis bioassay is desirable. In addition, studies are needed to determine the teratogenic and reproductive effects of these isomers, and additional investigations should be conducted to examine their mutagenic properties in mammalian cells.

DICHLOROMETHANE

methylene chloride; methane, dichloro-
CAS No. 75-09-2

CH_2Cl_2

Dichloromethane was evaluated in the first and third volumes of *Drinking Water and Health* (National Research Council, 1977, pp. 743-745; 1980, pp. 124-128). The following material, which became available after the 1980 report was prepared, updates and, in some instances, reevaluates the information contained in the previous reviews. Also included are some references that were not assessed in the earlier report.

Metabolism

DiVincenzo and Kaplan (1981) measured blood carboxyhemoglobin in workers and volunteers exposed as long as 7.5 hours to atmospheric concentrations of 50, 100, 150, or 200 ppm (174, 347, 522, or 696 mg/m^3) for up to 5 consecutive days. As much as 34% of the absorbed dichloromethane was expired as carbon dioxide, and less than 5% was exhaled as

unchanged dichloromethane. Maximum carboxyhemoglobin levels of 6.8% were induced at exposure levels of 200 ppm, whereas the average peak carboxyhemoglobin level in exposed workers was 3.9%. The carboxyhemoglobin levels of workers exposed to carbon monoxide at the threshold limit value (TLV) of 35 ppm (121 mg/m^3) is approximately 6.5%, or 3% greater than those in workers exposed to dichloromethane.

The metabolism of orally administered ^{14}C-dichloromethane in rats resulted in saturation kinetics up to 50 mg/kg, and both ^{14}CO and $^{14}CO_2$ was found in the expired air (McKenna and Zempel, 1981). Eighty-eight percent of a 1 mg/kg dose was metabolized. Ahmed *et al*. (1980) reported that the metabolism of dichloromethane *in vivo* and *in vitro* was mediated by the cytochrome P450-dependent mixed-function oxidase system.

Health Aspects

Observations in Humans

No new data were found by the committee.

Observations in Other Species

New data found by the committee are summarized below.

Mutagenicity Dichloromethane was found to be mutagenic in the Ames *Salmonella* assay when tested in an open container inside a desiccator, but not mutagenic when incorporated directly into the agar. Mutagenic activity was detected with strains TA1535 and TA100 when used at 0.5 ml per desiccator, the only dose level used (Nestmann *et al*., 1980). The investigators did not specify whether or not the response was obtained in the presence and/or absence of a mammalian metabolic activation system. When 104 mM and 157 mM concentrations were incubated in suspension with logarithmically growing *Saccharomyces cerevisiae* D7 cells for 1 hour at 37°C, dichloromethane also induced gene mutation, recombination, and mitotic gene conversion. No exogenous mammalian metabolic activation system was required to induce the genetic effects (Callen *et al*., 1980).

Nestmann *et al*. (1981) identified dichloromethane as the volatile mutagenic component in six paint and varnish removers assayed for mutagenicity in 9-liter desiccators in the Ames *Salmonella* assay. Mutagenicity was observed in strains TA1535, TA100, and TA98. All experiments were performed with an Aroclor 1254-induced rat liver metabolic activation system only. Nestmann and colleagues found the chemical to be nonmutagenic when assayed in the HGPRT forward mutation assay with Chinese ham

ster ovary cells, and it did not increase unscheduled DNA synthesis (UDS) in cultured primary human fibroblasts (AH) and hamster (V79) cells. No exogenous metabolic activation system was included in these experiments.

A marginal increase in sister chromatid exchange (SCE) was observed in V79 cells with and without a rat liver metabolic activation system when tested at concentrations of 1%, 2%, 3%, and 4% for 1 hour at 37°C (Jongen *et al*., 1981). An aspecific (nongenetic) inhibition of DNA synthesis was observed in cultured primary human fibroblasts (AH) and hamster (V79) cells. These experiments were performed with concentrations ranging from 0.5% and 5%. The cells were examined between 30 minutes and 3.5 hours after treatment. The investigators did not state whether the DNA synthesis experiments were performed with and/or without an exogenous metabolic activation system.

In summary, dichloromethane is a volatile mutagenic substance in two bacterial test systems. It was nonmutagenic when tested in several mammalian test systems.

Carcinogenicity In a National Toxicology Program (1982c) bioassay, dichloromethane was tested for carcinogenicity in both sexes of the $B6C3F_1$ mouse and the Fischer 344 rat. Dichloromethane doses of 500 or 1,000 mg/kg in corn oil were administered by gavage to 50 rats of each sex per dose level; doses of 500 or 1,000 mg/kg were similarly administered to mice for 5 days/week for 2 years. There were also corresponding vehicle and untreated control groups of 50 rats and 50 mice of each sex.

Dichloromethane was carcinogenic in both male and female rats. Hepatic neoplastic nodules and adrenal cortical adenomas were observed in both sexes, and pancreatic acinar-cell adenomas were found in males. The compound caused hepatocellular carcinomas in both sexes of mice. There may have been an association of thyroid C-cell carcinomas in male rats and leukemia and alveolar/bronchiolar adenomas in female mice. The National Toxicology Program Technical Review Subcommittee, which evaluated this bioassay, concluded that there may have been excessive mortality due to gavage errors and also that the maximum tolerated dose may have been exceeded.

Teratogenicity Hardin and Manson (1980) exposed rats via inhalation to 4,500 ppm (15,620 mg/m^3) for 6 hours/day before and during the first 17 days of gestation. Some fetal weight reduction occurred, but no increases in malformations were found. In the same treatment group, behavioral studies were done by Bornschein *et al*. (1980). Postnatal growth, activity, and avoidance learning were not impaired, but behavioral habituation was more rapid in the exposed group.

These limited data indicate that dichloromethane is not teratogenic to rats.

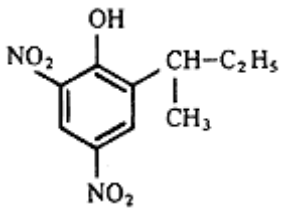
Conclusions and Recommendations

Because dichloromethane was carcinogenic in both rats and mice, no chronic SNARL has been calculated. Some of the uncertainties involving the cancer bioassay must be resolved before a final assessment is made concerning the potential risk to humans.

Dichloromethane is mutagenic in two bacterial test systems, but is not considered to be teratogenic to rats. Its metabolism is mediated by mixed-function oxidases with saturation kinetics exhibited by doses up to 50 mg/kg. This dose-dependent metabolism may necessitate a closer look at the pharmacokinetics, especially with respect to the doses used in the carcinogenicity bioassays.

DINOSEB

phenol, 2-*sec*-butyl-4,6-dinitro-
CAS No. 88-85-7



Dinoseb has been in use since 1945 as a herbicide and insecticide. Its primary application is in the control of annual weeds in many cereal and vegetable crops. This compound is slightly soluble in water (52 mg/liter at 25°C), but it can form salts with inorganic and organic bases, some of which are more soluble in water.

Metabolism

Dinoseb is believed to enhance metabolic activity by uncoupling oxidative phosphorylation and disrupting adenosine triphosphate synthesis (Brody, 1955), culminating, in extreme cases, in hyperthermia. In ruminants, dinoseb, an organonitro compound, is reduced to an amine, which may then cause the oxidation of hemoglobin to methemoglobin. There have been several reports that dinoseb causes methemoglobinemia and hemoly

sis in such animals (Fröslie 1971a,b, 1973; Karlog *et al*., 1978). Elevated temperature (32°C) enhanced the acute toxicity of dinoseb in mice, as reflected by an approximate 30% reduction in the LD_{50} (Preache and Gibson, 1975).

Dinoseb is metabolized via oxidation of either of the two methyl groups on the *sec*-butyl side chain, conjugation of the phenolic products, and the formation of many uncharacterized metabolites. In rats, but not in mice, there is a reduction of either of the two phenolic groups and acetylation of the metabolically formed *p*-amino group (Bandal and Casida, 1972).

Health Aspects

Observations in Humans

Dinoseb intoxication of humans accompanied by clinical symptoms of fatigue, sweating, and psychological alterations was described by Smith (1981). Hayes (1982) reported that a tractor driver required hospitalization because of localized pain and swelling that occurred after one eye was accidentally contaminated with diluted dinoseb spray. Visual impairment, which persisted for 3 days, was followed by a complete recovery.

Observations in Other Species

Acute Effects The estimated oral LD_{50} 24 hours after a single exposure have been determined by Bough *et al*. (1965) for the male mouse (20-40 mg/kg), male rat (25-40 mg/kg), female guinea pig (20-40 mg/kg), and male chick (40-80 mg/kg). Signs of poisoning were prostration, rapid respiration, and convulsions, which preceded death. These investigators also observed that dinoseb was rapidly absorbed through the shaved skin of mice, as measured by blood determinations. Doses of 100 mg/kg and 500 mg/kg produced 20% and 90% lethality, respectively, within 24 hours after exposure.

Dinoseb added to the diet of rats resulted in food refusal at approximately 56.7 mg/kg. A level of about 22.7 mg/kg diet caused a small but significant decrease in growth rate and a slight elevation in blood urea nitrogen (BUN). At about 11.3 mg/kg, only a slight increase in BUN was noted (Spencer *et al*., 1948).

Chronic Effects Spencer *et al*. (1948) fed dinoseb at dosages from 5.6 to 22.7 mg/kg bw/day to rats for 6 months. There was no effect on growth, BUN, organ weights, or histological findings at the lowest dose tested.

Hall *et al*. (1978, abstract) exposed Sherman rats to dinoseb in their diet at levels of 0, 50, 100, 150, 200, 300, 400, and 500 mg/kg for as long as

153 days. There was excessive mortality after 21 days in the 300 to 500 mg/kg dose groups. Dose-dependent decreases in growth were observed in the other exposed groups along with decreases in the organ weights of the liver, spleen, heart, lungs, and brain. Blood alkaline phosphatase, alanine aminotransferase, potassium, and BUN were increased, whereas lactic dehydrogenase and cholinesterase were depressed. Diffuse tubular atrophy of the tests was noted at 200 mg/kg.

Mutagenicity Mutagenicity was not observed when tested in eight *Salmonella* histidine-requiring tester strains and in a bacteriophage T4 mutation assay (Andersen *et al*., 1972). No metabolic activation system was included in the assay procedures. Mitotic gene conversion was observed in a yeast *Saccharomyces cerevisiae* assay at concentrations ranging from 185 to 1,665 ppm (Parry, 1973). No exogenous metabolic activation system was required to elicit the response. Myhr (1973) found that dinoseb strongly inhibited RNA and protein synthesis in HeLa cells exposed to 350 μg/ml for 30 minutes. A possible mechanism leading to such inhibition is the uncoupling of oxidative phosphorylation.

In summary, dinoseb elicited a mutagenic response in one microbial mutagenicity assay, but negative results were obtained in two other microbial assays.

Carcinogenicity Dinoseb did not produce a significant increase in tumors in two strains of mice when administered at the highest tolerated dose for 18 months (Innes *et al*., 1969).

Teratogenicity Dinoseb was administered orally, subcutaneously, or intraperitoneally to pregnant Swiss-Webster mice in doses up to 20 mg/kg/day (Gibson, 1973). Several schedules were used to administer the doses at different times during organogenesis. At subcutaneous and intraperitoneal doses of 17.7 mg/kg, maternal and embryo toxicity occurred. The following malformations were observed in the surviving embryos: skeletal defects, cleft palate, hydrocephalus, and adrenal agenesis. Oral administration of 20 or 32 mg/kg/day produced no gross or soft tissue defects, but some maternal toxicity was reported.

Gibson and Rao (1973) demonstrated that dinoseb does not readily cross the placenta, since levels in the fetuses never exceeded 2.5% of maternal plasma levels. Elimination was also dependent on route of exposure; following oral doses, it was approximately 4 times faster. In the fetuses, peak levels were reached much earlier after intraperitoneal exposure. The authors concluded that these differences may be responsible for the teratogenicity and fetotoxicity observed following intraperitoneal doses but not after oral exposures.

McCormack *et al*. (1980) studied postnatal renal function in rats exposed on days 10 to 12 of gestation by intraperitoneal injection of 16 mg/kg. The dilated renal tubules and pelvics seen in the neonatal period were either not found or were reduced by the forty-second postnatal day and renal function was normal. Beaudoin and Fisher (1981) administered 10 mg/kg intraperitoneally to rats on day 9 of pregnancy and studied the embryos *in vitro* after day 10. Little or no effect was observed on growth and development of the embryos.

These data indicate that dinoseb is teratogenic to mice after parenteral doses, but teratogenicity was not observed after oral exposures.

Conclusions and Recommendations

Suggested No-Adverse-Response Level (SNARL)

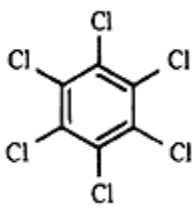
Chronic Exposure Based on the data of Spencer *et al*. (1948), a chronic SNARL can be calculated using the lowest no-observed-effect level in rats of 5.6 mg/kg/day. An uncertainty factor of 1,000 is used because this study was only of 6 months duration. Assuming that a 70-kg human consumes 2 liters of water daily and that 20% of the intake is derived from water, one may calculate the SNARL as:

$$\frac{5.6\ \text{mg/kg} \times 2 \times 70\ \text{kg}}{2\ \text{liters} \times 1{,}000} = 0.039\ \text{mg/liter, or } 39\ \mu\text{g/liter.}$$

Limited human data indicate that high exposure to dinoseb can result in a variety of physical and psychological symptoms. Since there are no data on chronic lifetime exposures, this information should be generated before limits for dinoseb exposure in drinking water are established.

HEXACHLOROBENZENE

benzene, hexachloro-
CAS No. 118-74-1



Hexachlorobenzene was evaluated in the first and third volumes of *Drinking Water and Health* (National Research Council, 1977, pp. 667-673;

1980, pp. 210-215). The following material, which became available after the 1980 report was prepared, updates and, in some instances, reevaluates the information contained in the previous reviews. Also included are some references that were not assessed in the earlier reports.

Metabolism

Hexachlorobenzene is slowly absorbed from the gastrointestinal tract. When hexachlorobenzene dissolved in rat bile was placed in rat intestinal loops, only 7% to 12% was found to be absorbed in 30 minutes (Turner and Shanks, 1980). The lymph as well as the blood are important in absorption. The ratios of concentrations in blood to those in lymph ranged from 115 to 1,019 in six replications, indicating that both pathways are important in absorption but that uptake by blood is greater than uptake by lymph. However, lymph uptake is important for by that route the first-pass effects of the liver are not a factor in the disposition of the compound.

The distribution of hexachlorobenzene administered to pregnant mice and rats either as single or multiple doses has been determined by Courtney *et al*. (1979) and Svendsgaard *et al*. (1979). In both species the amount found in fetuses and placentas was dose dependent at levels ranging from 10 to 100 mg/kg given orally. The concentrations were generally higher in the placentas than in the fetuses. In mice the concentrations in both maternal and fetal tissues were higher after multiple low doses than after single high doses. At equivalent concentrations, the amounts in the fetuses of the two species were similar.

A preliminary report on the pharmacokinetics of hexachlorobenzene in the beagle dog indicates that an intravenous bolus is rapidly distributed to the highly perfused tissues, but that it quickly disappears between 2 and 8 hours later and more slowly thereafter (Sundlof, 1981). Adipose tissue contained the highest concentration. This was also observed following the oral administration of 10 or 100 mg/kg. Most of the compound was eliminated unchanged via the bile. In rodents, however, 96% of the material in the bile was composed of metabolites rather than parent compound. Smaller amounts were excreted in the urine, which contained no parent compound.

Debets *et al*. (1980) suggested that hexachlorobenzene must be metabolized before it can exert its porphyrinogenic action because inducers of drug metabolism increased the accumulation of porphyrins in chick-embryo liver cell cultures and inhibitors decreased the formation of porphyrins.

Koss *et al*. (1979) reported that pentachlorothiophenol was present in the tissues of rats exposed to hexachlorobenzene. This metabolite is be

lieved to come from pentachlorophenylmercapturic acid, a urinary metabolite of mercapturic acid (Renner *et al*., 1978) that is possibly produced in the gastrointestinal tract by bacterial action, followed by reabsorption of the pentachlorothiophenol. Diethyl maleate, which lowers tissue glutathione levels, caused increased hepatotoxicity and porphyria in female rats fed hexachlorobenzene (0.1% in the diet) (Kerklaan *et al*., 1979). The administration of glutathione offered protection against these effects. These findings illustrate the possible importance of mercapturic acid formation as a detoxification pathway.

Pharmacokinetic studies in three rhesus monkeys given a single intravenous administration of hexachlorobenzene in doses ranging from 0.22 to 0.38 mg/kg indicated that cumulative fecal and urinary elimination after 1 year accounted for only 28.2% and 1.6% of the dose, respectively (Yang *et al*., 1978). Fat, bone marrow, adrenal glands, and liver contained high concentrations 100 days after administration, and concentrations in fat and bone marrow were still significant after 1 year.

Bailey *et al*. (1980) gave lactating rhesus monkeys oral doses of hexachlorobenzene at 60 mg/kg/day to study the transfer to their offspring. They noted that the concentration of hexachlorobenzene was 17 to 20 times higher in the milk than in maternal blood, whereas levels in the blood of the infants were 2 to 5 times higher than in the mother.

The direct excretion of hexachlorobenzene through the intestine, as opposed to biliary excretion, has been shown to be an important pathway for fecal elimination from the rat (Richter and Schäfer, 1981). Its importance as a route of elimination was demonstrated in a study by Rozman *et al*. (1981), who observed that *n*-hexadecane administered at 5% in the diet caused a 4- to 13-fold increase in the amount of hexachlorobenzene eliminated via this pathway in rats and rhesus monkeys. The rats had been given single oral doses of 100 mg/kg bw and the monkeys had received 0.11 mg/kg bw in pellets daily for 750 days or single or triple oral doses of 100 mg/kg each (Rozman *et al*., 1981).

Health Aspects

Observations in Humans

Hexachlorobenzene-exposed workers in a plant manufacturing chlorinated solvents were examined for blood levels of the compound and possible effects on their health (Currier *et al*., 1980). Mean blood levels measured each year for 4 years ranged from 160 to 312 ppb for groups of 44 to 50 workers. In comparison to another group of workers manufacturing

polyethylene and not exposed to hexachlorobenzene or similar compounds, there were no differences in urinary porphyrin excretion or in other clinical parameters, including serum enzymes and hemoglobin, and there was no evidence of porphyria cutanea tarda. A small change in hematocrit was observed, but it was not considered to be clinically significant.

Observations in Other Species

Acute Effects The committee found no reports extending previous reviews.

Chronic Effects The porphyrinogenic effects of hexachlorobenzene continue to be of concern. Determinations of urinary and fecal porphyrins indicated that iron overload potentiated the effect in female rats fed 0.2% hexachlorobenzene in the diet (Blekkenhorst *et al*., 1980). Smith *et al*. (1980) reported that porphyria developed at different rates in different lobes of the livers in female rats fed diets containing 0.01% hexachlorobenzene. In comparison to the other lobes, the caudate lobe initially had lower porphyrin levels, less depression of uroporphyrinogen decarboxylase, and less elevation of δ-aminolevulinate synthetase activity; in the advanced stages of porphyria, no differences were found among the lobes. Mechanism studies in rats given 100 mg/kg concentrations of hexachlorobenzene orally every other day suggest that its porphyrinogenic effects are exerted following conversion to an active form that binds covalently to proteins, resulting in products that inhibit uroporphyrinogen decarboxylase (Koss *et al*., 1980).

Information on the effects of hexachlorobenzene on xenobiotic metabolism has been extended to include guinea pigs (Lake *et al*., 1980). The administration of 30 mg/kg intraperitoneally for 7 days resulted in increases in ethylmorphine *N*-demethylase, 7-ethoxycoumarin *O*-deethylase, aniline hydroxylase, and cytochrome P450 levels. Increases in acetanilide hydroxylase and acetanilide esterase were observed following the oral administration of 0.1 mM/kg/day for 14 days to male rats (Carlson *et al*., 1979). Such inducing effects may influence hormone metabolism. Hexachlorobenzene concentrations of 250 ppm in the diet of mice for 21 days resulted in an increase in the metabolism of testosterone (Elissalde and Clark, 1979). There were increases in 6β-hydroxytestosterone and 7α-hydroxytestosterone, but a decrease in 16α-hydroxytestosterone formation. In female rats, hexachlorobenzene given at 80 ppm in the diet for 8 weeks resulted in increased excretion of 17β-estradiol in bile, but uterine weights,

estradiol metabolism, and bile flow rate were not affected (Mendoza and Watanabe, 1978).

Loose *et al*. (1979) reported that hexachlorobenzene given to mice at 167 ppm diet depressed antibody synthesis and lowered serum IgA concentrations. Gram-negative endotoxin sensitivity was increased 32-fold. Mean survival times following inoculation with *Plasmodium berghei* (NYU-2) was also decreased. In contrast, the administration of doses as high as 2,000 ppm diet for 3 weeks increased serum IgM but not IgG concentrations in rats (Vos *et al*., 1979). There was also a 3-fold increase in primary and secondary IgM and IgG titers in response to tetanus toxoid and an increase in the number of splenic lymphocytes. Cell-mediated immunity was not affected, as measured by resistance to *Listeria monocytogenes* infection, rejection of skin grafts, or delayed hypersensitivity to tuberculin. The *in vitro* response of thymus cells to mitogens was also unchanged.

In a four-generation reproduction study in rats, hexachlorobenzene was administered in the diet at levels of 0, 10, 20, 40, 80, 120, 320, and 640 ppm (Grant *et al*., 1977). The two highest levels were toxic to the females in the F_o generation, causing a substantial number of deaths. The viability index (i.e., the number of pups surviving 5 days/number of pups born alive × 100) for the pups in these groups was zero and only 55% for the F_{1b} generation group given 160 ppm. The lactation index (i.e., the number of pups weaned/number of pups alive on day 5 less culls × 100) was also lower in both the 120 and 80 ppm F_{1b} groups. At 80 ppm and higher doses, there were decreases in the pups' weight at birth and at the fifth and twenty-first days of life.

In female rats given oral doses of hexachlorobenze at 178 μmol/kg (50.7 mg/kg bw) every other day for 15 weeks, there was an apparent equilibrium between intake and elimination after 9 weeks of exposure (Koss *et al*., 1978). The elimination rate decreased over time. The biological half-life 38 weeks after the exposure ceased was estimated to be 4 to 5 months. Body weights of the animals did not differ from those of the controls, and although the relative weight of the liver, spleen, kidneys, and adrenal glands increased during the treatment period, they returned to normal by the end of the 38-week recovery period. Porphyrin, δ-aminolevulinic acid, and porphobilinogen levels in the urine were elevated during the exposure, but decreased to normal or near normal levels by the end of the recovery period; however, the porphyrin level in the liver was elevated during both periods. Böger *et al*. (1979) treated female rats orally twice per week with doses ranging from 0.5 to 32 mg/kg bw for 203 days. Dose-dependent deposition of hexachlorobenzene in the liver and an increase in hepatocyte size were observed. Porphyrin deposits, siderosomes, and changes in the size and shape of mitochondria were found in the hepatocytes.

Mutagenicity In a dominant lethal test, adult male rats received 0.70 and 221 mg/kg doses of hexachlorobenzene daily for 5 consecutive days (Simon *et al*., 1979). There was a dose-dependent reduction in the reproductive performance (mating index) in males, but no difference in the percentage of inseminated females who become pregnant (fertility index). No dominant lethal effects were observed. Guerzoni *et al*. (1976, abstract) reported mutagenic activity in a yeast *Saccharomyces cerevisiae* assay at 100 ppm. Hexachlorobenzene was also found to be positive in a DNA-cell-binding (DCB) assay in the presence of lysozyme and rat liver extract (Kubinski *et al*., 1981). This assay was based on the observation that nucleic acids, including DNA, form complexes with proteins and with other nucleic acid molecules in the presence of active carcinogens.

Pretreatment of male and female Wistar rats and pregnant Wister rats with intraperitoneal 10 mg/kg doses of hexachlorobenzene resulted in increased mutagenicity of 2,4-diaminoanisole when tested in the Ames *Salmonella* assay in the presence of the liver S9 fraction derived from the pretreated animals. An increase was also observed with the kidney S9 fraction, but not with the lung or the fetal liver S9 fraction (Dybing and Aune, 1977). Hexachlorobenzene-induced liver S9 fraction from male rats diminished the mutagenic activity of tris (2,3-dibromopropy) phosphate in the *Salmonella* plate incorporation assay with strain TA100 (Soderlund *et al*., 1979).

In summary, hexachlorobenzene was negative in a dominant lethal study, but mutagenic activity was observed in one microbial assay. The compound was also found to bind to DNA in a DNA-cell-binding assay.

Carcinogenicity The third volume of *Drinking Water and Health* (National Research Council, 1980, pp. 210-215) cited a study by Cabral *et al.* (1977) indicating that hexachlorobenzene was carcinogenic in the Syrian golden hamster, causing a significantly high dose-related incidence of hepatomas, liver hemangioendotheliomas, and thyroid adenomas.

In a follow-up carcinogenicity study, Cabral *et al*. (1979) fed groups of 30 to 50 Swiss mice of each sex diets containing 0, 50, 100, or 200 ppm (6, 12, or 24 mg/kg) concentrations of hexachlorobenzene for 101 to 120 weeks. Another group of 30 male and 30 female mice was fed 300 ppm of the test material for 15 weeks and was then maintained on hexachlorobenzene-free diets for the duration of the study. A dose-and time-related decrease in survival was observed after 30 weeks on test. The males were more sensitive to the toxicity of hexachlorobenzene. The reduced survival in both sexes was associated with tremors and convulsions. A decreased weight gain, more pronounced in males, was noted in both sexes of the dosed animals. The incidence of liver-cell tumors for the groups receiving

either 50, 100, or 200 ppm hexachlorobenzene in the diet is given in the section below. No liver cell tumors were present in the control animals or in the 50 ppm treated group. No other compound-related tumors were detected.

This carcinogenicity study confirmed the earlier findings and established that hexachlorobenzene is carcinogenic in both sexes of two experimental models, i.e., the Syrian golden hamster and the Swiss mouse. Furthermore, the study suggests a difference in species sensitivity as indicated by the multitumor type response of the hamster and the single-tumor type response of the mouse.

Carcinogenic Risk Estimate In the study by Cabral *et al*. (1979), there was a dose-related incidence of liver cell tumors in both sexes of the exposed outbred Swiss mice. These tumor incidences are summarized in Table II-4.

Each set of data showing a statistically significant increase was used to estimate a risk and 95% confidence of lifetime risk to humans after a daily consumption of 1 liter of water containing the compound in a concentration of 1 μg/liter. The risk estimates are based on the multistage model for carcinogenesis described earlier in this chapter for chlorobenzene. The conversion of animal doses to human doses is again based on body surface area, assuming the following weights: humans, 70 kg; rats, 400 g; and mice, 33 g. The conversion formula is: animal consumption = human consumption × (human weight/animal weight)$^{1/3}$.

Using the data from Cabral *et al*. (1979), the committee estimated the lifetime risk and upper 95% confidence estimate of lifetime risk after daily consumption of 1 liter of water containing the compound in a concentration of 1 μg/liter (Table II-5).

In a previous volume of *Drinking Water and Health*, the risk estimates from male and female hamsters were averaged to yield one composite number. If one averages the data in Table II-5, the estimated upper 95% confidence estimate of lifetime risk per μg/liter is 1.85×10^{-6}. This is somewhat different from the 2.9×10^{-5} upper 95% confidence estimate of

TABLE II-4 Tumor Incidence in Mice Exposed to Hexachlorobenzene[a]

Animal	Sex	Tumor Site	Dose Levels, mg/kg/day	Tumor Rates
Swiss mouse	Male	Liver	0, 6, 12, 24	0/47, 0/30, 3/29, 7/44
Swiss mouse	Female	Liver	0, 6, 12, 24	0/49, 0/30, 3/30, 14/41

[a] From Cabral *et al*., 1979.

TABLE II-5 Carcinogenic Risk Estimates for Hexachlorobenzene[a]

Animals	Sex	Estimated Human Lifetime Risk[b]	Upper 95% Confidence Estimate of Lifetime Cancer Risk per μg/liter
Swiss mouse	Male	3.75×10^{-7}	1.92×10^{-6}
Swiss mouse	Female	3.18×10^{-9}	1.78×10^{-6}

[a] Based on data from Cabral *et al*., 1979.
[b] Assuming daily consumption of 1 liter of water containing the compound in a concentration of 1 μg/liter.

lifetime risk per μg/liter, which was calculated in the third volume of *Drinking Water and Health* (National Research Council, 1980, p. 214).

Using the criteria for interpreting animal carcinogenicity data as outlined in Chapter 1, the committee based the above calculations on *limited evidence*.

Teratogenicity Khera (1974) gave rats single oral doses of hexachlorobenzene ranging from 10 to 120 mg/kg/day during several periods of organogenesis until day 21 of gestation. At the higher doses, extra fourteenth ribs were found, but this was associated with maternal toxicity. The fetal weights and survival rates did not differ from those of controls, and no other defects were found. Dominant lethal studies were negative. Courtney *et al*. (1976) found cleft palates, clubfoot, reduced kidney weight, and renal agenesis in the fetuses of mice given 100 mg/kg. The transplacental passage of hexachlorobenzene has been found in the rat and mouse (Svendsgaard *et al*., 1979), swine (Hansen *et al*., 1979), rat (Villeneuve and Hierlihy, 1975), rabbit (Villeneuve *et al*., 1974), and human (Astolfi *et al*., 1974).

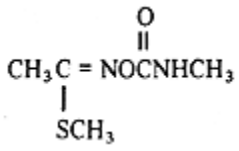
The data indicate that hexachlorobenzene is not teratogenic in the rat, but teratogenic effects in mice have been observed. Its potential for such effects in humans cannot be estimated.

Conclusions and Recommendations

This committee agrees with the findings in earlier reports that the acute toxicity of hexachlorobenzene appears to be low. Because hexachlorobenzene tends to be stored in adipose tissue for prolonged periods, additional perinatal studies are needed. Of major concern is its porphyrinogenic effect in humans, as indicated by studies in animals and some data on humans. There is a great need for epidemiological data, especially in view of its reported carcinogenic effects in two species of test animals.

METHOMYL

ethanimidothioic acid, *N*-[[(methylamino)carbonyl]oxy]-, methyl ester
CAS No. 16752-77-5



Methomyl was evaluated in the first volume of *Drinking Water and Health* (National Research Council, 1977, pp. 635-643). The following material, which became available after the 1977 report was prepared, updates and, in some instances, reevaluates the information contained in the previous reviews. Also included are some references that were not assessed in the earlier report.

Metabolism

Methomyl labelled with carbon-14 was rapidly metabolized and eliminated from the rat as carbon dioxide, acetonitrile, and unidentified urinary metabolites (Harvey *et al*., 1973). It binds reversibly with blood cholinesterase, which, in combination with its rapid rate of metabolism, may explain its high acute oral toxicity and much lower chronic oral toxicity (Kaplan and Sherman, 1977).

Health Aspects

Observations in Humans

A growing number of case histories have indicated that methomyl is highly toxic to humans following acute exposure, frequently requiring hospitalization as a result of blurred vision (Liddle *et al*., 1979; Morse *et al*., 1979; Smith, 1977).

A committee of the National Research Council (1982) found no evidence that long-term health effects resulted from short-term exposure to a variety of anticholinesterase compounds. That committee did not specifically review methomyl, which is a member of that broad class of chemicals. Nonetheless, this conclusion provides reassurance that short-term exposure to methomyl is unlikely to produce long-term adverse effects on human health.

Observations in Other Species

Acute Effects The oral LD_{50} of pure methomyl was 17 mg/kg for fasted male rats, 23.5 mg/kg for fasted female rats, and 37 mg/kg for rats on normal diets (Antäl *et al*., 1979). The minimum lethal dose of methomyl was 15 mg/kg for the guinea pig, 30 mg/kg for the dog, and 40 mg/kg for the monkey (Kaplan and Sherman, 1977).

Chronic Effects A decreased weight gain was observed in male mice fed a nutritionally complete diet containing a methomyl concentration of 250 ppm for 90 days, whereas lower concentrations (10-125 ppm) had no effect (Kaplan and Sherman, 1977). Further evaluation revealed that the entire range of treatments caused no clinical, hematological, biochemical, urinary, or pathological effects. Kaplan and Sherman also observed no evidence of toxicity in similar studies in dogs exposed for 90 days to doses up to 400 ppm in a nutritionally adequate diet.

In a 22-month study of rats exposed to methomyl in a nutritionally adequate diet, there was a decreased hemoglobin level at 200 to 400 ppm along with a significantly ($p < 0.05$) higher testis to body weight ratio in the 400-ppm males and histopathological alterations in the kidneys of males and females at 400 ppm and in the spleens of females at 200 and 400 ppm (Kaplan and Sherman, 1977). In dogs exposed to methomyl at 400 to 1,000 ppm, histopathological alterations were found in the kidneys and spleen. Consumption of diets containing 1,000 ppm was sufficiently toxic to result in lethality to several dogs in the treatment group. These authors suggested a chronic "no-effect" level of 100 ppm for rats and dogs.

Methomyl (200 mg/kg) and ethanol (10% aqueous solution) may interact to enhance the toxicity of methomyl in rats. Antäl *et al*. (1979) reported that this interaction produced decreased growth, an increased ratio of adrenal gland weight to body weight in both sexes, and several sex-specific changes, including increased hepatic triglyceride and free fatty acid levels in males and increased relative weight of kidneys and fasting glucose levels in females.

Mutagenicity Methomyl induced anaphase bridge formation, premature chromosome condensation, and a variety of mitogenetic effects in the broad bean *Vicia faba*. It was also found to induce mutations in the Ames *Salmonella* assay in the absence of a metabolic activation system (Gopalan and Njagi, 1981, abstract). A transplacental host-mediated hamster cell assay was used to study the ability of methomyl and its nitrosated derivative to induce morphological transformation in fetal cell cultures. These cell cultures were also examined for growth in soft agar (0.3%) and for

their ability to induce tumors in nude mice. Only the nitrosated methomyl was found to elicit cell transformation and growth in soft agar (0.3%) (Quarles *et al*., 1979b) as well as tumorigenicity in nude mice (Quarles *et al*., 1979a).

The data indicate that methomyl is mutagenic in one microbial mutagenicity assay. It also interferes with the cell cycle of one plant system. Negative results were obtained in a transplacental host-mediated hamster cell assay and in a transformation assay.

Carcinogenicity No new data were found by the committee.

Teratogenicity New Zealand white rabbits were fed methomyl at dietary levels of 0, 50, and 100 ppm on days 8 through 16 of gestation. When the fetuses were examined, there was no evidence of teratogenic effects. Alizarin-stained skeletons revealed no abnormalities in bone structure. A three-generation reproduction study in rats showed that methomyl did not have any adverse effects on various reproductive performance indices that could be attributed to feeding dietary levels of 50 and 100 ppm (Kaplan and Sherman, 1977).

After reviewing the data, the committee concluded that methomyl is not teratogenic to rats or white rabbits.

Conclusions and Recommendations

Suggested No-Adverse-Response Level (SNARL)

Chronic Exposure The findings from a chronic oral exposure study by Kaplan and Sherman (1977) suggested that 100 ppm is a "no-effect" level for rats (5 mg/kg bw) and dogs (2.5 mg/kg bw). Using this level from the dog for a 70-kg human consuming 2 liters of water daily, which contributes 20% of total intake, and an uncertainty factor of 100, one may calculate the chronic SNARL as:

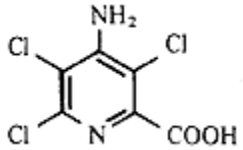
$$\frac{2.5\ \text{mg/kg} \times 70\ \text{kg} \times 0.2}{100 \times 2\ \text{liters}} = 0.175\ \text{mg/liter}.$$

The toxicity of methomyl has been characterized in acute, subacute, and chronic toxicological investigations, principally in rats and dogs. Additional information has been provided in clinical toxicology literature based on occupational exposures. Of greatest significance in the chronic animal studies was the consistent occurrence of histopathological changes in the kidneys of rats and dogs.

Future research in animal models should be directed toward evaluating the physiological significance, if any, of the histopathological changes induced by methomyl in kidney tissue.

PICLORAM

2-pyridinecarboxylic acid, 4-amino-3,5,6-trichloro-
CAS No. 1918-02-1



Picloram is a broad spectrum, persistent herbicide used on forage grasses. It is readily soluble in organic solvents (20,000 ppm in acetone) and water (430 ppm) (Johnson, 1971); it is degraded by ultraviolet light (Wirthgen and Raffke, 1977); and it is decomposed in water to negligible levels within 180 days (Johnson, 1971). In the study by Johnson (1971), a standing pond was sprayed at a rate of 4 pounds/acre, which resulted in 2,400 ppb at time zero, 700 ppb at day 1, and 6 ppb at 180 days.

Metabolism

Picloram is rapidly absorbed from the gastrointestinal tract and is excreted virtually unchanged in the urine and feces of male Fischer 344 rats within 48 hours (Nolan *et al*., 1980). Following a 10 mg/kg ^{14}C-picloram intravenous dose, the isotope was cleared biphasically and excreted in the urine. The half-time for rapid and slow clearance from plasma was 6.3 and 128 minutes, respectively. Oral administration of the same dose resulted in comparable half-times of 29 minutes and 3.8 hours, respectively. At higher (1,400 mg/kg) oral doses, the plasma levels remained constant for 3 hours, then slowly declined.

Balance studies in rats indicated that 98.4% of the dose was recovered. Urinary excretion resulted in an 80% to 84% recovery, fecal excretion resulted in approximately 15% recovery, less than 0.5% was recovered in the bile, and virtually no radioactivity was recovered as trapped $^{14}CO_2$ or as other volatile compounds (Nolan *et al*., 1980).

Health Aspects

Observations in Humans

The committee found no reports of adverse effects in humans.

Observations in Other Species

Acute Effects The acute oral LD_{50} of picloram has been reported to be approximately 1.5 g/kg in mice, 4 to 8 g/kg in rats, and 2 to 3.5 g/kg in rabbits (Belov, 1971; Johnson, 1971; Nolan *et al*., 1980).

Single oral doses of 720 mg/kg in sheep and 540 mg/kg in cattle did not result in overt signs of toxicity. However, a toxic synergism between picloram and (2,4-dichlorophenoxy)acetic acid (2,4-D) appears to exist in sheep. A 36 mg/kg dose of picloram plus 2,4-D at 134 mg/kg resulted in death of livestock (Jackson, 1966).

Chronic Effects Lifetime daily exposure of rats and dogs to diets containing 150 mg/kg bw doses of picloram resulted in no observable gross or microscopic signs of toxicity. In multigeneration studies conducted in rats at dietary concentrations up to 3,000 ppm, there was no evidence of adverse effects at the highest level tested (Johnson, 1971).

Inhalation exposure of rats to 150 mg/liter for 15 days resulted in no obvious signs of toxicity (Belov, 1971).

Mutagenicity In an *in vivo* cytogenetics study in male and female Sprague-Dawley rats, picloram administered orally at three dose levels (20, 200, and 2,000 mg/kg bw) produced no cytogenetic aberrations in bone marrow cells (Mensik *et al*., 1976). The herbicide was found to be nonmutagenic in the Ames *Salmonella* assay (Andersen *et al*., 1972; Carere *et al*., 1978), but was mutagenic in *Saccharomyces cerevisiae* at 1 ppm (Guerzoni *et al*., 1976, abstract) and in the *Streptomyces coelicolor* forward mutation spot test (Carere *et al*., 1978). No genetic effects were observed when picloram was tested for its ability to induce gene conversion, point mutations in the gene conferring resistance to 8-azaguanine, mitotic nondisjunction, and haploidization in *Aspergillus nidulans* (Bignami *et al*., 1977).

In summary, picloram was mutagenic in two out of four microbial test systems, but was nonmutagenic in an *in vivo* cytogenetics test with male and female rats.

Carcinogenicity In a National Cancer Institute (1978) carcinogenicity bioassay of picloram, the test material was administered for 80 weeks to both sexes of Osborne Mendel rats at dietary levels of approximately 7,435 or 14,875 ppm. In addition, male and female B6C3F_1 mice were fed picloram at levels of approximately 2,500 or 5,000 ppm in their diet. The histopathological findings indicated that there were no excess tumors in the male or female mice or in the male rats receiving the test material in their diets. However, the incidence of neoplastic nodules in the livers of female rats was significantly associated with picloram administration and there was an increase in the incidence of C-cell adenomas of the thyroid, which did not show an association with picloram administration. No other tumor at any other site was considered to be related to the exposure. The report concluded that "the findings are suggestive of the ability of the compound to induce benign tumors in the livers of female Osborne Mendel rats."

Although these data have been reviewed by another scientist (Rueber, 1981), who viewed the results differently, the first analysis done by the National Cancer Institute appears to be the most objective and is supported by a review of the data by Robens (1978, abstract).

These differences in interpretation, based on different diagnoses of identical slides, must be resolved to arrive at a more definitive assessment of the potential carcinogenicity of picloram.

Teratogenicity Multigeneration studies, in which rats were exposed to picloram from gestation through reproductive cycles to levels as high as 3,000 ppm diet, produced no evidence of effects on fertility, gestation, viability of pups, lactation, or skeletal development. Pregnant rats receiving doses of 1,000 mg/kg/day during organogenesis were normal, but there was a slight increase in embryo resorption. A dose of 2,000 mg/kg/day was toxic to the mothers, but did not induce malformations in the pups. The dose of 750 mg/kg/day was not toxic to the mothers or the fetuses (Johnson, 1971).

Thompson *et al*. (1972) found no teratogenic action when this compound was fed to rats in concentrations as high as 1,000 mg/kg bw on gestational days 6 through 15. No neonatal adverse affects were noted.

From these limited data, it can only be concluded that picloram is not teratogenic in the rat.

Conclusions and Recommendations

Suggested No-Adverse-Response Level (SNARL)

Chronic Exposure The report by Johnson (1971) indicated that a 150 mg/kg dose of picloram can be regarded as a no-observed-effect dose.

Using this dose for a 70-kg human drinking 2 liters of water daily and an uncertainty factor of 1,000, and assuming that 20% of exposure is provided by water, one can calculate the chronic SNARL as:

$$\frac{150 \text{ mg/kg} \times 70 \text{ kg} \times 0.2}{1{,}000 \times 2 \text{ liters}} = 1.05 \text{ mg/liter.}$$

The uncertainty factor of 1,000 was used because the issue of carcinogenicity has not yet been resolved and also because the Johnson (1971) study does not provide enough information for a complete judgment of its adequacy.

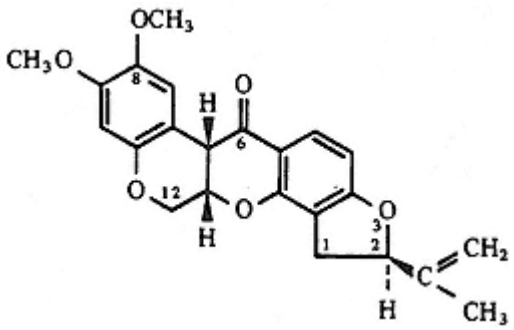
Picloram is relatively nontoxic in rats fed concentrations as high as 3,000 ppm in the diet through three generations. It is not teratogenic in the rat or mutagenic in two out of four bacterial tester strains with or without microsomal activation.

The results of carcinogenicity testing in rats and mice are equivocal. At levels of 14,875 ppm, benign hepatomas were observed in female rats. Picloram-related tumors were not reported in male rats or mice of both sexes.

ROTENONE

[1]benzopyrano[3,4-*b*]furo [2,3-*h*][1]benzopyran-6(6*H*)-one, 1,2,12,12a-tetrahydro-8,9-dimethoxy-2-(1-methylethenyl)-, [2R-(2 , 6a ,2a)-

CAS No. 83-79-4



In the form of ground derris root, rotenone has been used as a nonpersistent insecticide to control pests on plants and animals and as a fish poison to manage or to eliminate undesirable species in reservoirs, lakes, and streams (Fukami *et al*., 1967). First isolated in 1895, world consumption is

now estimated to be 10,000 to 20,000 kg/year (Haley, 1978). When exposed to light and air, rotenone undergoes rapid decomposition. Colorless solutions in organic solvents become successively yellow, orange, and, finally, deep red as the result of oxidation, and they may deposit crystals containing dehydrorotenone and rotenone (Windholz, 1976).

Metabolism

A literature review by Haley (1978) indicates that rotenone functions as an inhibitor of the mitochondrial oxidative phosphorylation-electron transport system, but the chemical also was found to be a potent *in vitro* antagonist of slow-reacting substance of anaphylaxis (Ashack *et al*., 1980).

Studies of the metabolism of rotenone have not been extensive. The committee found no literature on the *in vivo* kinetics of rotenone absorption or excretion. *In vitro* studies with microsomal preparations and *in vivo* studies with mice and houseflies indicated that there are hydroxylation metabolites of rotenone designated as rotenolone I; rotenolone II; dehydrorotenone I; dehydroxyrotenone; 8'-hydroxyrotenolone; 6',7'-dehydro-6',7'-dihydrorotenone; and other uncharacterized polar compounds (Fukami *et al*., 1967; Haley, 1978).

Health Aspects

Observations in Humans

Toxicity in humans has not been well studied. Large inhaled or ingested doses may cause numbness of oral mucous membranes, nausea and vomiting, muscle tremors, convulsions, tachypnea, and respiratory paralysis at lethal doses. The lethal oral dose has been estimated at 0.3 to 0.5 g/kg (Gosselin *et al*., 1976). Chronic poisoning may result in fatty changes in the liver and kidney. Direct contact may cause irritation of the skin or conjunctiva. No human fatalities have been reported (Haley, 1978; Windholz, 1976).

Observations in Other Species

Acute Effects In rats, the intravenous, intraperitoneal, and oral LD_{50} values for rotenone in ethanol or acetone solution were reported to be 0.20, 1.60, and 60 mg/kg, respectively (Santi and Tóth, 1965). Depending on the route of administration, toxic signs appeared within 2 to 20 minutes. Death from respiratory failure occurred within minutes after intravenous injection and within 1 day after intraperitoneal or oral administration.

These workers suggested that both the lethal and pharmacological effects of rotenone are due to inhibited mitochondrial metabolism. Haley (1978) reported that the acute oral toxicity of rotenone is more variable and less toxic. This investigator reported acute toxicity doses as 0.6 g/kg in rats, 1.5 g/kg in guinea pigs, and 3 g/kg in rabbits. In this study, dogs were not affected at 2 g/kg, but oral ingestion near the feeding time produced emesis.

Short-term administration of rotenone in sunflower oil, injected intraperitoneally in doses of 0.1 mg/kg/day for 5 days into female rats, produced a marked elevation in serum growth hormone concentration and a decrease in serum prolactin (Gosálvez *et al*., 1979). These alterations and transient elevations in concentrations of estrogens, progesterone, and corticosterone suggested to the authors that rotenone was stimulating the hypophysis and that the physiopathogeny of rotenone-induced mammary tumors is indirect and hormonal.

Subchronic Effects A 28-day repeated dose and 6-month (26-week) study in beagle dogs has been completed by Ellis *et al*. (1980). In this study, animals were dosed daily with the rotenone preweighed into gelatin capsules on a weekly basis for each animal. The capsules were stored at freezer temperature until administered to the dogs. The chemical was analyzed at greater than 99% purity; the 6-month study was conducted with only one lot of chemical. In the 28-day range-finding studies, one male and one female each received one of five levels of rotenone: 0.08, 0.4,2,10, or 50 mg/kg/day. The animals were observed daily, and their feed consumption and body weights were determined weekly. At 10 and 50 mg/kg the primary toxic effect was emesis, which was more pronounced and prolonged in females; at the high dose, excessive salivation was noted in the female. Despite the emesis, all the dogs generally gained weight, but the number of animals was too small and duration of exposure too short to draw definite conclusions about weight gain.

In the 6-month study, groups consisting of six male and six female beagles received rotenone at dose levels of 0, 0.4, 2, or 10 mg/kg/day. The doses were administered approximately 1 hour before daily feeding. Animals were observed daily, body weight was determined weekly, and both feed and water consumption were estimated 6 days/week. Hematological and clinical chemistry and urinalysis were performed twice during a pretreatment period and after 2, 4, 6, 13, 17, 21, and 26 weeks during the dosing period. Animals were necropsied at the end of the 26-week exposure, major organs were weighed, and approximately 35 tissues from each animal were retained and examined histologically.

The principal toxic sign was emesis, which occurred primarily during

the first week at the high-dose level (10 mg/kg/day); males appeared to be more affected than the females in this phase of the study. The dogs receiving the high dose had a higher incidence of diarrhea and soft stools throughout the study. The body weight of high-dose males and females decreased during the first 2 months, but remained stable thereafter. At 2 mg/kg/day, body weights of females were consistently lower than those of controls after 2 months. Effects on feed consumption generally paralleled body weight responses. At high doses, hematology and blood biochemistry appeared to be adversely affected in high dose males and females. In comparison to controls, hemoglobin and hematocrit values were significantly reduced. Erythrocyte counts were also generally lower than in controls, but seldom was the difference statistically significant. Other consistent effects were decreases in glucose, total lipids, and cholestrol. Urinalysis failed to reveal consistent effects that could be attributed to the test chemical. At necropsy, average absolute major organ weights were generally similar in all groups. Relative to body weight, the weights of the kidney (males), heart (females), and brain (males and females) were significantly higher in the high-dose group than in controls, but these differences were attributed to decreased body weight in the high-dose group. At necropsy, the most frequent lesions observed were hemorrhagic patches in one or more sections of the small intestine. This lesion was more prevalent in the high-dose dogs but was also observed in controls; hence, it could not be definitely associated with exposure to the test chemical. The only microscopically observed lesion that might have been related to the rotenone treatment was mild hemorrhagic enteritis of the small intestine in one low-and one high-dose female; however, the incidence was not dose-dependent and was much too low to be of significance. Other findings in all dose groups were not associated with exposure to the compound.

Chronic Effects The committee found no recent studies on chronic effects of rotenone. Studies conducted during the 1930's and 1940's were reviewed by Haley (1978), but the quality of the studies and their results are questionable. In general, these early studies indicated that chronic ingestion of cubé, derris root, or rotenone by rats led to suppressed growth, and 75 ppm caused liver injury. Rats maintained on high-fat diets containing derris root or rotenone for 140 days had normal growth curves and no histological evidence of organ damage (Haley, 1978).

In a 2-year study, rats and dogs were given cubé powder, the commercial natural product (Hansen *et al*., 1965), which contained 5.8% rotenone by analysis. The powder was added to the diet of rats at levels of 0, 50, 100, 250, 500, or 1,000 ppm and to the diet of dogs at levels of 0, 50, 150, or 400 ppm. Each experimental group consisted of 25 male and 25 female rats

and two male and two female beagle dogs. Unfortunately, the diets were not analyzed to verify rotenone content, the stability of the compound in the diets was not verified, and the frequency of the diet preparation was not stated. Otherwise, the basic experimental design was not unreasonable for a chronic study conducted at that time. The number of animals, especially rats, was too small, based on present standards for a carcinogenic evaluation, which was a major purpose of the study. Results of this study appeared to be reasonably straightforward. The test powder at all levels, except 50 ppm, inhibited the growth of the rats. Histological lesions in rats were attributable to the test material, and the incidence of gross mammary tumors, nephritis, and pituitary lesions at higher dose levels were lower than in the controls. No clinical or hematological effects were noted in the dogs.

Mutagenicity No unscheduled DNA synthesis (UDS) was observed in human fibroblast cultures (VA4) in the presence and absence of a rat S9 liver enzyme activation system when rotenone was tested at 1, 10, and 1,000 mM concentrations (Ahmed *et al*., 1977). Negative results were also obtained in a rat UDS hepatocyte assay (Probst and Hill, 1980). The colony-forming ability of continuously cultivated bovine cells (T4) derived from normal ovarian tissue was reduced to 50% in the presence of 3.5×10^{-7} M concentration of rotenone (Malcolm *et al*., 1973). Alkali-labile single strand DNA breakage was observed when mouse L1210 leukemia cells were exposed to 10^{-7} M rotenone. Hilton and Walker (1977) suggested that the DNA strand breaks might be an indirect effect of rotenone's effect on adenosine triphosphate (ATP) depletion, which could result in increased nucleolytic activity. Tomkins *et al*. (1980, abstract) observed no sister chromatid exchanges (SCE) in Chinese hamster ovary cells in the presence or absence of a rat liver S9 metabolic activation system. The maximum dose level used was the dose that reduced the proportion of dividing cells to 50%.

Rotenone added to Chinese hamster cells *in vitro* increased the mitotic index, and mitotic cells contained monopolar spindles with chromosomes grouped around centriole pairs near the cell center (Haley, 1978). The compound was also found to arrest mitosis in cultured mammalian cells by inhibition of the spindle microtubule assembly (Barham and Brinkley, 1976; De Brabander *et al*., 1976; Meisner and Sorensen, 1966). A 4.6×10^{-7} M (0.18 μg/ml) concentration of rotenone inhibited the rate of rat neurotubulin polymerization *in vitro* by 50% (De Brabander *et al*., 1976). The effective inhibition of the *in vitro* formation of microtubules from tubulin has been suggested by Marshall and Himes (1978) to be the mechanism by which rotenone inhibits mitosis.

In summary, the data regarding the mutagenicity of rotenone are inconclusive.

Carcinogenicity The potential carcinogenicity of rotenone was evaluated by Innes *et al*. (1969) in two strains of mice by oral intubation of the test materials in a 0.5% suspension in a gelatin vehicle. A dose of 1 mg/kg was administered daily to 7-day-old mice until they were 4 weeks old. The weanlings were then fed diets containing rotenone concentrations of 3 mg/kg for approximately 18 months. Histological examination of major organs and of grossly observed lesions supported the conclusion that oral administration of rotenone did not cause a significant increase in tumors.

In the early 1970's, a brief communication by Gosálvez and Merchan (1973) indicated that mammary tumors were produced in albino rats (strain unidentified) given rotenone in sunflower oil by daily intraperitoneal injection of 1.7 mg/kg for 42 days. The rotenone was reported as having less than 1% impurities, which consisted mainly of other rotenoids. Mammary tumors developed in the dosed animals within 6 to 11 months; no tumors of this type were present in controls after 19 months. The tumors were diagnosed as adenomas with interstitial fibrosis and "areas with adenocarcinomatous transformation." They were encapsulated, and some were successfully transplanted to normal rats. In a later study, rotenone administered to female Wistar rats in the diet at concentrations of 5, 10, or 20 ppm for 8 to 12 months resulted in a 40% incidence of mammary tumors. However, higher concentrations of rotenone (50 or 100 ppm) did not increase mammary tumors in treated rats above the 5% incidence observed in controls (Haley, 1978). In other publications, the same group of investigators described the morphology of rotenone-induced breast tumors (Merchan *et al*., 1978) and reported a possible mechanism for the induction of the tumors (Gosálvez *et al*., 1979).

Clarification of these findings was attempted by the U.S. Environmental Protection Agency through contractual laboratory testing and evaluation of the carcinogenic potential of rotenone in Syrian golden hamsters and Wistar and Sprague-Dawley rats (Leber and Persing, 1979; Leber and Thake, 1979). These studies were conducted with 98% pure rotenone containing traces of other rotenoids. In the hamster study, histological examinations were conducted on 30 tissue/organ samples from approximately 30 animals of each sex and experimental group that had received rotenone at doses of 0, 125, 250, 500, or 1,000 ppm in the diet for as long as 18 months. There was no evidence that rotenone was carcinogenic to the hamsters.

In the Sprague-Dawley rat study, rotenone in corn oil was administered daily by intraperitoneal injection to 25 animals of each sex at doses of 0,

1.7, or 3.0 mg/kg bw for 42 days. Fifteen animals of each sex were used as vehicle controls. The animals were observed for an additional 18 months after treatment until death or sacrifice. In the Wistar rat study, rotenone in corn oil was administered by gavage to 25 animals of each sex at doses of 0, 1.7, or 3.0 mg/kg bw for 42 days. Fifteen animals of each sex were used as vehicle controls. The Wistar rats were observed for an additional 12 months after treatment until death or sacrifice. Histological examination was performed on more than 30 tissue/organ sites sampled from animals from each of these two studies. There was no evidence that intraperitoneal or oral administration of rotenone in corn oil to Sprague-Dawley or Wistar rats induced mammary neoplasia or statistically significant neoplasias at any other organ site.

Evidence of the possible carcinogenicity of rotenone is limited to positive findings in one sex of one strain of rats and to one tumor type and site (i.e., adenoma of the mammary glands). The weight of evidence is further limited by the lack of findings of carcinogenicity in three comparable studies using the Syrian golden hamster and the Wistar and Sprague-Dawley rats and by limited evidence in the literature indicating that rotenone is not mutagenic. The conflicting evidence suggests that there were uncontrolled variables in the testing of rotenone, e.g., the purity of the test material, the sensitivity of the strains used, and the possible effects of the sunflower oil. These types of questions and the differences in test results must be resolved before the potential carcinogenicity of rotenone can be assessed.

Teratogenicity Khera *et al*. (1982) tested technical grade rotenone (87% rotenone and 13% other cubé extractives) for teratogenicity in pregnant female Wistar rats. The compound was administered by gavage in corn oil at doses of 2.5, 5.0, and 10 mg/kg bw. The 10 mg/kg dose killed 12 of the 20 rats after two to nine exposures. At this dose there was also a significant decrease in the number of live fetuses per dam and an increase in the proportion of resorptions. The incidence of skeletal malformations was not different from those of controls at all doses. There was, however, an increased frequency ($p < 0.05$) of skeletal aberrations such as an extra rib, delayed ossification of sternebrae, and missing sternebrae in the 5 mg/kg dose group. The 2.5 mg/kg dose produced no observed maternal toxicity or any adverse effect on fetal development. These authors concluded that, in light of the high dosages used, the effects noted may be of little or no significance to the rat and that additional studies should be conducted in a nonrodent species.

It is not possible to make a definitive assessment of the potential teratogenicity of rotenone based on this one negative study in rats.

Conclusions and Recommendations

Suggested No-Adverse-Response Level (SNARL)

Chronic Exposure Until the unconfirmed report of mammary tumors in female rats can be resolved, results of the 6-month study by Ellis *et al*. (1980) can be used to calculate a chronic SNARL. Using the lowest apparent no-effect level in dogs (2 mg/kg) and an uncertainty factor of 1,000 (because this is less than a lifetime study), and assuming that a 70-kg human consumes 2 liters of water daily and that 20% of intake is provided by the water, one may calculate the SNARL as:

$$\frac{2\ \text{mg/kg} \times 70\ \text{kg} \times 0.2}{2\ \text{liters} \times 1{,}000} = 14\ \mu\text{g/liter}.$$

Because results of acute toxicity studies have been variable, additional studies with the purified chemical, with several animal species, and with several routes of administration appear warranted. Additional teratological evaluation should also be considered, assuming that humans may be exposed to the chemical through drinking water. In addition, pharmacokinetic metabolism studies are needed, since rates of absorption and routes and rates of excretion would undoubtedly assist in the evaluation of toxic responses. Since rotenone enters surface waters through direct application in fishery management, definitive studies should be conducted to determine the fate (rates and routes of degradation) of the chemical in water under various environmental conditions. Since rotenone is quite sensitive to degradation following exposure to air and sunlight, it is necessary to determine the extent of these reactions and the ultimate products to which humans would be exposed.

Of greatest concern is the uncertainty of the chemical nature of the rotenone being tested. The compound's potential carcinogenicity must then be resolved. Carcinogenesis bioassays have produced equivocal results that cannot be resolved on the basis of current information. More recent studies have been negative.

TETRACHLOROETHYLENE

ethene, tetrachloro-
CAS No. 127-18-4
$Cl_2C = CCl_2$

Tetrachloroethylene was evaluated in the first and third volumes of *Drinking Water and Health* (National Research Council, 1977, pp. 769-770;

1980, pp. 134-142). The following material, which became available after the 1980 report was prepared, updates and, in some instances, reevaluates the information contained in the previous reviews. Also included are some references that were not assessed in the earlier reports. Tetrachloroethylene has also been the subject of a monograph by the International Agency for Research on Cancer (1979b), in which toxicology data on animals and humans are evaluated.

Metabolism

Recent metabolism studies have been prompted by an interest in the relative reactivity of the metabolites of chlorinated ethylenes. The symmetrical chlorinated ethylenes, including tetrachloroethylene, are postulated to be more resistant to metabolism and to the formation of reactive intermediates than are the unsymmetrical members of the series (Politzer *et al*., 1981). Tetrachloroethylene epoxide has been synthesized and the mechanism of rearrangement that was studied *in vitro* was consistent with the *in vivo* metabolism of tetrachloroethylene through an epoxide intermediate to trichloroacetic acid (Henschler and Bonse, 1978).

As noted in the third volume of *Drinking Water and Health*, only a small portion of systemic tetrachloroethylene is metabolized. Data from balance studies that permit good quantification of the disposition of tetrachloroethylene are generally lacking. In a study of mice given a single oral 500 mg/kg dose of ^{14}C-tetrachloroethylene, Schumann *et al*. (1980) reported that approximately 75% of the dose was expired as unchanged tetrachloroethylene. Water-soluble metabolites in the urine accounted for the other major portion of the dose. There were two additional facets of this study that deserve attention: (1) a lower dose in mice—10 ppm (69 mg/m^3) administered for 6 hours—resulted in a much larger proportion of the dose being metabolized and (2) observations of relative hepatic macromolecular binding of tetrachloroethylene metabolites indicated that the metabolism of tetrachloroethylene proceeds at a more rapid rate and to a greater extent in mice than in rats. *In vitro* studies with rat hepatic microsomes conducted by Costa and Ivanetich (1980) have snown that the maximum rate of metabolism for tetrachloroethylene is approximately 30-fold lower than the corresponding rate for trichloroethylene. This observation and those of Schumann and colleagues are all in keeping with the hypothesis that the hepatotoxicity of the chlorinated ethylenes is inversely related to the stability of the compound to biotransformation. If this is so, then it is of fundamental importance that the relative rates of metabolism for laboratory animals and humans be established before data from animals can be extrapolated with any confidence to humans.

Health Aspects

Observations in Humans

Two fatalities resulting from acute inhalation exposures to tetrachloroethylene have recently been reported. In each case, a male employee in a dry-cleaning establishment was overcome by tetrachloroethylene vapors. Postmortem blood levels of 4.5 mg/liter (Levine *et al*., 1981) and 44 mg/liter (Lukaszewski, 1979) were reported. The lower value is not far above the maximum blood level of approximately 2.5 mg/liter reported by Stewart *et al*. (1961) in humans exposed for 3 hours to a tetrachloroethylene concentration of approximately 200 ppm (1,374 mg/m^3) in air. This lends further credence to previous observations that sustained atmospheric concentrations exceeding 200 ppm can lead to narcosis and death.

The committee found no reports that extended or amplified the findings in earlier *Drinking Water and Health* reports on the effects of graded doses of tetrachloroethylene in humans.

Observations in Other Species

Acute Effects In a study of the toxicity of several solvents in mice pretreated with polybrominated biphenyls or Aroclor 1254, Kluwe *et al*. (1979) were unable to produce functional lesions of the liver or kidneys by administering a single maximal sublethal dose of tetrachloroethylene. This observation is consistent with earlier reports that tetrachloroethylene has a low potential for producing acute organ damage in animals.

Chronic Effects Schumann *et al*. (1980) gave 11 consecutive daily oral doses of tetrachloroethylene at levels of 100, 250, or 1,000 mg/kg to B6C3F$_1$ mice and Sprague-Dawley rats. Although body weight was not affected by treatment, relative liver weight was significantly increased in mice at all except the lowest doses and in rats at 1,000 mg/kg. Histological examination revealed hepatic changes only in the rats receiving 1,000 mg/kg/day, and these were considered minimal. In mice, however, hepatocellular swelling in the centrilobular region and an accentuated lobular pattern were observed at all dose levels.

Mutagenicity Negative results were obtained with strain TA100 in the standard plate *Salmonella* assay when tested with tetrachloroethylene up to 4×10^{-3} M concentration in the presence and absence of liver S9 frac

tion derived from phenobarbital-induced mice (Bartsch *et al*., 1979). When incubated with log phase *Saccharomyces cerevisiae* D7 cells for 1 hour at 37°C at concentrations of 4.9 and 6.6 mM, recombinations, mitotic gene conversions, and gene reversions were observed. A concentration of 8.2 mM was toxic to 99.9% of the cells. No exogenous mammalian activation system was needed to induce the genetic end points (Callen *et al*., 1980).

No chromosome aberrations, increases in sister chromatid exchange (SCE), or changes in cell-cycle kinetics were found in peripheral blood lymphocytes obtained from six workers exposed to tetrachloroethylene levels as high as 92 ppm (632 mg/m^3) from 2 months to 18 years or from four workers exposed to 10 to 40 ppm (69-276 mg/m^3) from 2 months to 18 years (Ikeda *et al*., 1980).

In summary, tetrachloroethylene was mutagenic in one of two microbial mutagenicity assays. Negative results were obtained in one *in vivo* cytogenetics study in humans.

Carcinogenicity As described in the third volume of *Drinking Water and Health*, a study by the National Cancer Institute (1977a) yielded positive findings of carcinogenicity in both sexes of B6C3F $_1$ mice. Only a preliminary report of a recent study by the National Toxicology Program (1982d) is now available. It is premature to make further judgments on this compound before the NTP study has undergone peer review and analysis. Thus, a risk estimate is not possible at present.

Teratogenicity Schwetz *et al*. (1975) exposed pregnant mice and rats to concentrations of 300 ppm (2,040 mg/m^3) for 7 hours/day during days 6 through 15 of gestation. No fetal toxicity or teratogenicity was found. Nelson *et al*. (1979) performed behavioral tests on the offspring of rats exposed to 100 ppm (680 mg/m^3) for 7 hours/day on days 14 to 20 of gestation and found no changes from the control pups. At exposure levels of 900 ppm (6,120 mg/m^3), the maternal animals gained less weight and the offspring performed less well on neuromotor tests and had lower levels of brain acetylcholine and dopamine. Pair-fed controls were not used.

The limited data indicate that tetrachloroethylene is not teratogenic in mice and rats.

Conclusions and Recommendations

Information made available since the last review of tetrachloroethylene in *Drinking Water and Health* (National Research Council, 1980) is not suffi

cient to enable the committee to make a more realistic assessment of no-adverse-response levels than that developed in 1980. The earlier recommendations for additional studies are also still valid. There also continues to be a need for further research to elucidate mechanisms of tetrachloroethylene toxicity and species differences in response to this compound. Data from carefully designed subchronic studies to determine effect and no-effect doses would permit much better estimation of the risk associated with low-level exposure of humans.

The question of carcinogenicity remains unresolved pending the complete analysis of the ongoing study by the National Toxicology Program.

1,1,1-TRICHLOROETHANE

ethane, 1,1,1-trichloro
CAS No. 71-55-6
$Cl_3C—CH_3$

1,1,1-Trichloroethane (methyl chloroform) was evaluated in the third volume of *Drinking Water and Health* (National Research Council, 1980, pp. 144-155). The following material, which became available after the 1980 report was prepared, updates and, in some instances, reevaluates the information contained in the previous review. Also included are some references that were not assessed in the earlier report.

Metabolism

There have been no studies of the metabolism of 1,1,1-trichloroethane following ingestion. Most reports continue to focus on the metabolic products of inhaled 1,1,1-trichloroethane, since occupational exposures to the compound occur by this route. The major metabolites of 1,1,1-trichloroethane are 2,2,2-trichloroethanol and 2,2,2-trichloroacetic acid. In humans, the appearance of trichloroethanol in the urine has been estimated to be a more accurate measure of intermittent exposure and metabolism than 1,1,1-trichloroethane exhaled in the breath (Caperos *et al*., 1982). The low partition coefficent of 1,1,1-trichloroethane in blood and the low rate of metabolism (3.5%) in humans combine to result in a rapid, but small uptake upon inhalation and a consequently rapid rate of excretion (Monster, 1979). A detailed pharmacokinetic model of 1,1,1-trichloroethane uptake and metabolism following inhalation by mice and rats has been suggested

by Schumann *et al*. (1982). In both animals, the utility of estimating the kinetics following oral ingestion at the trace levels found in drinking water has yet to be proven.

1,1,1-Trichloroethane is oxidized by the cytochrome P450 system to metabolites that bind covalently to cellular macromolecules, as evidenced by the dependence of the reaction on reduced nicotinamide adenine dinucleotide phosphate (NAPDPH) and the inhibition by carbon monoxide (Ivanetich and Van den Honert, 1981). Metyrapone and SKF 525-A also inhibit the reaction. The activity is dependent on cytochrome P450 rather than on cytochrome P448, since phenobarbital enhances both metabolism and binding, whereas -naphthoflavone does not. 2,2,2-Trichloroethanol is the major metabolite found in *in vitro* studies by Ivanetich and Van den Honert (1981). The identity of the reactive intermediate remains unknown.

Fasting increases the metabolism of 1,1,1-trichloroethane in the livers of rats without measurable effects on the cytochrome P450 or protein content. Differences between the sexes are present after 1 day of fasting, but decline after 3 days of fasting (Nakajima and Sato, 1979). The role of such nutritional effects on chronic low level ingestion is not known.

Health Aspects

Observations in Humans

No new data were found by the committee.

Observations in Other Species

Acute Effects Cardiac arrhythmias observed in laboratory animals exposed to 1,1,1-trichloroethane are similar to those seen in humans after self-intoxication. In rabbits, pretreatment with phenobarbital has reduced blood levels of 1,1,1-trichloroethane and the incidence of cardiac arrhythmias, whereas pretreatment with the mixed-function oxidase inhibitors SKF 525-A and 2,4-dichloro-6-phenylphenoxyethyldiethylamine hydrogen bromide (Lilly, 18947) increased blood levels of 1,1,1-trichloroethane and the incidence of arrhythmias (Carlson, 1981). 1,1,1-Trichloroethane, rather than its metabolites, is responsible for the arrhythmias (Carlson, 1981).

Sprague-Dawley rats exposed by inhalation to 4,345 mg/m^3 (800 ppm) 1,1,1-trichloroethane for 4 weeks had increased liver weights, but no appreciable increases in cytochrome P450; however, androstenedione metab

olism *in vitro* was depressed (Toftgard *et al*., 1981). Similar inhalation exposure of rats to 1,1,1-trichloroethane did not appear to influence the hepatic metabolism of biphenyl or benzo(*a*)pyrene (Nilsen and Toftgaard, 1980). The metabolism of aminopyrine is inhibited to a greater extent than aniline hydroxylation (Bolt *et al*., 1980). This could be explained by the fact that 1,1,1-trichloroethane binds readily to cytochrome P450, as evidenced by its type I binding characteristics (National Research Council, 1980). 1,1,1-Trichloroethane is likely to interfere with monooxygenase activity, but the dose-response characteristics of this competitive inhibition have not been reported and it is not known if trace concentrations of 1,1,1-trichloroethane, such as those occurring in drinking water, have similar inhibitory effects.

When 1,1,1-trichloroethane is perfused into rat liver, a number of intracellular enzymes are released (Noviakovia *et al*., 1981), but similar effects have not been observed *in vivo* (National Research Council, 1980). 1,1,1-Trichloroethane is not a potent hepatotoxicant.

Chronic Effects No new data were found by the committee.

Mutagenicity 1,1,1-Trichloroethane was mutagenic in the Ames *Salmonella* assay in strains TA1535 and TA100 when tested at concentrations of 0.1, 0.5, and 1.0 ml volumes in an open glass dish inside desiccators (Nestmann *et al*., 1980). The investigators did not state whether the mutagenic response was obtained in the presence and/or absence of a metabolic activation system.

Based on these data, 1,1,1-trichloroethane is mutagenic in one bacterial test system.

Carcinogenicity 1,1,1-Trichloroethane was tested for carcinogenicity in both sexes of the $B6C3F_1$ mouse and the Fischer 344 rat (National Toxicology Program, 1982e). Doses of 3,000 or 1,500 mg/kg bw were administered by gavage to both sexes of mice. Rats were given doses of 750 or 375 mg/kg. The test material was administered in corn oil to groups of 50 rats and 50 mice of each sex 5 days/week for 2 years. There were also vehicle controls of 50 rats and 50 mice of each sex. Low doses were used to overcome the consequences of poor survival due to the toxicity previously encountered in both Osborne-Mendel rats and $B6C3F_1$ mice. The results in both sexes of rats confirmed the findings of an earlier study (National Cancer Institute, 1977b) that the compound was not carcinogenic when administered by gavage to the Fischer 344 rat. In the $B6C3F_1$ mouse, however, the results indicate a possible compound-related increased incidence

of hepatocellular carcinomas in both sexes. The incidence and types of tumors are presented in the following section.

Carcinogenic Risk Estimate In a study by the National Toxicology Program (1982e), there was an increased incidence of hepatocellular carcinomas in the exposed mice of both sexes. An NTP review committee concluded that 1,1,1-trichloroethane was carcinogenic for female $B6C3F_1$ mice, causing an increase in hepatocellular carcinoma. The association in male $B6C3F_1$ mice was considered equivocal. The tumor incidences are summarized in Table II-6.

Each set of data showing a statistically significant increase was used to estimate a lifetime risk and an upper 95% confidence estimate of lifetime risk in humans following a daily consumption of 1 liter of water containing the compound in a concentration of 1 μg/liter. The estimates of lifetime risk are based on the multistage model for carcinogenesis described earlier in this chapter for chlorobenzene.

The conversion of animal doses to human doses is again based on body surface area, assuming the following weights: humans, 70 kg; rats, 400 g; and mice, 33 g. The conversion formula is: animal consumption = human consumption × (human weight/animal weight)$^{1/3}$. The human dose estimates were also reduced by a factor of 5/7 to take into account the fact that the test animals were only gavaged 5 days per week.

Using the data from the study by the National Toxicology Program (1982e), the committee estimated the lifetime risk and upper 95% confidence estimate of lifetime risk in humans after a daily consumption of 1 liter of water containing the compound in a concentration of 1 μg/liter (Table II-7). In previous volumes of *Drinking Water and Health*, the risk estimates from male and female rats and mice were averaged to yield one composite number. If one averages the data in Table II-7, the estimated upper 95% confidence estimate of lifetime risk per μg/liter is 2.98×10^{-8}.

Using the criteria for interpreting animal carcinogenicity data as out

TABLE II-6 Tumor Incidence in 1,1,1-Trichloroethane-Exposed Mice[a]

Animal	Sex	Tumor Site	Dose Levels, mg/kg/day	Tumor Rates
$B6C3F_1$ mouse	Male	Liver	0, 1,500, 3,000	16/50, 24/50, 20/50
$B6C3F_1$ mouse	Female	Liver	0, 1,500, 3,000	3/49, 5/49, 10/49

[a] Based on data from the National Toxicology Program, 1982e.

TABLE II-7 Carcinogenic Risk Estimates for 1,1,1-Trichloroethane[a]

Animal	Sex	Estimated Human Lifetime Risk[b]	Upper 95% Confidence Estimate of Lifetime Cancer Risk per μg/liter
B6C3F$_1$ mouse	Male	1.27×10^{-8}	3.70×10^{-8}
B6C3F$_1$ mouse	Female	1.09×10^{-8}	2.26×10^{-8}

[a] Based on data from the National Toxicology Program, 1982e.
[b] Assuming a daily consumption of 1 liter of water containing the compound in a concentration of 1 μg/liter.

lined in Chapter I, the committee based all the above calculations on *limited* evidence.

Teratogenicity Schwetz *et al*. (1975) exposed pregnant mice and rats to 1,1,1-trichloroethane vapor at concentrations of 875 ppm (4,827 mg/m^3). Both groups of animals were exposed for 7 hours/day on days 6 through 15 of gestation. No fetal toxicity or teratogenicity was found. Lane *et al*. (1982) exposed mice to 1,000 mg/kg concentrations of 1,1,1-trichloroethane in drinking water and found no adverse effects on reproduction.

The limited data indicate that 1,1,1-trichloroethane is not teratogenic in mice or rats.

Conclusions and Recommendations

Because virtually all of the studies of 1,1,1-trichloroethane have been conducted with inhalation exposures, it is difficult to make judgments concerning the effects following oral exposure. Data on the metabolism and pharmacokinetics of 1,1,1-trichloroethane in laboratory animals and humans are still needed.

When 1,1,1-trichloroethane was reviewed in the third volume of *Drinking Water and Health*, the only available carcinogenicity study was negative. Therefore, the committee calculated a chronic SNARL based on the lowest dose used in the negative cancer bioassay. Subsequent retesting has produced *limited evidence* that 1,1,1-trichloroethane is carcinogenic to mice but not to rats. Until such time as data are available to differentiate the effects of the doses in mice, rats, and humans, it must be assumed that 1,1,1-trichloroethane is carcinogenic in humans. Therefore, the carcinogenic risk estimate given above, even though based on *limited evidence*, should supercede the chronic SNARL published in the 1980 review.

TRICHLOROETHYLENE

ethene, trichloro-
CAS No. 79-01-6
$ClHC = CCl_2$

Trichloroethylene was evaluated in the first and third volumes of *Drinking Water and Health* (National Research Council, 1977, pp. 777-781; 1980, pp. 155-166). The following material, which became available after the 1980 report was prepared, updates and, in some instances, reevaluates the information contained in the previous reviews. Also included are some references that were not assessed in the earlier reports. Trichloroethylene has also been the subject of a monograph by the International Agency for Research on Cancer (1979a), in which toxicity data on animals and humans are evaluated.

Metabolism

The continuing high level of interest in the metabolic disposition of trichloroethylene has undoubtedly been stimulated by the increasing accumulation of evidence that its metabolic products are the cause of functional impairment and tissue damage in various organs. Henschler and Bonse (1978) postulated that the first step in the metabolism of this compound and other chlorinated ethylenes is the formation of an epoxide, a highly reactive intermediate with alkylating properties. Furthermore, their studies of synthetic epoxides indicate that the ethylenes with unsymmetrical chlorine substitution (a series to which trichloroethylene belongs) are more highly electrophilic than the analogs with symmetrically positioned chlorine atoms. In the rat, trichloroethylene induces its own metabolism by hepatic microsomes, an effect that increases the toxicity of the agent and, paradoxically, results in the destruction of the enzymes that catalyze the metabolism (Pessayre *et al*., 1979b, 1980).

Trichloroethylene appears to be activated by a phenobarbital-induced form of cytochrome P450 to an active species that can bind and inactivate the cytochrome (Costa *et al*., 1980). In both rats and mice, the induction of hepatic microsomal enzymes led to a greater degree of trichloroethylene bioactivation and increasing quantities of trichloroethylene-related materials covalently bound not only to hemes and cytochromes but also to various macromolecules, proteins, lipids, and DNA (Pessayre *et al*., 1980; Sipes and Gandolfi, 1980; Stott *et al*., 1982). These studies extend and confirm previously postulated mechanisms of trichloroethylene toxicity.

For a more complete review of the literature and discussion of the data, the reader should consult the report of Stott *et al*. (1982).

In rodents there is a good correlation between the rate and extent of trichloroethylene metabolism and the degree of observed hepatic toxicity. The importance of dose in the interpretation of the biochemical effects of trichloroethylene is underscored by the work of Stott *et al*. (1982), which showed that little alkylation of DNA by trichloroethylene metabolites occurs in the absence of cytotoxicity. In the same study, the investigators demonstrated that $B6C3F_1$ mice are capable of metabolizing more trichloroethylene to a toxic intermediate than are Osborne-Mendel rats. Extending this finding one step further, the authors cited the kinetic work of Monster *et al*. (1976) and Filser and Bolt (1979) as a basis for concluding that humans metabolize approximately 20 times less trichloroethylene than rats, on a weight basis. Since the metabolism of trichloroethylene is an important factor in its toxicity, one must view toxicity data cautiously when making interspecies comparisons.

Dichloroacetic acid has been shown to be a urinary metabolite of trichloroethylene in the mouse (Hathway, 1980). If confirmed, this finding would help to explain the relatively high toxicity of trichloroethylene in this species. A highly reactive intermediate almost certainly precedes the formation of the dichloroacetic acid. Studies by Nomiyama and Nomiyama (1979a,b) emphasize the differences in the rate and disposition of trichloroethylene in humans, rats, and rabbits. Although the disparity among the doses given introduces some uncertainty in the interpretation of the data, it is clear that the metabolism of trichloroethylene proceeds at a much slower rate in humans than in the rat or rabbit. In addition, a much greater proportion of trichloroethylene is excreted as trichloroacetic acid in the urine of humans than in the urine of rats or rabbits. Trichloroethanol is a major metabolite in rats, rabbits, and humans. Using an *in vitro* rat liver microsomal system, Leighty and Fentiman (1981) demonstrated that trichloroethanol is conjugated to palmitic acid. Whether this conjugation occurs *in vivo* and whether it occurs to an extent that would be important in the disposition of trichloroethylene remains to be established.

Health Aspects

Observations in Humans

Konietzko and Reill (1980) monitored 10 serum enzymes in 20 male volunteers for signs of toxicity following 4-hour exposures to 95 ppm (511 mg/m^3) doses of trichloroethylene. The dose was calculated to be equivalent to an 8-hour maximum allowable concentration (MAC) 50-ppm (268 mg/m^3)

exposure. No effects on serum enzyme activities were observed at the end of the exposure period or at either of two points during the first 24 hours. At the end of the 4-hour exposure, the following mean blood concentrations were measured: trichloroethylene, 5.8 μg/ml; trichloroethanol, 3.4 μg/ml; and trichloroacetic acid, 7.2 μg/ml. At 24 hours, trichloroacetic acid concentrations had risen to 15.3 μg/ml, whereas the concentrations of unchanged trichloroethylene had dropped to 0.18 μg/ml. The results of this study lend further support to the conclusion that trichloroethylene is not toxic in humans at exposure levels > 100 ppm (535 mg/m^3).

Observations in Other Species

Acute Effects The committee found no new reports that would amplify or extend the data cited in the previous reviews.

Chronic Effects Studies in which mice were given trichloroethylene by gavage 5 days/week for 3 weeks indicated that 250 mg/kg caused minimal hepatotoxic effects (Stott *et al*., 1982). The effects of this treatment were evident only upon histopathological examination of the liver. Daily doses of 500 mg/kg or greater caused an increase in liver weight and centrilobular hypertrophy of the liver. In contrast, a 3-week exposure of rats to trichloroethylene doses of 1,000 mg/kg/day resulted in no liver damage.

Mutagenicity Trichloroethylene was nonmutagenic in the Ames *Salmonella* assay when tested with TA100 in a 10-liter desiccator. Exposure levels were as high as 20% in air (v/v) for up to 16 hours. The assay was performed in the presence and absence of a phenobarbital-induced liver S9 fraction from male mice (Bartsch *et al*., 1979). Chloral hydrate—a metabolite of trichloroethylene—was found to be mutagenic in strain TA100 in the *Salmonella* standard plate incorporation assay in doses ranging from 0.5 to 10.0 mg/plate. The mutagenic activity was enhanced in the presence of rat liver S9 mix. Negative results were obtained with the frameshift mutant TA98 (Waskell, 1978). When tested in *Saccharomyces cerevisiae* D7, trichloroethylene induced both point mutations and mitotic gene conversion. These effects were observed only in the presence of mouse liver S10 at doses ranging from 10 to 40 mM concentrations. A mutagenic response was also obtained in the host-mediated assay with mice; both point mutations and gene conversion were observed in S. *cerevisiae* D7, and gene conversion in S. *cerevisiae* D4, when recovered from the liver and kidneys after both acute and subacute dosing. S. *cerevisiae* recovered from lung tissue showed little, if any, genetic effects (Bronzetti *et al*., 1980).

In another study with S. *cerevisiae* D7, no exogenous mammalian meta

bolic activation was necessary to elicit recombinations, mitotic gene conversions, and gene reversions after incubation for 1 hour at 37°C at a concentration of 15 mM. The highest dose used, a 22 mM concentration, was toxic to 99.7% of the cells. The investigators found that the yeast cytochrome P450 system mediated the metabolic conversion (Callen *et al*., 1980). No mutagenic effects were observed in a dominant lethal assay with NMRI—Han/BGA male mice exposed for 24 hours to 50-, 202-, and 450- ppm (268, 1,086, and 2,420 mg/m^3) concentrations of trichloroethylene. The following parameters were examined: fertilization rate, postimplantation loss, preimplanation loss, and dominant lethal mutations (Slacik-Erben *et al*., 1980).

In summary, trichloroethylene was mutagenic in one microbial mutagenicity assay and in a host-mediated assay in mice. Negative results were obtained in a dominant lethal assay in mice.

Carcinogenicity A draft technical report of a study by the National Toxicology Program (1982a) described a bioassay of trichloroethylene for carcinogenicity in both sexes of B6C3F$_1$ mice and Fischer 344 rats. The results for Marshall, ACI, and Osborne-Mendel rats are also contained in that report. Positive findings of carcinogenicity in B6C3F$_1$ mice in a previous study by the National Cancer Institute (1976) were discussed in the first and third volumes of *Drinking Water and Health* (National Research Council, 1977, pp. 778-779; 1980, pp. 163-164). In the more recent study, the B6C3F$_1$ mouse was used primarily as a laboratory positive control. The B6C3F$_1$ mouse and the Osborne-Mendel rat were also used to clarify questions raised by the earlier study concerning the housing of the test animals in the same room with animals receiving a variety of other volatile chemicals and by the presence of epichlorohydrin as a stabilizer in the test compound. A single 1,000 mg/kg bw dose of trichloroethylene in corn oil was administered by gavage to 50 mice of each sex, and doses of 1,000 and 500 mg/kg bw were given in the same manner to 50 rats of each sex 5 days/week for 2 years. For each group of test animals there were corresponding groups of controls composed of 50 animals of each sex. The trichloroethylene was stabilized with an amine antioxidant (diisopropylamine) and contained no detectable traces of 1,2-epoxybutane or epichlorohydrin. The sensitivity of the analytical method used to detect epichlorohydrin was 0.001%. Each group of animals was housed in a separate room.

The results observed in the mice support the previous National Cancer Institute (1976) findings that trichloroethylene significantly increased the incidence of hepatocellular carcinomas in B6C3F$_1$ mice of both sexes.

In general, the primary findings indicate that the Marshall, ACI, Osborne-Mendel, and Fischer 344 rats have a much lower sensitivity to the

carcinogenicity of trichloroethylene than was observed for the B6C3F $_1$ mouse. The tumor rates and sites are detailed in the following section on risk estimation.

In a chronic inhalation study of trichloroethylene stabilized with an amine antioxidant, Henschler *et al*. (1980) found no evidence of carcinogenicity in mice, rats, or Syrian hamsters.

In an epidemiological study of cancer deaths among a small study group of 518 men occupationally exposed to trichloroethylene, there was no statistically significant excess of cancer deaths (Axelson *et al*., 1978). The authors noted that the study could not conclusively rule out an increased cancer risk to humans, especially with respect to types of neoplasms that are rare in humans, including liver tumors.

Carcinogenic Risk Estimate In the study by the National Toxicology Program (1982a), there was an increased incidence of hepatocellular carcinomas in the exposed mice. The tumor incidences are summarized in Table II-8.

Each set of data showing a statistically significant increase was used to estimate lifetime risk and an upper 95% confidence estimate of lifetime risk in humans after a daily consumption of 1 liter of water containing the compound in concentrations of 1 μg/liter. The estimates of risk are based on the multistage model for carcinogenesis described earlier in this chapter for chlorobenzene.

The conversion of animal to human doses is again based on body surface area, assuming the following weights: humans, 70 kg; rats, 400 g; and mice, 33 g. The conversion formula is: animal consumption = human consumption × (human weight/animal weight)$^{1/3}$. The human dose estimates were also reduced by a factor of 5/7 to take into account the fact that the test animals were only gavaged 5 days per week.

Using the data from the study by the National Toxicology Program (1982a), the committee estimated the lifetime risk and upper 95% confidence estimate of lifetime risk to humans after a daily consumption of

TABLE II-8 Tumor Incidence in Trichloroethylene-Exposed Mice[a]

Animals	Sex	Tumor Site	Dose, mg/kg/day	Tumor Rates
B6C3F$_1$ mouse	Male	Liver	0, 1,000	8/48, 30/50
B6C3F$_1$ mouse	Female	Liver	0, 1,000	2/48, 13/49

[a] Based on data from the National Toxicology Program, 1982a.

1 liter of water containing the compound in a concentration of 1 μg/liter (Table II-9).

The National Toxicology Program's Technical Reports Review Subcommittee reviewed the data from the bioassay of the B6C3F$_1$ mice exposed to trichloroethylene. This group questioned certain aspects of the entire bioassay, i.e., that an excess number of deaths were attributed to gavage errors and that the maximum tolerated dose (MTD) was exceeded in both doses given to the rats. This matter is now undergoing further review. In addition, that subcommittee has not as yet approved the trichloroethylene studies involving Marshall, Osborne-Mendel, and ACI rats.

In previous volumes of *Drinking Water and Health*, the risk estimates from male and female rats and mice were averaged to yield one composite number. If one averages the data in Table II-9, the estimated upper 95% confidence estimate of lifetime risk per μg/liter is 3.3×10^{-7}. This is similar to the 1.1×10^{-7} upper 95% confidence estimate of lifetime risk per μg/liter, which was calculated in the first volume of *Drinking Water and Health* (National Research Council, 1977, p. 794).

Using the criteria for interpreting animal carcinogenicity data as outlined in Chapter I, the committee based the above calculations on *limited* evidence.

Teratogenicity Schwetz *et al*. (1975) exposed pregnant mice and rats to 300 ppm (1,630 mg/m^3) for 7 hours/day on days 6 through 15 of gestation. No fetal toxicity or teratogenicity was found. Dorfmueller *et al*. (1979) exposed female rats to 1,800 ppm (9,800 mg/m^3) for 6 hours daily for 2 weeks before pregnancy and for the first 20 days of gestation. They found anomalies of skeletal and soft tissues, which were considered to be indicative of developmental delay. No behavioral effects were observed in the off-spring of the treated animals. Examination of sperm from mice exposed by inhalation to 0.3% for 4 hours daily for 5 days revealed increased abnormalities after 28 days (Land *et al*., 1981).

TABLE II-9 Carcinogenic Risk Estimates for Trichloroethylene[a]

Animal	Sex	Estimated Human Lifetime Risk[b]	Upper 95% Confidence Estimate of Lifetime Cancer Risk per μg/liter
B6C3F$_1$ mouse	Male	3.77×10^{-7}	5.48×10^{-7}
B6C3F$_1$ mouse	Female	6.84×10^{-8}	1.12×10^{-7}

[a] Based on data from the National Toxicology Program, 1982a.
[b] Assuming a daily consumption of 1 liter of water containing the compound in a concentration of 1 μg/liter.

Rather limited data indicate that trichloroethylene does not have significant teratogenic potential in mice and rats.

Conclusions and Recommendations

Information that became available since the last review of trichloroethylene in *Drinking Water and Health* (National Research Council, 1980) is not sufficient to permit any more realistic assessment of suggested no-adverse-response levels than those made in Volume 3. Moreover, the additional studies recommended in the last review identify needs that are still present. A greater understanding of the differences among various species will be necessary before data obtained in studies in animals can be extrapolated to humans with more confidence.

The current risk estimation for exposure to trichloroethylene is very similar to that calculated in Volume 1 of *Drinking Water and Health*. Furthermore, this reconfirmation is important when and if limits for trichloroethylene in drinking water are established. It is also important that the uncertainties surrounding the carcinogenicity bioassay be resolved prior to reaching any decision on the potential for adverse health effects in humans following exposure to trichloroethylene. A chronic SNARL for trichloroethylene is not calculated because of its carcinogenicity in animals, but acute SNARL's were presented in Volume 3.

VINYL CHLORIDE (MONOCHLOROETHYLENE)

ethene, chloro-
CAS No. 75-01-4
$H_2C = CHCl$

Vinyl chloride was evaluated in the first volume of *Drinking Water and Health* (National Research Council, 1977, pp. 783-787). The following material, which became available after the 1977 report was prepared, updates and, in some instances, reevaluates the information contained in the previous review. Also included are some references that were not assessed in the earlier report.

Metabolism

After oral and inhalation exposures, vinyl chloride is rapidly absorbed, and its uptake can be saturated by either route (Bolt, 1978; Watanabe *et al*., 1976a,b). Pharmacokinetics data indicate a dose-dependent metabo

lism of vinyl chloride resulting in exhalation of the monomer at saturation (Gehring and Blau, 1977; Radwan and Henschler, 1977) and excretion of the sulfur-containing products thioglycolic and *N*-acetyl-*S*-(2-hydroxyethyl)-cysteine in the urine (Watanabe *et al*., 1976a,b).

Nonlinear (pseudo zero-order) kinetics apply at inhalation exposures to 250 ppm (640 mg/m^3) or greater, and the lungs provide the most rapid route of elimination. However, when saturation has been reached, vinyl chloride is eliminated by metabolism and urinary excretion (Bolt, 1978). Oral doses less than 250 mg/kg result in 70% to 75% of the vinyl chloride being excreted as a urinary metabolite and 4% to 5% in the expired air, whereas equivalent intravenous doses (which simulate inhalation exposure) result in 99% elimination of the compound in the expired air and less than 1% in the urine (Green and Hathway, 1975; Watanabe *et al*., 1976a,b).

Vinyl chloride is metabolized through the reactive epoxide (oxirane) intermediate, which binds macromolecules (Bergman, 1982) and depletes glutathione reserves (Du *et al*., 1982; Hefner *et al*., 1975). This reactive intermediate is the product of cytochrome P450 activation and can be enhanced by preexposure to inducers of mixed-function oxidases (Pessayre *et al*., 1979a).

Health Aspects

Observations in Humans

No new data were found by the committee.

Observations in Other Species

Acute Effects No new data were found by the committee.

Chronic Effects Several long-term inhalation toxicity and carcinogenicity studies have been reported. The chronic toxicity of vinyl chloride in the diet and in soy oil has recently been studied by Feron *et al*. (1981). In this experiment, the levels of exposure ranged from 1.7 to 300 mg/kg/day. These authors reported a decreased blood-clotting time, enlargement of liver and spleen, and a shift from angiosarcoma at 300 mg/kg to angiosarcoma and hepatocellular carcinoma at 14.1 and 5 mg/kg to the exclusive development of adenocarcinomas at 1.7 mg/kg. The no-observed-effect level is less than 1.7 mg/kg.

Mutagenicity The following investigators reported vinyl chloride to be mutagenic in the Ames *Salmonella* assay: Hällström *et al*. (1981), Bartsch

et al. (1975, 1979), and Simmon *et al*. (1977). The assays were performed in desiccators. Metabolic activation was not required to elicit the mutagenic response, but inclusion of liver S9 fraction from humans, rats, or mice, in addition to S9 derived from *Drosophila* Karsnas and Hikone larvae, enhanced the mutagenic response. Greim *et al*. (1975) reported vinyl chloride to be mutagenic in an *E. coli* suspension assay.

A positive response was obtained in a micronucleus test with male mice exposed by inhalation to 5% vinyl chloride for 4 hours (Jenssen and Remel, 1980). Negative results were obtained in a dominant lethal study with male mice exposed to 3,000, 10,000, and 30,000 ppm (7,670, 25,566, and 76,700 mg/m^3) for 6 hours/day for 5 days (Anderson *et al*., 1977). The frequency of sister chromatid exchanges and induced chromosome aberrations was increased in bone marrow cells of Chinese hamsters exposed to vinyl chloride at 1.25%, 2.5%, or 5% (v/v) for 6, 12, or 24 hours (Basler and Röhrborn, 1980). Chinese hamster V79 cells showed an increase in resistance to 8-azaguanine and ouabain after exposure for 5, 10, or 15 hours at 37°C to vinyl chloride at 5%, 10%, 20%, and 30% (v/v). The mutagenic response was observed only in the presence of a liver S15 fraction derived from phenobarbital-pretreated male rats (Drevon and Kuroki, 1979). No detectable mutation induction in two strains of *Neurospora crassa* was observed after their conidia were treated with vinyl chloride in an ethanol solution or in its gaseous form in the presence and absence of uninduced rat liver S9 fraction (Drozdowicz and Huang, 1977). Mutagenesis or gene conversion were observed in several yeast assays: *Saccharomyces pombe* and *S. cerevisiae* D4 (Loprieno *et al*., 1976) and S. *cerevisiae* D7 (Eckardt *et al*., 1981).

No chromosome aberrations were observed in peripheral lymphocytes from workers exposed for 2 years to maximum vinyl chloride levels of 4 ppm (19.2 mg/m^3) (Rössner *et al*., 1980). Peripheral lymphocytes obtained from workers occupationally exposed to levels of vinyl chloride greater than 5 ppm (12.8 mg/m^3) were found to have a higher incidence of chromosome aberrations than those obtained from a control group (Anderson *et al*., 1981). When concentrations of vinyl chloride were reduced to levels less than 5 ppm, chromosome aberrations in peripheral lymphocytes returned to control values in workers occupationally exposed to the chemical (D. Anderson *et al*., 1980).

In summary, vinyl chloride was mutagenic in several microbial mutagenicity test systems and in one *in vitro* mammalian mutagenesis assay. Positive results were also obtained in a mouse micronucleus test. Increases in the incidence of chromosome aberrations were observed in lymphocytes of occupationally exposed workers. Chromosome aberrations and sister chromatid exchange were seen in one animal species exposed to vinyl chloride.

Carcinogenicity Vinyl chloride is a proven carcinogen in mice, hamsters, and rats. In most experiments, the animals were exposed to vinyl chloride by the inhalation route, in concentrations ranging from 1 to 30,000 ppm (2.6-76, 687 mg/m^3) in air. Exposures were usually administered 4 hours/day 5 days/week for 17 to 52 weeks. In some studies, vinyl chloride in olive oil was administered by gavage 5 times a week during 1 year. In comparatively few studies did investigators examine the effects of vinyl chloride given by intraperitoneal subcutaneous injection (Maltoni *et al*., 1981).

Vinyl chloride produced tumors in mice, hamsters, and rats, regardless of the route of administration selected. Angiosarcomas of the liver were found in all animals examined. Some types of tumors were observed in one type of animal only, e.g., brain tumors, hepatomas, nephroblastomas, or sebaceous cutaneous carcinomas were found in rats only, whereas lung tumors (adenomas and adenocarcinomas) were found in mice only. In most experiments there was an indication of a dose response. Important determinants of the tumor response were duration and schedule of treatments. Newborn animals appeared to be especially sensitive to the carcinogenic effect of vinyl chloride, and there was evidence for transplacental carcinogenesis. The lowest dose of vinyl chloride to have a carcinogenic effect was claimed to be 50 ppm (130 mg/m^3) in air for an unspecified exposure time in Sprague-Dawley rats (Maltoni *et al*., 1981). A review of all other carcinogenicity studies on vinyl chloride largely confirmed these conclusions (International Agency for Research on Cancer, 1979a).

Adult Sprague-Dawley rats were exposed to 940 ppm (2,403 mg/m^3) concentrations of vinyl chloride by inhalation for 7 hours/day, 5 days/week for 24 weeks. Exposure was begun when the animals were 6, 18, 32, or 52 weeks old (Groth *et al*., 1981). The investigators found that older adult animals and females were more likely to develop angiosarcomas than were young or male animals. If confirmed, this observation would indicate that older animals are more susceptible to the carcinogenicity of vinyl chloride and that the latency period for the development of angiosarcoma could be shorter than in young animals. In another study, animals exposed to vinyl chloride by inhalation [600 ppm (1,534 mg/m^3), 4 hours/day, 5 days/week for 1 year] and to 5% ethanol in drinking water had an incidence of hepatic tumors (angiosarcoma and hepatocellular carcinoma) that was twice the incidence in animals exposed to vinyl chloride alone. Exposure to vinyl chloride and ethanol together also produced more hepatocellular carcinomas than exposure to vinyl chloride alone (Radike *et al*., 1981).

Hehir *et al*. (1981) examined single 1-hour exposures to various concentrations of vinyl chloride in air [50 to 50,000 ppm (128 to 127,812 mg/m^3)] to determine if they would elicit a carcinogenic response. In Fischer 344

rats, there was no evidence for a chemically induced tumor response. In ICR mice, only the 50,000-ppm doses produced an unequivocal increase in bronchial alveolar adenomas and carcinomas, whereas the A/J mice had pulmonary neoplasia following multiple inhalation exposure to 50 ppm. It was not clear whether dose fractionation had a significant influence on tumor incidence. Since the two experiments (single dose exposure and dose fractionation) were conducted in two different mouse strains (ICR and A/J), which are not equally susceptible to the development of lung tumors (Shimkin and Stoner, 1975), and since no data on tumor multiplicity were reported, the results are difficult to interpret. However, from this and some other experiments (Maltoni *et al*., 1981; Suzuki, 1981), it can be concluded that vinyl chloride inhalation produces lung tumors in ICR, A/J, CDI, CD, and NMRI mice.

Analysis of all available data on humans led the International Agency for Research on Cancer (1979a) to conclude that exposure to vinyl chloride results in an increased carcinogenic risk to humans. Organs most likely to be affected were the liver, brain, lung, and hemato- and lymphopoietic systems. In a more recent review of epidemiological studies of workers exposed to vinyl chloride, essentially the same conclusion was reached (Infante, 1981; Vianna *et al*., 1981).

Thus, there is convincing evidence that exposure of animals or of humans to high doses of vinyl chloride produces cancer. However, for many chemicals, vinyl chloride among them, the toxicity and carcinogenicity are mediated through a metabolic intermediate rather than through the parent compound. This could necessitate an estimate of how much of a chemical would indeed undergo biotransformation during exposure of animals before meaningful dose-effect relationships could be obtained or cross-species comparisons could be made. For vinyl chloride in particular it was shown that the incidence of angiosarcoma in rats was related to the amount of vinyl chloride metabolized rather than to the concentration of exposure (Gehring *et al*., 1978; Watanabe *et al*., 1977). Pharmacokinetic considerations should thus apply in extrapolating results from animal studies to humans. This has been done in several attempts to estimate risks from vinyl chloride exposure (M. W. Anderson *et al*., 1980; Gehring *et al*., 1978; Van Ryzin and Rai, 1980). It must be remembered, however, that in the final analysis the scientific rationale behind this approach relates only to a possible initial event in carcinogenesis, the interaction of a chemical with DNA. Carcinogenesis is more likely a multistage process rather than a one-hit event (Farber and Cameron, 1980), and pharmacokinetic considerations may not necessarily apply to all stages.

Teratogenicity John *et al.* (1981) exposed rodents and rabbits by inhalation to vinyl chloride ranging from doses of 50 to 2,500 ppm (128 to 6,390

mg/m^3) during major organogenesis and found no adverse fetal effects. Ungváry *et al*. (1978) exposed rats to 1,500 ppm (3,840 mg/m^3) during pregnancy and observed that there was increased fetal mortality but no malformations.

Although Infante *et al*. (1976a,b) reported increased rates of malformations in one city where a vinyl chloride plant was located, subsequent studies by Edmonds *et al*. (1978) revealed that the parents of these children had not been workers in the plant nor had they been living closer to the manufacturing source than had the controls. Infante *et al*. (1976b) reported a significant increase in fetal loss among the wives whose husbands had been exposed. As a "control" group, they used workers in rubber plants. Sanotskii *et al*. (1980) did not find an increase in spontaneous abortions among the wives of vinyl chloride workers.

In summary, vinyl chloride does not appear to be teratogenic in rats or rabbits. The data on humans are not adequate for judgment to be made.

Conclusions and Recommendations

A SNARL for chronic exposure was not calculated because orally administered vinyl chloride is an established carcinogen in humans. It is also carcinogenic in mice, hamsters, and rats, in which angiosarcomas were found, regardless of route. The older animals and females appeared to be more susceptible. The cancer risk estimate for vinyl chloride can be found in Volume 1 of *Drinking Water and Health*.

URANIUM (U)

Uranium was evaluated in the third volume of *Drinking Water and Health* (National Research Council, 1980, pp. 173-178). That review was devoted exclusively to the element's chemical toxicity. In the following review, the committee also considers its radiological effects and provides updates and, in some instances, reevaluations of the information on chemical toxicity contained in the previous volume. Included are some references that were not assessed in the earlier report.

Uranium is ubiquitously distributed throughout the earth's crust. It has a complex radioactive decay scheme resulting in the emission of different radiations and the production of several radioactive daughter products. Because its abundance in the crust varies geographically, uranium is a highly variable source of contamination of drinking waters that may be directly consumed by humans and incorporated into their diet. In this brief review, the committee discusses the potential for radiation and chemical toxicity from the ingestion of natural uranium and clarifies the difference

between radiation toxicity and the rather well-studied chemical toxicity of this element. For the purpose of brevity and conciseness, not all of the primary references have been cited; rather, the committee has provided several references that are representative and sufficiently broad to cover the needs of this report.

Natural uranium is present in soils and rocks in concentrations generally varying between 0.5 and 5 ppm. The average is approximately 1.8 ppm in most soils. Higher concentrations are present in salic rock, in granite, and in sedimentary shale. More than 99% of uranium is present as the isotope uranium-238. Another 0.72% occurs as the fissionable isotope uranium-235, and 0.0054% by weight of uranium as uranium-234. The half-lives are: uranium-238, 10^9 years; uranium-235, 10^8 years; and uranium-234, 10^5 years. The radioactivity of the three isotopes in natural uranium averages approximately 7.35×10^5 disintegrations per minute per gram, almost all of which is from uranium-238 (National Council on Radiation Protection and Measurements, 1975).

Uranium-238 is generally found to be in equilibrium with thorium-234, palladium-234, and uranium-234, so that a gram of natural uranium would contain 0.33 μCi of each of the four nuclides. Furthermore, uranium-238 and uranium-234 are generally in disequilibrium in nature. The fractionation of 234 from 238 is believed to occur by the displacement of the daughter atom uranium-234 from the crystal lattice by recoil, which renders uranium-234 potentially capable of being oxidized to the hexavalent stage when it is more easily leached into the water phase than the parent 238. Because of the disequilibrium, the 234:238 uranium activity concentration ratio can vary among water samples. Reported values range from 0.7 up to 9 (United Nations, 1977).

The activity concentration of uranium-238 in tap water is usually reported to be less than 0.03 pCi/liter. Thus, the contribution of drinking water to total dietary intake is generally small. A report by the United Nations (1977), however, states that concentrations of uranium found in Russian tap water have been as high as 70 pCi/liter and that concentrations of approximately 1,000 pCi/liter have been measured in several wells in Finland. The highest concentration actually reported was 5,000 pCi/liter. The very high concentration of uranium in the water from those wells was attributed to small, localized uranium-rich deposits (Asikainen and Kahlos, 1980; Kahlos and Asikainen, 1980).

Although uranium may adhere to inhaled particles, inhalation is only a minor route of entry into humans. The United Nations (1977) estimated that the daily adult intake via inhalation is approximately 1×10^{-3} pCi. The major route of entry is ingestion.

The National Council on Radiation Protection and Measurements

(1975) assumed a 1.8 kg/day food intake level for a "reference man" and, in limited measurements, determined that drinking water provides 2% of the 1 pCi of uranium ingested daily. Approximately 20% of the daily intake is provided by grains and cereal products; 20% by meat, fish, and eggs; 20% by green vegetables, fruits, and pulses; and 20% by root vegetables. As much as 10% may be present in milk and dairy products.

Metabolism

The data indicate that approximately 1 pCi (~ 1 μg) of uranium is consumed daily and that from 2% to 3% of that amount is derived from drinking water (National Council on Radiation Protection and Measurements, 1975). To a large extent, the uptake and fate of ingested uranium are controlled by the total quantity ingested and, to a lesser extent, on the particular chemical form. In general, the smaller the amount ingested, the greater the fraction absorbed (Durbin and Wrenn, 1975). For the purposes of this report, it will be assumed that the quantity of uranium ingested by humans is very small and, therefore, the maximum uptake possible occurs. Hursh and Spoor (1973) cite data indicating that between 12% and 30% of the ingested uranium is absorbed from the intestinal tract into the blood-stream. An intermediate figure of 20% is assumed in the following discussion.

Of the absorbed uranium, approximately 80% is excreted, 10% goes to the kidneys, and the remaining 10% is deposited in the skeleton. The kidney retention is believed to be brief, with a biological half-life of 1 to 2 weeks. The uranium deposited in the skeleton is divided disproportionately between the spongy and compact bone compartments. Approximately 80% of the skeletal mass is assumed to be compact bone in which ~ 95% of the deposited uranium has a short effective half-life ranging from 1 month to 1 year, whereas only ~ 2% of the absorbed uranium in the remaining 20% of the skeleton may have an average half-life of about 10 years (Roswell and Wrenn, 1980). Because of efficient renal clearance of circulating uranium, redistribution of uranium deposits is inefficient and the body burden of uranium probably reflects recent dietary intakes.

Natural uranium bicarbonate complexed with proteins is filtered by the kidney glomeruli. The plasma equilibrium is shifted from the proteinate to the bicarbonate until all uranium not deposited in the skeleton has passed through the glomeruli. Once in the kidney tubules, the bicarbonate complex is partially dissociated to conserve sodium. With the accompanying renal reabsorption of water, the urine becomes more acidic, the uranium more concentrated, and further bicarbonate disassociation occurs. The freed uranium can bind to the luminal surfaces of the cells lining the proxi

mal renal tubules and, with sufficient time and dosage, can cause tubular damage (Durbin and Wrenn, 1975). Hodge (1951) noted, "The uranium-inhibited reaction is located on the cell surface, is rate limiting in the presence of uranium, is chemical in nature, is an enzymatic not a diffusion process, is specific, and probably involves the reaction of uranium with the phosphates of adenosine triphosphate." Therefore, the mechanism of toxicity is likely to be suppression of cellular respiration. The site of action is invariably confined to the proximal convoluted tubules of the kidney (Hursh and Spoor, 1973).

Health Aspects

Observations in Humans

The committee found no reports of radiological toxicity in humans exposed to natural uranium by ingestion. Furthermore, as Hursh and Spoor (1973) noted, "The implication that the contamination of drinking water by uranium is an uncommon and relatively unimportant hazard is confirmed by the dearth of precise and unambiguous information in the literature." The excellent summarized history of uranium poisoning by Hodge (1973) indicates that renal injury occurred in uranium-treated diabetics in the last century and early in this century. These patients were generally given hundreds of milligrams per day over extended periods. There are no population studies, and only specific clinical assessments have been reported. Recent studies in humans are confined to those in which much lower concentrations of uranium were used. The aim of these studies was to determine the absorption and urinary excretion of oral doses to assist in interpreting reports of early therapeutic administrations.

Luessenhop *et al.* (1958) investigated the effects of intravenous administration of uranyl nitrate [$UO_2(NO_3)_2$] in humans. Five volunteers with terminal brain cancer received doses that ranged from 0.097 to 0.28 mg/kg (average, 0.15 mg/kg). They noted the following distribution-excretion pattern: from 6% to 14% to the skeletal system, 16% to the kidneys, and 49% to 84% excreted in the urine. These percentages are comparable to those found in studies of small animals. The most sensitive indicator of renal damage was an increase in urinary catalase. Other chemical signs included albuminuria, urinary casts, and a suggestion that there was interference with the renal capacity for reabsorption of sodium and chloride and the secretion of potassium. These authors concluded that, of the common laboratory animals, rats appear to correspond most closely in sensitivity to humans in regard to intravenous tolerance to uranium.

Observations in Other Species

Acute Effects The renal injury associated with uranium toxicity usually manifests itself several days after exposure. In rats, cellular necrosis appears in the lower portion of the proximal convoluted tubules as demonstrated by hyaline casts or casts containing shed necrotic cells. Accompanying the pathological changes are functional changes in the kidney, characterized by proteinuria, impaired Diodrast and *p*-aminohippuric acid clearance, and increased clearance of amino acids and glucose (Stone *et al*., 1961).

In studies in dogs, Thompson and Nechay (1981) demonstrated that uranium as uranyl nitrate [$UO_2(NO_3)_2$] produced a 74% inhibition of renal calcium Ca^{2+} ATPase and an 84% inhibition of renal magnesium Mg^{2+} ATPase at concentrations of 3×10^{-5} M and 1×10^{-5} M, respectively. They postulated that uranium dioxide (UO_2^{2+}) may compete with ATP for binding sites on the Ca^{2+} and Mg^{2+} ATPase and that this may be a factor in its renal toxicity.

Nomiyama *et al*. (1974) demonstrated in rabbits that early renal injury could be detected by increase of specific enzymes in the urine following one intravenous injection of uranyl acetate (0.2 mg of uranium/kg bw). Urinary alkaline phosphatase, glutamic oxaloacetic transaminase, and glutamic-pyruvic transaminase all increased significantly before changes were observed in routine urinalyses or renal function tests. Thus, assays for selected urinary enzymes may be more sensitive indicators of early renal injury.

An interesting phenomenon observed after acute exposure to uranium is the development of tolerance (mainly in rats) to the effects of repeated exposure. This tolerance may be related to the ability of "uranium-conditioned" animals to excrete uranium more efficiently or to a mechanism in which the renal tubular epithelium does not bind and retain uranium as well as "unconditioned" epithelium (Durbin and Wrenn, 1975).

Maynard and Hodge (1949) also studied the acquired uranium tolerance in rats and concluded that "prolonged exposure to uranium may produce conditions under which injection of an ordinary damaging dose of uranium results in no appreciable renal tubular necrosis."

Chronic Effects Many studies have been conducted to compare the toxicity of natural uranium and some of its isotopes after continuous ingestion. Rodents and dogs continuously fed a wide range of natural uranium concentrations developed no radiogenic cancers. At the higher levels, renal

damage predominates as the toxic end point. As pointed out by Durbin and Wrenn (1975), "Because uranium has a long residence time in bone, the radiation dose is limiting for all uranium isotopes except ^{235}U and ^{238}U and all mixtures of uranium isotopes containing at least 91.5% ^{238}U by weight (12-fold enrichment in ^{235}U by the gaseous diffusion process has at least an equal amount of ^{234}U)."

For example, in a classic toxicity study by Finkel (1953), injections of natural uranium in concentrations as high as 1 mg/kg induced no malignant bone tumors, whereas uranium-233 doses of 1 mg/kg proved to be a maximally effective bone carcinogen, comparable to uranium-232 at 5×10^{-4} mg/kg. More importantly, the uranium-232 activity was approximately 10 μCi/kg, as was that of the uranium-233, whereas the natural uranium contained a maximum of 7×10^{-4} μCi/kg.

Maynard and Hodge (1949) conducted 2-year studies in which rats were continuously fed different uranium compounds ranging from 0.1% to 20% of the dietary mass. The lowest dietary levels producing retardation of growth were: uranyl fluoride, 0.1%; uranyl nitrate hexahydrate, 0.5%; and uranium tetrafluoride, 20%. Uranium dioxide at 20%, the highest level tested, produced no effect. The only major pathological effect observed as a result of chronic oral exposure was necrosis of the renal tubular epithelium, involving predominantly the proximal convoluted tubule. The same effects were frequently observed in dogs and rabbits exposed for only 30 days. Although chemical toxicity was observed, primarily in the kidney, no radiogenic effects were noted.

The same authors also fed various uranium compounds at graded doses of 0.0002 to 0.2 g/kg/day to dogs over a 1-year period. With the exception of one dog fed the highest level (0.2 g/kg/day) of uranyl nitrate, all the dogs survived, gained weight, and were healthy and active. Only at dietary doses greater than 0.02 g/kg/day were any effects of uranium toxicity noted. Moderate elevations in blood nonprotein nitrogen and urea nitrogen were observed as well as transient urinary sugar and proteinuria. Pathological effects were not described.

The investigators observed interesting differences in response between dogs dosed for 1 year and rats dosed for 2 years. Rats were more resistant to the toxicity of uranyl fluoride (UO_2F_2) by a factor of approximately 25. For uranyl nitrate [$UO_2(NO_3)_2 \cdot 6H_2O$], the rats were more resistant by a factor of about 20, and for uranium tetrafluoride (UF_4), by a factor of 4.

In summary, studies in laboratory animals have produced radiation-related cancers only when high specific-activity isotopes of uranium were used. There have been no reports of cancers resulting from the ingestion of natural uranium in laboratory animals.

Mutagenicity No data were found by the committee.
Teratogenicity No data were found by the committee.

Conclusions and Recommendations

Suggested No-Adverse-Response Level (SNARL)

Chronic Exposure Because there is no evidence that naturally occurring uranium-238 is carcinogenic, a chronic exposure SNARL will be calculated. Two-year studies in rats and a 1-year study in dogs by Maynard and Hodge (1949) have indicated that the dog is the more sensitive species. In chronic exposures, the highest dietary level tolerated by dogs with no decreases in body weight gain or symptoms of renal involvement was 1 mg/kg/day. Luessenhop *et al*. (1958) found 0.15 mg/kg to be the minimum-observed-effect dose in humans, but this dose was administered intravenously. This amount is approximately the same as that absorbed (assuming 20% gastrointestinal absorption) following an oral dose of 1 mg/kg. Using an uncertainty factor of 100, and assuming that a 70-kg adult consumes 2 liters of water daily and that 10% of the uranium intake is provided by the water, one may calculate a chronic SNARL as:

$$\frac{1.0\ \text{mg/kg} \times 70\ \text{kg} \times 0.1}{100 \times 2\ \text{liters}} = 0.035\ \text{mg/liter, or } 35\ \mu\text{g/liter.}$$

If one assumes that the isotopic ratios in natural uranium are in equilibrium, then there would be 0.33 pCi/μg of uranium-238. A value of 35 μg/liter would be equivalent to 11.6 pCi/liter, which is about twice the 5 pCi/liter established as the current maximum contaminant level (MCL) for *radium* (U.S. Environmental Protection Agency, 1975a). Ingestion of water containing radium at 5 pCi/liter is estimated to carry a risk of between 0.7 to 3.0 fatal cancers per year per million persons exposed. There is currently no standard for uranium.

The total absence of carcinogenic effects from ingested natural uranium in either animals or humans makes it difficult to develop an appropriate model for the radiotoxicity of that element. Furthermore, the fact that drinking water rarely contributes more than 2% to 5% of the total uranium ingested daily leads to the conclusion that a radiation risk model for natural uranium is inappropriate and unjustified on the basis of present knowledge. When natural uranium is subjected to an enrichment process resulting in specific activity exceeding 10^6 disintegrations per minute per gram, it is possible that a radiotoxicity model could be developed (U.S.

Energy Research and Development Administration, 1976). This assumption is based primarily on the findings of Finkel (1953), who studied uranium-233 and natural uranium, and on the radioactivity rather than the mass relationships of the different uranium isotopes tested in laboratory animals. Finkel reported that the radioactivity, in terms of alpha disintegration rate, reached levels comparable to those seen in radium-226 toxicity. For purposes of this discussion, the effectiveness of uranium-233 and uranium-235 can be regarded as fairly comparable to that of radium-226, based on ingested μCi per unit time.

Because of its low specific activity, natural uranium does not pose a problem of radiotoxicity in drinking water. Assessment of uranium toxicity in drinking water should be based on its chemical toxicity and not on radiation toxicity. However, when the specific activity of uranium in drinking water has been altered so that it is greater than that of natural uranium, potential radiotoxicity should be given attention equal to that of the chemical toxicity. The committee also recommends that toxicological assessment of uranium in water be based solely on its renal toxicity in all instances except when industrial processes result in a marked enrichment of shorter-lived uranium isotopes.

Additional research should be carried out to determine with greater precision the dynamics of uranium absorption from the gastrointestinal tract for different chemical forms of uranium.

Conclusions

Chronic Toxicity

Table II-10 lists the compounds reviewed in this volume for which there were sufficient data to calculate either a chronic SNARL or cancer risk estimate. The statistical methodology for the cancer risk estimate is described for chlorobenzene on page 21. Further details on methodology can be found in Volumes 1 and 3 of *Drinking Water and Health* (National Research Council, 1977, 1980). It is important to stress that the reader should refer back to the discussion on individual compounds for specific details.

Mutagenicity As described in Chapter 1, a chemical was judged to be a mutagen when it could be shown that it was mutagenic in any one short-term test. The data summary in Table II-11 is based on this criterion. It is important to stress that the reader should refer back to the discussion on individual compounds for specific details.

TABLE II-10 Summation of Chronic Exposure Levels and Carcinogenicity Risk Estimate for Chemicals Reviewed

Chemical	Suggested No-Adverse-Response Level (SNARL), mg/liter, for Chronic Exposure	Upper 95% Confidence Estimate of Lifetime Cancer Risk per μg/liter
Aldicarb	0.007[a]	
Chlorobenzene		2.13×10^{-7}[b]
o-Dichlorobenzene	0.3[c]	
p-Dichlorobenzene	0.094[a,c]	
1,1-Dichloroethylene	0.10	
Dinoseb	0.039	
Hexachlorobenzene		1.85×10^{-6}[a]
Methomyl	0.175	
Picloram	1.05	
Rotenone	0.014	
Tetrachloroethylene	[d]	[d]
1,1,1-Trichloroethane		2.98×10^{-8}[b]
Trichloroethylene		3.3×10^{-7}[b]
Uranium	0.035	

[a] This is the suggested no-adverse-effect level calculated in Volume 1 of *Drinking Water and Health* (National Research Council, 1977) and continues to be the recommended SNARL.
[b] Based on *limited* evidence (see Chapter 1) from ongoing studies being conducted by the National Toxicology Program (1982a,d,e). The studies on these compounds have undergone peer review and the results are in press at this writing.
[c] This SNARL must be reviewed when the cancer bioassay is completed and reviewed (see text for details).
[d] Neither a SNARL nor a cancer risk estimate has been calculated by the committee pending the outcome of an ongoing study being conducted by the National Toxicology Program (1982d).

Teratogenicity The only compound reviewed in this volume that showed teratogenic potential following oral exposure was hexachlorobenzene. Dinoseb was teratogenic following intraperitoneal, but not oral exposure. The data for rotenone and trichloroethylene are inconclusive.

Research Recommendations

Although specific research recommendations are given for many of the compounds reviewed, the major areas are summarized here.

1. The most urgent need is for comparative data on various aspects of metabolism in laboratory animals and humans. Only with such data can relevant animal models be used to predict more accurately the potentially adverse health effects in humans.
2. In conjunction with the above recommendation, these kinds of data ultimately need to be included in the mathematical models now used to estimate cancer risk.

TABLE II-11 Mutagenicity Studies of Chemicals Reviewed in this Volume

Chemical	Mutagenicity[a]
Aldicarb	−
Carbofuran	−
Carbon tetrachloride	+
Chlorobenzene	ND[b]
o-Dichlorobenzene	−[c]
p-Dichlorobenzene	ND
1,2-Dichloroethane	+
1,1-Dichloroethylene	+
cis-1,2-Dichloroethylene	−
trans-1,2-Dichloroethylene	−
Dichloromethane	+
Dinoseb	+
Hexachlorobenzene	+
Methomyl	+
Picloram	+
Rotenone	−[c]
Tetrachloroethylene	+
1,1,1-Trichloroethane	+
Trichloroethylene	+
Vinyl chloride	+
Uranium	ND

[a] See text for details.
[b] ND = no data.
[c] Inconclusive data, see text for details.

3. Data on reproductive effects including teratogenicity should be generated for the majority of the compounds reviewed.

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III

Epidemiology of the Adverse Health Effects of Arsenic and Asbestos in Drinking Water

Both arsenic and asbestos were reviewed in earlier volumes of the *Drinking Water and Health* series. The discussions in this chapter are limited primarily to important new epidemiological data that became available after Volumes 1 and 3 had been completed and to reassessments of some older studies of effects of arsenic and asbestos in human populations.

ARSENIC

Arsenic was evaluated in the first and third volumes of *Drinking Water and Health* (National Research Council, 1977a, pp. 316-344; 1980, pp. 337-345). It was also comprehensively evaluated in 1977 by another committee (National Research Council, 1977b). Epidemiological studies reviewed in those volumes have provided conflicting associations between the presence of arsenic in drinking water and the development of skin cancer. Some studies discussed below were reviewed in previous volumes, while several were published after the previous volumes were prepared. One of the new reports evaluated by the committee is a review of the carcinogenicity data on arsenic, which was prepared by the International Agency for Research on Cancer (1980). That agency concluded that, although there is inadequate evidence for the carcinogenicity of arsenic compounds in animals, there is "*sufficient evidence* that inorganic arsenic compounds are skin and lung carcinogens in humans." This statement presumably applies to all sources of arsenic, including drinking water. It is based on the conflict

ing results from only two epidemiological studies (Morton *et al*., 1976; Tseng, 1977; Tseng *et al*., 1968).

The adverse health effects of exposure to arsenic compounds have been examined in epidemiological investigations for nearly a century. Investigators have gathered and examined data from a variety of sources, including patients given prescribed medicinal arsenic compounds, several occupationally exposed groups, and populations exposed to high levels of arsenic in drinking water supplies (Arguello *et al*., 1939). Although arsenic exposure has been associated primarily with skin cancer, associations with other cancers, cardiovascular dysfunctions, and a peripheral vascular disorder known as "blackfoot" disease have also been found.

Epidemiological investigations in Europe (Geyer, 1898), in several South American countries, e.g., Argentina (Arguello *et al*., 1939) and Chile (Borgono *et al*., 1977; Zaldivar, 1974), and in Taiwan (Tseng, 1977; Tseng *et al*., 1968) have suggested an association between the effects of chronic exposure to high levels of arsenic in drinking water and the occurrence of a variety of skin disorders, including skin cancer. The most significant of these reports was published by Tseng and colleagues, who not only correlated a high prevalence of skin cancer and blackfoot disease with the arsenic contents of drinking water supplies in Taiwan but also reported a dose-response relationship between both skin cancer and blackfoot disease and the duration of water intake based on a detailed house-to-house medical survey of approximately 40,000 people out of a total exposed population of about 100,000 individuals (Tseng, 1977; Tseng *et al*., 1968). The inhabitants of the area with endemic arsenic exposure began using artesian wells with high arsenic levels approximately 45 years before the study by Tseng *et al.* (1968). Most of these people were engaged in farming, fishing, or salt production, and their socioeconomic status was considered to be poor. Their diet was low in animal protein and fat, but high in carbohydrates. Their habits and customs were not considered different from those of persons living in other parts of Taiwan. The overall prevalence rates for skin cancer, hyperpigmentation, and keratosis were 10.6, 183.5, and 71.0 per 1,000, respectively. The skin cancers observed were atypical, in that approximately three-quarters of them were located on parts of the body not usually exposed to sunlight and more than 99% of the patients had more than one lesion.

The control population consisted of 7,500 persons living in a low arsenic exposure area, where drinking water concentrations of the element ranged from less than 1 ppb (μg/liter) up to 17 ppb. Approximately two-thirds (~ 5,000) of the controls lived on the nearby island of Matsu. Most of them were fisherman. The remainder of the controls, who lived in villages near the endemic exposure area, were farmers and salt workers. The sex and

age distribution in the control group was similar to that in the exposed group. Not a single case of melanosis, keratosis, or skin cancer was observed in the control population.

More than 45% of the wells serving as drinking water supplies for the "exposed inhabitants" had arsenic levels in excess of 400 ppb, compared with the U.S. Environmental Protection Agency (EPA) national drinking water standard of 0.05 mg/liter (50 ppb) (U.S. Environmental Protection Agency, 1976). In attempts to establish a dose-response relationship, investigators compared three groups, based on drinking water levels of arsenic: < 300 ppb, 300 to 600 ppb, and > 600 ppb. There was a definite ascending gradient or prevalence of effects from the low to high arsenic groups for both sexes in three different age groups, but no calculations of statistical significance across exposed groups were presented.

In three epidemiological studies conducted in the United States, investigators found no positive relationship between high levels of arsenic in drinking water and adverse health effects. More than 200 residents of Fairbanks, Alaska, exposed to drinking water containing mean arsenic levels of 224 ppb displayed no increases in skin disorders, although the longest exposure in the study population was only 10 years (Harrington *et al*., 1978). Similarly, Morton *et al*. (1976) did not note any increase in the incidence of skin cancer between 1958 and 1971 in Lane County, Oregon, where the arsenic content of the drinking water supplies was relatively high; however, only 5% of the arsenic levels of those supplies exceeded 100 ppb. In a recent study in Utah, Southwick *et al.* (1981) compared the health status of 145 people consuming drinking water containing arsenic levels of approximately 200 ppb and a matched control group of 105 participants from a neighboring community, where drinking water levels of arsenic averaged 20 ppb. The investigators did not find any cutaneous manifestations of arsenic toxicity. In addition, there was no excess of cancer incidence and death rates in the exposed community.

There are several possible explanations for the apparently conflicting results discussed above. One is the striking difference in the arsenic levels of the drinking water supplies surveyed; the average arsenic content of the supplies in Taiwan greatly exceeds those of the U.S. communities studied. Moreover, the duration of exposure was probably shorter and the amount of arsenic ingested much less in the U.S. studies than in the Taiwan studies, where personal mobility is greatly limited. Furthermore, lack of adequate nutrition and exposure to other environmental pollutants may have exacerbated the effects of arsenic exposure in Taiwan. The differences in exposure to sunlight between Taiwan and Alaska may have been a factor in the observed absence of skin disorders in the Fairbanks study (Harrington

et al., 1978) and the Utah study (Southwick *et al*., 1981). None of the investigators attempted to determine which arsenic compounds were present in the drinking water supplies surveyed. Since arsenic toxicity varies from compound to compound, this may have been a factor in the conflicting results of the different studies.

Unfortunately, the Taiwan studies did not include analyses of drinking water constituents other than arsenic in the water sources of the exposed and control groups. This lack of assessment reduces the extent to which confidence can be placed in the postulated association between arsenic levels and observed skin cancer. This is particularly relevant since Lu (1978) recently reported the presence of ergot alkaloids in the Taiwan well water associated with blackfoot disease. These alkaloids may cause symptoms similar to those of blackfoot disease. Other aspects of the Taiwan study might also be questioned. For example, there is a possibility that the observers might have been biased by knowledge of the high-or low-exposure areas. Moreover, only about 30% of the skin cancers were verified by biopsy. The extent to which these factors may affect the interpretation of the findings is uncertain.

The assessment of arsenic for adverse health effects is confounded by several issues. First, it is always of great value when epidemiological associations are supported with animal studies; however, animal studies have generally not indicated that arsenic is carcinogenic (International Agency for Research on Cancer, 1980; U.S. Environmental Protection Agency, 1979). Second, arsenic is now recognized as an essential nutrient in several mammalian species (e.g., the rat, chick, guinea pig, and goat) and possibly in humans as well. Extrapolation from data on animals suggests that 25 to 50 μg of arsenic per person may be the daily requirement for humans (U.S. Environmental Protection Agency, 1979).

When deriving the current maximum contaminant level (MCL) for arsenic, the EPA took into account the consumption of arsenic in food as well as its association with skin cancer in the Taiwan study (U.S. Environmental Protection Agency, 1976). Using data from Schroeder and Balassa (1966), the agency estimated that humans consumed approximately 900 μg of arsenic daily from food. The 50 μg/liter MCL (0.05 mg/liter) for arsenic in drinking water was based on the assumption that not more than 10% of the total ingested arsenic would be provided by that source. After the interim arsenic standard was established, the estimate of dietary arsenic consumption was greatly reduced from 900 to approximately 70 μg/day (U.S. Environmental Protection Agency, 1976, 1977, 1979). Because the rationale behind the drinking water standard (U.S. Environmental Protection Agency, 1976) is to keep the arsenic exposure from drinking water at 10%

of total ingested arsenic, the more recently recognized lower dietary exposure may necessitate a reevaluation of the current drinking water standard.

An important related issue is that the EPA has decided that there is no threshold dose for chemical carcinogens. Consequently, there is no safe exposure level to a carcinogen. Various biostatistical models have been used to predict the risk of cancer occurrence when human exposures are much lower than those in the observable range for which dose-response relationships are known. The EPA Carcinogen Assessment Group used a modified "one-hit" model to apply this type of downward extrapolation to the Taiwan data (U.S. Environmental Protection Agency, 1981). The results indicated that the lifetime risk of skin cancer from drinking water containing 20 ppb (or approximately one-half of the federal standard) would be 1 per 100. This prediction is at variance with the negative findings in the U.S. epidemiological studies cited above. Although the negative epidemiological studies on U.S. populations raise questions about the cancer risk estimations based on the Taiwan data, it is not possible to determine whether such discrepancies are caused by inadequacies of the Taiwan studies, the specific risk assessment model used, or a combination of both.

Conclusions and Recommendations

The epidemiological studies on U.S. populations have failed to confirm the association between arsenic in drinking water and the incidence of cancer observed in Taiwan. Furthermore, only 0.4% of the drinking water samples taken from public water supplies in the United States have exceeded a total arsenic concentration of 0.01 mg/liter (National Research Council, 1980). This finding is in sharp contrast to the *average* concentration of 0.4 mg/liter (400 ppb) reported in the Taiwan studies. It is therefore the opinion of this committee that 0.05 mg/liter provides a sufficient margin of safety, but that further experimental research and epidemiological evaluations of the association of elevated levels of arsenic in drinking water and skin cancer be undertaken.

Continued study of those U.S. populations exposed to high levels of arsenic in their drinking water appear warranted. Efforts should also be directed toward discovering the differences that exist between the chemical species of arsenic in Taiwan and those in the United States or the presence of any other potentially confounding factors. Moreover, the development of an appropriate animal model for carcinogenicity studies would help to resolve the equivocal epidemiological studies. In addition, there should be investigations about interactions of arsenic and other environmental fac

tors that may account for the observed differences in clinical studies as well as the effects of diet, race, and climate.

Research should also be designed to evaluate the possible essentiality of arsenic for humans—a requirement that has been demonstrated in four mammalian species. In the absence of new data, the conclusion reached in the third volume of *Drinking Water and Health* remains valid, i.e., "If 0.05 mg/kg of dietary [total] arsenic is also a nutritionally desirable level for people, then the adequate human diet should provide a daily intake of approximately 25 to 50 μg. The current American diet does not meet this presumed requirement" (National Research Council, 1980). The unresolved status of this issue is further reason for maintaining the current MCL for arsenic.

ASBESTOS

Asbestos fibers in drinking water and their putative health effects were reviewed in the first volume of *Drinking Water and Health* (National Research Council, 1977, pp. 144-168). At that time, there were only limited data from which to evaluate the potential adverse health effects of orally ingested asbestos. A number of research recommendations suggested in that volume have been, to some extent, fulfilled. Advances have been made in the detection, identification, and quantification of asbestos fibers in drinking water. Several chronic feeding studies completed since that time have failed to show an effect between the ingestion of various fiber types and the development of cancer at any site.

There have also been a number of epidemiological studies in which the exposure to asbestos in drinking water and the incidence of cancer at selected sites have been investigated. This review is limited to a discussion and evaluation of those studies and the development of a model to predict the risks, if any, from such exposure.

Background

A marked increase in the incidence rates of lung cancer and both pleural and peritoneal mesothelioma has been observed in workers exposed to asbestos through inhalation (International Agency for Research on Cancer, 1977). An excess of gastrointestinal tract cancers has also been found in these occupationally exposed groups.

The general population may be exposed to asbestos fibers in "air, beverages, drinking water, food and pharmaceutical and dental preparations and by consumer use of asbestos containing products" (International

Agency for Research on Cancer, 1977). The possible effects of such exposure from drinking water became a matter of some public concern when the Duluth, Minnesota, water supply obtained from Lake Superior was found to be heavily contaminated with asbestos fibers.

The potential hazard from asbestos in drinking water was considered by the American Water Works Association Research Foundation (1974). However, the report of this group refers only to asbestos fibers released into the water from asbestos-cement pipe. It concluded, "Calculations comparing the probable ingestion exposure in occupational groups to that likely to occur as a result of ingestion of potable water from asbestos-cement pipe systems suggest that the probability of risk to health from the use of such systems is small approaching zero." This conclusion has been questioned (McCabe and Millette, 1979; U.S. Environmental Protection Agency, 1979).

Exposure to Asbestos from Drinking Water

The results of an extensive EPA survey of asbestos concentrations in drinking water have recently been published by Millette *et al*. (1979). A summary of the data is given in Table III-1. More than 20% of the cities surveyed had water containing more than 1 million fibers per liter, as measured by transmission electron microscopy (TEM), and almost 11% of them had water containing more than 10 million fibers per liter. (This EPA survey was not a representative sampling of U.S. water supplies; therefore, caution should be exercised when drawing conclusions from the table.) The asbestos in these supplies was derived from a variety of sources: mining

TABLE III-1 Asbestos Concentrations in Drinking Water from 365 Cities in 43 States, Puerto Rico, and the District of Columbia, as Measured by Transmission Electron Microscopy[a]

Asbestos Concentration, 10^6 fibers/liter	Number of Cities	Percentage of Samples
Below detectable limits	110	30.1
Not statistically significant	90	24.7
Less than 1	90	24.7
From 1 to 10	34	9.3
Greater than 10	41	11.2
TOTAL	365	100.0

[a] Data from Millette *et al*., 1979.

process discharge into Lake Superior, natural erosion of serpentine rock in the Bay Area of California and in Seattle, asbestos-cement roofs, and asbestos-cement pipe. Some of the water supplies surveyed are now being filtered to reduce the asbestos fiber levels.

Asbestos concentrations in water have also been reported as mass per liter, but this measurement is not considered useful in the evaluation of possible carcinogenic effects (see Harington, 1981). Experiments in which gelatin pellets of asbestos were implanted into the pleura of rats indicate that the physical dimensions of the fibers, but not their type, are important and that the long, thin fibers are the most effective inducers of mesotheliomas (Stanton *et al*., 1981). The relevance of this work to the carcinogenicity of asbestos in humans has been reviewed by Selikoff and Lee (1978) and more recently in great detail by Harington (1981). Harington concluded that the "Stanton hypothesis," emphasizing the fiber dimensions as important determinants of carcinogenicity, appeared to hold true for the very limited, relevant data on humans. However, the critical fiber dimensions in humans, at least for mesothelioma, are probably much smaller than those suggested in the rat experiments, the most carcinogenic fibers apparently being those with diameters less than 0.05 μm and lengths greater than 3 μm (Harington, 1981).

The size distribution of asbestos fibers in water varies by source (Millette *et al*., 1980). The smallest fibers are found in water contaminated by the natural erosion of serpentine rock. These fibers have an average width of approximately 0.04 μm and an average length of 1 μm, compared to an average width of 0.1 μm and an average length of 1 μm for fibers from the Whitekloff asbestos mine, which was associated with a high incidence of mesothelioma (Harington, 1981). Thus, the average aspect ratio (length: width) for the waterborne fibers from natural sources is approximately 3 times greater than that for the airborne fibers found in the Whitekloff mine. Approximately 10% of the waterborne fibers are longer than 3 μm. Water contaminated by asbestos from asbestos-cement pipe contained fibers with an average diameter of 0.044 μm and an average length of 4.3 μm, which gives an average aspect ratio of 121 (Millette *et al*., 1980). Approximately 30% of these fibers were longer than 3 μm. The committee concluded that there appears to be no reason to consider fibers from either source as free from risk in comparison to fibers found in occupational settings.

Harington (1981) very tentatively concluded that fiber dimensions "may in time be applicable to regulatory practice," essentially agreeing with Selikoff and Lee (1978) that "there seems to be little basis … at present … for seeking to base selective control measures on such hypotheses," i.e., fiber dimensions. The committee concurs with these authors.

Estimating the Cancer Risk from Swallowed Asbestos Following Occupational Exposure

There have been a number of epidemiological studies of gastrointestinal (GI) cancer (in this report this term refers only to cancer of the esophagus, stomach, small intestine, colon, and rectum) associated with occupational exposure to asbestos. The reported relative risks (RR's) of observed to expected cases in these studies are not very large (between 1.5 and 3), and a number of other studies have failed to detect any risk of GI cancer (Advisory Committee on Asbestos, 1979). Thus, one must consider the possibility that the studies with positive results might have been affected by unrecorded biases. However, careful review of these studies by many authors and by a Working Group of the International Agency for Research on Cancer concluded that the association was one of cause and effect (Advisory Committee on Asbestos, 1979; International Agency for Research on Cancer, 1977; Miller, 1978). The committee concurs with these reviews.

Assuming that the exposed and unexposed workers have the same general risk factors for GI cancers and that their observed GI cancer rates are r_e and r_u, respectively, then the GI cancer burden from the exposure can be expressed either as a ratio of rates, or relative risk, i.e., RR = r_e/r_u, or a difference of rates, i.e., DR = $r_e - r_u$. For general risk assessment purposes, these can be expressed on a per unit exposure basis by dividing RR or DR by the exposure dose.

Both RR and DR are valid measures of the risk to the occupational group, but they implicitly make very different assumptions about the risks to individuals with different risk factors. The relative risk (or multiplicative) index (RR) implicitly assumes that the risk of GI cancer is increased in proportion to the individual's underlying risk. The difference of risk (or additive) index (DR) implicitly assumes that the amount of increased risk of GI cancer is independent of the individual's underlying GI cancer risk.

None of the occupational studies of exposure to asbestos and GI cancer provided data that would enable the committee to distinguish between these possible models (i.e., the multiplicative or additive models, or something intermediate). In fact, because of the limited data and lack of any known strong risk factors for GI cancer, except for increasing age, it is difficult to know how the studies could shed light on this issue. However, selection of a model is of critical importance in risk assessment and cannot be avoided. In the absence of evidence to the contrary, this committee has usually selected the additive model. For asbestos, however, some information suggests that the multiplicative model is to be preferred. The data relating lung cancer risk to joint exposures to asbestos and cigarettes are inadequately described by the additive model, whereas the multiplicative

model is generally regarded as providing a reasonable description of the data (see Saracci, 1977). The results of Seidman *et al*. (1979), who studied lung cancer in persons exposed for a limited time in an amosite asbestos factory in New Jersey, indicate that the excess lung cancer increased with age at exposure. Therefore, the additive model is clearly inadequate, but the data are again reasonably compatible with a multiplicative model. Unfortunately, the observations by Seidman *et al*. (1979) may have been severely confounded by possible age-related differences in cigarette-smoking habits, but a very similar pattern of risk was also observed for other "asbestos disease," i.e., asbestosis and other noninfectious pulmonary diseases, mesotheliomas, and cancers of the esophagus, stomach, colorectum, larynx, buccal cavity, pharynx, and kidney. The relative risks associated with exposure to asbestos are similar for all subsites in the GI tract (Miller, 1978; National Research Council, 1977). Although the above information may not constitute proof for the correctness of the multiplicative model, there is clearly no basis for favoring an alternative model for risk assessment.

Studies of Asbestos Workers

Table III-2 shows the results of five cohort studies of GI cancers in asbestos workers. The reports of Newhouse and Berry (1979) and Henderson and Enterline (1979) only give data for GI and certain other sites combined (see footnote to Table III-2). The committee adjusted the RR's to refer only to GI cancer by assuming that all excess deaths occurred in this grouping.

To make the observed RR's of use in estimating the possible consequences of ingesting asbestos fibers in drinking water, it is essential to have some measure of the amount of asbestos swallowed by these workers. Such estimates can only be approximate because so few measurements of airborne asbestos have been made in the workplace (and then, often years after actual exposure) and because those measurements then had to be applied to broad categories of employees (Peto, 1979). Table III-3 shows the estimates derived either from the published studies or from personal communication with the authors.

Converting Risk to Asbestos Workers to Risk from Swallowed Asbestos

In converting the observed risk of GI cancers in asbestos workers to risk of GI cancer from ingested asbestos fibers, a number of steps should be clearly distinguished.

TABLE III-2 Results of Five Cohort Studies of Gastrointestinal Tract Cancer in Asbestos Workers

Exposed Group	Cancer Site Codes[a]	Deaths		Ratio, O/E	RR[b]	Reference
		Observed (O)	Expected (E)			
U.S. and Canadian insulation workers	150-154	94	59.4	1.58	1.58	Selikoff *et al.*, 1979
N.Y. and N.J. insulation workers	150-154	43	15.1	2.85	2.85	Selikoff *et al.*, 1979
U.S. factory workers	150-154	32	21.5	1.49	1.49	Seidman *et al.*, 1979; U.S. EPA, 1979
London factory workers	150-158[c]	40	34.0	1.18	1.32	Newhouse and Berry, 1979
U.S. factory workers	150-159[c]	55	39.9	1.38	1.55	Henderson and Enterline, 1979

[a] 150 = esophagus
151 = stomach
152 = small intestine
153 = large intestine
154 = rectum
155 = liver
156 = gallbladder
157 = pancreas
158 = retroperitoneum and peritoneum
159 = gastrointestinal tract, not otherwise specified
[b] Standardized mortality ratio = 100 × RR (relative risk) for cancer site codes 150-154. (See text.)
[c] Excluding mesotheliomas.

Step 1: Measurement of Dose and Adoption of Risk Model

Since the committee is interested in effects at low doses, a model relating relative risk to dose must be used. The standard linear dose-effect model may be written:

$$RR = 1 + a \times \text{dose}, \qquad (1)$$

where a is a constant to be estimated. For asbestos workers, dose is a function of both intensity and duration of exposure. Intensity of exposure is measured in terms of the number of fibers that can be seen with the light microscope (LM) per milliliter of air. Dose is the simple product of inten

TABLE III-3 Average Asbestos Exposure Estimated for the Workers in Studies Shown in Table III-2 and Calculation of Increment in Relative Risk Based on Doses Measured with the Light Microscope

	Exposure				
RR	Intensity (LM fibers/ml air)	Duration (years)	Cumulative Dose (LM fibers/ml air) (years)	Relative Risk per Unit Cumulative Dose[a]	Reference
1.58	15	34	510	0.00114	Selikoff *et al*., 1979
2.85	15	40	600	0.00308	Selikoff *et al*., 1979
1.4	40	1.9	76	0.00645	Seidman *et al*., 1979; U.S. EPA, 1979
1.32	10-30	–	170[b]	0.00188	Newhouse and Berry, 1979
1.55	–	–	498[c]	0.00110	Henderson and Enterline, 1979

[a] Increase in RR for each year of exposure to one fiber identified by the light microscope (parameter *a* in equations 1 and 2).
[b] Calculated from Newhouse and Berry (1979) using the method from U.S. Environmental Protection Agency, 1979.
[c] Calculated from Table 2 of Henderson and Enterline (1979), assuming 1 mppcf (million particles per cubic foot) = 2 fibers/ml.

sity of exposure and duration of such exposure, usually measured in years (Y), and expressed as numbers of LM fibers/ml air times Y. It is not self-evident that equal doses measured in this way must have equal effects (e.g., cigarette smoking measured as pack-years does not have a fixed effect on lung cancer incidence but is greater at a low intensity for a long time); however, authors of all studies on asbestos-induced cancer concur that measurement of dose on this scale fits the data reasonably well and no alternative model has been seriously proposed. In particular, there is evidence that even the briefest exposures (less than 6 months) have very long-term effects on lung cancer rates with no diminution of the associated RR with the passage of time (up to 35 years) after exposure (Seidman *et al*., 1979). A linear dose-effect relationship for lung cancer and exposure to asbestos is most clearly shown by results reported by Henderson and Enterline (1979). For GI cancers, too few data have been published to establish or refute linearity, even at high doses. Peto (1979) has discussed these issues at some length.

Assuming the dose-effect model given by equation (1), one may estimate the value of parameter *a* as follows:

$$a = (\mathrm{RR} - 1)/\mathrm{dose}. \qquad (2)$$

The calculated values of *a* from the different studies are given in the Table III-3.

In the dose-effect model given by equations (1) and (2), dose is used to mean the cumulative dose calculated up to the age at which the cancer is diagnosed. This may be considered as the correct dose for a no-latent-period model, and as having general applicability if the dose was received over a brief period some time before the associated cancer was recorded. This would result in an overestimation of the dose to workers still occupationally exposed to asbestos when their cancer rates were being observed. This overestimation will generally be small, and as long as we adopt the same convention when calculating the risk from asbestos in the water supply, the error will have a very small effect on the computation of risk.

Step 2: Conversion of Dose of Asbestos Inhaled to Dose of Asbestos Swallowed

Since the excess GI cancers in the workers are assumed to be caused by the asbestos fibers that these workers swallowed rather than simply inhaled, the dose calculated in Step 1 must be converted to fibers swallowed. The committee estimated that

$$\text{breathing 1 LM fiber/ml for 1 year} = 588 \times 10^6 \text{ LM fibers swallowed.} \qquad (3)$$

where 588×10^6 is the product of 10^6 (ml in m^3), times 8 (m^3 of air breathed per day at work), times 5 (days worked per week), times 49 (number of weeks worked per year), times 0.3 (proportion of inhaled fibers that are subsequently swallowed). Only this last factor of 0.3 needs discussion.

Studies of short-term (30-minute) inhalation exposures of rats to Union Internationale Contre le Cancer (UICC) standard reference samples of asbestos indicated that an average of 40% of the various types and sizes of inhaled material is deposited somewhere in the respiratory tract (Morgan *et al.*, 1975). Although there is a lack of relevant data on the deposition of asbestos fibers in humans, Dement (1979) used a mathematical model of fiber behavior to calculate that roughly 28% of inhaled chrysotile fibers is deposited in humans and that approximately twice as much amosite is de

posited. Morgan *et al*. (1975) did not find such a large difference in their study on rats, although they did find that the deposition of one form of chrysotile was 25% less than that of amosite. Evans and his colleagues (1973) reported that approximately 65% of the material deposited in rats is cleared through the GI tract within a month. Their results suggest that this clearance process continues until almost all the deposited material is cleared. Relevant data on clearance in humans are again lacking, but the work of Cohen *et al*. (1979) on the clearance of ferrosoferric oxide (Fe_3O_4) dust from experimentally exposed men suggests that lung clearance in humans is similar to that in rats and that most of the deposited dust will be cleared in humans. For rats, then, approximately 40% of inhaled asbestos will enter the GI tract and a somewhat lower figure of 30% appears to be a reasonable estimate for humans. Note: no allowance has been made for the possibility that asbestos is ingested directly. Neglect of this could result in an overestimation of the effect of a unit dose of swallowed asbestos.

Step 3: Conversion of Number of Fibers Seen by Light Microscope to Number of Fibers Seen by Transmission Electron Microscope

Asbestos contamination of drinking water is measured in terms of number of fibers seen with the transmission electron microscope (TEM). To convert from light microscope (LM) measurements to TEM measurements, the committee has used the following equation:

$$1 \text{ LM fiber} = 50 \text{ TEM fibers.} \qquad (4)$$

This equation is based on the report of Lynch *et al*. (1970), who found that a conversion factor of 50 is roughly appropriate for asbestos exposure from textile manufacturing, friction work (i.e., mixing, grinding, cutting, and drilling), and pipe manufacturing. Conversion factors larger than this have been reported in the literature, e.g., McCabe and Millette (1979) used 100 and the U.S. Environmental Protection Agency (1979) used 200 in the criteria document, but these estimates do not appear to be based on the industrial exposure data considered in this chapter.

The relative risk equation (1), which applies to measurements made by the LM, may thus be expressed as follows for doses swallowed, as measured by the TEM:

$$\begin{aligned} RR &= 1 + [a/(588 \times 10^6 \times 50)] \times \text{dose (in TEM fibers swallowed)} \\ &= 1 + (a/0.0294) \times \text{dose (in TEM fibers swallowed}/10^{12}) \qquad (5) \\ &= 1 + b \times \text{dose (in TEM fibers swallowed}/10^{12}). \end{aligned}$$

Estimated values of b derived from the studies listed in Table III-3 are given in Table III-4. These values vary from 0.039 to 0.22; a "best" value (obtained by weighting the individual estimates of b inversely proportional to their estimated variance) is approximately 0.05.

Thus, the RR for GI cancer for a person who has swallowed $h \times 10^{12}$ TEM fibers can be estimated as follows:

$$RR = 1 + 0.05 \times h. \qquad (6)$$

Predicting the Results of Epidemiological Studies of GI Cancer Risk from Asbestos-Contaminated Drinking Water

Equation (6) makes direct estimates of relative risks observable in epidemiological studies correlating exposure to asbestos in drinking water and GI cancer mortality rates. It is also reasonable to assume that the relative risks derived from this equation will be approximately correct if applied to studies of cancer incidence.

A man who has been drinking water containing $d \times 10^6$ TEM fibers/liter for n years has consumed $h \times 10^{12}$ TEM fibers, where

$h = n \times 365.25 \times 2 \times d \times 10^{-6}$.

His relative risk of GI cancer in this nth year of exposure is:

$$RR = 1 + 0.05 \times h \qquad (6)$$
$$= 1 + 3.6525 \times 10^{-5} \times n \times d. \qquad (7)$$

For example, if n = 20 years and $d = 15 \times 10^6$ TEM fibers/liter, then the associated relative risk is:

$$RR = 1 + 3.6525 \times 10^{-5} \times 20 \times 15$$
$$= 1.011.$$

Equation (7) is directly applicable to epidemiological studies in which contamination of drinking water took place for only a limited time, e.g., in Duluth. To make equation (7) applicable to epidemiological studies in areas where the contamination has been present for a very long time, the different durations of exposure of different age segments of the population first need to be evaluated. The associated relative risks must then be calculated from equation (7), and then some "average" determined. The exact

TABLE III-4 Estimates of Increment in Relative Risk of Gastrointestinal Cancers per Unit Exposure

a, Increment per LM Fibers/ml Air Times Years	*b*, Increment per 10^{12} TEM Fibers Swallowed	Reference
0.00114	0.0388	Selikoff *et al*., 1979
0.00308	0.1048	Selikoff *et al*., 1979
0.00645	0.2194	Seidman *et al*., 1979; U.S. Environmental Protection Agency, 1979
0.00188	0.0640	Newhouse and Berry, 1979
0.00110	0.0374	Henderson and Enterline, 1979

form of this average would depend on the type of statistic used to describe the overall relative risk of GI cancer in the exposed community.

Converting RR's to Lifetime GI Cancer Risks

As discussed above, expressing the risk from any agent in terms of relative risks implies that the effect of the agent is to multiply whatever "normal" or background cancer rate exists. Table III-5 shows the results of calculations based on equation (6), indicating the consequences for U.S. white males who drink 2 liters of water containing $d \times 10^6$ TEM fibers/liter daily throughout life. To understand this table, consider a man aged 57.5 years. He has consumed water containing $57.5 \times 365.25 \times 2 \times d \times 10^6$ TEM fibers, or 42.0×10^9 TEM fibers up to this point in his life, and his relative risk of GI cancer is currently:

$$\begin{aligned} \mathrm{RR} &= 1 + 0.05 \times 0.0420 \times d \\ &= 1 + 0.0021 \times d. \end{aligned}$$

The additional RR is thus $0.0021d$, and the additional probability of GI cancer occurrence in this, his 58th year is:

$0.0021d \times 131.1/10^5 = 0.2754d/10^5$,

where $131.1/10^5$ is the 1970 GI cancer incidence rate in the United States for white males in the 55- to 59-year age group (Cutler and Young, 1975). Summing these additional probabilities of GI cancer deaths up to age 70 gives us a lifetime risk of $9.1060d/10^5$.

TABLE III-5 Gastrointestinal Cancer Incidence in White Males[a] and Calculation of Additional Risk from Swallowing $2 \times d \times 10^6$ TEM Fibers Daily Throughout Life. Additional Relative Risk Per 10^{12} TEM Fibers Swallowed Assumed to be 0.05.

Age Group, years	Incidence Rate per 100,000/ year[b]	Asbestos Swallowed, 10^9 TEM Fibers	Additional Relative Risk	Additional Incidence Rate
10-14	0.1	9.13*d*	0.00046*d*	0.0000*d*
15-19	0.9	12.78*d*	0.00064*d*	0.0006*d*
20-24	1.2	16.44*d*	0.00082*d*	0.0009*d*
25-29	2.5	20.09*d*	0.00100*d*	0.0025*d*
30-34	5.0	23.74*d*	0.00118*d*	0.0058*d*
35-39	8.5	27.39*d*	0.00136*d*	0.0116*d*
40-44	20.2	31.05*d*	0.00156*d*	0.0313*d*
45-49	43.1	34.70*d*	0.00174*d*	0.0748*d*
50-54	79.1	38.35*d*	0.00192*d*	0.1517*d*
55-59	131.1	42.00*d*	0.00210*d*	0.2754*d*
60-64	209.1	45.66*d*	0.00228*d*	0.4774*d*
65-69	320.1	49.31*d*	0.00246*d*	0.7892*d*
TOTAL	4,104.5			9.1060*d*

[a] Codes 150-154, as defined in Table III-2.
[b] Cutler and Young, 1975.

Expressing this in the more usual terms, the committee estimates that drinking water containing $(1/9.1060) \times 10^6 = 0.11 \times 10^6$ TEM fibers/liter may lead to one GI cancer case per 10^5 persons exposed over a 70-year lifespan. The equivalent figure for U.S. white women is 0.17×10^6 TEM fibers/liter.

Epidemiological Studies of Cancer Risk from Asbestos-Contaminated Drinking Water

Some epidemiological studies of communities with asbestos-contaminated drinking water are reviewed in this section. Their findings are compared to the predictions made from equations (6) and (7).

Duluth

Electron microscope studies revealed that during 1973 the Duluth, Minnesota, water supply contained from 1×10^6 to 30×10^6 TEM fibers/liter, depending on weather conditions on Lake Superior (Cook *et al*., 1974). From details of x-ray diffraction studies reported in the paper by Cook and his colleagues, one may estimate the mean number of asbestos fibers in the

drinking water over the year to be approximately 8×10^6 TEM fibers/liter. The contamination of Lake Superior and, hence, of the Duluth water supply was due to the dumping of industrial waste, a practice that had begun in 1955 (Masson *et al*., 1974).

Masson and his colleagues (1974) studied the cancer death rates in Duluth over four 5-year periods (1950-1954, 1955-1959, 1960-1964, and 1965-1969) by specific site of cancer and for males and females separately. They compared these rates to those of the entire state of Minnesota and to those of Hennepin County alone, which includes Minneapolis. They summarized their results as follows: "If the asbestos fibers had induced cancer at a particular [body] site [in Duluth], one would expect the mortality rates for that site to have increased in males and females [compared to the comparison groups], especially in the most recent five-year period (1965 to 1969). … The only site that fell into this category was cancer of the rectum." In commenting on this result, they concluded that this effect "was observed by chance."

Levy and his colleagues (1976) compared GI cancer incidence rates in Duluth for the 3-year period 1969 to 1971 to such rates in the cities of Minneapolis and St. Paul, but could not study changes that occurred since the asbestos dumping began. They concluded, "Although some differences in GI cancer incidence occurred among the three cities in 1969-1971, there was no consistent pattern of statistically significant differences observed." This study adds very little to the finding of Masson *et al*. (1974).

It it is assumed that Duluth drinking water contained some 8×10^6 TEM fibers/liter (the observed average value in 1973) ever since contamination began in 1955, equation (7) predicts the following relative risk for GI cancer in 1970—the mid-year in the study by Levy *et al*. (1976):

$$\begin{aligned} \mathrm{RR} &= 1 + 3.6525 \times 10^{-5} \times 8 \times 15 \\ &= 1.004. \end{aligned}$$

This relative risk is an overestimate of the true relative risk predicted by equation (7) since it assumes that the level of asbestos contamination reached the 1973 level immediately after dumping began, which is not likely. Predicted relative risks for the findings of Masson *et al*. (1974) will be smaller than this since the exposure periods were shorter. Relative risks of this order of magnitude are far too small for any epidemiological study to detect, let alone differentiate from possible confounding variables.

Connecticut

Harrington and his colleagues (1978) investigated the use of asbestos-cement pipe and the incidence of GI cancers in Connecticut for the period

1935 to 1973. The authors summarized their study as follows: "The age adjusted sex specific incidence data for stomach, colon, and rectal cancer for Connecticut townships for the period 1935 to 1973 were used to investigate whether asbestos cement pipe usage for domestic drinking water is associated with gastrointestinal cancer. The townships were grouped according to the Assessment of Exposure (AOE) and Risk Factor (RF) for asbestos. These are composite indices of asbestos exposure including factors relating to the age of the pipe, the ability of water to leach asbestos from the pipe, and the length of pipes used by the population. No association was noted between these asbestos risk scores and gastrointestinal tumor incidence."

This study is fundamentally flawed by the lack of data on actual levels of exposure to asbestos. We know only that measurements of asbestos fiber levels in the drinking water of the exposed populations "ranged from below detectable limits (10,000 fibers per liter) to 700,000 fibers per liter," and that asbestos-cement pipe was introduced "around 1950." Equation (7) would predict as an outside maximum possible observable relative risk:

$$\begin{aligned} RR &= 1 + 3.6525 \times 10^{-5} \times 24 \times 0.7 \\ &= 1.0006, \end{aligned}$$

where 24 (the period from 1950 to 1973) is the maximum duration of exposure and 0.7×10^6 TEM fibers/liter is the maximum observed asbestos contamination of the water supplies. As for Duluth, such relative risks are far too small to be sensibly detected by any epidemiological study.

San Francisco Bay Area

Drinking water in certain parts of the San Francisco Bay Area is contaminated with asbestos from naturally occurring serpentine rock. Concentrations as high as 180×10^6 TEM fibers/liter have been recorded by Kanarek *et al*. (1980). These investigators compared the observed to expected ratios of cancer incidence for 1969 to 1971 for the 722 census tracts of the San Francisco-Oakland Standard Metropolitan Statistical Area with the measured asbestos counts in tract drinking water. Their results for all GI tract tumors combined are given in Table III-6. The statistical significance of the observed ratios apparently disappeared when adjustment was made for individual tract values of "median family income, median school years completed, marital status, asbestos industry workers, [and] country of origin." The adjusted ratios are not given in the paper.

Based on the extreme assumption that the mean duration of relevant water exposure of cancer cases is 60 years, equation (7) predicts ratios of 1.00, 1.00, 1.02, and 1.06 at the four levels of asbestos contamination

TABLE III-6 Ratio[a] of Observed to Expected Gastrointestinal Cancer[b] Incidence for White Males and Females for Census Tract Groupings by Chrysotile Asbestos Fiber Counts in San Francisco Bay Area, from 1969 to 1971[c]

	Asbestos TEM Fibers/liter × 10^6			
Sex	~ 0.15	~ 0.6	~ 8	~ 26
Male	1.00	1.01	1.11	1.28
Female	1.00	1.02	1.11	1.19

[a] Adjusted to 1.00 for lowest asbestos exposure tracts.
[b] Codes 150-154, as defined in Table 3-2.
[c] From Kanarek *et al*., 1980.

shown in the table. The observed ratios are some 4-fold higher than these predicted values, but, as noted above, the statistical significance of the observed values disappeared after adjustments were made for various risk-modifying factors, and the adjusted ratios may be reasonably close to these predicted values. However, the predicted values themselves are likely to be too high, in that the 60-year mean exposure assumption is equivalent to the unlikely assumption that the population in the older, high-cancer-incidence age range had lived in their 1970 census tracts most of their lives. As pointed out by Kanarek and his colleagues, roughly one-half of the individual census tracts lost more than one-half of their residents between 1965 and 1970.

Lung cancer in males was found to be strongly related to asbestos in the water supply, even after adjustment for the risk-modifying factors mentioned above (Kanarek *et al*., 1980). The authors attributed this to "the carcinogenic potential of ingested asbestos fibers which migrate to the lungs. …" It is, however, at least as likely that this observed lung cancer effect merely demonstrates that the adjustments for other risk-modifying factors are not adequate. This possibility is strengthened by their observation that cancer of the endometrium has a negative association with the asbestos content of the water supply.

In summary, this study of cancer incidence in the San Francisco Bay Area has produced results that are not incompatible with predictions made from equation (7).

Puget Sound Area

Drinking water in certain parts of the Puget Sound area of western Washington State is also naturally contaminated with chrysotile asbestos (Polissar *et al*., 1982). The Sultan River source is especially affected; average

asbestos concentrations of 206×10^6 TEM fibers/liter were found in grab samples of tap water obtained from this source.

Polissar and his colleagues compared the cancer incidence data for 1974 to 1977 and the cancer mortality data for 1955 to 1975 from the census tracts with a Sultan River water source to such data from census tracts in the Puget Sound area with average asbestos concentrations in tap water of 7×10^6 TEM fibers/liter. Table III-7 shows their results for GI cancers. No effect of asbestos concentration is evident. However, a proportional incidence analysis of GI cancer in which long-term (> 30 years) residents were compared with short-term (< 30 years) residents of Everett—the main city served by Sultan River water—did show proportional incidence ratios of 1.49 and 1.39 for males and females, respectively.

It is evident from the "Average Annual Population at Risk" rows in Tables 3 and 4 in Polissar *et al*. (1982) that there was tremendous population growth in the census tracts served by the Sultan River between 1955 and 1977. The average annual total population from 1955 to 1975 was 45,272, and from 1974 to 1977 it was 156,099, i.e., the 1975 population appears to have been roughly 3.5 times the size of the 1965 population. In these Sultan River water tracts, the average duration of residence for persons in the cancer age range may therefore be as short as 5 years. Using this figure, equation (7) predicts a relative risk of 1.04.

If we assume that the cases with long-term (more than 30 years) residence in Everett had been there for 60 years and that cases with short-term residence had been there for 5 years, then equation (7) predicts a relative risk of 1.41, which is in close agreement with the observed proportional incidence ratios.

TABLE III-7 Odds Ratios for Gastrointestinal Cancer Incidence (1974-1977) and Mortality (1955-1975) for Males and Females in Census Tracts with a Sultan River Water Source[a]

Sex	Type of Data	Odds Ratio[b]
Male	Incidence	1.10
	Mortality	0.79
Female	Incidence	0.97
	Mortality	0.86

[a] From Polissar *et al*., 1982.
[b] Calculated from Tables 3, 4, and 7 in Polissar *et al*., 1982; odds ratio for census tracts with a Sultan River water source versus census tracts in the Seattle-Everett-Tacoma metropolitan areas, which have the Cedar River, Tolt River, Green River, or Lakewood Wells as their water sources.

Within the inherent limitations of the study design, therefore, the results of this study are compatible with the predictions of equation (7).

EPA Criteria Document Methodology

In the Ambient Water Quality Criteria Document, the U.S. Environmental Protection Agency (1979) considers much the same data discussed in this chapter, but uses a very different method of calculating the "additional lifetime cancer risk of 1 in 100,000." The agency's method of calculation can be analyzed by dividing it into a number of distinct steps:

Step 1: Conversion of LM Fibers/ml Air to TEM Fibers Swallowed

(This is the equivalent of Steps 3 and 4 in the committee's method.) A man breathing 1 LM fibers/ml air at work is considered by the EPA to swallow an average of

$200 \times 8 \times 10^6 \times 5/7 = 1{,}142.9 \times 10^6$ TEM fibers/day,

where 1 LM fiber = 200 TEM fibers, 8 = m^3 of air breathed per day at work, 10^6 = ml in m^3, and 5/7 = proportion of working days in a week. (The committee used a conversion factor of 50 for LM to TEM, a factor of 49/52 to represent working weeks in a year, and 0.3 as the proportion of inhaled asbestos fibers that are subsequently swallowed. As a result, the risks calculated by the committee are only 7.07% of those calculated by the EPA.)

Step 2: Conversion of LM Fibers/ml Air to TEM Fibers/Liter in Drinking Water for a 70-Year Exposure

1 LM fiber/ml air at work for 1 year

$= 1{,}142.9 \times 10^6$ TEM fibers swallowed daily for 1 year
$= (1{,}142.9 \times 10^6)/2$ TEM fibers/liter drinking water for 1 year
$= 571.4 \times 10^6$ TEM fibers/liter for 1 year
$= (571.4 \times 10^6)/70$ TEM fibers/liter for a lifetime of 70 years
$= 8.163 \times 10^6$ TEM fibers/liter lifetime exposure.

(The committee's method agrees with the principles of this calculation.) For example, the 510 LM fibers/ml air-year result of Selikoff and his colleagues (1979) given in the first row of their Table 3 becomes the equivalent

of $510 \times 8.163 \times 10^6 = 4{,}163.6 \times 10^6$ TEM fibers/liter in drinking water for 70 years.

Step 3: Conversion of TEM Fibers/Liter for 70 years to Additional Lifetime Cancer Risk per 100,000

The EPA assumes that the number of TEM fibers/liter over 70 years calculated in Steps 1 and 2 will have the same effect as the original occupational exposure on GI cancer and peritoneal mesothelioma. In the criteria document, this effect ( ) is considered to be:

= (number of excess deaths from GI cancer or peritoneal mesothelioma)/(total expected number of deaths from all causes),

where the expected numbers are calculated in the absence of asbestos exposure. The EPA then equates with an additional lifetime cancer risk of . The required TEM fibers/liter for an additional lifetime cancer risk of 1 in 100,000 is then obtained by simple proportion, i.e.:

/(calculated TEM fibers/liter over 70 years)

$= (1 \times 10^{-5})$/(required TEM fibers/liter over 70 years).

Thus,

required TEM fibers/liter

= (calculated TEM fibers/liter over 70 years)/($10^5 \times$).

For example, the 510 LM fibers/ml air-year result of Selikoff *et al.* (1979) given in the first row of their Table 3 was associated with 148.9 excess deaths from GI cancer (39.9) or peritoneal mesothelioma (109). As given in Table 30 of the criteria document, the total number of expected deaths from all causes was 1,660.96,

x = 148.9/1,660.96

= 0.0896

and

required TEM fibers/liter over 70 years

$= (4, 163.6 \times 10^6)/(10^5 \times 0.0896)$

$= 0.536 \times 10^6$ TEM fibers/liter.

(Note: if the conversion factors in Step 1 were replaced by the committee's estimates, then the last figure would need to be multiplied by 7.07%, giving required water quality of $0.536 \times 10^6 \times 7.07\% = 0.038 \times 10^6$ TEM fibers/liter.)

The critical assumption in Step 3 is equating excess deaths as a proportion of all deaths with additional lifetime cancer risk. This assumption is demonstrably false. Suppose that all causes of death except GI cancer and peritoneal mesothelioma were somehow eliminated from the population. By definition, this should make no difference to the calculation of lifetime risk from GI cancer or peritoneal mesothelioma from asbestos exposure, since these sites were not altered. But X will be drastically changed. In the study by Selikoff *et al.* (1979), the total expected number of deaths from all causes is reduced from 1,660.96 to 59.1, so that X changes from 0.0896 to 2.519, or a 28.1-fold increase. The required TEM fibers/liter is reduced from 0.536×10^6 to 0.019×10^6.

Animal Experiments

When there are few or no epidemiological data on which to base estimates of a compound's carcinogenicity in humans, one recourse is the use of data from experiments in animals. Although this is not the case for asbestos, confirmatory evidence that ingested asbestos is a GI tract carcinogen in animals would add some weight to the epidemiological data.

Asbestos has been shown to cause mesotheliomas in rats and hamsters when implanted into the pleural cavity and when inhaled (Port, 1980; Wagner *et al.*, 1980), but results of long-term asbestos ingestion studies in rats and hamsters have not produced any convincing evidence of an increase in GI tract tumors in either species. Table III-8 gives details of four feeding studies with substantial numbers of animals that provided little, if any, evidence of an effect on GI tract tumors.

Equation (6) suggests that the relative risk of GI cancers from asbestos exposure of humans may be written as:

$$RR = 1 + 0.05 \times h,$$

where $h \times 10^{12}$ is the number of TEM fibers swallowed. If we assume that the daily dose to an animal over a lifetime had an effect equivalent to that in humans exposed for 70 years, then the "Maximum Daily Dose" column of Table III-8 may be multiplied by $1.28 \times 10^{-9} = 0.05 \times 70 \times 365.25 \times 10^{-12}$ to give the expected excess relative risk (RR – 1) as shown in the table. On this basis only the experiments with chrysotile should have definitely produced a positive result, i.e., an increase in GI tumors.

TABLE III-8 Recent Long-Term Asbestos-Feeding Experiments

Animal	Route	Maximum Daily Dose, TEM fibers	Equivalent Daily Dose for Humans, TEM fibers[a]	Expected Excess Relative Risk (1)[b]	(2)[c]	Reference
Syrian golden hamsters	Drinking water	175×10^6 amosite	82×10^9	0.22	105	Smith *et al*., 1980
Fischer 344 rats	Diet	258×10^9 chrysotile	36×10^{12}	330	46,080	Donham *et al*., 1980
Syrian golden hamsters	Diet	699×10^9 chrysotile	445×10^{12}	895	569,600	National Toxicology Program, 1981a
Syrian golden hamsters	Diet	398×10^6 amosite	253×10^9	0.51	324	National Toxicology Program, 1981b

[a] Calculated to give equal daily per kg bw doses.
[b] Based on daily dose equivalence between animals and humans.
[c] Based on daily dose per kg bw equivalence between animals and humans.

The usual method of converting experimental results in animals to humans is based on mass of the test compound per kilogram of body weight or per square meter of body surface area. If we use the body weight correction, then the equivalent asbestos doses to humans are, of course, greatly increased (see column "Equivalent Daily Dose for Humans"), and the expected excess relative risks are more than 100 for all the experiments.

The reasons for the negative results in animals are unknown. It may be that the methods used to extrapolate data from humans to animals are totally wrong for physical carcinogens such as asbestos.

Summary and Conclusions

An excess of GI tract cancers has been observed in some, but not all, occupational groups exposed to asbestos. The reasons for the inconsistent results have not been established, but careful reviews of all the studies indicate that the results of the positive studies are real and that the demonstrated association of asbestos exposure and GI tract cancer is most likely one of cause and effect.

For asbestos this committee considers a multiplicative model for carcinogenic risk assessment to be the most defensible. This model assumes that the risk of GI tract cancer in persons exposed to asbestos at some given level will be some fixed multiple of their normal or background GI tract cancer rate. Many data on the relationship between asbestos exposure and cancer suggest that this multiplying factor, or relative risk (RR), may be accurately represented as a linear function of asbestos dose where asbestos dose is measured in terms of cumulative fiber exposure.

Data from a number of occupational studies suggest that the RR for GI tract cancer is $1 + 0.05 \times \text{dose} \times 10^{-12}$, where dose is the number of fibers swallowed, as counted by TEM. Using this equation, and assuming a daily consumption of 2 liters of water, the committee calculated that drinking water containing 0.11×10^6 TEM fibers/liter may lead to one additional GI tract cancer per 100,000 men exposed over a lifetime of 70 years. For women, drinking water containing 0.17×10^6 TEM fibers/liter may lead to one additional GI tract cancer per 100,000 exposed over a lifetime of 70 years.

Peritoneal mesotheliomas are associated with occupational exposure to asbestos, and indeed asbestos may be virtually the sole cause of this tumor. If peritoneal mesotheliomas are caused by asbestos fibers migrating from the gastrointestinal tract, which must be considered a definite possibility, then risk of such tumors needs to be included in evaluating the total cancer risk from swallowed asbestos. In the five studies shown in Table III-2, approximately 165 peritoneal mesotheliomas were observed; this compares to

the excess of approximately 94 other cancers shown in the table. If these tumors are simply included in the "Observed" column of Table III-2 and the calculations redone, then the risk estimates will be approximately 2.75 times as great. A multiplicative model for risk estimation is, however, not applicable in the absence of an underlying risk, so that for peritoneal mesotheliomas an additive model is required. The data from the studies of asbestos workers does not allow one to construct such a model. For the present, therefore, the estimates of risk given above should simply be considered as possible underestimates of the true risk.

The designs of the epidemiological studies of cancer rates in populations exposed to asbestos in drinking water all have major deficiencies, but the above estimates of risk are quite compatible with the results of these studies. Adequate animal feeding studies conducted to date have failed to confirm a cancer risk from ingested asbestos. Further work aimed at understanding the inconsistent results of GI cancer excess in occupationally exposed groups is clearly warranted.

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Appendix

1977 Amendment to the Safe Drinking Water Act

Appendix A in the first volume of the *Drinking Water and Health* series (National Research Council, 1977, p. 905) is entitled Legislation and Terms of Reference of the Study. It describes the purpose of the legislation, gives an abridged summary of it, explains why it was needed, and describes what subjects are to be addressed in the National Academy of Sciences study mandated by the Safe Drinking Water Act of 1974 (PL 93-523).

Section 1412(e)(2) of the 1974 act called for results of the National Academy of Sciences study to be reported to Congress no later than 2 years after the date of enactment of the title. The Safe Drinking Water Amendments of 1977 authorized continuation of the agreement with the National Academy of Sciences to revise the study "reflecting new information which has become available since the most recent previous report [and which] shall be reported to Congress each two years thereafter."

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