Drinking Water and Health, Volume 3

Safe Drinking Water CommitteeBoard on Toxicology and Environmental Health HazardsNational Research Council

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SAFE DRINKING WATER COMMITTEE
Board on Toxicology and Environmental Health Hazards
Assembly of Life Sciences
National Research Council

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This report has been reviewed by a group other than the authors according to procedures approved by a Report Review Committee consisting of members of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine.

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Preface

In 1975 the National Academy of Sciences-National Research Council initiated a series of studies to meet the congressional mandate of the Safe Drinking Water Act (PL 93-523). Results of these studies were published in *Drinking Water and Health* (National Academy of Sciences, 1977). Amendments to the act in 1977 called for revisions of the studies "reflecting new information which has become available since the most recent previous report [and which] shall be reported to the Congress each two years thereafter" (see Appendix).

Results of studies completed by the Safe Drinking Water Committee since 1977 are contained in this book and a companion volume entitled *Drinking Water and Health,* Volume 2. This book provides an evaluation of several epidemiological studies relating to drinking water, elaboration of previous studies of risk estimation (National Academy of Sciences. 1977), a toxicological assessment of selected drinking water contaminants, and an examination of the contribution of drinking water to the mineral nutrition in humans. *Drinking Water and Health,* Volume 2, contains an assessment of processes and chemicals for the disinfection of drinking water, identification of the by-products resulting from their use, and an evaluation of granular activated carbon for removal of organic and other contaminants from drinking water.

The general approach to the study, the considerations that enter into evaluation of health effects, and the reasons for the selection of subjects are discussed in the following paragraphs. The findings of the study are

summarized at the end of each chapter and briefly in the Executive Summary.

Economic considerations are not a part of this study.

The epidemiology chapter provides a critical assessment of the most recent studies on cancer frequency and organic constituents of drinking water. It points out deficiencies in the evidence and the probabilities of false associations, and discusses the potential for further research on this subject. Little new information was found on the subject of water hardness and cardiovascular disease beyond the recent study on the relationship of trace elements to cardiovascular disease (National Academy of Sciences, 1979).

Reliable and direct information on the toxicity to humans of most chemicals is very difficult to obtain. Usually, it must be based on accidental or occupational exposures which, by their very nature, are uncontrolled. The value and limitations of using acute exposure of subprimate animal species for assessment of risk from accidental spills and discharges are examined. Data from chronic exposure of animals are used to determine acceptable daily intakes (ADI) and risk estimates. The appropriateness of using one or the other of these expressions and the use of safety factors or a particular model for carcinogenic risk estimation are analyzed. Some important quantitative aspects of interspecies toxicology are summarized.

The health effects resulting from chemical, particulate, and radioactive contaminants in drinking water were evaluated in *Drinking Water and Health* (National Academy of Sciences, 1977). Radioactive contaminants are not considered in this study. Asbestos, one of the particulates examined in the first study, will be reevaluated when the several studies now underway are completed. The number of volatile organic compounds identified in drinking water has increased from 300 at the time of the first study to over 700 at present. Limitations on time, manpower, and scientific information have permitted an in-depth evaluation of only a few of these compounds that have recently been found in drinking water.

The evaluation process for each chemical consisted of reviews of both acute and chronic toxicity and, when the data were judged to be adequate, a suggested no-adverse-response level (SNARL) for 24-hr, 7day, and chronic exposure. Risk estimates were calculated for those chemicals suspected of being carcinogens (National Academy of Sciences, 1977).

Special attention was given to a list of compounds prepared by the Subcommittee on Chemistry of Disinfectants and Products of the Safe

Drinking Water Committee. This list consists of compounds that are formed as a result of chlorination or other methods of disinfecting water. Several other compounds were reviewed because of their involvement in potential spill situations or because sufficient new data had become available to justify a reevaluation of several chemicals examined in the first study.

Previous studies by the Safe Drinking Water Committee have had as their principal concern the identification and toxicological evaluation of substances found in drinking water. Quantitation of these adverse consequences forms the scientific base for establishment of standards. The last chapter of this volume is a departure from these previous studies. It is a review of the contribution of drinking water to mineral nutrition in humans. It focuses on both the benefits and the adverse effects of selected elements in drinking water, in cases in which symptoms of both deficiency and toxicity are known to occur. Quantitative differences between the amount of an element required for adequate nutrition and the smallest amount exhibiting toxicological symptoms are examined.

The differences in water intake between young and adult humans were investigated. Infants (7 kg) consume approximately one-third as much water on the average as an adult, but their body weight is only approximately one-tenth of adult weight and their food intake is obviously lower. For this reason, the water intake of an infant may contribute a significant quantity of a given element (National Academy of Sciences, 1974). Variations in water intake among adults are considered in *Drinking Water and Health* (National Academy of Sciences, 1977).

Requirements for nutrients are discussed in terms of recommended dietary allowances (RDA's) (National Academy of Sciences, 1974) or those intakes that have been judged adequate and safe (National Academy of Sciences, 1980), not minimal intakes necessary for survival.

It is a pleasure to express, on behalf of the committee and the subcommittees, a special note of thanks to the staff: Dr. Riley D. Housewright. Dr. Robert J. Golden, Dr. Roy Widdus, and Ms. Frances M. Peter whose informed and tireless efforts aided the committee in planning, conducting, and editing the study. We are grateful to Mr. David Goff, Ms. Virginia White, and Ms. Edna Paulson who assisted in an extensive search of the scientific literature.

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JOHN DOULL, CHAIRMAN

SAFE DRINKING WATER COMMITTEE

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I

Executive Summary

EPIDEMIOLOGY

A review of 12 epidemiological studies failed either to support or to refute the results of positive animal bioassays suggesting that certain trihalomethanes (THM's), e.g., chloroform, may cause cancer in humans. Any association between THM's and bladder cancer "was small and had a large margin of error"—not only because of statistical variances but also, much more importantly, because of the very nature of the studies. All of the epidemiological studies were handicapped by the extreme difficulty of identifying a very small effect in a population. The methodological complexities inherent in epidemiological studies of human populations exposed to multiple contaminants at low concentrations (ppb) in drinking water make it virtually impossible to establish a causal link between THM's and an increase in cancer of the bladder or of any other site. Small differences in cigarette consumption between two population groups could account for the observed associations.

Any causal relationships between THM's and bladder cancer are weakened by imprecise exposure data. In most of the studies, THM concentrations in different water sources were only inferred, rather than actually measured. In addition there are difficulties in controlling for a multitude of factors that are known to affect cancer incidence: cigarette smoking, diet, occupation, use of alcohol and drugs, socioeconomic status, ethnicity, and nonaqueous sources of THM's.

Data regarding drinking water hardness and cardiovascular disease

(CVD) were also examined. Current knowledge is derived largely from "ecological" epidemiological studies, in which individual exposures or risk factors have generally not been considered. In general, when studies encompass large geographical areas, hard water is correlated with low cardiovascular disease rates. This correlation breaks down when smaller areas are considered or when the studied populations are grouped by altitude or the proximity of a seacoast. Some noncardiovascular ailments are also associated occasionally with soft water areas, raising the possibility that soft water may merely be a concomitant of some more basic risk factor.

Given the current status of knowledge regarding water hardness and the incidence of cardiovascular disease, it is not appropriate at this time to recommend a national policy to modify the hardness or softness of public water supplies. The data do not indicate clearly which (if any) additions to soft water would benefit human health.

RISK ESTIMATION

Chapter III, Problems of Risk Estimation, examines the prediction of risks to human health using acute and chronic toxicity data from laboratory animals.

The concentration of most potentially toxic chemicals in drinking water is usually so low that it is difficult to predict potentially adverse effects from drinking the water. In cases of noncarcinogenic toxicity, the preferred procedure would be to make a risk estimate based on extrapolation to low dose levels from experimental curves obtained from much larger doses for which effects can be readily measured. In most instances, such data are not available, and the acceptable daily intake (ADI) approach should be used until better data are obtained. In the ADI approach, "safety factors" based on the quality of the data are applied to the highest no-observable-effect dose found in animal studies.

The Subcommittee on Risk Assessment believes that the ADI approach is not applicable to carcinogenic toxicity and that high dose to low dose extrapolation methods should be used for known or suspected carcinogens. Six models were evaluated for low dose carcinogenic risk estimation. They were the dichotomous response model; linear, no-threshold model; tolerance distribution model; logistic model; "hitness" model; and time-to-tumor-occurrence model. Because of the uncertainties involved in the true shapes of the dose-response curves that are used for extrapolation, a multistage model was judged to be the most useful. Such a model has more biological meaning than other models, e.g., the

probit or logistic model. Moreover, it tends to be conservative in that at low doses it will give higher estimates of the unknown risk than will many others.

More confidence would be placed in mathematical models for extrapolation if they incorporated biological characteristics such as pharmacokinetic data and time-to-occurrence of tumors. Until such data are available, the extrapolation from animals to humans should be done on the basis of surface area.

Epidemiological risk assessment is also evaluated in this report. The subcommittee considered descriptive and analytic (prospective cohort studies and case-control studies) epidemiological approaches.

TOXICOLOGY

Chapter IV, Toxicity of Selected Drinking Water Contaminants, is an evaluation of the health effects associated with the products of water disinfection that were identified by the Subcommittee on Chemistry of Disinfectants and Products. A second group of compounds evaluated was selected for one or more of the following reasons:

- They were judged to be of concern because of potential spill situations.
- They have been identified in drinking water subsequent to the 1977 report (300 volatile organic compounds were known then; 700 are known at present).
- Newly available data justify reexamination of several chemicals dealt with in 1977 in Drinking Water and Health (National Academy of Sciences, 1977).

In addition to providing information on chronic toxicity, the subcommittee evaluated the potential acute toxicity insofar as justified by the available data. These data provide a basis for making judgments of possible health effects resulting from accidental spills of chemicals into drinking water. To this end, the subcommittee provided a suggested no-adverse-response level (SNARL) for acute exposures of 24 hr and 7 days as well as for chronic exposures to single compounds. The safety (uncertainty) factor used in the calculations reflects the degree of confidence in the data. No estimates were given for mixtures of contaminants because of unknown interactions.

The information provided on each compound includes its metabolism, health effects (acute and chronic) on humans and other species,

mutagenicity, carcinogenicity, teratogenicity, and carcinogenic risk estimate.

NUTRITION

The Subcommittee on Nutrition has examined the contribution of selected inorganic elements in drinking water to the optimal nutrition of human populations. It studied the benefits of the presence of selected inorganic elements in water and their adverse effects in those cases in which symptoms of both deficiency and toxicity are known. Previous studies by the Safe Drinking Water Committee are limited to an evaluation of the adverse effects.

Recommended dietary allowances (RDA's) for various nutrients are discussed in the report. Estimates are made of adequate and safe intakes of nutrients for which a RDA has not been established. The contribution that drinking water can make to the requirement for these nutrients is calculated.

At their typical levels in drinking water, the nutrients reviewed in this report usually make a small, but by no means negligible, contribution to the mineral nutrition of humans.

When the intake of a particular nutrient by the general population or a particular group is marginal, the contribution by water may be important in preventing deficiency and ill health. This may be the case for magnesium, fluoride, iron, copper, zinc, vanadium, and chromium.

For the overwhelming majority of the nutrients studied, the risk of toxicity to normal individuals from typical levels in drinking water is negligible. When the level of a nutrient in drinking water is typical and reduction of total intake is prudent (e.g., for iodine or sodium), reduction from sources other than water are likely to be the option by which the largest initial reduction can be made.

The accurate assessment of the contribution of drinking water to nutrition is hampered by a lack of information on the speciation and bioavailability of elements. Additionally, water treatment practices, e.g., the addition of phosphates for corrosion control, as well as the use of chelators in food preparation, may alter nutrient composition of water.

REFERENCE

National Academy of Sciences. 1977. Drinking Water and Health. Safe Drinking Water Committee, National Academy of Sciences, Washington, D.C. 939 pp .

II

Epidemiological Studies

CANCER FREQUENCY AND CERTAIN ORGANIC CONSTITUENTS OF DRINKING WATER

After comparing cancer rates among people in Louisiana whose drinking water comes from the Mississippi River with rates in populations served by water from other sources, Harris (1974) concluded that something in the river water led to increased cancer rates. He suggested that the lower Mississippi contained many chemical pollutants, some, or some combination, of which were carcinogenic.

The initial study was challenged by DeRouen and Diem (1975a, 1977), and several other hypotheses were put forth. At that time the Environmental Protection Agency (EPA) was monitoring a large number of chemicals in U.S. water supplies—among them, chloroform and the other trihalomethanes (THM's). The National Cancer Institute (NCI) also studied the biological effects of chloroform in its large animal bioassay program and subsequently demonstrated an increase in liver and kidney tumors among animals exposed to high doses of chloroform (Page and Saffioti, 1976).

Given these several pieces of information, the EPA asked a number of research groups to determine whether there was indeed a relationship between cancer rates and chloroform and other THM's in water supplies. Even though the liver and kidney were the only sites at increased risk in the animal experiments, the various research groups attempted to evaluate the cancer risk at many other sites as well. The

rationale for this is that exposure to a single carcinogen may result in cancer at different sites in different experimental animals and in humans. For example, vinyl chloride has produced nephroblastoma in rats but not in humans. There is also ample evidence of chemicals that produce cancer in some species but not in others; for example, 2-naphthylamine is a potent bladder carcinogen in humans, dogs, and monkeys but is inactive in rats and mice.

Most of the EPA-requested studies used indirect evidence of the presence of THM in water supplies. Populations receiving surface water, which often contains organic materials and is sometimes treated with chlorine, were contrasted with those receiving groundwater. Because groundwater usually contains less organic matter and is less often chlorinated in the purification process than surface water, it is less likely to contain THM's.

Two of the studies used direct measurements of chloroform and other THM concentrations, which had been obtained by the EPA in two recent analytical surveys in a regression equation for U.S. county mortality rates with allowance being made for various demographic factors by including further linear regression terms.

The above epidemiological studies (published and unpublished), which comprise to our knowledge the sum total of epidemiological work on this subject, were reviewed by the subcommittee. None of the studies reviewed was adequately able to take into account many well-established risk factors for cancer rates at different sites. For example, for bladder cancer one needs to control for occupation, cigarette smoking, use of alcohol and drugs, nonaqueous sources of THM, coffee consumption, socioeconomic status, and ethnicity. The studies also had to assume that present exposures to THM reflected lifetime exposures of the populations studied: this is not only because THM concentrations in water have only recently been measurable, but also because the necessary information on migration patterns with associated THM concentrations is not readily available.

The subcommittee has summarized the various studies, made a critical assessment, pointed out where it believes the evidence is deficient, and discussed the potential for further research on this subject. The probabilities of false associations when many comparisons are made were also considered when final conclusions were made.

Summary and Conclusions

The studies that the subcommittee reviewed were divided into two groups: those in which nonspecific measures of exposure to putative carcinogens in water (e.g., the use of surface water versus groundwater) were examined and those in which water quality was characterized by measurements of THM concentrations. The subcommittee gave greater weight to the conclusions of the latter group of studies because crude measures of exposure, which lead to comparisons of cancer between surface-water users and groundwater users, must be of limited value. They do not permit the quantitation of exposure to contaminants in water consumed, which is needed to determine dose-response relationships between THM concentrations and cancer frequencies and to estimate the effects of reducing THM concentrations.

The conclusions drawn in the second group of studies, in which many cancer sites were examined, suggest that higher concentrations of THM's in drinking water may be associated with an increased frequency of cancer of the bladder. The results do not establish causality, and the quantitative estimates of increased or decreased risk are extremely crude. The effects of certain potentially important confounding factors, such as cigarette smoking, have not been determined.

The bladder is not one of the sites found to be at increased risk in experiments on animals exposed to chloroform. Tumors of the liver and kidney developed in laboratory animals.

The positive association found for bladder cancer was small and had a large margin of error—not only statistical, but, much more importantly, because of the very nature of the studies. Hogan *et al.* (1978, unpublished) found regression coefficients of bladder cancer mortality(deaths/10^5/year) on chloroform concentration (μg/liter) in drinking water to be approximately 0.003 for males and 0.0021 for females. Thus, if there is a causal relationship, an increase in chloroform concentration of 100 μg/liter might lead to increases of 0.3 deaths/10^5/year from bladder cancer in males and 0.2 deaths/10^5/year in females. These compare with the U.S. National Mortality Rates (1950-1969) for cancer of the bladder of 6.8 in males and 2.4 in females. Thus, a decrease of 100 μg/liter of chloroform in water may lead to a 4.4% decrease in bladder cancer death rates in males and 6.7% in females.

These changes, which have been shown in case-control studies to be explicable by as little as one to two cigarettes per day differences in average cigarette consumption, would probably be too small to be distinguished from possible confounding effects by any epidemiological study. Situations that could be more favorable for investigation should be sought so that further epidemiological work might be more rewarding.

STUDIES BASED ON INDIRECT MEASURES OF WATER QUALITY

Mississippi River-Louisiana, First Study

The first papers reporting relationships between cancer frequency and water quality were those of the Environmental Defense Fund (Harris, 1974, unpublished; Page *et al.,* 1976). These two papers were based on the published cancer mortality data by county in the United States (Mason *et al.,* 1974). In each Louisiana parish the proportion of water that was obtained from the Mississippi River was related, in a regression analysis, to cancer mortality. The study was controlled for urban-rural status, income, and employment in certain potentially hazardous industries. Significant positive correlation coefficients were found in two of the four race-sex groups for genitourinary cancer, and in all four groups for gastrointestinal cancer.

Mississippi River-Louisiana, Second Study

Using the same water quality data and county mortality data as Harris (1974, unpublished), DeRouen and Diem (1975b, unpublished) reported a major difference between northern and southern regions of Louisiana—a potentially important confounding factor for the cancer-water association. This was also noted by Buncher (1975, unpublished). DeRouen and Diem's analyses of cancer mortality and water source were restricted to southern Louisiana where parishes using only Mississippi River water were located. DeRouen and Diem compared cancer mortality at 16 anatomic sites in four race-sex groups (52 site-sex-race groups in all) between persons who received river water and those who did not. They found seven positive associations ($P < 0.05$) of mortality with use of river water in one or more sex-race groups for seven sites or site groups. Similarly, significant negative associations were found for seven sites. The consistency of associations in both sex and racial groups suggested positive associations for cancer of the bladder, breast, (i.e., lower mortality in populations using river water) for cancer of the liver and corpus uteri. Cancer of the lung and cervix uteri had a significant positive association in one population and a significant negative association in another.

Ohio River Area, First Study

A similar approach was used on data from the Ohio River area. Buncher (1975, unpublished) studied the four-state area comprised of Ohio, West

Virginia, Kentucky, and Indiana; Kuzma *et al.* (1977) confined their attention to Ohio. In the Ohio study, white males and females in all Ohio counties were compared according to the predominance of surface water or groundwater in their supplies. Sites with statistically significant ($P < 0.05$) excess mortality associated with surface water were the stomach in males and females and the bladder in males. In two additional site-sex groups, the liver and breast in females were positive at the 20% level. These rates were adjusted for age, county population, percent urbanization, median income, an index of manufacturing activity, and an index of agricultural, forestry, and fishing activity.

Ohio River Area, Second Study

Harris *et al.* (1977, unpublished) reviewed the Ohio studies. Although they generally agreed with the findings of Kuzma *et al.* (1977). they failed to confirm the positive association for liver and breast cancer in females. In addition, they reported positive associations for the esophagus in males (not studied by Kuzma) and the pancreas in males.

They studied the effect of water source on cancer mortality by regression analysis similar to that used in the first Ohio area study, but they used as a water variable the percentage of a county population that received surface water.

Upstate New York

Alavanja *et al.* (1977, unpublished) conducted a case-control study of persons dying with gastrointestinal or genitourinary cancer in several counties in upstate New York. The study was first confined to women to decrease the potentially confounding factor of occupational exposures, and the cancer sites were selected on the basis of indications from prior reports. Consequently, this may be thought of as an hypothesis-testing study. Each cancer case was individually matched with a woman dying of a cause other than cancer.

Matching variables were county, age, race, and birthplace. Statistical analysis procedures did not take the matching into account, but it is impossible to judge the effect of this omission. Water supplies were determined from addresses and were characterized as chlorinated or not chlorinated and as surface water or groundwater. A doubling of risk was found to be associated with chlorinated supplies in urban areas. A 50% increase in risk was found to be associated with chlorinated groundwater when compared with nonchlorinated water in rural areas. There was no

increase (risk ratio, 1.01) in rural areas supplied with chlorinated surface water in comparison with rural areas using nonchlorinated water.

In 1978, Alavanja's study was expanded to include men. He reported:

> Males living in the chlorinated water areas of Erie, Rensselaer and Schenectady counties and females living in the chlorinated water areas of Erie and Schenectady counties are at a greater risk of gastrointestinal [GI] and urinary tract [UT] cancer mortality than are individuals living in nonchlorinated water areas. Moreover, this excess risk of GI and UT cancer mortality is not due to a disparity in the age. race or ethnic distribution of the population or to an urban/rural factor, or hazardous occupation, or to inorganic carcinogens (Cd, As, Be, Pb, Ni, NO_3) or a surface water/groundwater difference.

In one county (Chautauqua) there was a significant deficit of both kinds of cancer mortality in chlorinated areas.

Because of the large population of Erie County, the data acquired there strongly influence the total risks reported. Urbanization is a potentially confounding variable in the study of effects of chlorination, and parceling out shares of the excess risk between urbanization and chlorination does not seem possible, although some attempts have been made. For example, urban and rural nonchlorinated areas were compared with urban and rural chlorinated areas.

Washington County, Maryland

Kruse (1977. unpublished) studied cancer of the kidney and liver in a population in Washington County, which includes the city of Hagerstown, Maryland. This population has been investigated extensively as an epidemiological community model, and the source of home drinking water had been determined in an earlier survey. This previously gathered information was used in a total population study designed to correlate water source, characterized as chlorinated or not chlorinated, with presence or absence of cancers at specific sites. The approach involved estimating the effect of water source through a multivariate analysis including eight additional potentially confounding demographic and sociological variables. There was an increased incidence of liver cancer associated with the chlorinated water supply, with a risk ratio of 1.5, but the increase was not statistically significant at the 20% level. Similarly, a decrease in incidence of cancer of the kidney with chlorination (risk ratio, 0.96) was not significantly associated with water source. There were only 91 cancer cases in this study.

Los Angeles County

In Los Angeles County, Mah *et al.* (1977, unpublished) correlated cancer mortality rates and incidence rates with chloroform content in drinking water. They divided the county into nine subareas categorized as having low, medium, or high chloroform content of the water. Eight cancer sites were studied: esophagus, stomach, colon, rectum, liver, lung, kidney, and bladder. Tabular and graphic inspection of data and limited analyses suggested no association of cancer rates with chloroform content of water, and the investigators believed no more detailed analysis was justified in view of certain limitations in the basic material, viz., absence of precise data on chloroform content, extensive use of bottled and other water transported from other sources by this population, and large population movements into and out of Los Angeles County in the last several decades.

Ohio River, Third Study

Salg (1977, unpublished) studied populations in areas served by water from the Ohio River, including all counties within the boundaries of the Ohio River Valley Basin as determined from water drainage maps. These counties lie within a seven-state area: Illinois, Indiana, Kentucky, Ohio, Pennsylvania, Tennessee, and West Virginia. The exposure variables, indicating possible intake of pollutants in the water supply, were the percent of county population served by surface water and the percent served by prechlorinated (defined as chlorination prior to filtration) water. The outcome variable was cancer mortality for 346 counties, specific for 19 anatomic sites of cancer. The main variables were investigated in a multivariate analysis using as possible confounding factors nine variables representing demographic and socioeconomic characteristics of the counties.

There were positive associations ($P < 0.2$) for 13 of the 19 site categories in one or more of four sex-race population groups. Similarly significant negative associations were found for seven site categories in one or more populations. In two or more population groups there were positive associations for cancer of the esophagus, rectum, breast, larynx, and for Hodgkin's disease.

Salg interpreted her findings to indicate the need for further study of carcinoma of the large intestine, rectum, and bladder. Large intestine cancers were found to be significantly increased in white males. Bladder cancer incidence was significantly elevated in white males and significantly decreased in nonwhite females.

Pittsburgh Study

Carlson and Andelman (1977, unpublished) conducted a study in the Pittsburgh region. They associated site, race, and sex-specific, age-adjusted cancer incidence rates by 1969-1971 census tract with drinking water, which was characterized by source of raw water (surface, ground, and river) and by water treatment plant.

Their paper is principally an investigation of statistical methodology. Their general conclusion is that significant associations, both positive and negative, are found between water quality and cancer incidence. The data are not summarized in a way that permits identification of cancers of specific sites.

New Jersey Study

Vasilenko and Magno (1975, unpublished) studied all 21 counties in New Jersey to determine the relation between water source and age-adjusted cancer mortality from lung, stomach, and urinary tract cancer of white males between 1950 and 1969. Water quality was estimated from the ratio of the number of households served by public systems and private water companies to the number served by individual wells. Confounding variables included in the analysis were urban-rural characteristics, income, education, migration, occupation, industrialization, and concentration of sulfur dioxide in the air.

In a multiple regression analysis, the water variable was positively associated with mortality from respiratory cancer and stomach cancer. A nonsignificant negative association was found for bladder cancer.

Summary

Nine of the ten studies described above showed a number of associations, some of which were statistically significant, between indirectly characterized water quality and cancer rates (incidence or mortality). One, the Los Angeles County study, reported no associations, but this study appeared to have greater limitations than any of the others.

Cancer rates at several sites were positively associated with water quality in one or another study, but no site consistently predominated. The bladder, stomach, large intestine, and rectum, which were cancer sites identified in a number of geographic areas, warrant further study.

The effects of certain important demographic variables were considered in a number of studies of this group, but other confounding factors, e.g., cigarette smoking, were not.

STUDIES BASED ON TRIHALOMETHANE CONCENTRATIONS

Three studies (Cantor *et al.,* 1977, unpublished; Hogan *et al.,* 1978, unpublished; McCabe, 1975, unpublished) have been conducted using measured THM concentrations. All three studies used cancer mortality data rather than incidence data. Although incidence data are usually preferable, the studies could not use these data as they are not generally available for the areas covered by the THM surveys.

Cantor *et al.* studied the relation between age-standardized cancer mortality for 1968 to 1971 of white men and women in U.S. counties that were categorized as urban on the basis that more than 50% of the county population lived in urban areas in 1970.

County THM concentrations were estimated from data obtained in two surveys conducted by the EPA (U.S. Environmental Protection Agency, 1975). The National Organics Reconnaissance Survey (NORS) sampled finished water in 80 water treatment plants across the country, and a survey conducted by EPA's Region V office covered 83 plants in Illinois, Indiana, Michigan. Minnesota, Ohio, and Wisconsin.

The analysis took into account the median school years completed by county inhabitants over age 25, foreign stock composition of county, county population, ratio of 1970 to 1950 county population, percent of county that is urban, percent of the county work force engaged in all manufacturing industries (U.S. Bureau of the Census, 1970), and major geographic region of the United States. An attempt was made by multivariate regression to explain the variability among counties of mortality rates for each site of cancer with sex-specific mortality rates greater than 1.5/100,000/yr. The residual mortality rates, which were "unexplained" by these other variables, were then correlated directly with measured THM levels (logarithms of the concentrations) for the 76 counties in which 50% or more of the population was served by the sampled water. To calculate the correlation coefficients, the data were weighted by the square root of the sex-specific person-years at risk in the population served by the sampled water supply as estimated by the product of percent of population served and population at risk. The statistical techniques used by the authors, particularly the weighting factors, are not standard and probably tend to decrease statistical significance.

The results of Cantor's analysis are shown in Tables II-1 and II-2. Among males, a significant ($P \leq 10\%$) positive correlation for the 76 counties was found between nonchloroform trihalomethane (NCTHM) concentration and bladder cancer. When the percent of county population served by the sampled water supply is increased to reduce the

TABLE II-1 Correlation Coefficients Between Residual Mortality Rates in White Males and Halomethane Levels in Drinking Water, by Percent of the County Population Served by the Sampled Supply[a]

		Correlation Coefficients and (*P*-Value for Two-Tailed *t*-Test) by County Population Served			
Site or Type of Malignancy	Halomethane Indicator[b]	50%-64%, 25 counties	65%-84%, 26 counties	85%-100%, 25 counties	50%-100%, 76 counties
Pancreas	Chloroform	0.04	-0.06	-0.32	-0.13
		(0.83)	(0.77)	(0.12)	(0.27)
Prostate	Chloroform	0.34	0.03	0.30	0.17
		(0.10)	(0.87)	(0.14)	(0.14)
Bladder	NCTHM	-0.22	0.29	0.38	0.19
		(0.29)	(0.15)	(0.06)	(0.10)
Kidney	Chloroform	-0.16	-0.11	0.42	0.07
		(0.44)	(0.60)	(0.04)	(0.55)
Brain	NCTHM	0.10	0.18	0.24	0.17
		(0.65)	(0.37)	(0.25)	(0.14)
Non-Hodgkin's lymphoma	NCTHM	-0.33	-0.19	0.36	-0.03
		(0.11)	(0.36)	(0.08)	(0.81)
Stomach[c]	Total THM	0.01	0.05	-0.14	-0.02
		(0.96)	(0.81)	(0.49)	(0.87)
Pancreas[c]	NCTHM	-0.12	-0.31	0.04	-0.16
		(0.57)	(0.12)	(0.84)	(0.18)
Lung[c]	Total THM	-0.02	0.02	0.15	0.07
		(0.94)	(0.90)	(0.46)	(0.56)

[a] From Cantor *et al.,* 1977.

[b] Abbreviations: NCTHM, nonchloroform trihalomethanes; THM, trihalomethanes.

[c] Included for comparison with results for females, for whom stronger associations with the listed indicator were observed.

TABLE II-2 Correlation Coefficients Between Residual Mortality Rates in White Females and Halomethane Levels in Drinking Water, by Percent of the County Population Served by the Sampled Supply[a]

		Correlation Coefficients and (*P*-Value for Two-Tailed *t*-Test) by County Population Served			
Site or Type of Malignancy	Halomethane Indicator[b]	50%-64%, 25 counties	65%-84%, 26 counties	85%-100%, 25 counties	50%-100%, 76 counties
Stomach	Total THM	0.01	-0.11	-0.36	-0.16
		(0.97)	(0.59)	(0.07)	(0.17)
Pancreas	NCTHM	-0.31	-0.12	0.31	-0.03
		(0.13)	(0.56)	(0.14)	(0.82)
Lung	Total THM	0.25	0.28	0.15	0.22
		(0.23)	0.17)	(0.46)	(0.05)
Bladder	NCTHM	-0.01	0.21	0.45	0.21
		(0.97)	(0.30)	(0.02)	(0.06)
Pancreas[c]	Chloroform	-0.25	0.20	-0.06	0.02
		(0.22)	(0.32)	(0.77)	(0.85)
Kidney[c]	Chloroform	-0.33	0.19	-0.04	-0.01
		(0.11)	(0.37)	(0.83)	(0.96)
Brain[c]	NCTHM	-0.07	-0.03	0.19	0.04
		(0.73)	(0.90)	(0.35)	(0.72)
Non-Hodgkin's lymphoma[c]	NCTHM	-0.36	0.26	-0.04	0.01
		(0.08)	(0.20)	(0.83)	(0.97)

[a] From Cantor *et al.,* 1977.

[b] Abbreviations: THM, trihalomethanes; NCTHM, nonchloroform trihalomethanes.

[c] Included for comparison with results for males, for whom stronger associations with the listed indicator were observed.

TABLE II-3 Weighted Regression Coefficients and Levels of Statistical Significance Between Chloroform Concentrations and Cancer Mortality in Men and Women[a]

	White males				White Females			
	NORS		Region V		NORS		Region V	
Site	β	P	β	P	β	P	β	P
Large intestine	0.0114	0.01	0.0115	0.01	0.0102	0.01	-0.0019	0.60
Rectum	0.0032	0.22	0.0062	0.03	0.0031	0.04	0.0007	0.75
Bladder	0.0034	0.10	0.0013	0.63	0.0017	0.04	0.0023	0.07
Liver	-0.0026	0.08	0.0048	0.03	-0.0020	0.21	0.0027	0.17
Stomach	-0.0096	0.04	-0.0023	0.65	-0.0069	0.01	0.0016	0.63
Esophagus	-0.0040	0.06	0.0016	0.53	-0.0005	0.35	0.0003	0.69
Tongue	-0.0045	0.12	0.0052	0.02	-0.0001	0.80	0.0000+	0.96

[a] From Hogan *et al.,* 1978.

misclassification of exposure, correlations with bladder and brain cancer tend to increase. Among women, positive correlations were found between total THM's and lung cancer and between NCTHM and bladder cancer. The lung cancer correlation coefficients did not show a dose-response relationship with the proportion of the population receiving the measured water. But the correlations with bladder cancer did: from 85% to 100% reached conventional significance (P = 0.02). A preliminary analysis showed a fairly strong association between halomethane levels and colon cancer rates, but control for composition of the population by ethnicity removed this association.

The authors computed correlation coefficients separately for three geographic regions (North, South, and Mountain Pacific) for the 51 counties in which 65% or more of the population received measured water. There were positive correlations between NCTHM and bladder cancer in males in all regions combined (r = 0.30, P = 0.03), and in females in each region separately as well as combined (combined r = 0.33, P = 0.02). For men in the North, a significant correlation was observed between NCTHM and bladder cancer (r = 0.52, P = 0.02).

Hogan *et al.* (1978, unpublished) conducted a similar study, in which they related earlier cancer mortality data to chloroform levels in finished water as determined by the same surveys. They used the National Cancer Institute's 20-year age-adjusted county cancer mortality rates for white men and women (Mason *et al.,* 1974) and county chloroform concentrations in drinking water estimated from the EPA's NORS and Region V surveys (U.S. Environmental Protection Agency, 1975). Regression equations were fitted to the mortality rates using, as independent variables, chloroform concentrations, 1960 county population, county population density, percent of county that is urban, percent of county population that is nonwhite, percent of county population that is foreign born, median number of school years completed by county residents over age 25, median family income of county, and percent of county work force engaged in manufacturing. Weighting was by total population exposed (both sexes and all races combined).

The results for sites where any association approached statistical significance are shown in Table II-3. The data are consistent with an increase in cancer rates of the rectum, the bladder, and possibly the large intestine with increased chloroform concentration.

McCabe showed that age-adjusted total cancer mortality rates correlated positively with estimated chloroform concentrations in 80 cities. Since no allowance appears to have been made for any of the confounding factors, it is inappropriate to draw conclusions from this study to compare with the Cantor and Hogan studies.

All studies were seriously limited by the absence of data on past exposures, which are the only ones that are directly relevant to cancer that has already been diagnosed. Similarly, all studies were deficient in identifying populations that were stable in the areas where the water quality was studied.

None of these papers discusses the nature of the correlation or regression coefficients obtained: in particular, whether the positive results found were due to one or two extreme observations or to a more relevant trend over the range of THM values. Similarly, none discusses the adequacy of using a simple linear regression equation to allow for certain confounding variables. Without such discussion interpretation must be especially circumspect.

Results of these studies demonstrate the problems of establishing relationships between health statistics and environmental variables, and lend emphasis to the caution with which they should be interpreted.

Prospects for Further Epidemiological Study

Adequate exploratory and hypothesis-generating work has been done. Studies raising suspicion of higher cancer rates among persons whose major water supplies came from surface waters with generally higher THM concentrations compared to rates among those whose major water sources were groundwaters have been followed by attempts to relate the cancer rates to actual THM concentrations. Further studies of this kind are unlikely to lead to more useful information, although an examination of age-specific data might prove fruitful. Consequently, future studies should be more specific and should examine possible confounding factors in detail.

When developing actual exposure data, i.e., THM concentrations in water consumed, investigators should gather information concerning duration of exposure to a particular water source. Further investigation of the validity and reliability of chemical analyses of water constituents would also be in order. Such epidemiological studies without experimental intervention usually cannot uncover small effects. Moreover, with so many confounding factors, it would be difficult to ascribe an effect to any factor with certainty.

For discussion, designs for further studies may be conveniently divided into two major types: case-control studies and cohort studies; including intervention studies.

Case-Control Studies These studies can start with cases (incidence, prevalence) or deaths. For certain investigations the information from

deaths may be adequate. However, the degree of detail necessary for investigating carcinogens in water makes it highly desirable that information be collected from the subjects themselves, rather than from near relatives or other proxies. Some populations are exposed to varying amounts of chloroform, e.g., users of certain cough medicines and toothpastes that contain high concentrations of chloroform. Such individuals should be identified among cases and controls.

The site most warranting a case-control study is the bladder, possibly followed by the colon and rectum. Confounding factors include occupation, cigarette smoking, use of alcohol and other drugs (including artificial sweeteners), nonaqueous sources of THM, coffee consumption, socioeconomic status, and ethnicity.

Cohort Studies Cohort studies, attempting to follow "exposed" and unexposed populations over time, may also be possible. Some natural experiments have been created by using different methods of water purification. Thus, a population from a community using activated charcoal filtration that is adequately and frequently regenerated might be compared over time with a population from a community not using that process. Due attention must be given to latency—the time difference between initial exposure and the appearance of disease. This applies to both retrospective and prospective studies.

Studies should be undertaken only after there is a clear understanding of the magnitudes of detectable differences. Because the effects anticipated from the usual concentrations of THM in drinking water are not expected to be great, large populations must be studied to demonstrate associations. Similarly, if there is no association, large populations must be studied to exclude the existence of the small effects that have been postulated.

Some useful data may be available from foreign countries—and the help of international agencies (World Health Organization-International Agency for Research on Cancer, North Atlantic Treaty Organization, U.S.-Japan Scientific Exchange, U.S.-USSR Scientific Exchange) should be sought.

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WATER HARDNESS AND CARDIOVASCULAR DISEASE

The Present Status of Knowledge

The following statement regarding water "hardness" appears in *Drinking Water and Health* (National Academy of Sciences, 1977), a report of the National Academy of Sciences-National Research Council Safe Drinking Water Committee (SDWC).

> Hardness may be defined as the sum of polyvalent cations present in water. The most common such cations are calcium and magnesium. Hardness is usually expressed in terms of the equivalent of calcium carbonate ($CaCO_3$). There are no distinctly defined levels of what constitutes a hard or soft water supply. Generally water with less than 75 mg/liter (ppm) of $CaCO_3$ is considered soft and above this concentration as hard.

The use of 75 mg/liter as the concentration separating soft and hard water was derived from practical considerations of effects, such as boiler scaling, rather than from observations on human health. This classification of waters as hard or soft is an oversimplification of the concept of hardness since the major contributors to hardness, calcium and magnesium, vary in relative proportions. Moreover, the classification does not describe quantitatively the possible presence of a wide range of polyvalent cations, including strontium and barium, which are sometimes found as trace elements in waters. Concentrations of vanadium, lithium, chromium, and manganese are higher in hard water. Cadmium, lead, copper, and zinc tend to occur in soft water at the tap, largely as a result of the greater corrosiveness (aggressiveness) of soft water in the distribution system. However, untreated hard water sometimes contains high levels of these elements. 'Therefore, the pattern is variable.

The chemical composition of water that is softened by municipal treatment or in the home will depend upon the softening method that is used. Ion-exchange methods raise the concentration of sodium in water, an effect that may have adverse consequences for health, at least for some individuals.

Current knowledge of the relationship of water hardness to cardiovascular disease is derived largely from "ecological" epidemiological studies, in which individual exposures or risk factors have generally not been considered. These studies were reviewed extensively by the National Academy of Sciences-National Research Council Panel on the Geochemistry of Water in Relation to Cardiovascular Disease (National Academy of Sciences, 1979). The status of knowledge in this field may be summarized as follows:

1. In general, when studies encompass large geographical areas, hard water is correlated with low cardiovascular disease rates. This correlation breaks down when smaller areas are considered or when the studied populations are grouped by altitude or the proximity of a seacoast. Some noncardiovascular ailments are also associated occasionally with soft water areas, raising the possibility that soft water may merely be a concomitant of some more basic risk factor.
2. Most studies have reported correlation coefficients and not risk estimates as a function of exposure. A few contain sufficient data for risk estimation: upper estimates of risk ratios for soft compared to hard water average approximately 1.25 for cardiovascular disease and 1.2 for arteriosclerotic, stroke, and hypertensive diseases (National Academy of Sciences. 1979).
3. From autopsy studies in Canada and the United Kingdom, investigators have reported low magnesium levels in various tissues (heart, diaphragm, and pectoral muscle) of persons who died from myocardial infarction compared to those who died from accidental causes. They observed similar deficits in persons from soft water areas as compared to hard water areas. However, conflicting data have been reported.

The SDWC was aware of detailed studies in progress in Canada and in the United Kingdom, but was unable to evaluate their significance since the results of these studies only became available late in the committee's deliberations.

Recommendations for Alteration of Current Practices

Given the current status of knowledge regarding water hardness and the incidence of cardiovascular disease, it is not appropriate at this time to recommend a national policy to modify the hardness or softness of public water supplies. The data do not indicate clearly which (if any) additions to soft water would benefit human health. However, certain

factors need to be considered carefully before individual decisions can be made.

Municipal authorities who are either softening their water supplies or are considering doing so should carefully assess the necessity for this treatment. If they decide that it is essential, they should select a method, such as lime softening, that does not elevate sodium levels.

Ion-exchange resins are often used to soften water, particularly in home water supplies. The ion-exchange process softens water by replacing calcium or magnesium with sodium. One atom of divalent calcium or magnesium is replaced by two atoms of sodium. In terms of actual weights of the elements, 100 mg of calcium would be replaced by about 115 mg of sodium. Thus, softening can result in increases in the concentration of sodium in the softened water. Avoiding such an increase might be prudent given the state of knowledge on effects of sodium consumption. The increase in the sodium concentrations in the water caused by ion-exchange softening is a direct function of the hardness level of the untreated water and the operating efficiency of the softening device. When a physician advises that an increase in sodium intake is undesirable, a "three-line system" should be used if soft water is desired for laundering and bathing. The line that supplies the drinking water taps bypasses the softener thus preventing the consumption of water with an increased sodium level.

Recommendations for Future Research Priorities

Further studies should be conducted on the relationship between water hardness and cardiovascular disease. Repetition of the types of studies that have generally been conducted to date is unlikely to yield the information that is necessary for the determination of a nationally applicable policy.

Epidemiological evidence should be gathered from studies of individual exposures to different water supplies, from studies of communities that have experienced changes in their water supplies, such as those resulting from discontinuation of softening and from changes in the composition of their drinking water. Studies to assess mortality rates should be controlled for such intercommunity differences as ethnicity and smoking. Some of these opportunities for studies could, with relatively little expenditure, be turned into very useful controlled prospective epidemiological studies if sufficient time is available for careful planning of control populations and maintenance of migration records.

The results of clinical research from two types of studies may provide useful information on the relationship of water hardness and cardiovas

cular disease. Tissue samples obtained during autopsy should be analyzed for a variety of elements, including magnesium, sodium, silicon, lead, and copper, and results examined for association with specific causes of death. Controlled trials of magnesium supplements (as fortified food or tablets) with groups of individuals at high risk of cardiovascular disease could also provide useful information.

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III

Problems of Risk Estimation

Historically, two approaches have been taken to estimate acceptable levels of exposure to various agents. One approach is based on the application of "safety factors" to levels of the chemical that did not produce an observed effect in animal studies. This approach gives rise to acceptable daily intakes (ADI's) for humans. The other, which has been used to estimate the risk to the population as a whole from low doses of radiation, is based on extrapolation of experimental dose-effect curves to lower dose levels where no data existed. This is called the "risk estimate" approach.

The risk estimate approach generally assumes that at all doses some organ, or targets within the organ, will be affected and that there is a finite probability for the occurrence of damage that can lead to ill health, i.e., there is no threshold. This probabilistic approach has been used not only for radiation but also to estimate the risks from carcinogens in drinking water (National Academy of Sciences, 1977).

To estimate risk, adequate dose-response curves must exist for the purpose of extrapolation. However, such data do not exist for the majority of chemicals that are found in drinking water. In these cases the Committee on Risk Estimation continued the ADI approach for noncarcinogens with the belief that it should be used until sufficient data accrue to make risk estimation feasible.

The rational determination of a permissible exposure to a toxic chemical in drinking water requires the ability to specify the quantitative nature of the exposure. Thus, the biological factors, such as absorption, distribution, metabolism, and excretion, that determine the toxic effects both in the animals used for testing and in humans should be assessed.

ACUTE EXPOSURE

While considerable attention has been paid to chronic exposure from chemicals in drinking water, the problem of acute exposures from accidental spills and discharges needs consideration. To protect exposed populations, health officials must be able to respond quickly following such occurrences. Observations on humans have provided some of the data on toxicity from acute exposures of 1 week or less. Clinical observations on the effects of both low concentrations and accidental high exposures, epidemiological observations on various segments of the population, and deliberately planned experiments provide a body of knowledge on dose-response relationships from which one can estimate risk. When such evidence is not available, recourse is often made to experimental exposures in various subprimate animal species. Although information derived from data on the most sensitive species may be desirable, the only data available often pertain to acute oral toxicities in rodents.

How the available data base is to be used in the assessment of acute risk is, of course, a matter of great concern. A wide range of "safety factors" (from 10 to 5,000) has been considered for use with chronic oral toxicity data to estimate an acceptable risk to the exposed population. Unfortunately, none of these safety factors, including the most conservative, has any relevant experimental verification in heterogeneous populations that are analogous to humans likely to be exposed acutely to contaminants in drinking water. The presumed absence of toxic effects at any particular level in an experimental system may not be adequate to protect especially sensitive population subgroups such as the fetus, infants, the infirm, or the aged.

To determine the safety factor to be applied to the acute toxicity data for a given situation, the following should be carefully evaluated:

- quality and quantity of data;
- most sensitive target organ(s) or body system(s) to be affected;
- interspecies and intraspecies variations;
- nature of the dose-response curve and the time-concentration relationships;
- nature and degree of severity of injury at which the effect of the exposure ceases to be reversible;
- potential interactions with other environmental chemicals or therapeutic drugs;
- identification of potential cumulative effects;

- known chronic or subchronic effects of similar or related compounds;
- identification of physiologic or pathologic states and functional abnormalities among the potentially exposed population; and
- possibility of chronic effects from repeated acute short-term exposure.

Because acute exposures to chemical contaminants have no demonstrable beneficial effect on health and because of the desirability of protecting sensitive members of our society, a conservative approach is advisable when establishing permissible levels of acute intake to insure an adequate supply of "safe" drinking water.

When compounds in drinking water appear in combination, as they often do, their joint effect may be additive, synergistic, or antagonistic. Some biochemical modeling has been done by Werkheiser (1971), who simulated the effect of antimetabolites on *de-novo* DNA synthesis, and observed considerable joint action ranging from potentiation through additivity to antagonism. In general, there is not likely to be sufficient information on mixtures of environmental contaminants. Consequently, estimates will out of necessity have to be based on a nonconservative assumption of additivity. The work of Smyth *et al.* (1969) on the joint action of 27 industrial chemicals is pertinent. They administered doses containing all possible pairs of the chemicals to rats by oral intubation. Comparison of the predicted LD_{50} to that observed in the rats that had received the 350 pairs of equivolume mixtures indicated the utility of a harmonic mean formula for estimation of relative hazard:

$$\frac{1}{\text{predicted } LD_{50}} = \frac{P_a}{LD_{50} \text{ of component A}} + \frac{P_b}{LD_{50} \text{ of component B}},$$

where P_a, P_b are the fractions of components A and B in the mixture.

The ratio of predicted LD_{50} to observed LD_{50} covered a range from 0.23 to 5.09. The majority of the time, the observed LD_{50} was well estimated by the formula (median range of 0.58 to 1.50 in predicted to observed LD_{50}). Comparable results were achieved for a selected subset of equitoxic mixtures. Data to extend the harmonic mean formula to multicomponent mixtures,

$$\frac{1}{\text{predicted } LD_{50}} = \sum_{i=1}^{N} \frac{P_i}{LD_{50} \text{ of component } i},$$

where $P_i = 1$, are generally lacking, and any attempt to predict acute toxicities of mixtures on this basis must allow for the possibility that a larger number of interactions is possible, and greater uncertainty should be anticipated. However, the use of such formulas for predictive purposes in the event of a spill of two or more agents into the drinking water may be the only way of estimating the toxicity of the mixture.

QUANTITATIVE EXTRAPOLATION OF TOXICITY FROM LABORATORY ANIMALS TO HUMANS

Reliable information on the toxicity of most chemicals to humans is very difficult to obtain. Usually, it must be based on accidental or occupational exposures which, by their very nature, are uncontrolled. Assembling information on the degree of exposure is difficult, and those who exhibit symptoms are much more likely to be studied than those who do not. This section summarizes some of the available information on the quantitative aspects of interspecies toxicology.

Ironically, the best quantitative data on interspecies toxicology, including humans, have been obtained from work on developing and evaluating anticancer drugs. These are usually cytotoxic agents that involve a variety of mechanisms of action. They are screened against experimental tumor systems in mice and, if sufficient activity is observed, subjected to extensive preclinical toxicology. Then, because of their generally low therapeutic indices, they must be used at or near maximally tolerated doses in the clinic. Thus, it is possible to obtain in ethical, well-controlled, and documented studies the toxic levels in several mammalian species including humans.

Pinkel (1958) examined appropriate therapeutic doses of several anticancer drugs in animals and humans and suggested that cancer chemotherapists consider body surface area as a criterion for dosage in both laboratory and clinical studies. Freireich *et al.* (1966) extended the observations of Pinkel to a group of 18 anticancer drugs. They generally confirmed Pinkel's hypothesis that the body surface area is a suitable normalizing factor for dose. They based their quantitation on the following toxicologic end points: the LD_{50}'s for rats or hamsters and the maximum tolerated dose for dogs, monkeys, and humans.

Dixon (1976) has questioned the usefulness of the mg/m^2 extrapolation if the clinical estimate is based on data from dogs and monkeys. In fact, when the more sensitive of these two species was used and the dose was expressed on a mg/kg basis, the correlation was excellent. Dixon

also observed that introduction of a new anticancer drug at one-tenth of the maximum tolerated dose (MTD) in the more sensitive species would be expected to be associated with a risk exceeding the human MTD of approximately 3%.

Goldsmith *et al.* (1975) argued that the mouse is a reasonably good predictor of human toxicity. The dog would have underpredicted human toxicity in 6 of 28 drugs, whereas the mouse would have underpredicted only 2 of 29. Overpredictions would have been approximately the same for dog and mouse. They did not argue that dose estimation should be based on rodent data alone but concluded that mouse data can be a useful addition to large animal data in estimation of the initial human dose for Phase I clinical trials. The adequacy of quantitation can be observed by comparing the ratio of the human dose (mg/m^2) arrived at in a clinical setting to the optimum dose in leukemic mice. The median ratio (for 30 drugs) was 1.5 with a range of 0.08 to 26.

In summary, the common practice among cancer chemotherapists of basing dose on body surface area is useful, particularly for extrapolation from small animals to humans, and is supported by a sizeable body of experimental evidence. Since body surface area is approximately proportional to the two-thirds power of body weight, the anticancer drugs are relatively *more* toxic to the larger animals than to the smaller ones. For example, by the Freireich criterion, the drug dosage given to a mouse (on a mg/kg basis) must be 12-fold greater than that given to a human.

CHRONIC EXPOSURE

Acceptable Daily Intake

The acceptable daily intake (ADI) of a chemical is defined as the dose that is anticipated to be without lifetime risk to humans when taken daily. It is not assumed that this dose guarantees absolute safety.

Determination of the ADI is often based on the application of laboratory animal toxicity data concerning chronic (long-term repeated) doses to the environmental doses to which humans are exposed. The use of safety (or uncertainty) factors in extrapolating animal toxicity data to acceptable exposure levels for humans has been the cornerstone of regulatory toxicology. The concept of a safety factor arose in the early days of food additive legislation when it became apparent that there was

no universally acceptable quantitative method for extrapolating from animals to humans.

The originators of the safety factor approach, Lehman and Fitzhugh (1954), founded the concept of the "100-fold safety factor" as a practical means of handling the uncertainties involved in extrapolation. They considered that animals might be more resistant to the toxic effects of chemicals than are humans. Hence, they applied a factor of 10 when extrapolating from animals to humans. They incorporated another factor of 10 to account for differential sensitivities within the human population. This concept of the 100-fold safety factor in regulatory toxicology pertaining to food additives has been endorsed by such international organizations as the World Health Organization (FAO/WHO Expert Committee on Food Additives, 1958). The 100-fold factor is usually applied to the highest no-adverse-effect dose measured in animal studies to establish the ADI for humans.

ADI's were first applied to food additives. Subsequently, the Joint FAO/WHO Expert Committee on Pesticide Residues (FAO/WHO, 1965) used the term ADI in its recommendations. In connection with environmental contaminants, the FAO/WHO Expert Committee on Food Additives (1972) specifically noted that the ADI concept is not applicable to heavy metals and lipophilic substances. These substances tend to accumulate in the body tissues after prolonged exposure. In some instances, different chemical forms of such metals as mercury are difficult to differentiate and may have vastly different toxicological properties. For contaminants, the WHO recommended use of "tolerable" intakes to signify permissibility rather than acceptability. "Tolerability" is applied only to those situations in which intake of a contaminant is unavoidably associated with consumption of otherwise nutritious food or with inhalation of air.

The FAO/WHO Expert Committee on Food Additives (1962) pointed out limitations and expressed reservations regarding use of the ADI. They recognized that animal species, strain, and sex differences, variations in susceptibility among exposed individuals, insufficient laboratory animal data, and a number of other matters must be considered when arriving at the ADI. Food additives or other environmental contaminants may be ingested by people of all ages throughout their lives. They are consumed by the sick as well as the healthy, and there may be wide variations among individual exposure patterns. Thus, it is not surprising to find that expert committees of the FAO/WHO do not steadfastly use the 100-fold factor, but at times modify the safety factor when there is a lack of available information regarding the particular substance under question. Thus, in 1962 the FAO/WHO

Expert Committee on Food Additives (FAO/WHO, 1962) introduced the terms "conditional" and "unconditional" ADI's. The "conditional" ADI's require a larger than 100-fold safety factor due to limitations or uncertainties regarding the available animal data or specifications with respect to the purity and identity of the chemical under consideration.

The ADI based on laboratory animal data is also dependent upon the interpretation of a no-adverse-effect level. For example, Edson and Noakes (1960), in their investigation of Diazinon, defined an adverse effect to be an important inhibition of red-cell cholinesterase (CHE) activity, where important meant at least a 20% reduction over the control values. This resulted in the determination by Edson and Noakes of a 5 mg/liter no-effect level since it produced only a 19% reduction of CHE activity in red cells. The meaning of a difference between 19% and 20% is questionable. This experimental determination of a no-effect level is also dependent upon the number of animals used in the bioassay. The likelihood of observing a no adverse effect at a given dose is statistically greater for experiments with few animals than for larger experiments. (This is due to the fact that statistical tests of hypothesis have increasing power with increasing sample sizes.) Therefore, small studies are likely to produce higher no-effect levels than large studies; yet the ADI concept does not explicitly take this into account.

Because of the uncertainties in this method of determining the ADI level, it is desirable to examine the feasibility of improving the use of the animal toxicity data for low exposure noncarcinogenic risk assessment. One approach is extrapolation to low doses from high dose animal toxicity data that show a dose response. The subcommittee has examined the potential of such extrapolation for providing estimates of noncarcinogenic toxic effects of drinking water contaminants at the low exposure levels that were determined by the ADI calculations in *Drinking Water and Health* (National Academy of Sciences, 1977). Each of the studies upon which those ADI's were based was reviewed to determine whether the data were of sufficient quality for the dose-response extrapolation.

The utility of dose-response extrapolation for noncarcinogenic toxic effects is subject to a number of limitations. Many of the experimental bioassays were conducted at dose levels that were too low to show any adverse effect. For example, Table VI-6 on the toxicity of Amiben and Table VI-54 on the toxicity of methyl methacrylate in *Drinking Water and Health* (National Academy of Sciences, 1977, pp. 520 and 748) show that the highest dose tested in all the bioassays of these chemicals did not produce any toxic effects. Therefore, no dose-response extrapolation could be used since no dose-response was observed. Another limitation is the lack of detail in the data reported in some of the published studies

of these contaminants. In many instances the numbers of animals tested were not given, the toxic effect was not quantified arithmetically (e.g., the effects were ranked by +, + +, or + + +), or the published report simply stated a no-observed-effect level without supporting data. An additional problem encountered when measuring noncarcinogenic effects is that the toxic response is often difficult to quantify. Behavioral effects are an example of such responses.

Even with quantifiable dose-response information, the results of extrapolation methodology are difficult to interpret, as illustrated by the two examples presented below. One is an example of a quantitative response; the other is an example of a dichotomous (yes-no) response. These two studies are among the best, in the sense of usable published data, for illustrating dose-response methodology.

The first example deals with the toxicity of hexachlorophene (HCP). Gaines *et al.* (1973) reported on a bioassay in rats. Ten rats of each sex (F_0 generation) were fed HCP in their diets at levels of 0, 1.16, and 5.8 mg/kg/day beginning at the age of 4 to 5 weeks. After treatment for 166 days, the F_0 rats were pair-mated to produce the F_1 generation. Ten rats of each sex of this F_1 generation were continued on the same treatment as their parents. The F_0 rats were sacrificed at 258 days and the F_1 rats at 145 days. The occurrence of brain lesions was found to be associated with high dietary levels of this chemical and the no-adverse-effect level was found to be 1.16 mg/kg. The combined subchronic toxicity results for both sexes of rats are shown in Table III-1. Because there was no apparent difference in sensitivities between the sexes, they were combined for this analysis. For *illustrative purposes only,* the data for the two generations were combined by using a crude measure of total exposure based on the product of the concentration in the diet and length of exposure. The following dose-response extrapolation is not necessarily meant to be meaningful. These assumptions, the combining of sexes and generations, along with this measure of total exposure, were made in order to construct a usable example of the extrapolation methodology.

A log-logistic dose-response model was fitted to these data; the dose was the total dose and the response was the occurrence of a brain lesion. It was assumed that the spontaneous occurrence of such lesions was nil. The fitted dose-response curve was then used to estimate the probability of a dose-induced brain lesion at the ADI level of 0.00116 mg/kg/day. This ADI was translated to a total lifetime dose in mg/kg-days for humans based on an assumption of a total population dietary intake of 666 kg/yr (Lehman, 1962) for a 70-kg human over a 70-year lifetime, producing 57 mg/kg-days of exposure. The estimated probability of brain lesion occurrence is 3.3×10^{-5}. However, this extrapolation is

based on the "best fitting" log-logistic dose-response curve. Many other estimates of the parameters in the model fit these data almost as well. Therefore, to incorporate the statistical variability into this extrapolation, an approximate 95% confidence interval on the estimated risk was calculated. This interval indicates that the extrapolated risk lies between 3×10^{-8} and 0.095, an interval so wide as to suggest that little is known quantitatively about the level of risk. This example shows that applying dose-response extrapolation techniques when the data are limited in experimental dose levels and sample sizes will not yield precise risk estimates.

TABLE III-1 Brain Lesions Observed in Ten Female and Ten Male Rats That Had Been Fed Hexachlorophene [a]

Generation	Days on Diet	Dietary Level, mg/kg	Total Dose, mg/kg x days	Number of Animals with Brain Lesions/ Total Treated
F_0	258	0	0	0/20
		1	258	0/20
		5	1290	10/20
F_1	145	0	0	0/20
		1	1450	0/20
		5	725	3/20

[a] From Gaines *et al.,* 1973.

The second example deals with the toxicity of 2,3,7,8-tetrachlorodibenzo*p*-dioxin (TCDD) in rats and mice (Kociba *et al.,* 1976; Vos *et al.,* 1974). The ADI in *Drinking Water and Health* (National Academy of Sciences, 1977) for TCDD of 0.0001 µg/kg/day was calculated from the results of the Kociba study by applying a safety factor of 100 to the noadverse-effect level of 0.01 µg/kg/day. The subchronic toxicity effect of TCDD upon the thymus weights of the rats and mice in the two studies is shown in Table III-2.

A general dose-response model relating dose, d, to thymus weight, W, is $W(d) = W_0(1-F(d))$, where W_0 represents the weight for unexposed animals and $F(d)$ is a dose-response curve for which $F(0) = 0$ and is monotonically increasing to a limit of unity. Therefore, $W(0) = W_0$ and $W(d_1) < W(d_2)$ when $d_1 > d_2$. A log-normal model and a log-logistic model, both of which have the commonly observed sigmoid appearance but have different extrapolation characteristics, were used for $F(d)$. These

TABLE III-2 Thymus Weights of Animals Treated with TCDD [a]

Rats (treated for 13 weeks)			Mice (treated for 6 weeks)	
	Thymus Weights, g (mean + SE of mean)			Thymus Weights, g (mean + SE of mean)
Dose, µg/kg/day	Male	Female	Dose, µg/kg/wk	Male
0.(5)[b]	0.52(±0.07)	0.40(±0.01)	0 (9)[b]	0.056(±0.003)
0.001(5)	0.51(±0.04)	0.37(±0.01)	0.2(9)	0.054(±0.006)
0.01(5)	0.50(±0.04)	0.34(±0.02)	1.0(9)	0.046(+0.003)
0.1(5)	0.41(±0.04)	0.23(±0.02)	5.0(9)	0.031(±0.002)
1.0(5)	0.13(±0.02)	0.09(±0.01)	25.0(14)	0.011(±0.002)

[a] From Vos *et al.,* 1974, and Kociba *et al.,* 1976.

[b] Number in parentheses indicates sample size.

models were fitted by the weighted least squares method to each of the three sets of data in Table III-2. The fitted models were then used to estimate the decrease in thymus weight at the ADI level of 0.0001 μg/kg/day. These estimated reductions are shown in Table III-3.

TABLE III-3 Estimated Reduction in Thymus Weight at a Continuous Daily Exposure to TCDD of 0.0001 μg/kg

	Estimated Reduction in Thymus Weight, %		
Model	Male Rats	Female Rats	Male Mice
Log normal	0.0004	0.19	0.00001
Log logistic	0.02	0.87	0.005

The variability of the extrapolations is illustrated in Table III-3. Although the female rat appears to be the most sensitive of these three groups, the two dose-response models give quite different results. At a daily exposure level of 0.0001 μg/kg, the average thymus weight of the most sensitive animal, the female rat, is estimated to be either 99.81% or 99.13% that of the unexposed animals. On the basis of average values, a reduction of this magnitude is much too small to be serious; however, one should be concerned with the effect of this difference in average values upon the proportion of the population at risk that would have seriously low thymus weights. For example. the female rat has an average thymus weight of 0.4 g, and the standard deviation of the distribution of thymus weights is approximately 0.04 g. To illustrate, we shall assume that a thymus weight of 0.25 g is small enough to cause physiological difficulties. Assuming a normal distribution of thymus weights in the population, then the proportion of animals with thymus weights of 0.25 g or less would be 0.0088% in the unexposed population and either 0.0092% or 0.0125% in a population exposed to the ADI based on the log-normal or log-logistic extrapolation model, respectively. Depending on the extrapolation model used, this implies that 0.0004% or 0.004% of the population would be affected by exposure to TCDD at the ADI value. These calculations serve as an example of the potential of what may be gained by the application of dose-response methodology to noncarcinogenic toxic responses.

The potential utility of dose-response extrapolation methodology for noncarcinogenic human risk assessment does exist but has been found to be of limited value for contaminants in drinking water. The models used to estimate risk require lifetime feeding studies which use appropriate numbers of animals of each sex and demonstrate some dose response. As noted above, this type of information is not now available for many of

the contaminants of drinking water. While both the risk estimate and ADI approach require some degree of value judgment, it is the belief of this subcommittee that the ADI methodology is the most useful for noncarcinogenic hazards given the general deficiencies in available data. In situations for which high-quality toxicological data are available, the risk estimate approach would be appropriate for the assessment of noncarcinogenic hazards. Consequently, in the absence of such data the application of safety factors to no-observed-effect levels that have been derived from laboratory animal toxicity data is the most feasible and currently acceptable method for the determination of human exposure limits to noncarcinogenic environmental contaminants.

To incorporate differential measures of uncertainty in these ADI calculations, the Safe Drinking Water Committee (National Academy of Sciences, 1977) used the following uncertainty factors: a factor of 10 when chronic human exposure data were available and were supported by chronic oral toxicity data in animal species; a factor of 100 when good chronic oral toxicity data were available in some animal species but not in humans; and a factor of 1,000 with limited chronic animal toxicity data.

It should be cautioned that even when complete toxicological data are available, including those from long-term tests, the ADI represents only a judgment regarding acceptable levels of human exposure, and is *not* an estimate of risk nor a guarantee of absolute safety. Furthermore, the ADI methodology does nothing to correct the deficiencies in mathematical extrapolation methods.

The subcommittee has recommended that the ADI concept should not be applied to carcinogens. The reasons for this recommendation are illustrated by the following examples.

A *theoretical* ADI may be computed for the known or suspected carcinogens that appear in Table VI-60 of *Drinking Water and Health* (National Academy of Sciences, 1977, p. 794). The calculations in Table III-4 were based on the lowest dose that showed a statistically significant increase over background, whereas a true ADI is always based on the highest no-observed-adverse-effect level. Furthermore, in every instance the minimum effect used for computation was cancer. While other kinds of minimum effects have been observed for a few of these compounds, carcinogenicity was the end point used to calculate the risk estimates. Therefore, to achieve consistency, it was used in all cases. If a "safety factor" is applied to this dose, then the risk at this value (mg/kg/day) can be determined by using the upper 95% confidence estimate of lifetime cancer risk per µg/liter from Table VI-60. (Although the selection of the 95% confidence interval can be considered to be arbitrary

and/or traditional, the need for working with a confidence interval is necessary for a conservative approach to public health.) Using this approach, the estimated risks range from a low of 0.07% for DDT to a high of 10% for δ-BHC (Lindane) (see Table III-4).

To demonstrate further the variability that would be associated with the ADI approach, an arbitrary risk value of 1×10^{-6} was chosen, and the daily dose (mg/kg/day) that would theoretically produce this level of risk was calculated. The doses (mg/kg/day) required to produce a risk of 1×10^{-6} range from 5.5×10^{-8} (Dieldrin) to 1.3×10^{-4} (carbon tetrachloride). These examples show the potentially great variability in risk calculated at supposedly equivalent levels of safety using the safety factor. Thus, applying ADI methods to carcinogens instead of risk estimates may very well be misleading with regard to regulatory actions.

MODELS FOR LOW DOSE CARCINOGENIC RISK ESTIMATION

Dichotomous Response Models

In many quantitative theories of carcinogenesis it is assumed that the process consists of one or more stages at the cellular level beginning with a single cell somatic mutation at which point the cancer is initiated. These stages may be cell mutations or other biochemical events that are either monocellular or multicellular in origin. Whittemore and Keller (1978) reviewed all these theories. Many of these theories lead to a mathematical model relating dose, *d,* to the probability of response, $P(d)$, by

$$P(d) = 1 - \exp\left(-(\lambda_0 + \lambda_1 d + \lambda_2 d^2 + \ldots + \lambda_k d^k)\right).$$

This model was used by the Safe Drinking Water Committee (National Academy of Sciences, 1977). Its background and its application to experimental animal data are discussed in more detail in the committee's 1977 report. In addition to this particular mathematical dose-response model, a number of other models have been proposed for the assessment of low exposure human risk. The most commonly used extrapolation models are described in the following section.

Linear, No-Threshold Model

Conceptually, the simplest model for high to low dose risk extrapolation is based on the assumption that risk is directly proportional to the environmental exposure level. In mathematical terms, the probability of

TABLE III-4 Estimated Human Cancer Risks That Would Be Associated with Known or Suspected Carcinogens Using a "Safety Factor" Approach

Compound	Lowest Minimum Effect Dose, mg/kg/day[a]	Doses Derived from Safety Factor Approach, mg/kg/day[b]	Upper 95% Confidence Estimate of Lifetime Cancer Risk from Dose in Column 2[c]	Dose Rate, mg/kg/day (associated with a lifetime cancer risk of 1×10^{-6})[d]
α-BHC (Benzene hexachloride)	50	0.10	1.0×10^{-2}	9.5×10^{-6}
ß-BHC	40	0.08	2.3×10^{-2}	3.4×10^{-6}
γ-BHC (Lindane)	80	0.16	1.0×10^{-1}	1.5×10^{-6}
BCEE [b*is* (2-Chloroethyl)ether]	100	0.20	1.7×10^{-2}	1.2×10^{-5}
Carbon tetrachloride	80	0.16	1.2×10^{-3}	1.3×10^{-4}
Chlordane	6	0.012	1.5×10^{-2}	7.9×10^{-7}

Compound	Lowest Minimum Effect Dose, mg/kg/day[a]	Doses Derived from Safety Factor Approach, mg/kg/day[b]	Upper 95% Confidence Estimate of Lifetime Cancer Risk from Dose in Column 2[c]	Dose Rate, mg/kg/day (associated with a lifetime cancer risk of 1 x 10^{-6})[d]
Chloroform	60	0.12	1.4 x 10^{2}	8.4 x 10^{-6}
DDT	0.4	0.0008	6.7 x 10^{-4}	1.2 x 10^{-6}
Dieldrin	0.02	0.00004	7.3 x 10^{-4}	5.5 x 10^{-8}
ETU (Ethylenethiourea)	215	0.43	6.6 x 10^{-2}	6.5 x 10^{-6}
Heptachlor	2.8	0.0056	1.6 x 10^{-2}	3.4 x 10^{-7}
Kepone	1.1	0.0022	6.8 x 10^{-3}	3.2 x 10^{-7}
PCB (Aroclor 1260)	5	0.01	2.3 x 10^{-3}	4.6 x 10^{-6}
Trichloroethylene	1169	2.3	1.8 x 10^{-2}	1.3 x 10^{-4}
Vinylchloride	16.65	0.033	1.1 x 10^{-3}	3.0 x 10^{-5}

[a] The minimum-effect dose used was that which produced a significant difference ($P = 0.05$) between the exposed animals and the controls. For consistency the minimum effect used in all instances was cancer because this was the end point used to compute the original risk estimates. See Drinking Water and Health (National Academy of Sciences, 1977) for further details on individual chemicals.

[b] This dose is derived from the application of a safety factor of 500 to the lowest dose at which some effect (cancer) was observed. It thus differs from a traditional ADI in which a safety factor is applied to the highest dose at which no effect is observed. Using a different safety factor would not change the conclusions based on this example.

[c] Calculated using the upper 95% confidence interval estimate of lifetime cancer risk given in Drinking Water and Health (National Academy of Sciences, 1977, p. 794). Assuming an average human adult weight of 70 kg, the risk for vinyl chloride is: 0.033 mg/kg/day x 70 kg x 1000 x 4.7 x 10^{-7} = 1.1 x 10^{-3}

[d] Calculated from values in the preceding columns, e.g., the vinyl chloride dose rate associated with a lifetime cancer risk of 1 x 10^{-6} is

$$\frac{0.033 \times 10^{-6}}{1.1 \times 10^{-3}} = 3.0 \times 10^{-5}.$$

a carcinogenic response, $P(d)$, is related to the daily dose level, d, by the equation $P(d) = \alpha d$.

This simple extrapolation model and the models described below assume the impossibility of proof of a population threshold level, i.e., a level of the carcinogen below which exposure will produce no response in any member of the population at risk. Under the assumption that the true dose-response relationship is convex (positive second derivative with the dose on the abscissa) in the low dose region, linear extrapolation from an experimental dose in this region of convexity will provide an overestimate to the true low dose risk. However, it is often not known whether the experimental dose is in this convex region.

Tolerance Distribution Models

The hypothesis of tolerance distribution, which was originally proposed for toxic responses other than carcinogenesis, is based on the assumption that each member of the population at risk has an individual tolerance for the toxic agent below which a dose will produce no response, whereas a dose as great or greater will certainly produce the response. Furthermore, it is assumed that these tolerances vary among the population members according to some probability distribution, F. The frequency distribution of tolerances, as measured on a linear scale, is seldom symmetrical since a few individuals with extremely high tolerances will provide the distribution with an extended "tail." However, a simple transformation of the scale of measurement, such as a logarithmic transformation, will often convert the distribution to near symmetry. This probability distribution of tolerances is also commonly assumed to involve two parameters, one of location, a, and one of scale, $\beta \leq 0$. Therefore, this distribution can be generally denoted by $F(\alpha + \beta \log z)$, where z denotes the tolerance level to a particular toxic agent. This probability distribution produces a dose-response relationship in the following manner. Assume that an individual from the population is selected at random and is given a dose of size d. Then the probability for this randomly selected individual response, $P(d)$, is the probability that his tolerance is less than d, i.e.,

$$P(d) = F(\alpha + \beta \log d) = \int_{-\infty}^{\alpha+\beta \log d} dF(x).$$

Therefore, the proportion of the population expected to respond to a specific dose of the toxic agent is indicated by the proportion of individuals having tolerances less than this dose level. These assumptions imply that the probability of response at a zero dose is equal to zero, i.e.,

$P(0) = 0$, and that the probability of response at an infinite dose is equal to one, i.e., $P(\infty) = 1$.

The incorporation into these models of spontaneous response, i.e., a response in the absence of exposure to the specific toxic agent, is discussed in a later section.

The results of many toxicity experiments have shown that the proportion of responders increases monotonically with dose and often shows an approximately sigmoid relationship with the logarithm of dose. This led to the development of the log-normal, or log-probit, model of dose response. This model assumes that the distribution of tolerances, *F,* is Gaussian (normal) against the logarithm of dose. This assumption of normality is not based on strong biological theory, but in the absence of evidence favoring a specific alternative tolerance distribution, the fact that the normal distribution accords fairly well with historical observation has made the assumption attractive. A history of the development of this model is given by Finney (1952).

Mantel and Bryan (1961) have suggested that a modified version of this model be used for extrapolation of carcinogenesis bioassays from high to low doses. They stated that since the normal probability model is not usually true at the extremes, i.e., there is often more probability mass in the tails of the distribution than the normal model would predict, then the slope of the relationship between the log dose and the probit of response will flatten out and become less steep as the dose level decreases. They proposed that high dose to low dose extrapolation be based on the observed data but with the use of a slope that is shallower than that observed. They suggested that the slope selected be no greater than the average true slope over the extrapolation range. A slope of one probit per 10-fold change in dose is commonly used for this Mantel-Bryan extrapolation.

Logistic Models

Another mathematical dose-response function, which was originally derived from chemical kinetic theory, is the logistic function

$$P(d) = F(\alpha + \beta \log d) = [1 + \exp(\alpha + \beta \log d)]^{-1}, \beta \leq 0.$$

This dose-response model is based on the assumption of a logistic distribution of the logarithms of the individual tolerances in the population and a theoretical description of certain chemical reactions. Reed and Berkson (1929) demonstrated the utility of this type of function to describe the time behavior of a number of different chemical reactions. One of its primary uses has been to describe autocatalytic

reactions. Berkson (1944) proposed its use as a dose-response model due to its theoretical basis in chemical kinetic theory and its sigmoid appearance which is similar to that of the integrated normal curve. If the response under consideration is measured in terms of this type of physicochemical reaction in a biological assay, then the logistic function does have a theoretical basis. However, in terms of a tolerance distribution, there is no theoretical justification for preferring either the logistic or normal distribution.

The normal and logistic models are the most attractive to the biologist since each is applicable in many contexts. Other tolerance distribution models have been suggested but have received little support and use because of their lack of a theoretical basis (Finney, 1971). All tolerance distribution models have little theoretical justification for a carcinogenic response.

"Hitness" Models

Dose-response models for radiation-induced carcinogenesis have been proposed on the basis of what is called the "target theory." This theory assumes that the site of action has some number ($N \leq 1$) of critical "targets" and that an event occurs if some number ($n \leq N$) of them are "hit" by k, or more, radiation particles. In addition, the probability of a hit is assumed to be proportional to the radiation dose. For this class of models, the probability of a response increases with increasing dosage, presumably due to an increased chance of a critical target being "hit" rather than to a variation among individual tolerances to the effect of such a dosage. Therefore, the proportion of the population expected to respond to a specific dose of the toxic agent is determined by the probability that the dose produces the required number of "hits" on the critical "targets." The most commonly used models in this class are the single-hit model ($N = 1$, $k = 1$) in which a single hit on a single critical target is necessary for the response, the two-hit model ($N = 1$, $k = 2$) in which either of two hits on a single target is responsible for the effect, and the two-target model ($N = 2$, $k = 1$) in which one hit on each of two targets is required. The single-hit theory has been used to describe high linear energy transfer (LET) radiation, while the two-target models describe low LET radiation (Kellerer and Rossi, 1971; Rossi and Kellerer, 1974). Brown (1976) argued in favor of a multievent theory of radiation-induced carcinogenesis which involves both a linear and quadratic dependence upon dose. He also suggested that the possibility of cell killing at high dose levels be included in the model. Other generalizations of this theory include variations among the targets in the

probability of a hit and variations in tolerance among the subjects at risk. These models are all summarized by Turner (1975).

All of these models assume the nonexistence of a population threshold, i.e., a level below which exposure will not produce a response in any member of the population at risk. A more complete discussion of this assumption can be found in *Drinking Water and Health* (National Academy of Sciences, 1977, Ch. II).

Models of Time to Tumor Occurrence

The dose-response models discussed in the previous section are based quantal (yes-no) observational data. However, for many experimental or observational studies, there is an additional piece of information that these quantal response models ignore. These data concern the distribution of times from initiation of exposure to response or, in cases of no response, the total length of time the subjects were observed without a response. Among others, Armitage and Doll (1961) defined this time between initial exposure and clinical appearance of the disease as the latent period. These data add information that aids the determination of the dose-response relationship, especially in experimental situations in which the response rates at the high dose levels are close to 100%.

Experiments in which most dose levels produce 100% or nearly 100% response will not provide much information on the dose-response relationship; however, examination of the times to response will often show a monotonic relation between their means, or medians, and the dose levels. In addition, the actual times to tumor occurrence are meaningful in the sense that tumors that appear early may be more biologically important and a greater potential hazard than the later tumors. Moreover, these response times permit the formulation of mathematical models that relate the dose level to the probability distribution of times to response. These models may then be used to estimate the expected numbers of responders in the population at risk at any time. Gail (1975) proposed three measures of the effect of dose upon life expectancy that could be used in place of its effect on cancer incidence.

The formulation of a mathematical time to response model consists of two parts. The first is the mathematical form for the probability distribution of the random variable of response time, T:

$$\text{probability } (T \leq t) = F(t;\theta),$$

where $F(\bullet)$ is some cumulative probability distribution function indexed by a vector of parameters, θ It is assumed that the mathematical form of

F is the same for each dose level and that one or more of the parameters are functions of dose. It is also assumed that all subjects will eventually respond, i.e., $F(\infty) = 1$; however, because of competing risks such as death without evidence of the disease, the subject may be removed from observation before the response occurs. This assumption is probably more valid for chronic exposure than for acute exposure.

The second assumption concerns the relation between the dose level, d, and one of the parameters, θ. A general empirical relation that has been proposed by Busvine (1938), among others, is

$$\theta_i = \alpha d^{\beta}, \text{ or } \log(\theta_i) = \log(\alpha) + \beta \log(d),$$

although there is no presumed biological basis for this relationship. However, when θ_i is the median time to response, an approximate linear relation between the logarithm of θ_1 and the logarithm of dose has often been observed.

A number of mathematical time to response models have been proposed. All of them have corresponded directly with dichotomous dose-response models (Chand and Hoel, 1974). One of the first models was proposed by Druckrey (1976). He assumed that the probability distribution of the response times was log normal, that the median response time was related to dose as above, and that the standard deviation was independent of dose. It can be shown that this model is related to the normal, or probit, quantal response model.

A Weibull model for the response time distribution was proposed by Pike (1966) and Peto *et al.* (1972). In this model the scale parameter is related to dose while the shape parameter is independent of dose. This model corresponds to an extreme value quantal response model in general, and when the parameter β in the above relationship is assumed to be unity, this model corresponds to a single-hit quantal response model. These and other models have also been studied by Shortley (1965) and Gart (1965).

Armitage and Doll (1961), Armitage (1974), and Peto (1974) have proposed that when exposure to a carcinogenic agent is at a constant rate continuously over time, there is a general model in which the hazard rate, i.e., the age-specific incidence rate, can be factored into the product of a function of dose, d, and a function of time, t:

$$H(t,d;\theta_1,\theta_2) = f(t;\theta_1)g(d;\theta_2).$$

The Weibull distribution, where $f(t;\theta_1) = \theta_1 t^{\theta_1 - 1}$, is a member of this general class. Hartley and Sielken (1977) developed the analysis of dose-response data using this model and applied it to some experimental results. They used the polynomial form of the multistage model for the

function of dose $g(d;\theta_2)$ and a general polynomial for the function of time $f(t;\theta_1)$. Crump (1978) also assumed this factorable hazard model with a polynomial for the function of dose and applied marginal likelihood analysis techniques which require no assumption on the form of the time function.

The utility of these time to response models requires additional research. They appear to provide improved estimates of the relation between dose and response. Their utility will depend upon the number of tumors found in the animal bioassay: high incidence tumors and their times to occurrence will provide substantially more information than low incidence tumors. In addition, the actual times to tumor occurrence are unavailable for many animal bioassays, either because the records were not kept or the tumor was of a type found only upon sacrifice at the termination of an experiment, e.g., after 2 years of exposure. These restrictions reduce the usefulness of such approaches for high to low dose extrapolation.

When using a model that is fit to the experimental result and then used for extrapolation, it is assumed that the dose-response relationship observed at these high dose levels will continue to hold throughout the entire spectrum of exposure levels. This assumption has been questioned by some toxicologists and other health scientists. The effective exposure level—the amount of the carcinogen actually reaching the target cells and molecules—may well be some complex function of the absorption, distribution, biotransformation, and excretion characteristics of the dose, each of which may depend upon and influence the level of the carcinogen to which the animals are environmentally exposed. The following section contains discussions of some pharmacokinetic considerations that are relevant to both low dose extrapolation and quantitation of differences between species.

PHARMACOKINETIC CONSIDERATIONS

A pharmacodynamic hypothesis for toxicity from foreign chemicals states that biological effects are manifestations of biochemical interactions between the foreign substances (or materials derived from them) and components of the body. It follows that these interactions result from the presence of a toxic material at the site of action and that the fundamental biochemical interaction depends in some way on the concentration of the toxic material and the length of time that it is present.

Actual mechanisms of toxicity are many and varied, and the kinetics

relating concentration with effect depend on the mechanism. It is commonly assumed in pharmacology that the biochemical effect of a reversible inhibitor depends on the existing free concentration of drug at the site of action. Irreversible biochemical reactions require joint consideration of concentration and duration of exposure. Whether or not any interaction at the biochemical level will be observable as a biological effect and the time course of that effect also depend on the nature of the interaction. An inhibitor may have to react with a significant fraction of receptor sites before any gross effect will be observed. The effect, then, may correlate directly with concentration, e.g., in certain central nervous system responses, or it may be delayed, e.g., if protein or DNA synthesis is involved. Irreversible reactions with DNA are of particular concern because, in principle, a single defective molecule that is not repaired or eliminated from the pool could lead to a mutation or to cancer. Furthermore, clinical expression could be delayed many years.

Dose Effects

A critical problem in the application of pharmacokinetic principles to risk assessment is the potential change in metabolism as concentrations decrease. Linear models are most frequently used for drug disposition; however, there are numerous examples of nonlinear behavior in a therapeutic range. While the potential of these models to predict both therapy and toxicity is generally recognized, relatively few models completely elucidate a complex reaction scheme. One of these is the model of Levy *et al.* (1972), who developed a detailed kinetic description of the elimination of salicylic acid and its metabolites. Two of the several steps (formation of salicyl phenolic glucuronide and salicylurate) exhibit saturation kinetics.

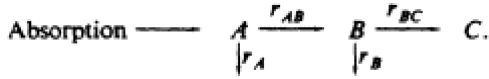
The presence of saturation kinetics, particularly as they affect various reaction paths differentially, has significant implications to the testing of environmental chemicals. Gehring and Blau (1977) have stressed the existence of nonlinear elimination of a variety of chemicals such as 2,4,5-trichlorophenoxyacetic acid 1,4-dioxane and its implication to toxicity testing. Nonlinear kinetics pose significant problems in quantitative extrapolation from "high" doses to "low" doses if the kinetic parameters are not measured.

Many of the physiological processes, e.g., biliary or urinary secretion, which underlie pharmacokinetics, have limited capacities. Similarly, various relevant biochemical interactions, such as protein binding, membrane transport, and metabolism, may exhibit nonlinear behavior at

sufficiently high concentrations. Some of these have been reviewed by Wagner (1974).

The nonlinearities are most often expressed in the form of a Langmuir or Michaelis-Menten expression, $kC/(K + C)$, where k is the capacity of the process for binding, transport, chemical reaction, etc., and K is the value of the concentration, C, at which the rate or binding is half maximal. Quite clearly, the concentration is "high" or "low" when compared with the constant, k. All processes that follow a Langmuir or Michaelis-Menten expression approach linearity with a constant or proportionality equal to k/K as C becomes small compared with K.

It is now well established that a foreign chemical may undergo metabolism to a more (or less) toxic form. The kinetic implications and consequences for extrapolation of animal data to humans were discussed by Gillette (1976, 1977). Most of the emphasis has been placed on organic chemicals since comparable concepts relating to precise chemical species of inorganic complexes in biological systems are not well studied. The diagram shows a simplified reaction scheme following the approach of Gillette (1976):



The parent chemical is represented by A, and the reactive intermediate is represented by B. Both are assumed to be at steady state with a constant rate of absorption of A. The steady-state analysis is instructive; more complex transient analysis would not lead to a different result. The various reaction rates are indicated by r as follows: r_A and r_B represent the summation of all processes that may be involved in the elimination of A and B or chemical conversion to nontoxic products; r_{AB} is the reaction rate for conversion of A to the reactive intermediate; r_{BC} represents the rate of irreversible reaction of the reactive intermediate with the site of toxicity, e.g., by covalent binding to protein molecules. Many of the various rates may be enzymatically mediated and kinetically saturable. Although a one-compartment description of the body is implied by the diagram, the various chemical reactions and excretory processes do not necessarily occur at the same site. B may be formed in the liver and, if sufficiently stable, could produce toxicity in other organs. Similarly, B might be conjugated in the liver and eliminated by the kidneys.

Quite clearly, if all of the processes are linear, the concentration of the reactive intermediate B (and thus exposure to toxicity) will be proportional to the rate of absorption of A. Saturation phenomena and enzyme

induction or inhibition produce quite variable results depending upon the steps where they act. If the pathways of r_A or r_B leading to nontoxic products and elimination start to saturate, then the production of *C* will increase more rapidly than the dose. However, if the pathways of r_{AB} leading to *C* start to saturate, then the production of *C* may increase less rapidly than dose. In a similar way, induction or inhibition of enzymatic or excretory processes may increase or decrease *C*. Thus, it appears impossible to make generalizations concerning the effect of dose on the formation of toxic intermediates over a wide range of doses; however, since all processes that follow Michaelis-Menten kinetics approach linearity at sufficiently low concentrations, exposure to *B* should be proportional to dose. It is unlikely that totally new biochemical pathways are invoked as concentrations increase; only the relative quantitative importance of the various pathways is at issue.

A slight variant of the reaction scheme shown above was considered by Cornfield (1977) to support the possibility of a threshold level for a carcinogenic compound:

$$D + S \underset{}{\overset{k}{\rightleftharpoons}} X$$

$$D + T \xrightarrow{k^*} Y.$$

In the above scheme, *D* represents a toxic substance that combines reversibly with a free substrate, *S*, to form an activated complex, *X*. *D* can also react irreversibly with a deactivator, *T*, to form a toxin-deactivator complex, *Y*. It was assumed that the probability of a toxic reaction is proportional to the amount of *X* and argued that as long as *T* is present in excess of *D* there would be no toxic reaction. That conclusion could not be valid for any finite values of the rate constants in a transient or steady-state analysis. If *D* is much less than *S* and *T,* both forward reactions are pseudo-first-order reactions, and exposure to *X* is proportional to the dose of *D.*

Species Considerations

Chemicals must be tested for toxicity in experimental systems and the results extrapolated to humans. The methodology for making this extrapolation rests, in part, on pharmacokinetic considerations of species similarities in absorption, distribution, binding, metabolism, and elimination.

It is generally conceded that no animal mimics "man" in all respects that are relevant to pharmacokinetics. Studies in humans themselves can be quite variable, reflecting intraspecies variations. Even if there were an

animal in which the pharmacokinetics of a particular substance mimicked an average human, there is no certainty that the mimicry would extend to another substance.

Species differences do not obviate the value of model systems; they do make interpretation of results more difficult and arbitrary. Models are used extensively in engineering. These may be physical or purely mathematical. They may be designed to simulate only a part of a complex system. In any event, the model is *never* the same as the prototype. Successful use of the results of a model rests on a knowledge, often largely empirical, of the similarities among species. Therefore, it is appropriate to consider both similarities and differences among species when examining pharmacokinetics that are relevant to risk assessment.

Adolph (1949) correlated a large number of quantitative properties of mammals with body weight. His correlation equation took the following form:

property = a(body weight) ^{k} .

In most cases the exponent was not unity, i.e., these properties were not linearly proportional to body weight. In all cases when the "property" was a physiologic or metabolic function, the exponent was less than unity. Representative values of k include: urine output, 0.82; creatinine clearance, 0.69; basal oxygen consumption, 0.734; nitrogen output, 0.735; oxygen consumption by liver slices, 0.77. This observation indicates that the function per unit of body weight goes *down* as body weight increases. For example, a 20-g mouse consumes approximately 9 times more oxygen per unit of body weight than does a 70-kg human. Some similar variation has been observed among tissue perfusion rates (Bischoff *et al.,* 1971), which are important to pharmacokinetics. Some of these concepts have been applied to correlate the history of plasma concentrations of methotrexate, an anticancer drug, among several mammalian species that eliminate the drug predominantly unchanged (Dedrick *et al.* 1970).

Purely physical interactions of environmental contaminants with biological tissues and fluids might not be expected to show great variation among species; however, much work needs to be done to establish an experimental basis for that hypothesis. For example, a heavy metal ion that reacts very strongly with a free sulfhydryl group may not discriminate greatly between species. Hughes (1957) reported less than a fourfold difference between the association constants of methylmercury iodide with human and bovine serum albumin. The ratio of the concentration of dieldrin in the adipose tissue to that in the blood has been reported to be 104 in rats and 169 in dogs (Walker *et al.,* 1969). The

corresponding ratio in humans is 136 (Hunter *et al.,* 1969). Physiologic modeling of dieldrin pharmacokinetics by Lindstrom *et al.* (1974) is based on the idea that equilibrium distribution into a tissue is proportional to the lipid content of that tissue.

The interspecies differences that may confound pharmacokinetic predictability most significantly are those concerning metabolism. Quinn *et al.* (1958) stated that humans usually metabolize drugs less rapidly than other animals. There are numerous exceptions to that generalization. Furthermore, as discussed above, toxicity may be related to the concentration of an active intermediate, and there are extraordinarily large qualitative and quantitative differences in metabolism.

As discussed by Williams (1974), foreign organic compounds tend to be metabolized in two phases. Phase I reactions lead to oxidation, reduction, and hydrolysis products. Phase II reactions lead to synthetic or conjugation products that are relatively polar and, thus, more easily excreted by the kidney and, in some cases, by the liver. Within this general framework, however, there are large interspecific variations. Williams points out that such variations in Phase I reactions are very common and often appear to be unpredictable. If an interspecific difference is found for a particular compound, similar compounds may have similar variations. Phase II reactions are much more limited in number than Phase I reactions. Eight principal conjugation reactions are common to humans and most other mammals. It may be possible to identify patterns in these reactions.

In part, because of the complexity of metabolism studies in whole animals and variables introduced by factors other than metabolism, a large number of studies have been conducted on tissue extracts. Some of the studies of hepatic and extrahepatic mixed-function oxidase have been reviewed by Bend and Hook (1977). They observed that most microsomes used in metabolism studies have come from rodent livers and that the cytochrome P-450 content of human microsomes has generally been reported to be lower than P-450 from rats, but with considerable variation. Cytochrome P-450 appears to have a role in most microsomal mixed-function oxidase reactions. Nelson *et al.* (1976) observed that levels (per milligram of protein) of cytochrome P-450, NADPH-cytochrome P-450 reductase, and NADPH-cytochrome-c reductase were lower in human microsomes than in those from the male rat and female pig. Kuntzman *et al.* (1966) determined that 3,4benzpyrene, pentobarbital, and 3-methyl-4-monomethylaminoazobenzene are metabolized more slowly by human liver enzymes than by preparations from male rats; dealkylation of acetophenetidin was more rapid with human liver preparations. Krasovskii (1976) examined

mammalian hepatic enzyme activities (per kilogram of body weight) and showed that the activity of 15 of 16 enzymes tended to decrease as body weight increased.

Biological knowledge is still too limited to permit confident extrapolation of pharmacokinetics of many metabolized substances from laboratory animals to humans. Both the qualitative and quantitative aspects of metabolism should be studied further. It may be possible to obtain adequate information from *in-vitro* studies on isolated cells (such as hepatocytes) or cell-free extracts and to use this information in conjunction with pharmacokinetic models that incorporate physiological and anatomical differences. A philosophy of "scale-up" design has been discussed by Dedrick (1973). Early work attempting to relate *in-vitro* to *in-vivo* metabolism has met with at least semiquantitative success for cytosine arabinoside (Dedrick *et al.,* 1973) and phenytoin (Collins *et al.* 1978).

In summary, no laboratory animal is fully equivalent to humans pharmacokinetically. There are, however, many similarities as well as differences. Pharmacokinetic analysis of appropriate experiments, including *in-vitro* studies, can considerably strengthen our ability to assess the risk to humans posed by foreign substances in the environment. The incorporation of pharmacokinetic models into the estimation of doseresponse relationships is worthwhile. The practical business concerning which techniques to use and their quantitative impact on the low dose estimation process has not yet been resolved unanimously.

INTERACTION IN RISK ESTIMATION

Most toxicological studies of the effects of chemicals on mammals are performed using one chemical at a time as the toxicant. However, the joint action of chemicals in the environment must also be addressed. There have been many experiments on cocarcinogenesis and initiationpromotion. Cocarcinogenesis involves the simultaneous administration of more than one chemical or the influence of modifying factors upon the carcinogenic process. Initiation-promotion involves a prior, often very low exposure to a carcinogenic agent, an initiator that induces an irreversible change in some of the cells in the target tissue. But the initiator produces little or no evidence of cancer without subsequent exposure to a promoter, an agent that is generally incapable of initiating the carcinogenic process but increases the cancer response when applied after initiation. Some chemicals are complete carcinogens, capable of

both initiation and promotion, whereas other chemicals produce only initiating action and still others, only promotion.

Dietary components (Carroll and Khor, 1970; Walters and Roe, 1964) and combinations of known carcinogens (Diechmann *et al.,* 1967) have been studied as modifying factors of carcinogenesis. Schmahl (1976) reported his results on a number of different studies in which animals were exposed either to two carcinogens having the same or different target organs or to one carcinogen and one noncarcinogenic stimulus. The joint effects of these agents interacted only with carcinogens having the same target site.

Some epidemiological observations illustrate joint action in humans. Selikoff *et al.* (1968) found a greater than additive effect of cigarette smoking and exposure to asbestos dust on lung cancer mortality. Lundin *et al.* (1969) reported a possibly similar effect of cigarette smoking and exposure to uranium. On the other hand, for oral cancer Rothman and Keller (1972) concluded that the joint effect of cigarette smoking and alcohol consumption upon the risk could be described by a simple additive model.

Theoretical Models for the Joint Actions of Two or More Agents

Bliss (1939) formulated one of the first theoretical developments of the joint action of two toxic ingredients. He defined three types of joint action: (1) independent joint action in which the toxicants act independently and have different modes of action; (2) similar joint action in which the toxicants have the same mode of action but act independently so that one component can be substituted at a constant proportion for the other; and (3) synergistic or antagonistic action in which one component either synergizes or antagonizes the action of the other toxicant. Bliss also used the probit dose-response model to relate the dose levels of the two toxicants to the probability of response.

Plackett and Hewlett, in a series of papers (Plackett and Hewlett, 1952; Hewlett and Plackett, 1959, 1964), proposed biological models for combinations of toxic agents, derived mathematical models from them, and tested their fit to experimental data. Their models were based on the existence of tolerances in biological systems, where the amount of the toxicant acting upon the system is the amount transmitted to the site of action. Plackett and Hewlett (1967) and Ashford and Colby (1974) have also developed a system of mathematical models for joint action based upon the concepts of routes of administration, sites of action, and physiological systems that may be affected. The action of a toxicant is assumed to take place as a result of the occupation of receptors, which is

governed by the laws of mass action dependent upon the concentrations of the toxicants at the site(s) of action.

The utility of another model for independent action, the application of harmonic mean calculation, is discussed in the section on acute exposure.

These theoretical models were all derived for noncarcinogenic responses and may not apply to carcinogenesis. For carcinogenic responses, Whittemore and Keller (1978) have proposed a mathematical model to describe the observed initiation-promotion phenomenon of some carcinogenesis in which tumors regress after termination of exposure to the promoting agent. They applied their model to data from experiments of Burns *et al.* (1976) on mouse skin papillomas and obtained excellent agreement between the model and the data.

Initiation-promotion and cocarcinogenesis may have regulatory implications different from those for complete carcinogens, i.e., when exposure to another factor is not required. A prolonged period of exposure to a promoter is often required before cancer appears. During this period, if the application of the promoter is stopped soon enough, the incidence of cancer can often be substantially reduced. In animal studies, this time sequence has been studied by Roe and Clack (1964). In humans, similar conclusions might be drawn from a study of lung cancer among British doctors who gave up smoking (Doll and Hill, 1964). The effects of promoting agents in the environment upon the total cancer burden of the population may be greater than the effects of initiating agents that induce mutations by interacting directly with cellular DNA. These initiating agents or complete carcinogens may be discovered through experimental *in-vitro* mutagenesis tests or through animal bioassays, while promoting agents whose mechanism of action may be nonmutagenic in nature, e.g., an agent that increases cell proliferation, may not be mutagenic *in vitro* and will often not be carcinogenic in a single agent animal bioassay.

Hamilton and Hoel (1978) proposed a modification of the multistage carcinogenesis model of Armitage and Doll to take into account multifactor exposures. For exposure to two agents, they assumed that no more than two stages in the process are affected. Their most general model allowed each agent to affect each of the two stages and allowed for modification of effect by one agent upon the other. They showed that the meaning of the term "interaction" is dependent upon the model of dose and response. The estimation of low exposure risk in a multifactor situation will be highly dependent upon the mechanisms of action by the joint agents.

The models of joint toxic action could be of benefit in the risk

assessment of low exposures; however, none has been adequately studied for this purpose. Their theoretical and practical implications need to be studied further before their utility can be assessed. High dose to low dose extrapolation for individual agents is an unresolved problem filled with many unknowns, and extrapolation of the actions of joint agents contains an additional major source of uncertainty.

Epidemiological Risk Assessment

Two major problems are encountered in the assessment of human risk based on the results of experimental animal bioassays: (1) the extrapolation from the observed risks at relatively high exposure levels, which are commonly used in animal bioassays, to low exposure levels that are comparable to human environmental exposure and (2) the extrapolation of estimated risks from laboratory animals to humans. The current methodologies that are used for these extrapolations suffer from a lack of basic knowledge concerning the disease process in animals and humans and from a paucity of relevant data. Although epidemiological studies on human populations that have been exposed to low levels of environmental contaminants are not faced with either of these problems, they are beset with difficulties of their own.

To measure the effects of factors that are related to specific human diseases, two general epidemiological approaches are available—descriptive and analytic. Descriptive studies are mainly observational. They can suggest possible relationships but seldom prove them. Analytic studies may also be used to test specific hypotheses or to measure specific effects.

Two commonly used types of analytic studies are: (1) prospective cohort studies in which populations exposed to various levels of a suspect agent are followed and their future disease incidence is compared, and (2) case-control studies in which the past exposure to a suspect agent is measured in a sample of individuals with and without the disease in question. Mathematical models of dose-response relationships can be fit to the results from either type of study to estimate the quantitative relation between exposure level and disease incidence. However, these studies also suffer from a number of limitations and difficulties. Both prospective cohort and case-control studies may produce biased results when confounding from other factors related to disease risk is not considered. Moreover, these studies lack the desirable experimental control of extraneous factors, which is found in animal bioassays, since many of the relevant risk factors are either unknown or cannot be adequately measured or controlled. Therefore, the quantitative results of such studies are always open to question. In addition, practical

limitations of prospective cohort studies are the long period between initiation of the study and the collection of statistically adequate numbers of disease cases, and the correspondingly high cost of such a long study. An important limitation of case-control studies is the questionable validity of retrospective information concerning exposure that may have occurred over a long period in the past.

Even with their inherent limitations and difficulties, these types of analytic epidemiological studies provide useful information for assessing the risk from environmental contaminants. However, there are no adequate analytic studies of this nature on the relationship between cancer incidence and exposure to drinking water contaminants.

There are a number of epidemiological studies of the relationship between cancer incidence and organic contaminants in drinking water. These studies are discussed in some detail in Chapter II. Three of the studies (Cantor *et al.,* 1977; Hogan *et al.,* 1979; McCabe, 1975) compared cancer mortality rates among counties across the United States in which trihalomethane (THM) concentrations in the drinking water supplies had been measured during two surveys conducted by the U.S. Environmental Protection Agency (1975). Multiple regression techniques were used to analyze the variability among counties in the sex-specific, age-adjusted mortality rates for each site of cancer. This methodology related the cancer mortality rate in the county, M, to a number of explanatory variables, X, $i = 1, \ldots, k$, and THM concentration, in the drinking water, W, by the following equation:

$$M = \alpha + \sum_{i=1}^{k} \beta_i X_i + \gamma W,$$

where the parameters α, β ($i = 1, \ldots, k$), and γ are to be estimated from the data. The effect of one unit of THM concentration was measured by the parameter γ, which represented the difference in cancer mortality between an unexposed county ($W = 0$) and a county with one unit of exposure ($W = 1$).

These studies, although useful for suggesting future analytic studies, are deficient in the quantification of cause-and-effect relationships. Hogan *et al.* (1979) discussed in some detail both the biological and statistical limitations of this methodology. The outcome, or dependent variable, cancer mortality, is an average aggregate rate for a heterogeneous collection of individuals from 1950 to 1969. Individual exposure patterns were unknown, and any secular changes might have been concealed by the aggregation. In 1975 the THM exposure data were collected for only one sample site in each county. Any quantitative conclusions from such a study would require the assumptions that the

relative sampled THM concentrations have been basically unchanged for up to 50 years and that the single site is representative of an entire county's drinking water supply. Another problem is that THM concentration may be correlated with those of other water contaminants. Thus, any estimated THM effect may represent the effect of some other causative agent.

In addition to the epidemiological limitations, there are other inferential validity problems with the statistical methodology. These problems include the exclusion of important cancer risk factors such as cigarette smoking, the erroneous inclusion of factors that are not related to cancer but are related to THM concentrations, and the assumption that THM concentrations are measured with small error. (Hogan *et al.* showed that there was considerable variation in the THM measurements between the two EPA surveys.) Quantitative indications produced by a multiple regression analysis of observational data on groups of individuals will provide necessary but insufficient data, and will suggest the need for confirmation by more controlled analytic epidemiological studies. Summarizing their opinion on these limitations, Hogan *et al.* stated: " . . . the quantitative, causal interpretation of results generated by an indirect study would appear to be a very tenuous and questionable practice in most instances."

Reliability of Quantitative Risk Estimates

As noted earlier, there is a paucity of data for the toxicological effects of many chemicals that could be found as contaminants in drinking water. This is true even at the high dose levels at which effects could be measured. At the low dose levels corresponding to expected human exposures, the attendant number of responses are so small that the performance of experiments with adequate statistical precision would require an inordinate number of laboratory animals. Furthermore, such studies would be confounded by the potential differences in response between the controlled test animal and the highly variable human population living in a complex environment.

Consequently, to estimate effects obtained at low levels of a given agent, an extrapolation must be made from the data that were obtained at higher doses. Unfortunately, the extrapolated risk for a given low dose is highly dependent upon the mathematical model chosen for such an enterprise.

To illustrate, consider the three common dose-response models: log normal, log logistic, and single hit. These three models give similar values over the range of doses that can be measured, i.e., 5% to 95% response,

rates. However, upon extrapolation to very low dose ranges, they would give very dissimilar estimates. This can be seen in Table III-5.

TABLE III-5 Expected Response Rates for Different Dose-Response Models as a Function of Dose[a]

	Percent Response		
Relative Dose	Log Normal	Log Logistic	Single Hit
16	98	96	99+
8	93	92	92
4	84	84	94
2	69	70	75
1	50	50	50
1/2	31	30	29
1/4	16	16	16
1/8	7	8	8
1/16	2	4	4
0.01	0.5	0.4	0.7
0.001	0.00035	0.026	0.07
0.0001	0.0000001	0.0016	0.007

[a] From Food and Drug Administration Advisory Committee on Protocols for Safety Evaluation, 1971.

Although the three models differ very little over a 256-fold dose range, they lead to increasingly divergent estimates upon extrapolation to very low levels. At a dose that is one-thousandth of the 50% response dose, the single-hit model gives an estimated response rate 200 times as large as that given by the log-normal model. A limited animal bioassay conducted at dose levels high enough to give observable response rates cannot discriminate among these various models, and these same models are substantively divergent at lower dose levels. These factors present major difficulties for high to low dose extrapolations. Therefore, the model must be selected on the basis of biological considerations. This decision may greatly affect an estimated risk at a low dose level and, hence, a resulting regulatory standard.

There is no unanimity concerning the proper way to incorporate the spontaneous, or background, response, i.e., responses that do not result from exposure to the chemical. One approach, which is used for carcinogens, assumes independent action between the chemical and the background. This is known as "Abbott's correction." The other method,

which was proposed by Albert and Altshuler (1973), assumes that the dose of the carcinogen is additive to the background. These two approaches can give substantially different results when extrapolating outside of the observed dose range. Table III-6 shows an example of this difference for a log-normal dose-response model with a slope of unity and an overall 50% response rate at a dose of one unit which includes a spontaneous, or background, rate of 5%.

TABLE III-6 Extrapolated Dose-Induced Response Rates for a Log-Normal Model with Two Different Corrections for Background

Dose Level	Independent Action	Additive to Background
1.0	0.47	0.45
0.1	0.14	0.13
0.01	0.019	0.018
0.001	0.13×10^{-2}	0.19×10^{-2}
0.0001	0.30×10^{-4}	0.19×10^{-3}
0.00001	0.20×10^{-6}	0.19×10^{-4}

As the mathematical theory predicts (Crump *et al.,* 1976), the model, assuming background additivity, approaches linearity at low dose levels.

When fitting dose-response models to carcinogenesis data, the effective exposure level, which is the amount of the carcinogen actually reaching the target cells and molecules, is likely to be some complex function of the absorption, distribution, biotransformation, and excretion characteristics of the host. Each characteristic may depend upon and influence the level of the carcinogen to which the animals are environmentally exposed. With the current state of knowledge, the *invivo* mechanisms that relate environmental chemical exposure levels to the levels that reach the target cells are usually not adequately quantified. Consequently, assumptions of proportionality between the environmental exposure level and the effective exposure level may be wrong. The proportionality assumption is doubtlessly an oversimplification of the true relationship in the absence of information on metabolic pathways, activation and deactivation systems, biological repair, and other pharmacokinetic considerations. However, this assumption is needed in order to apply the extrapolation models that are currently available.

The Safe Drinking Water Committee (National Academy of Sciences, 1977) used a probabilistic multistage model to estimate the carcinogenic

risk from exposure to low doses. This model was chosen over others because it is based on a plausible biological mechanism of carcinogens, i.e., a single cell somatic mutation model for the initiation of cancer. Because the other models are more empirical, they were thought to be less desirable.

At low doses, the multistage model is often mathematically equivalent to the linear or single-hit model. Therefore, its use for extrapolation is consistent with the conservative linear risk estimation. If the precise mechanism of carcinogenesis is represented by a threshold or log-normal dose-response relationship, the multistage model may considerably overestimate the risk at low dose levels. However, this possibility cannot be reasonably quantified.

In the committee's report (National Academy of Sciences, 1977) risk calculations were made for each contaminant of drinking water that had been shown to be carcinogenic in an appropriate animal bioassay. The calculations were based on available carcinogenicity data and an average value was reported. To estimate quantitatively the cumulative carcinogenic risk of several carcinogens, or multiple responses due to the same carcinogen, e.g., liver and bladder tumors induced by 2-acetylaminofluorene (2-AAF), the individual risks might be added. This assumes that interactions are not present and that the risks are small enough so that adjustments for joint probabilities are not needed. If interactions leading to synergism or antagonism are found, then adjustments must be made when cumulative effects are estimated.

Estimates obtained from each model have wide ranges of variability. This results from the statistical variability of the observations; the biological variability among strains, sexes, and organs of the laboratory animals; and the differences within the experimental range among different species.

For the various compounds considered in *Drinking Water and Health* (National Academy of Sciences, 1977), the statistical upper 95% confidence limit on risk was typically a factor of 2 to 10 over the actual risk estimate. Occasionally, the multistage models that are the best fit to a data set do not contain a linear term. When this is true, the upper confidence limit would be orders of magnitude greater than the estimate itself.

Variability of low dose risks among strains and species of rodents has also been empirically estimated for a few compounds. Roughly, it appears as though risk estimates may be a factor of 10 higher or lower than the median risk level (e.g., see Table 6.22 on chloroform, p. 192 in *Chloroform, Carbon Tetrachloride, and Other Halomethanes,* National Academy of Sciences, 1978).

Finally, for six human carcinogens considered by the NAS study on pest control (*Contemporary Pest Control Practices and Prospects,* National Academy of Sciences, 1975), one finds that on an *equivalent daily dose rate basis,* the most sensitive rodent comes within a factor of 10 in predicting effects in humans. This assumes the use of the most sensitive species, the appropriate sex, and the appropriate site of action. In this report, comparisons were based on total lifetime dose per unit *body weight.* At times, this resulted in the rodent appearing to be more sensitive than humans.

In conclusion, if the estimates of risk from low doses of carcinogens are made with reasonable data and reasonable models, the variability noted above results in a precision of 1 to 2 orders of magnitude in the estimates.

CONCLUSION AND SUMMARY

Finished drinking water may contain small amounts of many potentially toxic chemicals. The concentration of most of them is often so low that it is very difficult to predict a potential observable effect. In these cases of noncarcinogenic toxicity, the preferred procedure is to make a risk estimate based on extrapolation to low dose levels from experimental curves obtained from much larger doses where effects can be readily measured. When such curves are not available, the ADI approach should be used until better data are available. In this approach, safety factors are applied to the highest no-observable-effect dose found in animal studies.

The subcommittee believes that the ADI approach is not applicable to carcinogenic toxicity and that high dose to low dose extrapolation methods should be used for carcinogens. Because of the uncertainties involved in the true shapes of the dose-response curves that are used for extrapolation, a multistage model might be fitted to the data. Such a model has more biological meaning than other models, e.g., the probit or logistic model. Moreover, it tends to be conservative in that, at low doses, it will give higher estimates of the unknown risk than will many others.

More confidence could be placed in mathematical models for extrapolation if they also incorporate the principles of pharmacokinetics and time to occurrence of tumors. Procedures for doing this are being studied and might be available soon. Much more effort is required in this area. Extrapolation from animals to humans should incorporate information on comparative pharmacokinetic data between the two species.

In the absence of such data the subcommittee suggests that the extrapolation be based on the surface area rule. When reliable, valid human data are available, they should be used in conjunction with information from animal bioassays. The weight given to the human data in this process will depend on its quality and sensitivity.

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IV

Toxicity of Selected Drinking Water Contaminants

CHEMICALS EVALUATED

The health effects of a large number of contaminants found in drinking water were evaluated in *Drinking Water and Health* (National Academy of Sciences, 1977).

The compounds evaluated in this chapter were selected for the following reasons:

1. Sufficient new data have become available to justify further attention to several chemicals examined in the first study.
2. New contaminants have been identified in drinking water subsequent to the first study.
3. Several compounds were judged to be of concern because of potential spill situations.
4. The chlorination of drinking water or the use of other disinfectants yields compounds that require toxicological evaluation. A list of such compounds was prepared by the Safe Drinking Water Subcommittee on Chemistry of Disinfectants and Products. They are evaluated in this chapter by the Subcommittee on Toxicology.

The 1977 study (National Academy of Sciences, 1977) examined the radioactive, particulate, and chemical contaminants found in drinking

water. Radioactive contaminants are not considered in this study. Asbestos was one of the particulates examined in the first study. A reevaluation of this contaminant will be justified when the several studies now underway are completed. The number of volatile organic compounds identified in drinking water supplies has increased from approximately 300 at the time of the first study to 700 at the present time and will continue to grow. Limitations of time, manpower, and scientific information have not permitted an in-depth evaluation of most of the compounds recently found in drinking water. It was the belief of this subcommittee that it could perform a more valuable service to the Environmental Protection Agency (EPA) in the future if it evaluated criteria documents that were prepared by the EPA or other groups contracted to conduct these tasks. It will be necessary for the EPA to develop a mechanism for a comprehensive search and review of the literature in order to make in-depth hazard assessments for these chemicals. It is the consensus of this subcommittee that this cannot be done appropriately by the National Academy of Sciences because time and staff requirements far exceed those available. Neither can it be expected that the scientists who donate their services on these subcommittees will have the resources or time to carry out the routine aspects of this task.

ACUTE EXPOSURES

In addition to providing information on chronic toxicity, the subcommittee has evaluated the potential acute toxicity insofar as justified by the available data. These data will provide a basis for making judgments of possible health effects resulting from accidental spills of chemicals into drinking water supplies. To this end the subcommittee has provided a suggested no-adverse-response level (SNARL) for acute exposures of 24 hr or 7 days. These values are calculated based on the assumption that 100% of the exposure to the chemical was supplied by drinking water during either the 24-hr or 7-day period. In those few cases where the chemical is a known or suspected carcinogen, the potential for carcinogenicity after an acute exposure has not been considered. These acute SNARL's were calculated only when there was human exposure data or sublethal animal data. LD_{50}'s were not used as a basis for calculation. Some 7-day values were derived by dividing the 24-hr SNARL by 7, but only when the data were *very good.* The converse was

not done nor were data obtained from studies of lifetime exposures used to establish acute SNARL's. In some cases in which data from inhalation exposures were used there was information on absorption/retention. The details concerning retention and absorption are given in the monographs for each chemical. It must be emphasized that these calculated acute SNARL's should not be used to estimate hazard from exposures exceeding 7 days. They 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 reflect the degree of confidence regarding the data as well as the combined judgment of the subcommittee members.

As in the previous report, the following assumptions were used when assigning an uncertainty factor to calculate either the acute or chronic SNARL's:

- An uncertainty (safety) factor of 10 was used when good chronic or acute human exposure data were available and supported by chronic or acute data in other species.
- A factor of 100 was used when good chronic or acute toxicity data were available for one or more species.
- A factor of 1,000 was used when the acute or chronic toxicity data were limited or incomplete.

CHRONIC EXPOSURES

When the chemical of concern was *not* a known or suspected carcinogen, the subcommittee calculated a SNARL for chronic exposure. In most cases, whenever chronic SNARLS's were estimated, data were available from studies lasting a major portion of the lifetime of the experimental animal. For these SNARL's an arbitrary assumption was made that 20% of the intake of the chemical of concern was from drinking water. Because of this assumption it would be inappropriate to use these values as though they were maximum contaminant intakes. A risk estimate rather than a chronic exposure SNARL was provided in those cases in which there was adequate evidence of carcinogenicity [see *Drinking Water and Health* (National Academy of Sciences, 1977) for details].

Table IV-1 summarizes the acute and chronic SNARL's as well as the carcinogenic risk estimates for the chemicals reviewed in this report.

TABLE IV-1 Summation of Acute and Chronic Exposure Levels and Carcinogenic Risk Estimates for Chemicals Reviewed

Chemical	Suggested No-Adverse-Response Level (SNARL), mg/liter, by Exposure Period[a]			Upper 95% Confidence Estimate of Lifetime Cancer Risk per µg/liter[b]
	24-hour	7-day	Chronic	
Acrylonitrile				1.3×10^{-6}
Benzene		12.6		
Benzene hexachloride	3.5	0.5		
Cadmium		0.08	0.005	
Carbon tetrachloride	14	2.0		
Dichlorodifluoromethane	350	5.6		
1,2-Dichloroethane				7.0×10^{-7}
Epichlorohydrin	0.84	0.53		
Ethylene dibromide				9.1×10^{-6}
Methylene chloride	35	5.0		
Polychlorinated biphenyl	0.35	0.05		
Tetrachloroethylene	172	24.5		1.4×10^{-7}
1,1,1 -Trichloroethane	490	70	3.8	
Trichloroethylene	105	15		
Trichlorofluoromethane	88	8		
Toluene	420	35	0.34	
Uranium	3.5	0.21		
Xylenes	21	11.2		
Bromide	1,400	224	2.3	
Catechol	2.2			
Chlorine dioxide			0.38	
Chlorite		0.21		
Chloroform	22	3.2		
Dibromochloromethane	18			
2,4-Dichlorophenol			0.7	
Hexachlorobenzene		0.03		2.9×10^{-5}
Iodide	115.5	16.5	1.19	
Resorcinol	11.7	0.5		

[a] See text for details on individual compounds.

[b] See *Drinking Water and Health* (National Academy of Sciences, 1977) for details.

CHEMICALS SELECTED BY EPA

Acrylonitrile

(CH_2=CHCN)

Acrylonitrile is an unsaturated synthetic organic compound that has a variety of applications. Primarily it is used in the production of acrylic and modacrylic fibers, nitrile rubber, and plastics. As a pot nt, highly effective fumigant it is used most often to protect grain, dried fruit, walnuts, and tobacco against insect pests. Annual production totals approximately 682 million kg (U.S. Environmental Protection Agency, 1978a).

Acrylonitrile, also known as 2-propenenitrile, vinyl cyanide, and cyanoethylene, is a colorless, highly flammable liquid with a mild, pungent odor resembling that of peach pits. It is manufactured by the reaction of propylene with ammonia in air. Its boiling point is 77.3°C. At 20°C its solubility in water is 7.35 g/100 ml, and the specific gravity of the liquid is 0.811 (Manufacturing Chemists Association, 1974).

The principal exposure of humans to acrylonitrile is likely to occur through atmospheric contamination. Patterson *et al.* (1976) estimated that the total emission from manufacturing processes in 1974 was 14 million kg. There is relatively little information on the movement, fate, and persistence of acrylonitrile in water. Zabezhinskaya *et al.* (1962) reported that at an initial concentration of 10 mg/liter, only 46% remained after 24 hr, 19% after 48 hr. and 5% after 96 hr. Under some conditions, the relatively high vapor pressure of acrylonitrile would probably promote the escape of the compound to the atmosphere.

Metabolism

The metabolism of acrylonitrile has not been studied systematically with radio-labeled material. Investigators studying the metabolism of acrylonitrile have concentrated on ascertaining the fate of the cyano group of the molecule. Brieger *et al.* (1952) reported that inhaled acrylonitrile is metabolized first to the free cyano group, and then to thiocyanate. They found high cyanate levels and high thiocyanate levels in the blood of rats, dogs, and monkeys that had been treated with acrylonitrile. In a study with both oral and intraperitoneal administration of acrylonitrile in rats, mice, and Chinese hamsters, Gut *et al.* (1975) observed that thiocyanate was eliminated in the urine and that acrylonitrile was bound strongly to components of the blood. There appeared to be an interspecific difference in the metabolic pattern: less cyanide formed in rats than in mice. Earlier studies (Dudley and Neal, 1942; Lawton *et al.,*

1943; Paulet *et al.,* 1966) indicated the same lack of agreement on the principal metabolic product of acrylonitrile. Some of the studies reported cyanide, and others, thiocyanate, as the principal breakdown product. The fate of the remainder of the molecule has not been established. The possibility of conjugation has been raised by Hashimoto and Kanai (1972) who reported binding of acrylonitrile with cysteine and glutathione with a concomitant decrease in sulfhydryl (SH) groups. Earlier work by these investigators indicated that a large amount of injected acrylonitrile was unchanged after treatment of rabbits, guinea pigs, and rats.

Health Aspects

Observations in Humans A recent report by the Dupont Corporation to government regulatory agencies stated that acrylonitrile may be a carcinogen (Anonymous, 1977). It also stated that preliminary results of an epidemiological study of workers in a polymerization operation with potential for exposure to acrylonitrile indicated excess cancer incidence and cancer mortality as compared with company and national experience. This study included about 470 males who began working in the polymerization area of the plant between 1950 and 1955 and who are still actively employed or have been retired from the company (Anonymous, 1977). The Dupont report emphasizes that the data are preliminary and that more exhaustive studies are under way.

A number of incidents of illnesses and fatalities have been caused by the industrial and structural pest control uses of acrylonitrile. The compound is an acute poison and a severe skin and eye irritant. It may be toxic when inhaled, ingested, or absorbed through intact skin. Symptoms of exposure include nasal and respiratory oppression, vomiting, nausea, weakness, fatigue, headache, and diarrhea (Patterson *et al.,* 1976). The symptoms of poisoning from acrylonitrile are very similar to those from cyanide. Such poisoning generally results from inhalation by workers of vapor in industrial settings where the concentration of acrylonitrile varies from 16 to 100 ppm. No fatalities have been reported under these conditions.

In contrast to the safety record of acrylonitrile in the chemical industry, the use of the compound in structural pest control has resulted in a number of fatalities (Davis, 1967; Davis *et al.,* 1973; Patterson *et al.,* 1976; Radimer *et al.,* 1974; Sartorelli, 1966). The reported fatalities generally resulted from direct exposure to acrylonitrile or from too rapid a return to a building that had been fumigated with the compound.

Death resulted following symptoms that are similar to those of cyanide poisoning. However, death has also occurred as the result of toxic epidermal necrolysis (Radimer *et al.,* 1974). Acrylonitrile, when used for fumigation, is generally combined with other materials such as methylene chloride and carbon tetrachloride. Consequently, it is somewhat difficult to disassociate the symptoms of one compound from another.

Observations in Other Species

Acute Effects Investigating acute poisoning after intravenous injection of acrylonitrile in dogs and rabbits, Paulet *et al.* (1961) observed considerable differences in interspecific response to cyanide intoxication. Symptoms of nervous disorders dominated the picture. Electroencephalographic records show that the higher nervous centers were affected. The investigators also found hyperglycemia and a decrease in the concentration of plasma inorganic phosphate. Acute oral toxicity values (LD_{50}'s) for acrylonitrile range from 27 to 128 mg/kg for mice (Benes and Cerna, 1959; Zeller *et al.,* 1969) and from 78 to 93 mg/kg for rats (Benes and Cerna, 1959; Smyth and Carpenter, 1948).

Acrylonitrile has also been characterized as a serious hazard in inhalation studies conducted by the Union Carbide Corporation in rats (Union Carbide Corporation, 1970). After breathing saturated air for 5 min, all exposed animals died. After breathing 100 ppm for 4 hr, all of six rats died; for 2 hr, one of six; and for 1 hr, none of six. After breathing 500 ppm for 4 hr, none of six died, and for 8 hr, one of six died (Union Carbide Corporation, 1970). Roudabush *et al.* (1965) reported that acute dermal LD_{50} values from acrylonitrile were 0.28 mg/kg when applied to the abraded skin of rabbits, 0.46 mg/kg on the intact skin of guinea pigs, and 0.84 mg/kg on the abraded skin of guinea pigs.

Subchronic and Chronic Effects There are surprisingly few long-term studies on the toxicity of acrylonitrile in laboratory animals. None of the few studies in the literature was designed to establish a no-adverse-effect or minimal-effect dosage level.

Dudley *et al.* (1942) conducted a three-part study of the inhalation toxicity of acrylonitrile. In a preliminary series, they exposed four rhesus monkeys and two dogs for 4 hr/day, 5 days/week for 4 weeks to an average concentration of 0.12 mg/liter (56 ppm) of acrylonitrile in air. These experiments indicated that dogs are more susceptible to acrylonitrile than are monkeys and that repeated exposure to concentrations of 0.12 mg/liter produces no signs of cumulative action. In the second part of their study, they exposed 16 rats, 16 guinea pigs, 3 rabbits, and 4 cats

in the same manner for 8 weeks to an average concentration of 0.22 mg/liter (100 ppm) of acrylonitrile in air. These experiments show that rats, guinea pigs, and rabbits tolerate repeated exposures to 0.22 mg/liter acrylonitrile in air over a period of 8 weeks; that cats are definitely more sensitive to acrylonitrile than are rodents; and that there is no evidence of cumulative action of acrylonitrile. In the third part of the study, they exposed 16 rats (8 adult, 8 young animals), 16 guinea pigs, 4 rabbits, 4 cats, and 2 rhesus monkeys in the same way to an average concentration of 0.33 mg/liter (153 ppm) acrylonitrile in air. These experiments showed that repeated exposures to 153 ppm were definitely toxic to guinea pigs, rats, and rabbits and were much more toxic to monkeys and cats. The exposures produced irritation of eyes and nose, loss of appetite, gastrointestinal disturbances, and incapacitating weakness of hind legs from which the animals recovered relatively rapidly. Even after exposure to such high concentrations no definite evidence of cumulative action was observed.

In a study on the effects of acrylonitrile on rats Barnes (1970) noted no adverse effects. Six rats were given 15 successive oral doses of 30 mg/kg, followed by seven doses of 50 mg/kg, and then 13 doses of 75 mg/kg over a period of 7 weeks. The investigators supplied no details on the types of observations that were made to assess toxicity.

Studies have also been conducted on the toxicity of acrylonitrile to adult rats following daily intraperitoneal administration of the compound (Knobloch *et al.,* 1971). Daily injection of 50 mg/kg for 3 weeks produced a statistically significant loss of body weight; leucocytosis; signs of damage and functional disturbances of liver and kidneys; increase in the weight of liver, kidneys, and heart; and histological damage. Microscopic examination of the organs of these animals showed slight damage of neuronal cells of the cortex and brain stem and parenchymal degeneration of liver and kidneys. Unfortunately, no other dosage rates were included in this study.

Following the reports of epidemiological evidence of the carcinogenicity of acrylonitrile, Norris (1977) initiated a 2-year feeding and inhalation study in rats. In the ingestion study, acrylonitrile was incorporated into the drinking water of laboratory rats at concentrations of 0. 35, 100, and 300 mg/liter (corresponding to 0, 4, 10, and 30 mg/kg/day). In the inhalation study, male and female rats were exposed to 0, 20, and 80 ppm acrylonitrile for 6 hr/day, 5 days/ week. In April 1977, interim results of the 2-year studies were reported (National Institute for Occupational Safety and Health, 1977; Norris, 1977). Rats ingesting 35 mg/liter acrylonitrile exhibited mild signs of toxicity while

those ingesting 100 and 300 mg/liter showed marked signs of toxicity. Norris (1977) reported that both male and female rats that ingested 100 or 300 mg/liter acrylonitrile for 12 months developed stomach papillomas (1 of 20 rats at 100 mg/liter and 12 of 20 at 300 mg/liter); central nervous system tumors (2 of 20 at 35 mg/liter, 2 of 20 at 100 mg/liter, and 3 of 20 at 300 mg/liter); and Zymbal gland carcinoma (2 of 20 at 100 mg/liter, and 2 of 20 at 300 mg/liter). No such tumors were seen in control animals. In the inhalation study, after 1 year of exposure to 80 ppm acrylonitrile. 26 rats died and three developed central nervous system tumors that were comparable to those reported in the ingestion study. Gross examination of other rats in this study, who were also exposed to 80 ppm acrylonitrile by inhalation, revealed an increased incidence of ear canal tumors and mammary region masses. In animals exposed to 20 ppm, there was an apparent increase in subcutaneous masses of the mammary region, although no ear canal or central nervous system tumors were observed. Other than neoplasms, signs of toxicity were limited to decreased water and food consumption and decreased body weight gain.

Mutagenicity The mutagenicity of acrylonitrile has been demonstrated in the *Salmonella typhimurium* test (Milvy and Wolff, 1977) and in *E. coli* WP2 strains (Venitt and Bushell, 1977). In the Ames (*Salmonella*) assay, acrylonitrile was active in the presence of a mouse liver homogenate, producing mutations in three tester strains. Bacteria were exposed by spotting the acrylonitrile on a lawn of *Salmonella;* by shaking a reaction mixture consisting of bacteria, liver homogenate, and acrylonitrile; and by exposing the homogenate and bacteria to an atmosphere containing acrylonitrile. By the latter method, mutagenicity was observed at exposures as low as 57 mg/liter. Acrylonitrile was also mutagenic in various DNA-repair strains of *E. coli* WP2. The effects were weak in plate incorporation tests, but assays using a simplified fluctuation test showed acrylonitrile to be significantly mutagenic at doses that were 20 to 40 times lower than those giving significant results in the plate test. Use of the different DNA-repair strains indicated that acrylonitrile causes DNA damage of the type that is exemplified by methyl methanesulfonate.

Carcinogenicity Acrylonitrile has given positive results in a rat feeding study. Epidemiological evidence also contributes strong evidence to implicate acrylonitrile as a carcinogen. These studies are discussed above.

Reproduction The subcommittee found no studies on reproductive effects of acrylonitrile.

Teratogenicity The subcommittee noted no studies reporting the teratogenicity of acrylonitrile.

Carcinogenic Risk Estimate The interim results of a 2-year ingestion study with acrylonitrile in the drinking water of rats give evidence of what appears to be an increase in cancer at several sites (Norris, 1977). Dose-response data (Norris, 1977) were used to estimate both the lifetime risk and an upper 95% confidence bound on the lifetime risk at the low dose level. These are estimates of lifetime human risks which have been corrected for species conversion on a dose/surface area basis. The risk estimates are expressed as a probability of cancer after a lifetime consumption of 1 liter/day of water containing 1 μg/liter of the compound under study. For example, a risk of 1×10^{-6} implies a lifetime probability of 2×10^{-5} of cancer if 2 liters/day were consumed and the concentration of the carcinogen was 10 μg/liter. This means that at a concentration of 10 μg/liter during a lifetime exposure this compound would be expected to produce one excess case of cancer for every 50,000 persons exposed. If the population of the United States is taken to be 220 million people this translates into 4,400 excess lifetime deaths from cancer or 62.8 per year.

For acrylonitrile at a concentration of 1 μg/liter, the estimated lifetime risk for humans is 6.7×10^{-7}. The upper 95% confidence estimate is 1.3×10^{-6}. Both of these estimates are the averaged risks calculated from the male and female rats. They are based on preliminary data of Norris (1977) and are subject to change when the study is completed.

Conclusions and Recommendations

Based on toxicological investigations, the Food and Agriculture Organization/World Health Organization (FAO/WHO, 1965) concluded that an acceptable daily intake of acrylonitrile for humans could not be determined. Both epidemiological and controlled feeding and inhalation studies since 1965 indicate that acrylonitrile is a carcinogen. Therefore, it would not seem possible to establish a long-term acceptable level for this compound in drinking water. Unfortunately, since LD_{50} studies indicate only the level of short-term toxicity, short-term exposure limits cannot be calculated.

Antimony

(Sb)

Antimony is a metal that is chiefly a by-product of base metal and silver ores. It has oxidation states of + 3 (trivalent) and + 5 (pentavalent) and forms compounds with halides, oxygen, sulfur, and organic anions such as tartrate, thioglycollate, and thioglycollamide. It is used industrially in flameproofing textiles, in vulcanizing of rubber, and in the manufacture of paint pigments, electronic semiconductors, thermoelectric devices, and fireworks (National Institute for Occupational Safety and Health, 1978; Robert and Boston, 1974). Antimony compounds also have medical application (Gross, 1974; Harvey, 1975). Organic antimonial compounds are used as parasiticides to treat different forms of schistosomiasis, bilharziasis, and leishmaniasis.

Most exposure of humans to antimony compounds occurs in industrial settings. Schroeder (1970) has estimated the human daily intake from all sources to be about 100 μg.

Metabolism

Trivalent and pentavalent antimony are differently distributed and excreted. Trivalent compounds have a great affinity for erythrocytes and, therefore, give low plasma concentrations. Pentavalent compounds tend to remain in the plasma. The trivalent form is excreted at a much slower rate in the urine than pentavalent antimony probably because it collects at much lower levels in plasma (National Institute for Occupational Safety and Health, 1978; Robert and Boston, 1974). After administration of a single therapeutic dose of trivalent antimony only 10% was recovered in 24 hr, whereas 50% of the pentavalent form was recovered in 24 hr (Harvey, 1975).

Most trivalent antimony (tartar emetic) is excreted in the feces, whereas the pentavalent forms are excreted mainly in the urine. The distribution of antimony to the tissues has not been thoroughly studied. However, in guinea pigs the trivalent compounds are found in high concentration in the thyroid and liver while the pentavalent forms are found in the liver and spleen after oral dosing. Abdallah and Saif (1962), in their studies of humans, showed that the highest concentrations of antimony occur in the liver, followed by the thyroid and heart. They administered sodium antimony dimercaptosuccinate (^{124}Sb) intravenously. The liver, heart, and thyroid retained antimony for 20 days. When three 100-mg doses of antimony were administered intramuscularly over 9 days there was still considerable antimony in these tissues 53 days later.

Antimonial compounds are absorbed very slowly from the gastrointestinal tract. Therefore, when used medicinally, they are administered parenterally (Harvey, 1975).

Health Aspects

Antimony is not essential to the life and health of humans or animals. It resembles arsenic both chemically and biologically, and symptoms of acute and chronic toxicity from antimony closely resemble those induced by arsenic. Generally, the trivalent compounds are more toxic than the pentavalent. Reports of toxic reactions to antimony are few. When they are observed, they are usually a result of industrial exposure or antimonial treatment of parasitic diseases (National Institute for Occupational Safety and Health, 1978).

Observations in Humans The major toxic symptoms that are associated with antimonial compounds in humans involve the gastrointestinal tract, heart, respiratory tract, skin, and liver. The most serious effects of these compounds, which are exerted on the heart, have been observed during antimonial therapy and during industrial exposure (National Institute for Occupational Safety and Health, 1978). Such cardiac alterations include changes in the electrocardiogram, primarily in the T-wave (suppressed amplitude, inversion of the T-wave, and prolongation of the Q-T interval); bradycardia; and fluctuations in blood pressure (Brieger *et al.,* 1954; National Institute for Occupational Safety and Health, 1978). Respiratory changes include irritation of the mucous membranes, upper respiratory tract irritation, and, more seriously, pneumonoconiosis. Pneumonia has also been cited as a side effect to the therapeutic use of antimony. Gastrointestinal symptoms include cramps, nausea, pain, anorexia, diarrhea, and vomiting.

There is little information concerning dose-response relationships. Many toxicities have occurred as the result of industrial exposure to inhaled antimony and are complicated by the presence of other agents (National Institute for Occupational Safety and Health, 1978; Robert and Boston, 1974). However, most toxic syndromes have been observed in patients receiving antimonial therapy, and many have been reproduced in animal studies.

Observations in Other Species

Acute Effects The LD_{50} values for different antimony compounds vary considerably. Bradley and Frederick (1941) administered antimony

compounds intraperitoneally to rats and found the following LD_{50} values: antimony potassium tartrate, 11 mg/kg; antimony trioxide, 3.25 g/kg; and antimony metal, 100 mg/kg. The oral LD_{50} for antimony potassium tartrate was 300 mg/kg. After the oral administration of antimony chloride to rats, Arzamastsev (1964) reported that the LD_{50} of trivalent antimony chloride ($SbCl_3$) was 675 mg/kg, and that of the pentavalent compound ($SbCl_5$), 1.115 g/kg. These compounds were somewhat more toxic in guinea pigs, i.e., the LD_{50} for $SbCl_3$ was 574 mg/kg, and for $SbCl_5$, 900 mg/kg.

Chronic Effects Effects of chronic feeding studies vary depending upon the antimonial compound under study. Arzamastsev (1964) gave trivalent antimony chloride *per os* to rats for 10 days at 135 mg/kg. Toxic symptoms included myocardial degeneration from which two rats died during the treatment. Studies were also conducted using guinea pigs. Ten-day studies at 12 and 20 mg/kg doses of trivalent antimony chloride produced blood changes (decreased hemoglobin, increased reticulocyte count). A longer term study was performed using guinea pigs given trivalent antimony chloride orally in doses ranging from 0.0025 to 2.5 mg/kg for 6 months. No toxic symptoms were observed with the lowest dose. Robert and Boston (1974) reported work done in 1927 by Flury in which antimony potassium tartrate was administered to rats for 85 days in oral doses up to 200 mg/rat at which time the rats died. Feeding antimony trioxide in doses less than 2 g/rat/day did not give rise to observable toxic symptoms.

Mutagenicity Paton and Allison (1972) investigated the effects of antimony sodium tartrate (2.3 nM) on human leukocytes *in vitro.* They examined 100 metaphases and observed that 12% of the cells displayed chromatid breaks. The authors did not draw any conclusions.

Carcinogenicity There is no direct evidence that antimony induces carcinogenicity. Some reports (National Institute for Occupational Safety and Health, 1978) suggest that deaths from lung cancer were higher in factory workers in Great Britain who had been exposed to antimony than in the general population. Because there were many complicating factors in this situation, no firm conclusions could be drawn. Kanisawa and Schroeder (1969) reported that 76 mice given 5 μg of antimony potassium tartrate per milliliter of drinking water throughout their lifetimes showed no increase in incidence of tumors.

Teratogenicity A report by Belyayeva (1967) is the only recent investigation concerning the effects of antimony on reproduction. This investigator compared reproductive indices between women working in an antimony metallurgical plant (located in the USSR) with a similar group that had not been exposed to antimony. The antimony workers had a greater incidence of spontaneous abortion, premature births, and gynecological problems (menstrual cycle disorders and inflammatory diseases) than did the control group. In animal studies, Belyayeva (1967) administered metallic antimony (≤ 5-μm diam) by inhalation at a dose of 50 mg/kg to 30 female rats. Only 15 rats became pregnant, some only after multiple matings. These rats produced fewer offspring (5.4 versus 7.8) than controls. Gross inspection of the placentas and newborns showed no morphological changes.

Conclusions and Recommendations

Suggested No-Adverse-Response Level (SNARL) The data from which to calculate either 24-hr or 7-day SNARL's are inadequate. If a chronic SNARL is calculated using the lowest no-observed-adverse-effect level of 0.0025 mg/kg, which was reported by Arzamastsev (1964), the resulting value is less than the estimated daily intake of antimony.

However, maximal environmental levels have been set by others as follows:

1. Trivalent antimony—0.05 mg/liter of potable water (Dawson, 1974) (based on USSR standard).
2. Antimony—0.05 mg/liter-maximum—permissible concentration, USSR (Stofen, 1973).
3. National Institute for Occupational Safety and Health (NIOSH) occupational standard—0.5 mg/m^3 for a 10-hr work shift in a 40-hr workweek.

The taste threshold for either trivalent or pentavalent antimony is 0.6 mg/liter (Arzamastsev, 1964).

Benzene

(C_6H_6)

This compound was evaluated in *Drinking Water and Health* (National Academy of Sciences, 1977, p. 688). The following material, which

became available after that 1977 publication, updates and, in some instances, reevaluates the information in the earlier report. Also included are some references that were not assessed in the original report.

Benzene is produced by petroleum refining, coal tar distillation, coal processing, and coal coking. In 1976 the U.S. production of benzene exceeded 0.5 million kg. It is used primarily as a chemical intermediate in the manufacture of styrene, cyclohexane, detergents, and pesticides.

Bowden (1972) reported that gasoline that is used in motor vehicles in the United States usually contains 0.8% by volume benzene, but the range extends to 2%. A survey of inhalation exposures for 12 personnel at service stations that dispensed gasoline containing 1.6% benzene by volume showed a maximum of 0.39 µg/liter (0.123 ppm) (Hartle and Young, 1976). Benzene is somewhat soluble in water (0.82 g/liter). In the National Organic Monitoring Survey (NOMS), which was conducted from March 1976 through January 1977, benzene analyses were performed on three samplings from community water supplies, which were representative of various types of sources and treatment processes. The numbers of positive benzene analyses per numbers of cities sampled were 0/111, 7/113 (0.4 µg/liter), and 4/16 (0.95 µg/liter) (U.S. Environmental Protection Agency, 1978c).

Metabolism

Whether administered by inhalation, orally, or by another route, benzene is eliminated rapidly by expiration and excretion in the urine. Parke and Williams (1953) administered ^{14}C-labeled benzene orally to rabbits (0.34-0.5 g/kg) and collected samples over 3 days. Benzene expired in air accounted for 43% of the dose; 34.5% was excreted in the urine as glucuronide or ethereal sulfate conjugates of the metabolic oxidation products phenol, quinol, catechol, and hydroxyquinol together with small amounts of other products; and 5% to 10% remained in the tissues. Benzene is metabolized similarly in humans (Laskin and Goldstein, 1977). It is readily absorbed through the lungs and has a retention rate of about 50% (Srbova *et al.,* 1950). Inhalation at 52 to 62 ppm for 4 hr resulted in 30% retention after 3 hr (Nomiyama and Nomiyama, 1974). Hunter and Blair (1972) reported that humans retained 230 mg after exposure to 80 to 100 µg/liter for 6 hr. Retained benzene is distributed in tissues according to their fat content. Bone marrow, which, *in toto,* is an organ about two-thirds of the size of the liver, has a high tissue blood partition coefficient for benzene. Metabolites are believed to be important in the development of hematotoxicity, partly because of the effect of altered liver metabolism upon leukopenia

and other hematopoietic responses. But little is known of the metabolic fate of benzene in the bone marrow (Snyder and Kocsis, 1975).

Health Aspects

Observations in Humans The toxicity to the hematopoietic system of chronic exposure of humans to benzene is well documented. Reported effects include myelocytic anemia, thrombocytopenia, or leukopenia (occurring either separately or in cases of pancytopenia) and leukemia, particularly acute myelogenous and monocytic leukemia. In many of these studies benzene was exposed with additional solvents at relatively high concentrations. Data on the level and duration of exposure are inadequate for deriving dose-response relationships of chronic benzene toxicity (Vigliani, 1976). Infante *et al.* (1977) reported a retrospective cohort study of two populations of workers who were involved in production of rubber sheeting (Pliofilm). Benzene was the only material in their work environment that was known to be associated with blood disorders. In both plants during 1940-1949, the occupational exposure of 561 workers to benzene was apparently well within the maximum allowable concentration of 100 ppm that was usually recommended. Vital status to 1975, which was obtained for 75% of the workers, showed a significant excess of leukemia in those exposed to benzene, indicating a 10-fold increase in risk of death from myeloid and monocytic leukemia.

The onset of leukemia is usually preceded by many observable effects on the hematopoietic system (Snyder and Kocsis, 1975). It is not known whether benzene causes leukemia as one aspect of its hematotoxic effects, whether the leukemia is a consequence of benzene-induced damage to immunological components of the bone marrow, or whether the leukemic effects are unrelated to the other hematopoietic manifestations (Laskin and Goldstein, 1977). The data suggest that benzene is a leukemogen in humans. The current U.S. standard sets maximum occupational exposure to benzene at 10 ppm as a time-weighted average for up to a 10-hr day. This standard is based on an accumulation of data indicating possible effects on the hematopoietic system from prolonged inhalation of benzene in the range of 20 to 60 ppm (National Institute for Occupational Safety and Health, 1974). However, in consideration of the evidence for benzene as a leukemogen, OSHA in February 1978 proposed the adoption of 1 ppm (3.2 μg/liter) benzene as a "lowest feasible level" in air as a time-weighted average for an 8-hr day with a ceiling of 5 ppm for any 15-min period (U.S. Department of Labor, 1978).

Effects of benzene on proliferation of bone marrow in humans include increased incidence of chromosomal aberrations with aneuploidy and breakage. In cases of frank hematopoietic toxicity, such changes might be due to the disease, but Forni *et al.* (1971) noted significant chromosome aberrations in cultured leukocytes that were obtained from workers who were exposed to benzene but showed no symptoms of benzene poisoning. Among workers who had recovered from other signs of benzene hematotoxicity, aneuploidy in leukocytes persisted for 10 years, but karyotypes appeared normal by 12 years (Pollini and Biscaldi, 1977).

Observations in Other Species

Acute Effects Six rabbits that were exposed by inhalation to 35,000 to 45,000 ppm underwent anesthesia after 3.7 min and showed other effects in the central nervous system until death, which ensued after 22.5 to 71 min (Carpenter *et al.,* 1944). Kimura *et al.* (1971) studied acute oral toxicity in Sprague-Dawley rats. They used 6 to 12 rats of both sexes per group in testing newborn and 14-day-old rats, and 6 male rats per group for the other ages. Single dose LD_{50} values (95% confidence level) for newborn, 14-day-old, young adult, and old adult rats were <0.9, 3.0 (1.8-5.0), 3.3 (2.6-4.2), and 4.9 (3.5-6.2) g/kg body weight, respectively.

Chronic Effects Leukopenia is the most commonly observed effect of chronic benzene intoxication in laboratory animals. Deichmann *et al.* (1963) reported that repeated inhalation of as little as 61 ppm benzene by rats at 5 hr/day. 4 days/week for 6 or more weeks causes a significant decrease in circulating leukocytes. However, at lower exposures, a fall in leukocyte concentration typically leads to increased cell proliferation which causes cyclical fluctuations. Moreover, there is normally wide variation among cell counts during diurnal cycles and among individual animals. Laskin and Goldstein (1977) concluded that nearly all reports of leukopenia in animals exposed to less than 1,000 ppm benzene are unlikely to stand up to statistical evaluation.

Benzene mixed with equal parts of olive oil was administered to rats by subcutaneous injection (Gerarde. 1956; Latta and Davies, 1941). Weight loss and leukopenia resulted from doses of 880 mg benzene/kg body weight, which were given daily for 14 days (Gerarde, 1956). and from doses of 1.32 g benzene/kg/body weight, which were given daily for 3 to 60 days (Latta and Davies. 1941). In Latta's group a rat that died after 10 days had hyperplastic bone marrow, and one that died at 21 days had acute leukopenia and hypoplastic bone marrow. Oral adminis

tration of benzene to rats in daily doses of 1, 10, 50, and 100 mg/kg body weight during 132 days over 6 months resulted in leukopenia and erythrocytopenia at the lowest minimal effect level of 50 mg/kg (Wolf *et al.,* 1956).

Mutagenicity Toxic effects on bone marrow cells of rats and other laboratory animals include changes in chromosome number and chromosome breakage that resemble those in humans. There was no clear evidence for dose-dependent response (Laskin and Goldstein, 1977). Lyon (1975) used the Ames assay, with *Salmonella typhimurium* strains TA-98 and TA-100, to test benzene for mutagenicity in doses ranging from 0.1 to 1.0 µl/plate, both without and with microsomal fractions at concentrations from 1 to 50 µl/plate. Postmitochondrial supernatant suspensions of microsomes were prepared from liver homogenates from normal rats and from rats that had been treated with phenobarbital and 3-methylcholanthrene (MCA), and from the bone marrow of normal and MCA-treated rats. Benzene was uniformly negative in all these assays and was also inactive in the dominant lethal assay in rats.

Carcinogenicity No benzene-induced carcinogenesis has been observed in an animal system. An extensive study with mice (Ward *et al.,* 1975) indicated some increase in granulocytic leukemia. After reviewing the data from that study, the National Academy of Sciences Safe Drinking Water Committee concluded that the increase is not statistically significant, even when time to response is incorporated into the analysis (National Academy of Sciences, 1977, p. 690).

Teratogenicity The subcommittee noted only one study of teratogenicity. In this study, Watanabe and Yoshida (1970) gave subcutaneous injections of acute toxic doses of benzene (3 ml/kg body weight) to pregnant mice on days 11 through 15 of gestation. Malformations were most prevalent in the group that was treated on the 13th day. Four of 15 litters, involving 10 of 127 fetuses, had cleft palate, agnathia, or micrognathia (Watanabe and Yoshida, 1970). Decreased white cell count and weight gain in the benzene-treated mice were the same whether the litters were normal or included malformed fetuses. As an indication of the toxicity of the dose used in this experiment, five male mice that received benzene at 3 ml/kg body weight survived, while four of five male mice that had been injected with 4 ml/kg died within 3 days.

Carcinogenic Risk Estimate As noted above, there are no data from animal models for use in extrapolation. Occupational studies on human

exposure (Aksoy *et al.,* 1972, 1974a,b, 1976; Ishimaru *et al.,* 1971; Thorpe, 1974) do not contain adequate information on degree of exposure or size of population at risk. In addition, the workers in benzene-related occupations typically were exposed to other chemicals, as in the study reported by Ott *et al.* (1978). Consequently, extrapolation of benzene-induced cancer risk from such data as these would be tenuous.

In a study by Infante *et al.* (1977) workers were exposed to benzene as the sole chemical suspected of affecting the hematopoietic system. In these cases, benzene concentrations apparently were high during the first years of exposure and were lower thereafter. There are no data indicating how often short exposures at elevated levels may have occurred. Estimates of actual exposure are inadequate for extrapolation for risk of benzene-induced leukemia.

Conclusions and Recommendations

The acute effects of benzene cover a wide range of signs and symptoms. The effects are transitory but may lead to more lasting chronic effects such as anemia. If exposure is continuous and great enough, leukemia is a strong possibility for susceptible members of the population. There are no dose-response data on animals, and the data on humans are inadequate to calculate a risk estimate for benzene with mathematical models.

Suggested No-Adverse-Response Level (SNARL)

Twenty-Four-Hour Exposure There are no adequate data from which this calculation can be made.

Seven-Day Exposure Wolf *et al.* (1956) administered benzene orally to rats 5 days/week for 6 months. They reported leukopenia and erythrocytopenia at the daily dose level of 50 mg/kg. Quantitative effects were not reported but this could be considered a minimal effect. Adjusting to a 7-day exposure, 50 mg/kg/day x 5/7 days = 36 mg/kg/day. applying a safety factor of 100, and assuming total exposure to be from 2 liters of drinking water per day during this period:

$$\frac{36 \text{ mg/kg} \times 70 \text{ kg}}{100 \times 2 \text{ liters}} = 12.6 \text{ mg/liter.}$$

This SNARL ignores the established mutagenicity and suspected carcinogenicity of benzene.

Chronic Exposure No chronic SNARL can be calculated because benzene is a suspected human carcinogen.

In summary, there is no adequate source of data (animal or human) on which to base a statistical extrapolation from high to low exposure. More studies on the role of metabolism and mode of action of benzene metabolites in the bone marrow are needed, particularly on their relationship to chromosome aberrations and mutation and to immunological systems. More data are also needed on the teratogenicity and cocarcinogenicity of benzene. If there are data on industrial exposure to benzene, then systematic monitoring should be started with a view to following the population groups at risk.

Before chronic limits for benzene in drinking water can be established, more extensive toxicological data must be gathered and evaluated. In the absence of such data, it is useful to consider what portion of the average dose of benzene is contributed by its presence in drinking water. Benzene is a natural constituent of fruit, fish, vegetables, dairy products, nuts, eggs, and rum, and has been found in cooked meat. In these forms, it provides humans with an estimated daily intake of 250 μg/day (Kraybill, 1977). The benzene content of ambient air at automobile service stations was recorded at 0.39 μg/liter (Hartle and Young, 1976). The general urban atmosphere contains much less: an unreferenced value was 0.05 μg/liter. At this concentration, assuming a daily inhalation of 24 m^3 and a retention of 50%, the dose from inhalation would be 600 μg/day. Based on the same assumptions, the current OSHA recommendation for a 10hr time-weighted average of 3.2 μg/liter (1 ppm) as a lowest feasible level in the workplace would allow a daily dose of 16 mg. From food and air the average urban dweller receives approximately 850 μg of benzene daily. In 1978 the level of benzene in U.S. drinking water was reported to be 1 μg/liter, or a 2-μg daily dose, assuming a 2 liter/day intake (U.S. Environmental Protection Agency, 1978c). Although such exposure is less than 0.3% of an average daily intake, major spills of benzene into drinking water could result in much higher exposures, since the solubility of benzene in water is 820 μg/liter.

Benzene Hexachloride and Lindane

(BHC) and ($C_6H_6Cl_6$)

These compounds were evaluated in *Drinking Water and Health* (National Academy of Sciences, 1977, p. 583). The following material, which became available after that 1977 publication, updates and, in some instances, reevaluates the information in the earlier report. Also included are some references that were not assessed in the original report.

Health Aspects

Observations in Humans In a study of the racial stratification of organochlorine insecticide residues, Kutz *et al.* (1977) found lindane residues in samples from Negroes more than twice as often as in samples from Caucasians. There was little racial difference noted for residues of *β*-benzene hexachloride (BHC).

Observations in Other Species

Acute Effects Kashyap *et al.* (1976) treated Wistar rats with technical BHC (15% gamma isomer) by intubation of a single dose as a 5% solution in olive oil. Ten rats per group were treated with 100, 200, 300, 400, and 500 mg/kg of body weight. The animals were observed for 48 hr and then sacrificed. The investigators observed mortality, physical symptoms, serum glutamic pyruvic transaminase (SGPT), prothrombin time, coagulation time, cholinesterase activity, and histology of the liver and other organs at autopsy. Results indicated a significant increase in SGPT at all treatment levels, which indicates liver damage. Prothrombin time and coagulation time had also increased, even in animals that had received the lowest doses.

Mutagenicity Ishidate and Odashima (1977) obtained negative results for lindane when screening for chromosomal aberrations in Chinese hamster cells *in vitro.* Published reports indicate that lindane does not have significant mutagenic potential (National Academy of Sciences, 1977).

Carcinogenicity Thorpe and Walker (1973) fed groups of male and female CFI mice diets containing *β*-BHC at 200 mg/kg or γ-BHC at 400 mg/kg. Liver tumors were observed in 24% of the male and 23% of the female controls, in 73% of the males and 43% of the females that had received 200-ppm *β*-BHC, and in 93% of the males and 60% of the females that had received 400-ppm γ-BHC. Lung metastases were found in some males that had received *β*- and γ-BHC and in some females that had received γ-BHC. The incidence of other tumors was not increased by exposure to either isomer. However, the dose of 400 ppm of γ-BHC may be higher than the maximum tolerated dose. Therefore, nonspecific toxicity may have biased the observed effects. Only 3% of the females and 17% of the males that were fed lindane survived for the duration of the experiment at this dose, which is approximately 70% of the acute oral LD_{50}.

Weisse and Herbst (1977) treated groups of 100 mice (Chbi:NMRI [SPF]) of both sexes with 12.5, 25, and 50 ppm lindane. There were 200 controls. The concentrations represent 2.4, 4.1, and 8.2 mg/kg body weight for males and 2.0, 3.9, and 7.8 mg/kg body weight for females. The study lasted 80 weeks. No lindane-related production of tumors was evident at these dosages. Electron microscopic examination of the livers produced no evidence of lindane-induced fine-structural hepatocellular alterations.

Conclusions and Recommendations

Suggested No-Adverse-Response Level (SNARL)

Twenty-Four-Hour Exposure The data of Kashyap *et al.* (1976) provide a minimal-adverse-response level of 100 mg/kg for technical BHC (15% lindane) in rats. The concentration suggested for a 24-hr SNARL assumes that 100% of the exposure to the compound is provided by drinking water during that period and that a 70-kg human consumes 2 liters/day of water. Applying an uncertainty factor of 1,000, the following calculation may be made:

$$\frac{100 \text{ mg/kg} \times 70 \text{ kg}}{1{,}000 \times 2 \text{ liters}} = 3.5 \text{ mg/liter.}$$

Seven-Day Exposure Since there are no data on subchronic oral administration of BHC to animals, it is possible to calculate a 7-day SNARL by dividing the 24-hr value by 7. This result is 0.5 mg/liter.

Chronic Exposure This value cannot be calculated because technical BHC is a carcinogen in animals.

Bis(2-Chloroethyl)ether

($ClCH_2CH_2OCH_2CH_2Cl$)

This compound was evaluated in *Drinking Water and Health* (National Academy of Sciences, 1977, p. 710). The following material, which became available after that 1977 publication, updates and, in some instances, reevaluates the information in the earlier report. Also included are some references that were not assessed in the original report.

Health Aspects

Observations in Humans No new data.

Observations in Other Species

Mutagenicity After giving three different doses to mice by gavage in the heritable translocation test, Jorgenson *et al.* (1977) determined that the mutagenic potential for this compound was negative. It was weakly mutagenic in a *Salmonella* assay (TA-100 without S-9 mix) when incorporated into agar and very mutagenic when assayed in desiccators or in suspension (Simmon *et al.,* 1978).

Carcinogenicity Theiss *et al.* (1977) tested for pulmonary tumor induction in strain A/St male mice by giving them intraperitoneal injections of three dose levels 3 times a week for a total of 24 injections. Upon sacrifice, 24 weeks after the first injection, results were negative. Survival at 20 mg/kg, which was given 24 times (total dose of 480 mg/kg), was 100%, whereas at 40 mg/kg, which was given 4 times (total dose of 160 mg/kg), it was 75%.

Conclusions and Recommendations

Suggested No-Adverse-Response Level (SNARL)

Acute Exposure Oral LD_{50} values in three laboratory species average approximately 125 mg/kg. Using that LD_{50} as the basis of extrapolation to humans, and assuming a 70-kg body weight and 2-liter consumption of water per day, the LD_{50} in humans would be 4.3 mg/ml. Because the solubility of the compound in water is approximately 10 mg/ml, an acutely toxic dose in humans is theoretically possible. Since only acute LD_{50} animal data (no sublethal acute dose levels) were available, a 24-hr or 7-day SNARL cannot be calculated.

Chronic Exposure Since the compound is a "suspected animal carcinogen" (*Drinking Water and Health,* Table VI-60, p. 794, National Academy of Sciences, 1977), calculation of a chronic SNARL would not be appropriate. Further studies should be conducted on this compound if it is found to be present in a large number of water supplies.

Bis(2-Chloropropyl)ether

($CH_3CHClCH_2OCH_2CHClCH_3$)

Bis(2-chloropropyl)ether is a by-product of the manufacture of propylene glycol. It is commonly dumped into streams where it is stable.

Metabolism

Smith *et al.* (1977a) conducted studies in rats and monkeys on blood levels, blood level kinetics, tissue distribution, and excretion. Half-life determinations were made for 2 days in the rat, and 5 hr (α phase) and 2 days (*β* phase) in the monkey. There were significant accumulations of bis(2-chloropropyl)ether in the livers of monkeys. Excretion was essentially complete in both species at 24 hr. Its metabolites, 1-chloro-9-propanol, propylene oxide, and 2(1-methyl-2-chloroethoxy)propionic acid, were all found in the urine.

Health Aspects

Observation in Humans No available data.

Observations in Other Species

Acute Effects Smyth *et al.* (1951) reported the acute oral LD_{50} in rats to be 240 mg/kg. In the guinea pig, Spector (1956) found it to be 450 mg/kg. In inhalation studies in rats, 700 ppm killed all five animals after 6 hr, two of five died after a 6-hr exposure to 350 ppm, and one of four died after an 8-hr exposure to 175 ppm (Hake and Rowe, 1963).

Chronic Effects Twenty-two gastric intubations in rats for 31 days at 10 mg/kg and 200 mg/kg resulted in decreased growth. The 200 mg/kg exposure also resulted in increases in the weights of the liver, kidney, and spleen (Hake and Rowe, 1963).

Mutagenicity A test for mutagenic potential by the heritable translocation test (Jorgenson *et al.,* 1977) was negative in mice that received three different oral doses by gavage. The compound was weakly mutagenic in the Ames *Salmonella* assay using the agar-incorporation system and very mutagenic in desiccator or suspension assay systems. The mutagenic activity was enhanced by the addition of an S-9 activation mix that was prepared from human liver (Simmon *et al.,* 1978).

Carcinogenicity No available data.
Teratogenicity No available data.

Conclusions and Recommendations

Suggested No-Adverse-Response Level (SNARL)

Acute Exposure Using the only available data, which is an LD_{50} of 240 mg/kg in the rat, and extrapolating directly to humans, assuming 70kg body weight and 2-liter consumption of water per day, the LD_{50} for humans would be 16.8 g. This amounts to 8.4 mg/ml. Since the solubility of this compound in water is 1.7 mg/ml, it is unlikely that an acutely lethal dose in humans would be possible. Data are inadequate for calculations of a 24-hr or 7-day SNARL.

Chronic Exposure There are insufficient data for estimates of chronic toxicity. Long-term oral experiments should be conducted in at least two species of animals so that chronic risk can be calculated. In view of the probable frequent contamination of water supplies by this compound and its structural similarity to other carcinogenic haloethers, additional short-and long-term toxicity data are needed.

Cadmium

(Cd)

Cadmium is a silvery-white metal that is found in the same periodic group (IIB) as mercury and zinc. In industry, it is used principally in electroplating, in the manufacture of pigments, as a plasticizer, in batteries, and in electrical conductors (Friberg *et al.,* 1974; National Institute for Occupational Safety and Health, 1976a). Although not abundant, cadmium is distributed widely through the crust of the earth and is found wherever zinc is found in nature. Cadmium in the environment originates primarily in industrial sources (Webb, 1975). Concentrations of cadmium in unpolluted fresh water vary around 1 µg/liter. The drinking water standard is 10 µg/liter. The major source of cadmium for the general population is diet. Average daily intake values may vary between 50 and 150 µg/day. Cigarette smoke contains approximately 1 ppm cadmium. Those who smoke one pack of cigarettes daily are exposed to 2 to 4 µg of cadmium per day from that source (Friberg *et al.,* 1974). Occupational exposures to cadmium are considerable, averaging approximately 0.02 to 0.05 mg/m^3.

Metabolism

After cadmium is ingested or inhaled, it is distributed to most tissues of the body, but is found in highest concentrations in the liver and kidney. Within the various organs cadmium is bound primarily to a unique low molecular weight protein, termed metallothionein (Friberg *et al.,* 1974; Webb, 1975). Following oral administration, approximately 1% to 2% of the dose is absorbed in laboratory animals, while estimates of absorption in humans range up to 5% (World Health Organization, 1977). Variations may be induced by many factors such as age, dietary calcium, and dietary protein. Excretion of cadmium occurs primarily via the kidney at a very slow rate. Consequently, the accumulation of cadmium in humans is considerable. In fact, cadmium levels in the newborn are virtually negligible but may reach body burdens of 30 mg by age 30 (Schroeder, 1965). The biological half-life of cadmium is quite long and is estimated to be on the order of decades in humans (World Health Organization, 1977).

Health Aspects

Cadmium is a very toxic element. It is not a trace element that is essential to nutrition. Toxicity from this element can result from acute or chronic exposure, and the toxic syndromes may differ. Certain toxic effects may be prevented by the administration of such trace elements as zinc and selenium. The health aspects of cadmium have been reviewed extensively (Fleischer *et al.,* 1974; National Institute for Occupational Safety and Health, 1976a; Friberg *et al.,* 1974; Webb, 1975; World Health Organization, 1977). The following synopsis was derived from these sources unless otherwise noted.

Observations in Humans Acute exposures to cadmium in humans produce different effects depending upon the route of exposure. Among the general population, ingestion of food and fluids that have been contaminated with cadmium resulted in acute gastrointestinal disturbances such as nausea, vomiting, abdominal pain, diarrhea, and tenesmus. This contamination has occurred when cadmium-plated vessels have been used to prepare lemonade or other acidic beverages. The acute effects of industrial exposure to cadmium are generally manifested as lung damage, which may result in death. Typically, the symptoms include chest pain and pulmonary edema. Pulmonary fibrosis has been observed in survivors, but there are few data pertaining to the

acute dose-response relationship for cadmium toxicity in humans. Estimates of lethal concentrations vary from 2,500 to 2,900 mg/m^3.

Chronic exposure to cadmium may also cause a wide variety of effects involving many organs and systems. The severity of the manifestations depend upon the magnitude and duration of exposure.

The two major effects of chronic cadmium toxicity in persons that have been occupationally exposed to cadmium are obstructive lung disease and renal dysfunction. The lung disorders are primarily suggestive of pulmonary emphysema. Dose-response relationships are uncertain because time-weighted averages are either not available or available only for short periods. The most common abnormality from chronic cadmium exposure involves renal toxicity characterized by proteinuria. Other disturbances of renal tubular function include glycosuria, amino aciduria, decreased urine concentrating ability, and abnormalities in renal processing of uric acid, calcium, and phosphorus. Proteinuria can occur along with these other changes. Therefore, it may be the earliest sign of renal dysfunction in cadmium toxicity. After prolonged exposures, the kidney is generally regarded as the critical organ. Autopsy data, as well as animal data, indicate that the critical level of cadmium that produces damage in the kidney is approximately 150 to 250 µg/g wet tissue. Other manifestations of chronic occupational cadmium exposure include olfactory changes such as anosmia, anemia, and osteomalacia.

Excessive exposure to cadmium has also occurred in the general population through the ingestion of food, e.g., rice, and of drinking water containing high concentrations of cadmium. Such exposure has been implicated in itai-itai disease, which is characterized by osteomalacia and renal tubular dysfunction. Although cadmium has been widely implicated in this disorder, there may be other disposing factors involved as well.

The role of cadmium as a predisposing factor in hypertension is controversial. While epidemiological investigations have found a positive correlation between cardiovascular disease (including both hypertension and atherosclerotic diseases) and ambient cadmium levels, no direct cause-effect relationships have been established. Moreover, workers that have been exposed to cadmium do not exhibit a higher prevalence of hypertension than do other groups.

Observations in Other Species

Acute Effects After acute exposure to relatively high doses of cadmium, toxic effects are seen primarily in the gonads (testicular

necrosis), liver (structural and functional changes), kidney (tubular damage), and central nervous system (hemorrhagic lesions of sensory ganglia). Sarcomas at the injection site of cadmium have also been noted (see section on carcinogenicity). The LD_{50} value in male rats after oral administration of cadmium varies from 175 to 225 mg/kg (Kotsonis and Klassen, 1977; Schnell *et al.,* 1978).

Carcinogenicity There has been considerable interest in the possibility that cadmium exposure may cause cancer. Studies by Gunn *et al.* (1963, 1967) have shown that injection of 0.03 mmol/kg cadmium chloride could induce subcutaneous sarcomas at the injection site as well as testicular tumors (interstitial cell tumors) in rats and mice. Concomitant administration of zinc prevented development of such tumors. No carcinogenic effects were found by Schroeder *et al.* (1965) who fed cadmium to mice in concentrations of 5 ppm in drinking water over their lifetime.

Several epidemiological surveys have suggested that there is an increased incidence of prostate cancer in cadmium workers when compared to the general population (Kipling and Waterhouse, 1967; Lemen *et al.,* 1976; Potts, 1965). However, because these studies had small samples, age groups varying from 60 to 75 years, and concurrent exposure to other environmental contaminants, firm conclusions concerning the carcinogenicity of cadmium in humans cannot be drawn.

Mutagenicity There is little information concerning the mutagenic effects of cadmium. Epstein *et al.* (1972) reported that cadmium does not produce an increase in dominant lethal mutations in mice. In a study by Gilliavod and Leonard (1975), male mice (BALB/c) received 1.75 mg/kg cadmium chloride intraperitoneally and then were mated. There was no increased incidence of dominant lethal mutations in the offspring. In the treated males, dividing spermatocytes did not have chromosomal rearrangements such as reciprocal translocations. Conflicting results have been reported for humans. Paton and Allison (1972) did not find that cadmium induced chromosomal damage *in vitro* in cultured lymphocytes, while Shiraishi *et al.* (1972) did report such damage. Bui *et al.* (1975) performed chromosomal analyses on Swedish workers who had been exposed to cadmium and Japanese itai-itai patients. They found no increased frequency of chromosome aberrations.

Teratogenicity When injected parenterally, cadmium induces teratogenic effects in laboratory rats, mice, and hamsters. Chernoff (1973) administered cadmium chloride (4-12 mg/kg) on days 13 to 16 of gestation in CD strain rats and found a dose-related increase in fetal

deaths, a decrease in fetal weight, and an increase in the rate of anomalies such as micrognathia, cleft palate, club foot, and small lungs. Ishizu *et al.* (1973) gave subcutaneous injections of cadmium chloride (0.33-0.35 mg/kg) to mice on day 7 of gestation. The most common anomaly was exencephaly in fetuses that were removed on day 18. Other anomalies included spina bifida, absence of tail, and malformations of ribs, skull, and vertebrae.

Conclusions and Recommendations

Suggested No-Adverse-Response Level (SNARL)

Twenty-Four-Hour Exposure There are no adequate data from which to calculate a 24-hr SNARL.

Seven-Day Exposure This 7-day SNARL is based on data of Loeser and Lorke (1977) who fed cadmium chloride to rats in their diets in concentrations of 1 to 30 ppm for 3 months without effect. Assuming that the rats consumed 20 g/day of food and that their average weight was 250 g, their exposure is calculated:

$$\frac{30 \text{ mg/kg/day} \times 0.02 \text{ kg/day}}{0.25 \text{ kg}} = 2.4 \text{ mg/kg.}$$

For a 70-kg human consuming 2 liters/day, using a safety factor of 1,000 and assuming that 100% of exposure is from water during this period, the 7-day SNARL is calculated:

$$\frac{2.4 \text{ mg/kg} \times 70 \text{ kg}}{1{,}000 \times 2 \text{ liters}} = 0.08 \text{ mg/liter.}$$

Chronic Exposure The SNARL for chronic exposures is based on data of Decker *et al.* (1958), who gave cadmium to rats in their drinking water at a concentration of 10 ppm for 1 year without effect. They did observe effects such as anemia after exposing rats in the same manner to 50 ppm of cadmium for 3 months. In this study the rats consumed an average of 30 ml/day. Assuming that their average weight was 400 g, their daily exposure is calculated:

$$\frac{10 \text{ mg/liter} \times 0.03 \text{ liter/day}}{0.4 \text{ kg}} = 0.75 \text{ mg/kg.}$$

Using a safety factor of 1,000 for a 70-kg human consuming 2

liters/day and assuming that 20% of exposure is from water, the chronic SNARL is calculated:

$$\frac{0.75 \text{ mg/kg} \times 70 \text{ kg} \times 0.2}{1{,}000 \times 2 \text{ liters}} = 0.005 \text{ mg/liter.}$$

Carbon Tetrachloride

(CCl_4)

This compound was evaluated in *Drinking Water and Health* (National Academy of Sciences, 1977, p. 703). The following material, which became available after that 1977 publication, updates and, in some instances, reevaluates existing information in the earlier report. Also included are some references that were not assessed in the original report.

Health Aspects

Observations in Humans Van Oettingen (1964) reported that accidental ingestion of 14 to 20 ml has repeatedly caused fatal poisonings within 3 to 5 days; however, larger doses have occasionally not been fatal. Gosselin *et al.* (1976) reported that the mean lethal dose of carbon tetrachloride for humans lies between 5 and 10 ml (oral) although as little as 2 ml has caused death. Symptoms and/or death following ingestion occur after a latent period of 24 to 36 hr (Gosselin *et al.,* 1976).

Observations in Other Species

Acute Effects A single oral gavage of 0.25 mg/kg in adult male rats produces increases in liver tyrosine α-ketoglutarate transaminase (200%) and plasma alanine α-ketoglutarate transaminase (120%) within 5 hr (Murphy and Malley, 1969). Oral doses of 2.5 ml/kg result in decreases in liver and lung microsomal cytochrome P-450 and in decreases in the rates of metabolism of several substrates for the mixed function oxidase system (Chen *et al.,* 1977). These effects have been observed as early as a few hours after administration. Carbon tetrachloride itself is not thought to be the active agent; rather, it is probably the CC13 free radical, which is produced via the cytochrome P-450 system. Carbon tetrachloride induces lipid peroxidation, which in turn is responsible for the morphological changes (Smuckler, 1976). The toxicity of carbon tetrachloride can be modulated by agents that alter levels of liver cytochrome P-450. Phenobarbital increases liver P-450 and enhances carbon tetrachloride

toxicity. On the other hand, 3-methylcholanthrene, which induces the formation of P-448, does not enhance carbon tetrachloride toxicity. In fact, there is evidence that it may even lower it (Suarez and Bhonsle, 1976). Cobaltous chloride, which inhibits P450 selectively, decreases carbon tetrachloride hepatotoxicity (Suarez and Bhonsle, 1976).

Doses of carbon tetrachloride that induce biochemical and morphological changes in rat liver also induce an increase in bile duct-pancreatic fluid flow (BDPF) by a mechanism that is not understood. However, the increase in BDPF appears to be unrelated to the time course and the extent of liver necrosis and elevation of serum glutamic-oxaloacetic transaminase (SGOT) (Harms *et al.,* 1976; Imamura *et al.,* 1977; Peterson and Fujimoto, 1976).

Chronic Effects In long-term studies of carbon tetrachloride toxicity, Alumot *et al.* (1976b) fed rats diets that had been fumigated with carbon tetrachloride. Doses of 150 to 275 ppm had no effect on the growth of weanlings, but 520 ppm depressed weight gain significantly within 6 weeks. Females of a similar age were unaffected by the treatment. Triglycerides and total lipids were significantly higher in animals that were treated with the carbon tetrachloride, with the exception of those that were fed 150 ppm. The results of growth, fertility, and biochemical tests of rats that received 80 and 200 ppm for 2 years were not significantly different from those of controls. Pathological studies were not conducted.

Conclusions and Recommendations

Suggested No-Adverse-Response Level (SNARL) The following calculations are for noncarcinogenic effects only.

Twenty-Four-Hour Exposure Animal data indicate that 0.25 ml/kg (0.4 g) is the lowest dose that produces a toxic effect. Using a safety factor of 1,000 and assuming that 100% of the exposure will come from 2 liters/day intake, the 24-hr SNARL is calculated as follows:

$$\frac{0.4 \text{ g/day} \times 70 \text{ kg}}{1{,}000 \times 2 \text{ liters}} = 14 \text{ mg/liter.}$$

Seven-Day Exposure Assuming that repeated daily intake of carbon tetrachloride produces cumulative effects, the 7-day SNARL value should be one-seventh of the 24-hr exposure limit. This is 2 mg/liter.

Chronic Exposure This value will not be calculated because carbon tetrachloride is a carcinogen in animals.

Ethylene Dibromide (1,2-Dibromoethane)

(BrCH=CHBr)

Ethylene dibromide, also known as 1,2-dibromoethane and ethylene bromide, is a clear, colorless, heavy liquid at room temperature and has a distinctive odor. The water solubility of ethylene dibromide is 0.43 g/100 g of water at 30ºC (National Institute for Occupational Safety and Health, 1977). Its half-life in water at 20°C (pH 7) is approximately 14 years. The U.S. production of ethylene dibromide increased from an estimated 29 million kg in 1940 to over 150 million kg in 1973. Approximately 85% of the ethylene dibromide that has been produced has been used in gasoline as a constituent of antiknock mixtures containing tetraethyl lead. It is also used as a fumigant-insecticide, nematocide, and specialty solvent for resins, gums, and waxes (U.S. Environmental Protection Agency, 1976).

Metabolism

Ethylene dibromide is a reactive molecule that may form covalent bonds under certain conditions. From the data of Edwards *et al.* (1970) and Plotnick and Conner (1976), an approximate biological half-life using ^{14}C-labeled ethylene dibromide in mice and in guinea pigs can be estimated to be less than 48 hr. Twenty-four hours after intraperitoneal administration of 30 mg/kg of ethylene dibromide to guinea pigs, 65% was excreted as metabolites in the urine and 12% unchanged in the expired air. Similar findings were reported for mice.

After administering intraperitoneal injections of ^{14}C-ethylene dibromide to rats and mice, Shih (1976) found that the compound was widely distributed, with concentrations in the liver, kidney, and small intestine. At 24 hr the liver and kidney contain irreversibly bound ^{14}C in RNA, DNA, and protein. Ethylene dibromide appeared to be a substrate for at least two different enzymes including glutathione-S-transferase.

Health Aspects

Observations in Humans There are several case reports of human exposure to ethylene dibromide. In one, death occurred 54 hr following oral ingestion of 140 mg/kg of ethylene dibromide by a 43-year-old female. Postmortem examination disclosed major toxic effects in the liver

and kidneys. Other reports indicate severe contact irritation both to the skin on typical exposure and lungs following inhalation. Systemic manifestations include malaise and vomiting.

Observations in Other Species

Acute Effects Single oral dose lethality studies in several animal species revealed LD_{50}'s ranging between 420 mg/kg and 55 mg/kg. Rabbits were the most sensitive and mice the least sensitive (Rowe *et al.,* 1952b). Schlinke (1969) produced symptoms and/or death in sheep and calves by giving them single oral doses of 25 to 50 mg/kg. A 10 mg/kg dose in calves produced no ill effects. No autopsies were performed.

Chronic Effects Chronic toxicity has been studied mostly by inhalation exposures. On the basis of data that were gathered from repeated inhalation exposures in animals, McCollister *et al.* (1956) estimated maximum safe concentrations and exposure times for humans that are exposed to undiluted ethylene dibromide to be 192 mg/m^3 for a 7-hr single exposure. In chronic feeding studies, Alumot *et al.* (1968) gave feed that was contaminated with ethylene dibromide to hens in order to assess effects on weight gain and egg production. Concentrations as low as 40 ppm of ethylene dibromide administered for 10 weeks produced one or more adverse effects. Other investigators have reported significant egg weight reduction in chickens that were fed 5 to 7.5 ppm ethylene dibromide in grain (Bondi *et al.,* 1955). Amir and Volcani (1965) observed abnormal spermatozoa in Friesian bulls that had been fed an average of 1 mg/kg/day of ethylene dibromide from the age of 4 days until they were approximately 24 months old.

Mutagenicity Buselmaier *et al.* (1972) reported that ethylene dibromide is mutagenic in the host-mediated assay and in *in-vitro* studies with *Salmonella typhimurium.* The authors concluded that ethylene dibromide did not require activation through *in-vivo* metabolism to exert its mutagenic effects on *Salmonella typhimurium,* nor did metabolism of ethylene dibromide sufficiently deactivate its mutagenic potential, since both the host-mediated assay and the *in-vitro* plate tests were positive. Ethylene dibromide was mutagenic in fruit flies *(Drosophila melanogaster)* in tests by Vogel and Chandler (1974).

Carcinogenicity A number of investigators have reported ethylene dibromide to be carcinogenic (National Institute for Occupational Safety and Health, 1977). Olson *et al.* (1973) exposed rats and mice via chronic

oral intubation 5 times per week to experimentally predetermined maximally tolerated doses (MTD) and one-half MTD. They found a high incidence of squamous cell carcinoma of the stomach of both species. The initial doses were 80 and 40 mg/kg/day for rats and 120 and 60 mg/kg/day for mice. Weisburger (1977) reported that oral ethylene dibromide caused stomach tumors with many metastases in both rats and mice.

Teratogenicity No available data.

Carcinogenic Risk Estimate In a recent study by Olson *et al.* (1973), rats and mice were gavaged with doses of ethylene dibromide chloroethylene ranging from 40 to 120 mg/kg body weight for as long as 78 weeks. There was a high incidence of metastatic carcinoma of the stomach in both the mice and the rats. Each set of dose-response data was used to estimate statistically both the lifetime risk and an upper 95% confidence limit on the lifetime risk at the low dose level. These are estimates of lifetime human risks that have been corrected for species conversion on a dose/surface area basis. The risk estimates are expressed as a probability of cancer after a lifetime consumption of 1 liter/day of water containing 1 µg/liter of the compound. For example, a risk of 1 x 10^{-6} implies a lifetime probability of 2 x 10^{-5} of cancer if 2 liters/day were consumed and if the concentration of the carcinogen was 10 µg/liter. This means that during a lifetime exposure, this compound would be expected to produce one excess case of cancer for every 50,000 persons exposed. Assuming the population of the United States to be 220 million people, this translates into 4,400 excess deaths from cancer or 62.8 per year.

For ethylene dibromide at a concentration of 1 µg/liter the estimated lifetime risk for humans is 7.0 x 10-^{6}. The upper 95% confidence estimate is 9.1 x 10^{-6}. Both of these estimates are the averaged risks calculated from the male and female mice.

Conclusions and Recommendations

Suggested No-Adverse-Response Level (SNARL)

Carcinogenicity and mutagenicity studies strongly implicate ethylene dibromide. Although dose studies in laboratory animals are limited, the carcinogenicity data seem to require that water contaminated with ethylene dibromide not be consumed. This would include acute spills, 7day, or chronic situations. Furthermore, the data concerning acute and

subacute noncarcinogenic actions were insufficient to permit estimates of SNARL's for 24-hr and 7-day exposure limits.

Dichlorodifluoromethane

(Cl_2CF_2)

Dichlorodifluoromethane (Freon 12) is a relatively inert, nonflammable liquid that is used mainly as a refrigerant. Its molecular weight is 120.92, its vapor pressure is 6.43 atm at 25ºC, and it is quite insoluble in water (5.7 ml/100 ml at 26°C).

Metabolism

Apparently there are no data regarding the metabolic fate of Freon 12 following oral exposure. The fluorocarbon compounds are lipid-soluble and thus are generally well absorbed through the lung. Absorption after ingestion is 35 to 48 times lower than after inhalation (Charlesworth, 1975). The fluorocarbons are particularly stable compounds that do not appear to be metabolized (Blake and Mergner, 1974). Inhalation experiments in laboratory animals have demonstrated that the half-life of fluorocarbons is very short (minutes) (Clayton, 1967) and that they are eliminated via the lung. Adir *et al.* (1975) reported that complete recovery of an inhaled dose in dogs occurs within 40 to 60 min.

Health Aspects

Observations in Humans The threshold limit value (TLV) for inhaled dichlorodifluoromethane recommended by the American Conference of Governmental Industrial Hygienists (ACGIH) is 1,000 ppm for 8 hr (American Conference of Governmental Industrial Hygienists, 1975). This is approximately 20% by volume. There are no data on the oral route of administration in humans.

Inhalation studies in humans (Adir *et al.,* 1975; Azar *et al.,* 1972) indicated good tolerance to the TLV level (1,000 ppm, 25 mg/min). Even a 10.000-ppm exposure resulted only in a small (7%) reduction in the standardized psychometer test score indicating that this high dose given for 2.5 hr would not pose a serious threat to an individual's health. At 1,000 ppm, the Freon 12 level in venous blood was 1.2 µg/ml. This is much less than the 22.8 µg/ml that is necessary to sensitize the dog's heart (Azar *et al.,* 1973).

Acute human inhalation exposures indicate that concentrations in

excess of 50,000 ppm (5%) should be avoided and that 100,000 ppm (10%) produces unconsciousness (Largent and Largent, 1955).

Observations in Other Species

Acute Effects The inhalation LD_{50} in mice during a 3-hr exposure was 62% by volume, or 3,348 mg/liter (Shugaev, 1963). The LD_{100} was 66% by volume, or 3,564 mg/liter. Shugaev observed the earliest symptoms (motor stimulation, placidity, rapid respiration, immobility) at 30% by volume, or 1,620 mg/liter. At higher doses the mice quickly developed tremors and were narcotized.

After acute exposure by *inhalation* to a 13.5% concentration for 30 s, the myocardium in unanesthetized dogs was sensitized to subsequent injection of epinephrine (Azar, 1971). In contrast, a 2.5% concentration that was inhaled 6 hr/day for 5 days resulted in no cardiac sensitization in dogs.

When rats were intubated with 1,000 mg/kg (maximum feasible dose), Freon 12 produced no deaths (Clayton, 1967). Symptomatology was not described.

Chronic Effects Sayers *et al.* (1930) conducted inhalation experiments in dogs, monkeys, and guinea pigs. They exposed the animals 6 to 8 hr/day. 5 to 6 days/week, for up to 12 weeks. They investigated overt symptoms, growth, complete blood count, mortality, and gross pathology. Although the reliability of the data must be questioned because of the very small number of animals that were tested, the authors concluded that 20% vapor was tolerated by these species with only transient symptomatology and no accumulative or permanent deleterious effects.

Inhalation studies with dichlorodifluoromethane were conducted in mice, rats, and dogs by Smith and Case (1973). Dosages were 970 mg/kg/day for 23 months (mice), 164 mg/kg/day for 93 days (rats), 700 mg/kg/day for 93 days (dogs), 560 mg/kg for 90 days (dogs), and 2,240 mg/kg/day for 1 year (dogs). There were no changes in electrocardiograms of the dogs, whose high doses were approximately 200 times greater than those normally given in clinical use. No microscopic changes in tissue were found in any of the species. Blood chemistry and hematology and urinalyses were all normal.

Clayton (1967) gave dichlorodifluoromethane to male and female rats and dogs in oral dose levels of 160 to 379 mg/kg (rats) and 84 to 85 mg/kg (dogs) for 90 days. He observed no effects on growth, hematology, behavior, or on tissue that had been examined both grossly and

microscopically. Other studies, in which Clayton gave male rats 430 mg/kg for 10 days, also produced no effects on tissue or toxicity.

Dichlorodifluoromethane was administered by gavage to rats at 15 or 150 mg/kg/day for 2 years and in food to beagle dogs at 8 or 80 mg/kg/day for 2 years (Sherman, 1974). Except for a slight adverse effect on growth of the rats that had received the high doses, no clinical, biochemical, urinary, hematological, or histopathological changes were found in either species. No evidence of a carcinogenic response was observed.

Mutagenicity In chronic exposure studies, male and female rats were given Freon 12 in doses of 15 or 150 mg/kg by intubation. The rats were then bred and evaluated for fertility, corpora lutea, implantation sites, resorption sites, and number of live fetuses per litter. No dominant lethality was found at either dose level (Sherman, 1974).

Carcinogenicity For 2 years, groups of 50 male and 50 female albino rats were given Freon 12 orally by intubation in doses of approximately 15 or 150 mg/kg daily. Control groups of 50 rats of each sex received the vehicle alone by intubation. Indices studied included growth, clinical signs, mortality, hematology, urine, blood biochemistry, organ weight, and tissue pathology. No evidence of a carcinogenic effect was found (Sherman, 1974).

Teratogenicity In studies of inseminated Wistar albino rats and albino rabbits, Paulet *et al.* (1974) administered a mixture of 90% Freon 12 (dichlorodifluoromethane) and 10% Freon 11 by inhalation for 2 hr/day. Rats were exposed on days 4 to 16 of gestation, and rabbits on days 5 to 20. The mixture was administered in a 20% concentration (200,000 ppm). No indications of any embryotoxic, fetotoxic, or teratogenic changes were found when dams were sacrificed and fetuses removed on day 20 of gestation (rats) or day 30 of gestation (rabbits). No oral teratology data were found.

Conclusions and Recommendations

Suggested No-Adverse-Response Level (SNARL)

Twenty-Four-Hour Exposure The above acute toxicity data characterize a compound of relatively low toxicity. For example, an oral single dose of 1,000 mg/kg in rats resulted in no mortality; inhalation in mice at 1,620 mg/liter of air produced pharmacologic symptoms but no

mortality; and inhalation by dogs of 135 mg/liter of air resulted in no cardiac sensitization even after 6 hr/day for 5 days.

The acute side effect of most concern is cardiac sensitization. The oral rat data indicate that a cardiac toxic dose may exceed the maximum (limited) water solubility (307.8 mg/liter). An uncertainty factor of 100 was used. Assuming 100% exposure from water during this period and a 2 liters/day intake by a 70-kg human:

$$\frac{1{,}000 \text{ mg/kg} \times 70 \text{ kg}}{100 \times 2 \text{ liters}} = 350 \text{ mg/liter.}$$

Seven-Day Exposure The 10-day experiment of Paulet *et al.* (1974) in male rats (cited above) showed that an oral dose of 430 mg/kg/day was free of toxicity. This would translate, by direct extrapolation, into 30,100 mg/kg/day for a 70-kg human, or 15,050 mg/liter of water, assuming a consumption of 2 liters/day. Even a 100-fold safety factor would allow for 150 mg/liter in water. Based on the 10-day rat study and using the same assumptions as above:

$$\frac{430 \text{ mg/kg} \times 70 \text{ kg}}{100 \times 2 \text{ liters}} = 150 \text{ mg/liter.}$$

Chronic Exposure Two-year studies in rats and dogs by the Haskell Laboratory indicate that the highest dose levels used (150 mg/kg/day in rats and 80 mg/kg/day in dogs) were free of any clinical, biochemical, hematological, or histopathological effects (including tumors). An uncertainty factor of 100 was used. A 20% intake from water and consumption of 2 liters/day of water by a 70-kg human was assumed:

$$\frac{80 \text{ mg/kg} \times 70 \text{ kg} \times 0.2}{100 \times 2 \text{ liters}} = 5.6 \text{ mg/liter.}$$

These data demonstrate that Freon 12 has remarkably low chronic toxicity even at high doses. Its very short half-life and rapid excretion may be a major factor in this low toxicity.

1,2-Dichloroethane

($ClCH_2$-CH_2Cl)

This compound was evaluated in *Drinking Water and Health* (National Academy of Sciences, 1977, p. 723). The following material, which became available after that 1977 publication, updates and, in some instances, reevaluates the information in the earlier report. Also included are some references that were not assessed in the original report.

Metabolism

The pharmacokinetics of 1,2-dichloroethane (DCE) has been the subject of a limited number of investigations. Morgan *et al.* (1970), in a study of the uptake and elimination of a series of chlorinated hydrocarbons in humans, found DCE vapor to be rapidly absorbed but rather slowly exhaled in relation to other compounds in the series. Morgan and his colleagues (1970, 1972) attributed rapid absorption and retention of the chlorinated hydrocarbons largely to their lipid solubility. Consequently, it appears that DCE has a potential for accumulation in the body. Apparently no data have been published regarding pharmacokinetics of ingested DCE in humans or in laboratory animals. The only *in-vivo* study of metabolism of DCE was conducted in mice by Yllner (1971). He found that DCE was metabolized through a 2-chloroethanol intermediate to chloroacetic acid and related conjugation products. The proportion of DCE that was metabolized, as opposed to the DCE that was exhaled unchanged, varied inversely with dose. In *in-vitro* studies Van Dyke and Wineman (1971) confirmed that DCE can be dechlorinated by a rat liver microsomal system. Despite reports that liver alcohol dehydrogenase in the rat (Johnson, 1967) and human (Blair and Vallee, 1966) can catalyze oxidation of 2-chloroethanol to chloroacetaldehyde, the nature of DCE metabolism and its role in DCE toxicity remain largely speculative.

Health Aspects

Observations in Humans

Acute Effects A number of reports of human fatalities resulting from ingestion of DCE have been reviewed by the National Institute for Occupational Safety and Health (1976c). Estimated quantities of the chemical consumed range from "a sip" to as much as 100 ml. Quantities from 20 to 50 ml were commonly reported to be lethal. These figures are compatible with the established oral LD_{50} in rats, which is 0.77 ml/kg (Smyth *et al.,* 1969).

Published case histories of acute human exposures to DCE reveal that symptoms and signs of poisoning are quite similar, whether the DCE was inhaled or ingested. Nausea and vomiting are experienced by many persons who have been subjected to large quantities of the chemical by either route of exposure. Another important, recurring clinical finding in such patients is the presence of hemorrhagic lesions of most organs. An explanation for the finding is offered by Martin *et al.* (1969), who

reported depletion of a number of clotting factors and thrombocytopenia in a DCE-poisoned patient. The hypocoagulability is attributed to disseminated intravascular coagulation. Schoenborn *et al.* (1970) were not successful in preventing loss of clotting factors and subsequent circulatory collapse by administering heparin to a patient 5.5 hr after DCE ingestion. However, Przezdziak and Bakula (1975) reported the successful use of heparin in the treatment of a man exhibiting both consumption coagulopathy and hepatic coma. Severe hepatotoxicity may augment hypocoagulability. Apparently, this occurred in the case of a 14-year-old who died from ingestion of approximately 15 ml of DCE (Yodaiken and Babcock, 1973).

Chronic Effects Accounts of chronic exposure of humans to low levels of DCE via inhalation and/or skin contact in occupational settings indicate that toxic manifestations may resemble those in persons who have been poisoned by acute exposures to high concentrations of DCE. The recommended U.S. standard for occupational exposure to DCE is 5 ppm. This standard is based largely upon reports of adverse effects in humans in European industrial settings (National Institute for Occupational Safety and Health, 1976c). Among other neurological abnormalities, these effects include nausea and vomiting, changes in appetite and mood, depression, gastric pain and upset, and liver and kidney dysfunction. Workers experiencing such difficulties were exposed for various lengths of time to DCE vapor at levels ranging from as little as 10 ppm to as much as 200 ppm. Several of these accounts, which were reviewed by National Institute for Occupational Safety and Health (1976c), apparently suffer from inadequate or inappropriately designed atmospheric sampling. However, there are a sufficient number of reports of adverse health effects to suggest that prolonged inhalation of 10 to 15 ppm DCE may be harmful. A single study, which was conducted under controlled laboratory conditions with humans, suggests that inhalation of as little as 1.5 to 3.0 ppm DCE for 30 s to 1 min can produce transient vasoconstriction and altered breathing patterns (Borisova, 1957).

Observations in Other Species

Acute Effects Studies of the acute toxicity of DCE in laboratory animals have for the most part involved inhalation exposures. The acute oral LD_{50} of 1,2-dichloroethane has been established at 0.77 (0.67-0.89) ml/kg in rats (Smyth *et al.,* 1969). LC_{50} values for vapor inhalation are 12,000 ppm in 0.53 hr, 3,000 ppm in 2.75 hr, and 1,000 ppm in 7.20 hr in

rats (Spencer *et al.,* 1951). In one study with rabbits, the LD_{50} for skin penetration was determined to be 3.89 (3.40-54.46) ml/kg. Signs of acute poisoning in animals are quite similar to those in humans. The toxic effects of single acute exposures to 1,2-dichloroethane were central nervous system depression, lung irritation, and injury to the liver, kidneys, and adrenals (Gohlke and Schmidt, 1972). A single study by Plaa and Larson (1965) concerns acute exposure of laboratory animals to DCE by a route of administration other than inhalation. These investigators gave the compound to mice by intraperitoneal injection and found modest renal injury only with a near-lethal dose (i.e., 0.4 ml/kg). The renal injury was indicated by alteration of only one of three variables tested.

Chronic Effects Based upon the chronic exposure studies of Heppel *et al.* (1946), Hofman *et al.* (1971), and Spencer *et al.* (1951), the highest noeffect vapor level in a variety of species would be approximately 100 ppm. Even at this level, increase in liver weight and diminished body weight gain have been noted in certain species (Hofman *et al.,* 1971; Spencer *et al.,* 1951). No mention was made in these studies of measurement of pharmacokinetic parameters.

Results of two long-term DCE feeding studies have recently been published. In the first, Alumot *et al.* (1976a) fed male and female Leghorn chickens diets containing 250 and 500 ppm DCE for up to 2 years. They observed effects during this time on growth, fertility, spermatogenesis, or clinical chemistry. There was some reduction in egg weight and egg production. Therefore, Alumot *et al.* (1976a) proposed 100 ppm as a maximum tolerance limit for contamination of poultry feed and 5 mg/kg body weight as an acceptable daily intake for chickens.

In the second chronic, low-level feeding study with aliphatic halocarbons, Alumot *et al.* (1976b) fed carbon tetrachloride or DCE to male and female rats for up to 2 years. Results of their fertility and reproduction studies are discussed in the section on teratogenicity. The minimum toxic dietary levels of carbon tetrachloride and DCE in the feed, as revealed by increases in hepatic total lipids and triglycerides after 5- to 7-week feeding periods, were 275 and 1,600 ppm, respectively. No significant alterations in body weight gain or in clinical chemistry indices were observed in male or female rats after 2 years of consumption of carbon tetrachloride (80 and 200 ppm) or DCE (250 and 500 ppm). Based on these established "no-effect levels," Alumot *et al.* (1976b) proposed that an acceptable daily intake of DCE for animals was 25 ml/kg body weight and a tolerance level for feed contamination was 250 ppm. These investigators also proposed a tolerance of 10 ppm for DCE in human

food, considering that a safety factor of at least 100 should be applied to data from experiments in animals.

Mutagenicity 1,2-Dichloroethane has been found to be a weak mutagen in several nonmammalian and *in-vitro* test systems. Brem *et al.* (1974) reported a mutagenic effect of 1,2-dichloroethane in *Salmonella typhimurium* (TA 1530, TA 1535 and TA 1538) and DNA polymerasedeficient *Escherichia coli.* However, it was the weakest of the series of haloalkanes tested. In other studies, DCE has been found to be weakly mutagenic in *Drosophila* (Shakarnis, 1969, 1970) and in two strains of S. *typhimurium* (McCann *et al.,* 1975b; Rannug and Ramel, 1977). Noting that DCE and 1,2-dibromoethane are mutagenic in *Drosophila,* Vogel and Chandler (1974) suggested that each compound, upon loss of a halogen atom, might act as a bifunctional alkylating agent capable of introducing cross-links into biological molecules such as DNA.

Although DCE itself is apparently a weak mutagen in certain nonmammalian test systems, certain of its metabolites would appear to be more hazardous. Two possible human metabolites, 2-chloroethanol and chloroacetaldehyde, are reported to be relatively potent mutagens in certain bacterial test systems, but not in others (McCann *et al.,* 1975a; Rannug *et al.,* 1976; Voogd and Van der Vet, 1969). McCann *et al.* (1975a) and Rannug *et al.* (1976) demonstrated that chloroacetaldehyde is many times more potent than 2-chloroethanol, while chloroacetate is inactive. McCann *et al.* (1975a) could enhance the mutagenicity of chloroethanol by incubating it with rat liver microsomes, but could not similarly activate DCE. However, Rannug and Ramel (1977) reported a fivefold increase in mutagenicity of DCE, when a 9,000-g, NADPH-deficient rat liver fraction is added to DCE in their *S. typhimurium* screening system.

Carcinogenicity There is recently published evidence that high doses of DCE may be carcinogenic in certain species of animals. Theiss *et al.* (1977), in their evaluation of the ability of DCE to produce pulmonary adenomas in Strain A male mice, reported that repeated intraperitoneal injections of 100 mg/kg body weight of the chemical elicited an increased tumor incidence at 24 weeks, although this incidence was not significantly (statistically) different from controls. At the National Cancer Institute (1978), female and male mice and rats were given large oral doses of DCE (50-300 mg/kg) daily (5 times/week) for 78 weeks. Mammary tumors were observed in DCE-treated female rats, while stomach tumors were seen in some DCE-exposed male rats. Male mice showed a dose-dependent increase over controls in incidence of

hepatocellular carcinoma, and both male and female mice that had been subjected to DCE exhibited an increase in lung tumors. McCann *et al.* (1975a) speculated that vinyl chloride, DCE, and 2-chloroethanol all may prove to be carcinogenic, since each may be metabolized via a similar pathway. They mentioned chloroacetaldehyde as a potential carcinogenic metabolite that is common to each of these three compounds.

Teratogenicity Although DCE and its metabolites have been demonstrated in certain nonmammalian test systems to be mutagenic, no one has apparently found DCE to be teratogenic *in vivo.* Heppel *et al.* (1946) failed to report abnormalities in the offspring of guinea pigs and rats that had been subjected to long-term DCE inhalation exposures during pregnancy. Alumot *et al.* fed chickens (1976a) and rats (1976b) diets containing 250 and 500 ppm DCE for up to 2 years. In the chickens, there was no effect on sperm count or motility and no influence on fertility of either sex. No alteration of fertility or reproduction was seen in the rats. The average size, weight, and mortality of offspring were unaffected by DCE.

Carcinogenic Risk Estimate In a recent study by the National Cancer Institute (1977), rats and mice were gavaged with doses of 1,2-dichloroethane ranging from 47 to 299 mg/kg/day for as long as 78 weeks. There was a dose-related incidence of squamous cell carcinomas of the forestomach in the male rats, but not in the females. The mice also exhibited dose-related cancer at several sites including mammary glands and endometrium, and there were alveolar/bronchiolar adenomas in both sexes. Each set of dose-response data was used to make statistical estimates of both the lifetime risk and an upper 95% confidence bound on the lifetime human risks. These estimates have been corrected for species conversion on the basis of dose/surface area. The risk estimates are expressed as a probability of cancer after a lifetime consumption of 1 liter/day of water containing 1 μg/liter of the compound. For example, a risk of 1×10^{-6} implies a lifetime probability of 2×10^{-5} of cancer if 2 liters/day were consumed and the concentration of the carcinogen was 10 μg/liter. At a concentration of 10 μg/liter during a lifetime exposure, this compound would be expected to produce one excess case of cancer for every 50,000 persons that are exposed. If the population of the United States is taken to be 220 million people, this translates into 4,400 excess lifetime deaths from cancer, or 62.8 per year.

At a DCE concentration of 1 μg/liter, the estimated lifetime risk for humans is 3.7×10^{-7}. The upper 95% confidence estimate is 7.0×10^{-7}.

Both of these estimates are the averaged risks, which have been calculated from the male and female rats and mice.

Conclusions and Recommendations

Suggested No-Adverse-Response Level (SNARL)
Twenty-Four-Hour Exposure/Seven-Day Exposure

DCE is acutely toxic to both humans and laboratory animals. Although its ingestion has proved fatal to a number of individuals, there is unfortunately little information pertaining to quantities of DCE consumed in nonfatal cases. Nor are there data on concentrations of the parent compound or its metabolites in the bodily fluids or organs of the poisoned victims. Thus, there is little basis for making a reasonable judgment as to the minimum toxic oral dose of DCE in humans. Little is known about potential toxic effects of exposure of animals to DCE by any route other than inhalation. While inhalation studies are valuable in determining manifestations of exposure to DCE and setting vapor exposure limits, inhalation toxicity studies include few data on uptake and distribution of the compound. Thus, it is very difficult to relate toxic effects at a given vapor concentration of DCE to injury that might result from ingestion of a given quantity of the chemical. The metabolism of DCE and the role of metabolism in activation or inactivation of the compound as a toxicant are largely speculative. Therefore, the subcommittee concluded that there is insufficient information to serve as a basis for recommending a 24-hr or a 7-day suggested no-adverse-response level (SNARL).

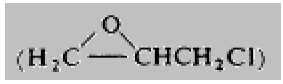
Definitive acute and subacute toxicity studies in animals should be conducted using several dissimilar animal species; employing a range of oral doses to characterize dose-response relationships and ascertain the maximum no-effect level for both single ingestions and multiple ingestions over a 24-hr and a 7-day period; examining a variety of test parameters which are valid indices of injury to known or suspected target organs/cells/biochemical systems (organs to be examined should include the heart, lung, kidney, adrenal, and liver); and investigating uptake, distribution, metabolism, and excretion of DCE and its major metabolites.

Chronic Exposure Chronic exposure to DCE is potentially hazardous. A variety of injurious effects in humans have been attributed to prolonged, low-level exposure to the chemical in occupational settings (National Institute for Occupational Safety and Health, 1976c). Unfortunately, it is difficult to relate findings in such accounts of inhalation

and/or dermal exposures to situations involving chronic oral intake of DCE. Although a number of inhalation studies utilizing several species of animals indicate that 100 ppm of DCE is essentially a "no-effect level" on chronic intermittent exposure, extrapolation to continuous ingestion is tenuous. When considered alone, data generated by the chronic feeding studies of Alumot *et al.* (1976a,b) appear to be the best and most applicable for calculation of a chronic SNARL. They reported "no-effect dietary levels" of 250 and 500 ppm for rats and chickens fed DCE-contaminated diets for 2 years. These investigations included evaluation of indices of growth, blood chemistry, fertility, and reproduction. These data should not serve as the only basis for setting a chronic SNARL since a number of recent reports suggest that DCE may be a mutagen and/or a carcinogen.

Additional long-term oral ingestion studies employing several species of animals are needed to determine if DCE is a carcinogen and, if so, which organs are involved in different species; the nature of uptake, metabolism, and accumulation of DCE and its metabolites; and minimum times and doses of DCE that are required to induce tumors. Populations of workers that have been exposed occupationally to DCE and to 2-chloroethanol might also be screened, as are vinyl chloride workers, for cancer. Vinyl chloride, 2-chloroethanol, and DCE may each be carcinogenic, since they may share a common metabolic pathway. Additional investigations need to be conducted using other indices of toxicity. Attention might be concentrated on histopathology and on sensitive tests to detect cardiac, adrenal, pulmonary, renal, and hepatic injury.

Epichlorohydrin

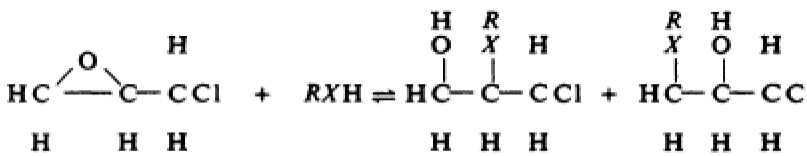


Epichlorohydrin (ECH) is an important industrial chemical that is used frequently as a solvent for resins, gums, cellulose, and paints and, especially, as a raw material for the manufacture of epoxy resins and synthetic glycerine (International Agency for Research on Cancer, 1976; National Institute for Occupational Safety and Health, 1976b; U.S. Environmental Protection Agency, 1977a). In agriculture, it is used as an insect fumigant (Anonymous, 1978). ECH is synthesized commercially from allyl chloride, allyl alcohol, dichlorohydrin-glycerine, or propylene. However, the major route of synthesis is apparently high temperature chlorination of propylene to allyl chloride, which is followed by chlorohydrination with hypochlorous acid to a mixture of isomeric glycerol chlorohydrins. These are subsequently dehydrochlorinated with alkali to yield the technical product (Lichtenwalter and Riesser, 1964). In

1975 the total U.S. production of ECH was about 250 million kg; 325 million kg was produced in 1978. In 1969, an estimated 58% of the ECH was used in the manufacture of synthetic glycerine and 42% was processed to refine ECH. Refined ECH is used in the manufacture of epoxy resins, surface active agents, pharmaceuticals, insecticides, agricultural chemicals, textile chemicals, coatings, adhesives, ion-exchange resins, solvents, plasticizers, glycidol esters, ethinyl-ethylenic alcohol, and fatty acid derivatives. Most epoxy resins are synthesized by alkylating bisphenol A with ECH. The compound is also a proposed intermediate in the metabolism of allyl chloride (Van Duuren, 1977).

ECH is a colorless liquid at room temperature. It has a distinctive odor, which has been described as "ethereal," "chloroform-like," and "garlic-like." The boiling point of ECH is 116°C at 20 mm of mercury. The low latent heat of vaporization of ECH (9,060 cal/mol) contributes to its relatively high volatility. ECH is soluble in water, 6.4% w/w at 20°C (International Agency for Research on Cancer, 1976).

ECH is characterized by two potentially reactive sites: the epoxide ring and the chlorine atom. The presence of the highly strained three membered ring makes ECH a relatively reactive compound. It is hydrolyzed slowly at room temperature, but hydrolysis is greatly accelerated by heat or traces of acid or base. An important feature of the chemistry of ECH is its ability to form compounds containing two functional groups. ECH reacts *in vivo* to form covalent bonds with alcohols, amines, thiols, and other nucleophilic biochemical constituents of the cell. The epoxide ring opens to form a new, stable, covalent carbon-hetero atom bond:



Reactions of epichlorohydrin with cellular nucleophiles (where *X* = an electronegative element such as O, N, or S, and *R* = an alkyl, aryl, or other organic group).

The initial reaction product(s) may undergo a second nucleophilic reaction to form stable, covalent cross-linking bonds between two molecules, either by direct displacement of the chlorine atom or through the formation of a nonstable, short-lived cyclic intermediate (Alexander *et al.,* 1952). These cross-linking bonds may have a high degree of chemical stability, such as that found typically in epoxy resins.

The half-life of ECH in water at pH 7 is about 36.3 hr (National

Institute for Occupational Safety and Health, 1976b). Presumably, ECH is hydrolyzed initially to form 1-chloro-2,3-propanediol (α-chlorohydrin). Neither ECH nor α-chlorohydrin is a strong nucleophile and could be expected to react further (Jones *et al.,* 1969). Reaction of ECH at the less reactive chlorine rather than the epoxide is also known to occur (International Agency for Research on Cancer, 1976).

ECH has a pronounced effect on the organoleptic properties of water. Not only does it impart a specific odor, but it can also be irritating to the mouth. The threshold for odor perception of the compound is 0.5 to 1.0 mg/liter (133 to 265 ppm), while the threshold for its irritant action is below this level at 0.1 mg/liter (26.5 ppm) (U.S. Environmental Protection Agency, 1977a). In light of the toxicity and cumulative effects of ECH, Fedyanina (1968) recommended 0.01 mg/liter as a maximum permissible concentration in reservoir water.

Metabolism

There has been little research on the metabolism of ECH (National Institute for Occupational Safety and Health, 1976b). Apparently, α-chlorohydrin is a hydrolysis product of ECH (Jones *et al.,* 1969; National Institute for Occupational Safety and Health, 1976b). Jones *et al.* (1969) demonstrated the *in-vivo* formation of α-chlorohydrin by hydrolysis of ECH, since Wistar rats dosed orally or intraperitoneally with α-chlorohydrin or ECH yield some of the same urinary metabolites, i.e., 2,3-dihydroxypropyl-S-cysteine and its N-acetate. Since ECH is a strong electrophile that is capable of reacting with cellular nucleophiles, it is probable that some ECH metabolites are also covalently bound to various tissue macromolecules.

While there are few published reports on the metabolism of ECH, the fate of its hydrolysis product, α-chlorohydrin, has been investigated in some detail. Jones (1975) showed that α-chlorohydrin is conjugated with glutathione in the rat and then excreted in the urine as S-(2,3-dihydroxypropyl)cysteine and the corresponding mercapturic acid, N-acetyl-S-(2,3dihydroxypropyl)cysteine. On the basis of the similar antifertility properties of α-chlorohydrin and glycidol (Jackson *et al.,* 1970) and of the results of *in-vivo* and *in-vitro* metabolism experiments (Jones, 1975), α-chlorohydrin is thought to be converted initially *in vivo* to the reactive intermediate glycidol (2,3-epoxypropanol). Glycidol is metabolized by conjugation with glutathione. In a subsequent report, Jones and Murcott (1976) showed that α-chlorohydrin is also oxidatively metabolized to *β*-chlorolactic acid and then to oxalic acid by the rat. In the 24 hr after administration of ^{36}Cl-labeled α-chlorohydrin, 16% of the radioactivity

was also eliminated in the urine as $^{36}Cl^-$. Presumably, this dechlorination occurs when α-chlorohydrin is converted to glycidol.

Health Aspects

Observations in Humans Several reports of industrial exposure to ECH have been documented (National Institute for Occupational Safety and Health, 1976b; U.S. Environmental Protection Agency, 1977a). Eye and throat irritation, nausea, vomiting, headache, and dyspnea were the initial effects after a worker inhaled an unknown amount of ECH. An enlarged liver was reported within 2 days of exposure, and bronchitis and liver damage were present 2 years later. ECH has also been associated with persistent burns following dermal exposure of workers (National Institute for Occupational Safety and Health, 1976b; U.S. Environmental Protection Agency, 1977a).

Several reports (Kucerova and Polivkova, 1976; Kucerova *et al.,* 1976, 1977; Sram *et al.,* 1976) have indicated that ECH has a dose-related mutagenic effect on human lymphocytes. Kilian *et al.* (1978) reported that an unknown substance (presumably a metabolite), which induced mutations in *Salmonella typhimurium,* was present in the urine of individuals who were accidently overexposed to ECH.

The hygienic standard for ECH [8-hr time-weighted average (TWA) in mg/m^3] is 5 in East Germany and 18 in West Germany. In the USSR, the maximum acceptable ceiling concentration is 1 mg/m^3 (Winnell, 1975). The United States recently lowered its standards to a TWA of not greater than 2 mg/m^3 for a 40-hr workweek, with a ceiling concentration of 19 mg/m^3 (National Institute for Occupational Safety and Health, 1976b). Previously, the U.S. standard was a TWA of 19 mg/m^3 (Winnell, 1975).

The National Institute for Occupational Safety and Health (1976b) estimates that 50,000 employees may currently be exposed to ECH in the United States.

Observations in Other Species

Acute Effects Reports of acute oral LD_{50} values for ECH range between 90 and 240 mg/kg for rats, mice, and guinea pigs (Christensen *et al.,* 1976; Lawrence *et al.,* 1972; National Institute for Occupational Safety and Health, 1976b). The acute LD_{50} in mice, rats, guinea pigs, and rabbits after intraperitoneal administration was between 118 and 165 mg/kg. The LD_{50} in rats and mice following intravenous injection was 154 and 178 mg/kg, respectively (Patty, 1963). The subcutaneous LD_{50}

of ECH in the mouse was 720 mg/kg and the dermal toxicity in the rabbit was 1,300 mg/kg (Christensen *et al.,* 1976). The acute toxicity of the ECH metabolite α-chlorohydrin was similar to that of ECH. Its oral LD_{50} for the rat and mouse was 150 and 160 mg/kg, respectively (Christensen *et al.,* 1976).

Because of the volatility of ECH and the many opportunities for frequent industrial exposure to the chemical, considerable work has also been done on its inhalation toxicity. Inhalation exposure of mice to 16,600 and 8,300 ppm ECH for 30 min produced 100% mortality, whereas no deaths were observed in mice that had been exposed to 2,370 ppm for 1 hr (National Institute for Occupational Safety and Health, 1976b). Exposure of rats to 250 ppm ECH for 4 hr killed two to four out of six rats (Carpenter *et al.,* 1949). The lowest published lethal concentration (LCLo) for a 30-min inhalation exposure of mice was 7,400 ppm (Christensen *et al.,* 1976).

Acute exposure to ECH can cause central nervous system depression, irritation of the respiratory tract, profuse nasal discharge, weight loss, leukocytosis, and kidney damage (International Agency for Research on Cancer, 1976; National Institute for Occupational Safety and Health, 1976b). Death generally results from depression of the respiratory center. Nephrotoxicity is a cumulative effect of ECH poisoning, and renal insufficiency occurs within 24-48 hr in approximately 80% of the rats that have been given 125 mg/kg of the compound. ECH produces extreme irritation when tested intradermally, dermally, or intraocularly in rabbits (Lawrence *et al.,* 1972).

Subacute Effects Gage (1959) exposed five groups of eight albino Wistar rats (four males and four females) to ECH vapor at 9, 17, 24, 56, and 120 ppm for 6 hr/day, 5 days/week, for a total of 11 to 19 days. To facilitate comparison of the data the National Institute for Occupational Safety and Health (1976b) calculated the total amount of inhaled ECH from the concentration of ECH in the inhaled air and the duration of exposure. This calculation does not include an estimated percent absorption of the inhaled ECH. All rats inhaling ECH at a concentration of 120 ppm for 11 exposures (total amount inhaled approximately 1,132 mg/kg) experienced labored breathing, profuse nasal discharge, a marked loss of weight, leukocytosis, and an elevation in urinary protein excretion.

Microscopic examination of the kidney and liver showed leukocyte infiltration, atrophy, and necrosis, while the lungs were congested and edematous. Rats that were exposed 8 times to ECH at 56 ppm (total amount inhaled approximately 417 mg/kg) had mild nasal irritation and

some abnormal lung histopathology. At the lowest concentration tested, 17 ppm for 19 days, which is equivalent to an exposure of 227 mg/kg, there were no apparent effects.

Lawrence *et al.* (1972) gave intraperitoneal injections of 11.2 and 22.4 mg/kg/day to groups of 12 Sprague-Dawley rats for 30 days. At the end of the exposure period hemoglobin values were decreased significantly at the high dose, whereas there was a significant increase at the lower dose. Leukocytosis was also observed in the group given the high dose. The kidney: body weight and brain: body weight ratios increased significantly at both ECH doses. Microscopic examination did not reveal any abnormalities in any organs except lungs where lesions were evident in all ECH-exposed groups.

In a second experiment male Sprague-Dawley rats received 0, 11.2, 22.4, and 56.0 mg/kg, injected intraperitoneally on Mondays, Wednesdays, and Fridays for 12 weeks (Lawrence *et al.,* 1972). At the highest dose level there was a significant reduction in body weight, hemoglobin, and hematocrit values. This was accompanied with an increase in the weights of the heart, kidney, and liver, and a decrease in brain weight. The only adverse effect in animals receiving 11.2 mg/kg was a reduced hematocrit.

Grigorowa *et al.* (1974) exposed two groups of 60 male albino rats to ECH at 15.6 or 156 ppm by inhalation for 4 hr/day for 8 days. At the highest exposure level (estimated at 89 mg/kg/day), there was a significant decrease in body weight. They observed no significant adverse effects after exposures to 15.6 ppm (8.9 mg/kg total daily intake). Some of the animals were exposed at 35°C for 45 min on each of the 8 days, but the investigators concluded that heat stress had no effect on the toxicity of ECH.

The toxicological properties of ECH were studied by Fedyanina (1968), who administered daily oral doses of 20 mg/kg to groups of 18 albino rats over 2 months. Body weights of control and exposed animals were the same throughout the treatment. Rats that were exposed to ECH showed decreased numbers of reticulocytes and leukocytes and an altered differential leukocyte count. Blood glutathione levels were increased and were primarily in the oxidized form.

Gage (1959) also exposed two groups of two rabbits to ECH vapor at concentrations of 16 and 35 ppm for approximately 6 hr/day, 5 days/week. At the higher levels, the animals were exposed 20 days (approximately 439 mg/kg total amount inhaled). Inhalation exposure to ECH at 35 ppm produced nasal irritation, but postmortem examination failed to show any abnormalities. The second group of rabbits was exposed for two periods at 16 ppm (total amount inhaled approximately

20 mg/kg), but this exposure was reduced to 9 ppm and continued for 20 more days (approximately 113 mg/kg total amount inhaled). Thus, the second group of rabbits is estimated to have inhaled a total dose of 133 mg/kg. Nasal irritation was observed during the period in which the animals were exposed to 16 ppm; however, following exposure for a longer period to 9 ppm, no adverse effects were detected.

Fedyanina (1968) also administered daily oral doses of 80 mg/kg ECH to 13 rabbits over 2 months. Two rabbits died toward the end of the experiment, and the weight of the experimental rabbits was significantly below that of the control animals. Exposed animals exhibited a reduced leukocyte count, an altered differential count, decreased blood glutathione levels, and various changes in blood chemistry.

Chronic Effects Kremneva and Tolgskaya (1961) exposed a group of 10 rats for 3 hr daily to ECH vapor at 5.2 to 15.6 ppm for up to 6.5 months. This exposure is equivalent to a daily ECH intake of between 2.2 and 6.7 mg/kg/day. No deaths or signs of intoxication were observed in the exposed animals, but the gain in body weight was less than that of controls. Exposed animals also showed evidence of hyperexcitability. The investigators observed no significant variations in the composition of the peripheral blood, but histopathological examination of the tissues showed some abnormal lung pathology.

Fomin (1966) exposed three groups of 15 male albino rats to ECH vapor for 24 hr/day for 98 days at concentrations of 0, 0.05, 0.5, and 5.2 ppm (equivalent to 17.2, 172, and 1,722 mg/kg total inhaled dose). Rats exposed to the highest dosage level, 5.2 ppm (17.6 mg/kg/day), showed reduced weight gains and prolonged latent periods in the motor defense reaction. The number of leukocytes in the blood was increased, and there was an elevated concentration of coproporphyrin in the urine. Gross and microscopic examination disclosed emphysema, bronchopneumonia, edematous areas, and an altered histopathology of the blood vessels in the lungs. Cloudy swelling in the epithelium of the convoluted tubules of the kidney, interstitial hemorrhage in the heart, and severe lesions in the brain were also evident. Animals inhaling ECH at the intermediate concentration of 0.52 ppm (1.76 mg/kg/day) showed leukocytosis and a decrease in blood nucleic acids, while animals exposed to the lowest concentration of ECH, 0.05 ppm (0.176 mg/kg/day), produced no abnormalities.

Fedyanina (1968) administered ECH orally to groups of 15 albino rats at doses of 0, 0.005, 0.05. and 5 mg/kg/day for 6 months. The author then studied the effects of ECH exposure on the conditioned reflexes of the animals using an "accelerated" variant of the motor-alimentary

method. Dose-dependent weakening of stimulation and inhibition processes were seen in animals receiving 5 and 0.05 mg/kg/day. Doses of 0.005 mg/kg/day only diminished the strength of reflexes to light stimulus in the ECH-exposed animals, suggesting that this dose is liminal. However, supplementary studies on the effect of doses of 0.005 and 0.0005 mg/kg/day revealed a decreased synthesis of hippuric acid after loading with sodium benzoate in animals that were treated with 0.005 mg/kg/day. The authors concluded that a daily dose of 0.0005 mg/kg ECH had no adverse effect on the functions that were investigated.

The same investigator (Fedyanina, 1968) also conducted longer studies in rabbits in which groups of 11 rabbits received daily oral doses of 0, 0.005, 0.05, and 0.5 mg/kg of ECH for 6 months. The most pronounced changes in the treated rabbits were a reduced reticulocyte count, reduced blood glutathione levels, and increased albumin in the plasma of animals that had been dosed with 0.5 mg/kg/day. Levels of oxidized and reduced glutathione in blood and ascorbic acid in the kidney and liver were reduced in animals that had received 0.05 mg/kg/day ECH. Rabbits that had been treated with the lowest concentration showed no significant changes.

Reproductive Studies ECH-induced sterility has been reported in male animals. Hahn (1970) gave oral doses of 15 mg/kg of ECH for 12 days (total dose of 180 mg/kg) to adult male rats of demonstrated fertility. Within 1 week males became infertile, but fertility was restored after 1 week of daily dosing when ECH was terminated. Histological examination of the testes, epididymis, prostate, and seminal vesicles showed no difference between treated and control animals.

Jones *et al.* (1969) investigated the antifertility effects of ECH on Wistar rats. A single oral or intraperitoneal dose of 50 mg/kg produced infertility effects resembling those produced by the ECH metabolite, α-chlorohydrin.

Cooper *et al.* (1974) gave oral doses to adult male rats. One group, which received five daily doses of 20 mg/kg (100 mg/kg total), displayed loss of fertility during the first 2 weeks, but recovered completely by the third week. A second group, which received five daily 50 mg/kg doses (total dose 250 mg/kg) in the same manner, was rendered completely sterile throughout a 10-week period. The last group of rats, which received one 100 mg/kg oral dose, experienced sterility within 1 week. By the 12th week following this treatment, permanent sterility had probably occurred.

There is considerable information regarding the antifertility properties

of the ECH metabolite α-chlorohydrin, which is an orally active, reversible male antifertility agent in the rat, hamster, guinea pig, ram, boar, and monkey (Brown-Woodman *et al.,* 1974;Cooper *et al.,* 1974; Coppola, 1969; Dixit *et al.,* 1974; Ericsson, 1970; Ericsson and Baker, 1970; Ericsson and Norland, 1970; Ericsson and Youngdale, 1970; Johnson and Pursel, 1973). However, in the rhesus monkey chronic administration of α-chlorohydrin also produces damage to bone marrow (Kirton *et al.,* 1970). The minimal effective daily oral dose of α-chlorohydrin in the male rat that is necessary to prevent fertilization in the female following mating was 2.5 mg/kg/day (Vickery *et al.,* 1974).

α-Chlorohydrin has two effects on the reproductive tract of the male rat. Five consecutive 10 mg/kg daily doses cause immediate and reversible infertility by inhibiting sperm glycolysis. Conversion of α-chlorohydrin to its 1-phosphate ester within the spermatozoa and inhibition of glycolysis by this ester through inactivation of phosphoglyceraldehyde dehydrogenase has been suggested as the cause of antifertility action of α-chlorohydrin (Mohri *et al.,* 1975). The low-dose effect may also involve the epoxide metabolite of α-chlorohydrin, glycidol (Jackson *et al.,* 1970).

A single high oral dose (100 mg/kg) of α-chlorohydrin in the rat produces epididymal lesions or spermatoceles (Cooper *et al.,* 1974). These lesions occlude the ductuli efferentes, blocking the passage of testicular sperm and producing prolonged or even permanent infertility. Jones and Murcott (1976) have proposed that this and observed renal toxicity are due to the *in-vivo* oxidation of *α*-chlorohydrin to *ß*-chlorolactic acid and, ultimately, oxalic acid.

Jackson and Robinson (1976) found that the R(-)isomer of α-chlorohydrin exhibited neither reversible antifertility activity nor permanent sterilizing capacity, but the toxicity of the R(-)isomer was higher than that of the racemic mixture DL-α-chlorohydrin. Conversely, the S(+)isomer of α-chlorohydrin has almost twice the antifertility activity of the racemic mixture and a reduced toxicity (Jackson *et al.,* 1977).

Mutagenicity ECH is considered a potent mutagen and its mutagenic properties have been the object of numerous investigations (Fishbein, 1976; National Institute for Occupational Safety and Health, 1976b; U.S. Environmental Protection Agency, 1977a.) One of the earliest studies on the mutagenicity of ECH was undertaken by Rapoport (1948) who reported that it induced 0.7% mutations in *Drosophila melanogaster.* The mutagenicity of ECH was subsequently shown in *Escherichia coli* (Strauss and Okubo, 1960), in *Klebsiella pneumoniae* (Voogd, 1973), and in plants (Loveless, 1951). A 150 mg/kg intraperitoneal dose did not

induce dominant lethal mutations in ICR/Ha Swiss mice (Epstein *et al.,* 1972).

Mukai and Hawryluk (1973) found that ECH induced a 20-fold increase in revertants over controls in both *E. coli* and *S. typhimurium.* In studies aimed primarily at testing the mutagenicity of vinyl chloride and its metabolites, Elmore *et al.* (1976) examined the mutagenicity of ECH since it is a methylene homolog of the chloroxirane metabolite of vinyl chloride. Tests with *S. typhimurium* strain TA-100 (a base-pair substitution mutant) showed that ECH was highly mutagenic. These and subsequent tests were conducted in the absence of a mammalian S-9 fraction. However, ECH was less active than its congener, the chloroxirane metabolite of vinyl chloride. In contrast, the chemical did not inhibit a recombination repair of a deficient strain of *Bacillus subtilis* (strain MC-1), whereas the chloroxirane compound was highly active. These workers suggested that ECH produced a different type of DNA lesion than did the chloroxirane compound. Similar studies by Sram *et al.* (1976) demonstrated high mutagenic activity of ECH in *S. typhimuri*um tester strains G-46 and TA-100 at exposures of 1 to 50 mM/hr. Three hours after doses of 50 and 100 mg/kg, ECH was found to increase the frequency of back mutations using *Salmonella* strains G-46, TA-100, and TA-1950 in a host-mediated assay.

ECH induced chromosome abnormalities in the bone marrow of mice when injected intraperitoneally in single doses ranging from 1 to 50 mg/kg or in five doses of 5 to 20 mg/kg, and when given orally in single doses ranging from 5 to 100 mg/kg or in five doses of 20 mg/kg (Sram *et al.,* 1976). The frequency of chromosome change was dose-dependent. ECH did not induce dominant lethal mutations in mice when injected intraperitoneally in doses ranging from 5 to 40 mg/kg or in five doses of 1 to 10 mg/kg, and when given orally in single doses ranging from 20 to 40 mg/kg or in five doses of 4 to 20 mg/kg.

Human peripheral lymphocytes that were exposed to 10^{-11} to 10^{-4} M ECH *in vitro* for 24 hr produced chromosomal abnormalities (Kucerova and Polivkova, 1976; Kucerova *et al.,* 1976; Sram *et al.,* 1976), but ECH was 4 to 5 times less mutagenic than the polyfunctional alkylating agent triethylenephosphoramide (TEPA) when tested in this test system at similar concentrations. The chromosomal changes were dose-dependent, and the most common type of aberration produced by ECH were chromatid breaks, followed by chromosomal breaks. Chromatid exchanges were rare and chromosomal exchanges extremely rare. A subsequent report by this group (Kucerova *et al.,* 1977) summarizes a prospective cytogenic study on 35 workers who had been occupationally

exposed to ECH. Blood samples were obtained from them after the first and second year of exposure and cultivated for 56 to 58 hr. The percentage of cells with chromosomal aberrations in these blood samples was 1.37 before exposure, 1.91 after the first year of exposure, and 2.69 after the second year of exposure. These differences were highly significant at $P < 0.0001$. Particularly frequent were chomatid and chromosomal breaks after exposure.

Kilian *et al.* (1978) noted that an unknown substance (presumably a metabolite) that can induce mutations in *S. typhimurium* was present in the urine of individuals who were accidentally overexposed to ECH. Mutagenic activity was also detected in the urine in mice after oral administration of 200 to 400 mg/kg ECH. The reactive constitutent in urine is likely to be α-chlorohydrin, which is a product of hydrolysis of ECH (National Institute for Occupational Safety and Health, 1976b). α-Chlorohydrin is a highly reactive electrophile that is capable of forming conjugates with sulfhydryl compounds *in vivo* (Jones *et al.,* 1969), and, as is the case with ECH, also has potent male antifertility activity (Cooper *et al.,* 1974; Dixit *et al.,* 1974; Vickery *et al.,* 1974).

Sram *et al.* (1976) estimated the genetic risk to humans from occupational exposure to ECH. The expected exposure to ECH for workers in the plastics industry was estimated to be approximately 7.1 mg/kg/year. In their evaluations of individual genetic risk of humans, based on their measurements of mutagenic activity, the investigators concluded that the 7.1 mg/kg yearly dose of ECH would result in detectable level of genetic injury.

α-Chlorohydrin is an alkylating agent *in vitro* (Jones *et al.,* 1969), but, like ECH (Sram *et al.,* 1976), it failed to produce dominant lethal mutations (Jones *et al.,* 1969).

Carcinogenicity Kotin and Falk (1963) reported that a single subcutaneous injection of ECH (0.5 mg) resulted in one skin papilloma, a single hepatoma, two lung adenomas (in one mouse), and four malignant lymphomas in a population of 30 mice. However, the control group also developed a number of hepatomas, lung adenomas, and lymphomas, but no skin papillomas. Therefore, results of this study were somewhat inconclusive.

Weil *et al.* (1963) reported that although skin painting with undiluted ECH (3 times/week for 25 months) produced no tumors in 40 mice, ECH was the most systematically toxic of 60 epoxy compounds that were tested. Van Duuren *et al.* (1972, 1974) investigated the effect of dermal application, subcutaneous injection, and intraperitoneal injection

of ECH on female mice. A 580-day skin painting study (2 mg of ECH applied 3 times/week to 50 mice) produced no papillomas or carcinomas. Another group of 50 mice received one skin application of 2.0 mg followed 2 weeks later with thrice-weekly applications of phorbol myristate acetate (PMA), a tumor promoter, for the duration of the study (385 days). Nine mice developed papillomas, and one developed a carcinoma. Three of 30 mice in a control group that received PMA alone developed papillomas. The subcutaneous injection of ECH (1.0 mg) into 50 mice weekly over 580 days resulted in the development of sarcomas in six mice and an adenocarcinoma in one. In the intraperitoneal experiment, 30 mice received weekly injections of 1.0 mg into the lower abdomen over 450 days. None developed local sarcomas, but 11 mice had papillary lung tumors.

On the basis of these studies, the International Agency for Research on Cancer (1976) believes that ECH is "carcinogenic in mice by subcutaneous injection" and that it is "active as an initiator in a twostage skin carcinogenesis study in mice." The lack of long-term oral ingestion or inhalation studies is a serious deficiency in any analysis of the carcinogenicity of ECH. Nevertheless, the experiments that have been cited do raise some concern about the risks of continuous exposure, especially in the workplace.

Nelson (1977, personal communication) reported the preliminary results of several long-term inhalation studies with ECH. In the first experiment, rats received 30 exposures of 6 hr each to 100 ppm ECH. Twenty-eight of the initial 40 rats had died by the time the preliminary results were released. Three of them had developed squamous cell cancers of the nasal epithelium, and a fourth had a squamous cell papilloma. A second experiment, started after the first, investigated the effect of chronic exposure to 100, 30, and 10 ppm ECH. At the time of Nelson's report, 2 of the 12 rats that were still alive in the 100-ppm group had developed nasal masses that resembled those observed during life in animals with subsequent histopathologically confirmed cancer. Nelson placed the results in perspective when he noted that the "occurrence of these tumors in relatively small numbers should be viewed against the background of experience of this laboratory in which thousands of rats had been used in inhalation studies without the observation of a single squamous cell carcinoma of the nose in control animals." Nelson's preliminary conclusion was that "ECH must be regarded as carcinogenic for the nasal epithelium of the rat."

Teratogenicity The subcommittee located no teratogenic studies with ECH (National Institute for Occupational Safety and Health, 1976b).

Conclusions and Recommendations

ECH is a highly reactive alkylating agent that has been shown to be mutagenic and carcinogenic in a variety of test systems. Although ECH is moderately toxic upon acute oral exposure, having LD_{50} values ranging between 90 and 240 mg/kg, subacute or chronic exposure to this chemical produces a variety of other toxic effects, depending on the route of exposure. Tissue damage occurs primarily in the lungs after inhalation exposure, but ECH that is absorbed via the lungs or from the gastrointestinal tract also elicits blood abnormalities including anemia, leucocytosis or leukemia, liver and kidney damage, and antifertility effects in male animals (Fomin, 1966; Lawrence *et al.,* 1972; National Institute for Occupational Safety and Health, 1976b). ECH is also a potent mutagen (Fishbein, 1976; Sram *et al.,* 1976) and carcinogen (International Agency for Research on Cancer, 1976; Nelson, 1977, personal communication). Because of the well-established carcinogenic and mutagenic properties of the compound, no long-term exposure estimate has been made.

Suggested No-Adverse-Response level (SNARL) Data that are suitable for use in estimating a 1- or 7-day no-effect exposure level for ECH are very sparse. Nevertheless, some attempts at such calculations have been made.

Twenty-Four-Hour Exposure Lawrence *et al.* (1972) observed a significant increase in pentobarbital-induced sleep in ICR mice that had been given a single intraperitoneal 17 mg/kg dose of ECH. Since intraperitoneal and oral LD_{50} values for ECH in the same strain of mice were 170 and 236 mg/kg, respectively, a comparable increase in pentobarbital-induced sleep would probably have occurred with an oral dose of 24 mg/kg. Using an uncertainty factor of 1,000 and assuming that all of the ECH intake is from 2 liters/day consumption of water during this period, the following calculation can be made to yield a suggested no-adverse-effect level (SNARL) in drinking water of 0.84 mg/liter for a 1-day exposure to ECH:

$$\frac{24 \text{ mg/kg} \times 70 \text{ kg}}{1{,}000 \times 2 \text{ liters}} = 0.84 \text{ mg/liter.}$$

Seven-Day Exposure Hahn (1970) found that male rats given daily 15 mg/kg oral doses of ECH became infertile after 1 week of treatment. Using an uncertainty factor of 1,000 and assuming that all of the ECH is

ingested from 2 liters/day of drinking water during this period, the following calculation can be made to yield a suggested no-adverse-effect level for drinking water of 0.53 mg/liter for a 7-day exposure to ECH:

$$\frac{15 \text{ mg/kg} \times 70 \text{ kg}}{1{,}000 \times 2 \text{ liters}} = 0.53 \text{ mg/liter.}$$

An almost identical estimate (0.46 mg/liter) may be obtained using the results of Gage (1959), who observed no significant adverse effects in rats receiving inhalation exposure to ECH at 13 mg/kg/day (National Institute for Occupational Safety and Health, 1976b).

The calculated 7-day and 1-day SNARL's in drinking water are only estimates that completely ignore the well-established mutagenic and suspected carcinogenic properties of ECH.

Most of the long-term exposure data on ECH are derived from inhalation experiments. Since estimation of long-term risk to humans from ECH in drinking water makes much use of such inhalation data, there is a pressing need for conducting subacute and chronic experiments in animals that have been orally exposed to ECH. Only then can assumptions used to extend inhalation results be validated.

Methylene Chloride

(CH_2Cl_2)

This compound was evaluated in *Drinking Water and Health* (National Academy of Sciences, 1977, p. 743). The following material, which became available after that 1977 publication, updates and, in some instances, reevaluates the information in the earlier report. Also included are some references that were not assessed in the original report.

Metabolism

Exposure to methylene chloride produces an elevation of blood carboxyhemoglobin levels in humans (Stewart *et al.,* 1972a,b), rabbits (Roth *et al.,* 1975), and rats (Rodkey and Collison, 1977). Biological conversion of methylene chloride to carbon monoxide has now been well established (Rodkey and Collison, 1977). DiVincenzo and Hamilton (1975) administered 412 to 930 mg/kg [^{14}C]-methylene chloride intraperitoneally in corn oil to Sprague-Dawley rats. Methylene chloride was largely eliminated unchanged in the expired air during the first 2 hr. After 24 hr, only 2% of the original dose remained in the body. This 2% was found mostly in the liver, kidneys, and adrenal glands. Rodkey and Collison (1977) administered methylene chloride by vaporization in a

closed rebreathing system to rats. Addition of methylene chloride to the gas phase caused an initial increase in carbon monoxide production to about 35 times the normal endogenous rate at all doses given (80-800 μM/kg). The amount of carbon monoxide that accumulated approached 50% of the dose of methylene chloride that was administered. The data suggest that a low dose of methylene chloride causes substrate saturation of the enzyme system involved in carbon monoxide production. Large doses of methylene chloride caused a progressive increase in the rate of carbon monoxide production over several hours, suggesting that substrate-induced enzyme formation occurs. The elevated carboxyhemoglobin levels resulting from methylene chloride exposure have a biological half-life that is twice that of the carboxyhemoglobin produced from exposure to carbon monoxide, because the absorbed methylene chloride is released slowly from storage sites in the body tissue.

The addition of methanol to paint remover formulations appears to extend the biological half-life of carboxyhemoglobin derived from methylene chloride. Elevation of carboxyhemoglobin, which is caused by inhalation of methylene chloride, was reduced significantly in rabbits pretreated with carbon tetrachloride (Roth *et al.,* 1975). The biological significance of carboxyhemoglobin levels derived from methylene chloride has not been fully defined. Stewart and Hake (1976) reported that blood carboxyhemoglobin elevations induced by methylene chloride persisted longer than when similar levels of carboxyhemoglobin were induced by breathing carbon monoxide.

The literature shows that there is a wide variation in concentrations of carboxyhemoglobin in persons that have been exposed to a given concentration of methylene chloride for a given period and a lack of consistent correlation in measurement for calculation of carboxyhemoglobin. This apparent lack of correlation may have originated in the variations in rate of carbon monoxide formation and the existence of other carboxyhemoglobin-forming events, such as smoking, and/or individual differences in carboxyhemoglobin-eliminating events, including pulmonary function.

Health Aspects

Observations in Humans Raleigh (1974) studied 562 workers, 103 of whom comprised the highest exposure group, having been exposed to methylene chloride at 50 to 100 ppm. There was no increase in the incidence of cardiovascular, gastrointestinal (including liver), genitourinary, or central nervous system disease in the exposed groups, as compared with a nonexposed worker population.

Winneke and Foder (1976) reported that the central nervous system function was impaired during methylene chloride exposure. Acute exposure to 500 ppm produced a state of reduced activity, which was accompanied by short periods of sleep. The effects of exposure to a paint remover (80% methylene chloride, 20% methanol by weight) during rest and exercise were reported by Stewart and Hake (1976). No untoward responses occurred during the 24-hr period following each exposure. None of the four subjects in this study found the paint remover vapor to be irritating to the eyes, nose, or throat. Nor were there observed abnormalities in electrocardiogram or blood chemistry values; however, the above-cited authors and others have commented on the potential risks of elevated carboxyhemoglobin levels to those individuals with preexisting cardiovascular stress (angina, myocardial ischemia, etc.). The difficulty in extrapolating from exposure levels of methylene chloride to symptoms is reminiscent of the occasional poor correlation between symptoms of carbon monoxide toxicity and carboxyhemoglobin levels.

Langehennig *et al.* (1976) reported an apparent paradox between greater than 20% carboxyhemoglobin levels resulting from methylene chloride exposure and lack of symptoms. A recent report by Goldbaum *et al.* (1976), which showed that administered carboxyhemoglobin does not produce manifestations of carbon monoxide toxicity, indicates that carboxyhemoglobin levels may not fully define the toxicity of methylene chloride.

Roberts and Marshall (1976) reported a case involving the ingestion of two pints of a paint remover that contained methylene chloride, methanol, cellulose acetate, triethanolamine, paraffin wax, and detergent. No carboxyhemoglobin levels were measured. The major acute features of the intoxication included hemolysis, hemorrhage of the gastrointestinal tract, and metabolic activation. They found no hepatic, renal, or cardiac injury.

The National Institute for Occupational Safety and Health (1976d) recommended that occupational exposure to methylene chloride not exceed 75 ppm, a time-weighted average exposure for up to a 10-hr workday.

Observations in Other Species

Acute Effects The acute oral LD_{50} values are 1.6 to 2.3 ml/kg for rats (Kimura *et al.,* 1971). In these same studies, Kimura *et al.* determined the approximate dose that induces the first observable signs of toxic actions (i.e., dyspnea, ataxia, cyanosis, and/or coma). For adult rats the oral dose was 0.001 ml/kg. The intraperitoneal LD_{50} values are 1.50 ml/kg

for mice and 0.95 ml/kg for dogs (Klaassen and Plaa, 1967). The toxic hazard to the eye from methylene chloride as liquid or vapor was assessed in rabbits by Ballantyne *et al.* (1976). They observed a small transient increase in corneal thickness and intraocular tension along with evidence of inflammation of the conjunctiva. The concentrations required for these effects were equivalent to a splash contamination of the eye with 0.01 ml of methylene chloride.

Chronic Effects In a chronic study, Bornmann and Loeser (1967) reported no adverse effects in rats that had been maintained on drinking water containing methylene chloride at 2.25 g/18 liters for 91 days. In a study on the effects of methylene chloride inhalation, Heppel *et al.* (1944) reported no adverse effects on dogs and rabbits in a 6-month exposure to 5,000 ppm; however, a slight weight reduction was observed in guinea pigs. Some liver injury was found after 7.5 weeks at 10,000 ppm. Studies by Balmer *et al.* (1976), involving exposure of guinea pigs for 5 days to approximately 500 ppm of methylene chloride plus high concentrations of ethanol, suggest that ethanol may potentiate the effects of methylene chloride in the liver (i.e., fatty livers).

Mutagenicity Methylene chloride was negative in a *Drosophila* mutagenicity test (Filippova *et al.,* 1967) but quite mutagenic in the *Salmonella typhimurium* (TA-100 without S-9 mix) assay (Simmon *et al.,* 1978).

Carcinogenicity Pulmonary tumor response in strain A mice was negative with doses of 160 to 800 mg/kg, which were given 16 to 17 times (Theiss *et al.,* 1977).

Teratogenicity Methylene chloride vapor was not teratogenic in rats and mice at 1,250 ppm (Schwetz *et al.,* 1975). Both species were exposed daily for 7 hr on days 6 through 15 of gestation. The investigators found no fetal toxicity or teratogenicity, although there was an increase in the incidence of variation in the development of the sternum.

Conclusions and Recommendations

Suggested No-Adverse-Response Level (SNARL)

Twenty-Four-Hour Exposure Data on oral dosing of animals indicate LD_{50} values ranging between 1.6 and 2.3 ml/kg, which is similar to the intraperitoneal LD_{50} values of 0.9 to 1.5 ml/kg. The cause of death in

these studies is not clear. The minimal-effect dose in rats is 1 ml/kg. The water solubility of methylene chloride is approximately 2 ml/dl, which means that humans could theoretically consume a lethal dose in an acute spill situation. Data on a no-effect oral dose do not exist. Using the minimal-effect acute oral dose for the rat (1 ml/kg), assuming 2 liters/day of drinking water as the only source, and employing a safety factor of 1,000:

$$\frac{1.3 \text{ g/kg} \times 70 \text{ kg}}{1{,}000 \times 2 \text{ liters}} = 35 \text{ mg/liter.}$$

Seven-Day Exposure No data are available for calculation. Using the acute 24-hr level of 35 mg/liter and dividing by 7 days:

$$\frac{35 \text{ mg/liter}}{7} = 5 \text{ mg/liter.}$$

Chronic Exposure This cannot be calculated due to a lack of adequate chronic (lifetime) exposure data.

Polychlorinated Biphenyls

($Cl_{2\text{-}5}H_{0\text{-}3}C_6\text{-}C_6H_{0\text{-}3}Cl_{2\text{-}5}$)

These compounds were evaluated in *Drinking Water and Health* (National Academy of Sciences, 1977, p. 756). The following material, which became available after that 1977 publication, updates and, in some instances, reevaluates information in the earlier report. Also included are some references that were not assessed in the original report.

Polychlorinated biphenyls (PCB's) are a mixture of chlorinated biphenyls that have been produced commercially by the chlorination of biphenyl. PCB's have important heat-resistant properties and have been used in the production of capacitors and transformers. Commercially prepared PCB mixtures often contain between 40 and 60 different chlorinated biphenyls (Aroclors). PCB's are well-known environmental contaminants, and the actual number of chlorinated biphenyls reaching the environment probably is nearly 100 (Pomerantz *et al.,* 1978). The chemistry of these materials has been reviewed by Hutzinger *et al.* (1974) and Pomerantz *et al.* (1978).

The environmental fate and occurrence of the PCB's are functions of their water solubility, volatility, bioaccumulation, photostability, and biodegradability. Some PCB mixtures are known to be contaminated with varying amounts of chlorinated naphthalenes and chlorinated

dibenzofurans. These substances have potent biological actions of their own, which may confound the observed toxicological properties of PCB mixtures.

In addition to their occurrence in surface water, PCB's have been detected in U.S. drinking water where their concentration is limited by their solubility (Deinzer *et al.,* 1978).

Metabolism

The biological and toxicological properties of PCB mixtures may vary, depending upon their isomeric composition. The metabolism and biochemical toxicity of the PCB's have recently been reviewed by Matthews *et al.* (1978). Radio-labeled chlorobiphenyls with increasing chlorine numbers have a corresponding increase in biological half-life in mammals. The rate of elimination of the chlorobiphenyls is related to the extent of metabolism (Matthews and Anderson, 1975). The lipophilicity of the chlorobiphenyls increases with increasing chlorine content and also is a factor in their biological disposition.

The degradation and elimination of PCB congeners depend upon the hepatic microsomal enzyme system. Two possible mechanisms for biotransformation have been suggested by Ecobichon (1976). The first and most rapid mechanism involves the formation of an arene oxide intermediate and requires the presence of unsubstituted adjacent carbon atoms in the nucleus. The second and much slower mechanism uses a different hydroxylation system for isolated unsubstituted positions, such as those found in highly chlorinated biphenyls. Two adjacent unsubstituted carbon atoms appear to be important in metabolism. Their presence facilitates the formation of arene oxides by the hepatic mixed-function oxidases.

Single-dose oral administration of PCB's to rodents, monkeys, swine, and sheep has indicated that intestinal absorption is rapid and approximately 90% complete (Albro and Fishbein, 1972; Allen and Norback, 1973; Berlin *et al.,* 1973; Borchard *et al.,* 1975). The feces provide the major route of excretion. Only traces of PCB's could be found in the urine of animals.

In mice, a single oral dose of [^{14}C]-pentachlorobiphenyl rapidly entered the circulation and was distributed to the liver, kidneys, lungs, and adrenals. Within 24 hr, radioactivity was redistributed to the fat. which remained the major reservoir of unchanged PCB's in the body until only traces remained after 32 days (Berlin *et al.,* 1975a,b).

Polychlorinated biphenyls are strong inducers of hepatic mixed-function oxidase enzymes. The potency increases with increasing

chlorination of the biphenyl rings. The enzyme-inducing properties of the PCB's are unusual in that the mixtures share the inducing properties of both phenobarbital and 3-methylcholanthrene (3MC) (Alvares *et al.,* 1973). Phenobarbital induction is characterized by an increase in cytochrome P450 and an increase in a wide range of enzyme activities, while 3MC induces a more specific range of enzyme activities and cytochrome P_1-450 (P-448), a cytochrome differing in several physical and chemical properties from cytochrome P-450.

These effects of the PCB's can modify the toxicity of other agents, suggesting that secondary toxication or detoxication reactions that occur in PCB-exposed animals will be a function of the level of exposure, the nature of the secondary agent, and the mechanisms by which the toxicity is produced.

Health Aspects

Observations in Humans PCB's are liver toxins and produce chloracne and, possibly, peripheral neuropathy in humans (Murai and Kuroiwa, 1971). Humans may be exposed to PCB's from occupational exposure, dietary exposure, or exposure to air or water containing PCB's. Cordle *et al.* (1978) have reviewed exposures of humans to PCB's and the reported effects.

Among the diseases that are attributed to PCB's is Yusho, an acute outbreak of which occurred in Japan in 1972. This disease has been ascribed to the ingestion of rice oil containing PCB's although Kuratsune *et al.* (1976) suggest that the rice oil was contaminated with polychlorinated dibenzofurans, which may have played a significant role in the observed toxicity. Consequently, the human health effects that occurred in the Yusho incident cannot be attributed entirely to the PCB's (Cordle *et al.,* 1978).

Recent surveys have indicated that PCB's can be found in the milk of nursing mothers. The highest reported level was 10.6 ppm. The mean for all samples was 1.8 ppm (U.S. Environmental Protection Agency, 1976).

Observations in Other Species

Acute Effects PCB's have low acute toxicity. Acute oral LD_{50} values in rats, rabbits, and mice range from 1 to 10 g/kg of body weight. Kimbrough *et al.* (1978) compiled LD_{50} values for a variety of PCB mixtures and pure isomers. In addition to the well-known effects on mixed-function oxidase enzymes, high doses of the PCB's may cause enlargement of the liver and liver cells. Other commonly reported effects

include decreased reproductive function, renal histopathological change, hepatic porphyria, and an increase in bile flow with a concomitant increase in biliary excretion of certain materials such as thyroxine, immunosuppression, thymus atrophy, and lymphopenia.

Subchronic and Chronic Effects Because of the bioaccumulation of PCB's, subchronic and chronic effects of these agents are of more concern than acute toxicity.

Tucker and Crabtree (1970) reported deaths in rats fed Aroclor at 1 g/kg for 28 to 53 days. Rabbits were given repeated daily oral doses of 300 mg of Aroclor 1221 and 1254 for 14 weeks. Aroclor 1254 produced liver enlargement and damage and one death. Aroclor 1221 produced only minor changes (Koller and Zinkl, 1973). Allen *et al.* (1974) administered doses of 25 mg/kg Aroclor 1248 in the diet of six rhesus monkeys for 2 months. This resulted in facial edema, loss of hair, and acne 1 month after onset of feeding. Aulerich *et al.* (1973) produced 100% mortality in mink within 6 months by feeding them diets containing PCB at 30 mg/kg (10 mg/kg each of Aroclor 1242, 1248, and 1254). In a study by Ringer *et al.* (1972), female mink that were fed a diet supplemented with Aroclor 1254 in 5 mg/kg doses for 9 months failed to produce offspring.

The oral administration to rats of Aroclor 1242, 1254, and 1260 at 1, 10, and 100 mg/kg for 18 months (Keplinger *et al.,* 1971) produced adverse effects only at the higher dosage. Aroclor 1242 and 1254 caused an increase in liver weight and a reduction in litter survival at 100 mg/kg. Kimbrough *et al.* (1972) reported experiments in which male rats survived Aroclor 1260 at 1 g/kg for 8 months, but 8 of 10 females died at this dosage. With both Aroclor 1254 and 1260, there was a significant dose-dependent increase in liver weight in male rats produced by doses as low as 20 mg/kg in the diet; in female rats, liver enlargement occurred only at 500 mg/kg and higher.

The dietary ingestion of 100 ppm of Aroclor 1248, 1254, or 1262 for 52 weeks by male Sprague-Dawley rats produced no effect on gross appearance or weight gain (Allen *et al.,* 1976). However, there was an increase in total serum lipids and cholesterol and a transient increase in triglycerides. These changes were accompanied by morphologic alterations in the liver such as generalized liver hypertrophy and focal areas of hepatocellular degeneration.

Zinkl (1977) produced varying degrees of dermatitis involving the ears and, later, the nose, tail, and feet of rats by feeding them continuously with Aroclor 1254. This lesion was found in 15 of 60 animals fed 100 ppm of the PCB mixture for 10 to 20 weeks. Oral administration of

Aroclor 1248 at 2.5 and 5.0 mg/kg produced periorbital edema, alopecia, erythema, and acneiform eruptions within 1 to 2 months (Allen, 1975; Allen and Norback, 1973; Allen *et al.,* 1974).

The feeding of Aroclor 1242 or 1254 to weanling swine and sheep at 20 ppm decreased food efficiency and rate of weight gain over 91 to 105 days of exposure. However, gross and/or microscopic lesions were minor (Hanson *et al.,* 1976).

Mutagenicity Aroclor 1242 and 1254 administered orally to Osborne Mendel rats in five daily doses of 125 or 250 mg/kg and 75, 150, or 300 mg/kg, respectively, failed to produce dominant lethal effects. Treated rats were mated with untreated females for 10 to 11 weeks following treatment (Green *et al.,* 1975a). Furthermore, Aroclor 1242 and 1254 failed to produce cytogenetic effects in bone marrow and spermatogonium cells of rats (Garthoff *et al.,* 1977; Green *et al.,* 1975b). Aroclor 1232, 1254, and 1268 were tested for their ability to inhibit testicular DNA synthesis in mice. These compounds were found to stimulate incorporation of tritiated thymidine into testicular DNA following a single intraperitoneal dose of 500 mg/kg. These changes were not considered significant evidence of an adverse effect (Seiler, 1977). Furthermore, administration of Aroclor 1254 at a dose of 50 mg/kg daily for 7 days failed to cause any significant chromosomal damage or alteration in spermatogenesis for up to 30 days following exposure (Dikshith *et al.,* 1975).

Carcinogenicity In a recent review of a number of carcinogenicity studies in which mice and rats had been fed various mixtures of PCB's, Kimbrough *et al.* (1978) described liver tumors produced by Aroclor 1254 (Calandra, 1975; Kimbrough and Linder, 1974). Kimbrough *et al* (1975) claimed that Aroclor 1260 produced hepatocellular carcinomas in rats. Tumor production, as noted in these studies, was the result of feeding dietary levels of 100 ppm or more.

The possible carcinogenicity of Aroclor 1254 was tested in Fischer 344 rats that received diets containing 25, 50, or 100 ppm for 104 to 105 weeks. Twenty-four rats of each sex received each diet, and matched controls consisted of 24 males and 24 females. After 2 years of exposure to PCB's, the treated rats showed a trend toward an increased incidence of lymphomas and leukemias, but the incidence was not statistically different from controls. Some treated animals were found to have hepatocellular adenomas and carcinomas, but these changes were not seen in controls. A high incidence of hyperplastic nodules was also observed in the animals that had been exposed to Aroclor 1254.

Hyperplastic nodules are regarded by many to be preneoplastic. The incidences of tumors were not significant. Consequently, the investigators concluded that Aroclor 1254 was not carcinogenic in Fischer 344 rats; however, the high incidence of hepatocellular proliferative lesions was believed to be related to administration of the chemical (National Cancer Institute, 1978).

Teratogenicity Several studies have demonstrated that PCB's can cross the placenta, but no studies have detected defects of embryo-fetal development (Curley *et al.,* 1973; Kato *et al.,* 1972). In contrast, other studies have reported that the placenta acts as an effective barrier to the transfer of PCB's (Baker *et al.,* 1977). In addition, Funatsu *et al.* (1972) and Miller (1971) have linked maternal ingestion of PCB with darkbrown staining of the skin of newborn babies.

PCB's have no known or clearly defined teratogenic effects in mammals although the transfer of PCB's across the placenta suggests the potential for some form of fetal toxicity (Kimbrough *et al.,* 1978).

Conclusions and Recommendations

Suggested No-Adverse-Response Level (SNARL)

Twenty-Four-Hour Exposure Because the PCB's are a complex mixture of isomers and impurities having various biological activities and environmental fates, it is risky to suggest no-adverse-response limits. However, one of the most sensitive indicators of exposure to the PCB's is the induction of the mixed-function oxidase enzymes of the liver of mammals. Using this effect, the minimal no-adverse-response to Aroclor 1254 in rats is 2 to 5 ppm in the diet, which is the apparent threshold for these effects (Grant *et al.,* 1974). Moreover, as little as 1 to 2 mg/kg of Aroclor 1254, given within 24 hr, is sufficient to stimulate mixed-function oxidase enzymes in rats (Bruckner *et al.,* 1977). If this end point is used as an indicator of minimal toxicity, the following 24-hr SNARL value may be calculated for the PCB class (without reference to any particular isomer or contaminant). Assuming that 100% of exposure is from water during this period and that a 70-kg human consumes 2 liters of water per day, and using an uncertainty factor of 100:

$$\frac{1 \text{ mg/kg} \times 70 \text{ kg}}{100 \times 2 \text{ liters}} = 0.35 \text{ mg/liter.}$$

The uncertainty factor of 100 was chosen because of the large number of studies that substantiate the effect and dosage described above.

Seven-Day Exposure Using the same argument, a 7-day SNARL may be calculated:

$$\frac{\text{24-hr SNARL}}{7} = \frac{0.35\ \text{mg/liter}}{7} = 0.05\ \text{mg/liter.}$$

Chronic Exposure A reliable chronic SNARL may not be calculated for the PCB's for the reasons noted above and also because certain PCB's are suspected carcinogens.

Although there are considerable data on the toxicity of mixtures of PCB's, there is a paucity of data on the pure congeners that are present in these mixtures. Whether chronic toxicity is related to the metabolism of the PCB's remains to be determined. Considerably more attention must be directed to the detection of impurities in PCB's at very low concentrations. Polychlorodibenzofurans may constitute only one of several significant contaminating compounds that are responsible for PCB toxicity. Populations at special risk—both the industrially exposed and those heavily exposed by the ingestion of contaminated foods—should be evaluated carefully.

Despite the lack of evidence in the United States that dietary PCB's have any deleterious effects on health, there is a growing concern about the long-range effects of the contamination of our ecosystem with these chemicals. There is an urgent need for epidemiological studies of exposed populations, more precise identification of all sources of PCB contamination, and efforts directed at the control of disposal of PCB's. Because of the demonstrated carcinogenic potential, studies on individual congeners—both those metabolized and those stored by humans-are urgently needed.

Tetrachloroethylene

($Cl_2C = CCl_2$)

This compound was evaluated in *Drinking Water and Health* (National Academy of Sciences, 1977, p. 769). The following material, which became available after that 1977 publication, updates and, in some instances, reevaluates information in the earlier report. Also included are some references that were not assessed in the original report.

Metabolism

The pharmacokinetics of tetrachloroethylene (TCE) has been studied extensively in humans who have been exposed to vapors of the chemical. Unfortunately, little work has been done to delineate the uptake,

distribution, metabolism, and excretion of oral doses of TCE. Studies have demonstrated that TCE is rapidly absorbed through the lung (Fernandez *et al.,* 1976) and skin (Stewart and Dodd, 1964). Since TCE is a small, uncharged, lipophilic molecule, one would expect that it would be rapidly and completely absorbed from the gastrointestinal tract as well. Blood levels of 2 to 4 ppm TCE have been measured in persons who have breathed 100 to 150 ppm continuously for several hours (Hake and Stewart, 1977). As discussed below, 100 ppm appears to be the minimum effective concentration in vapor that is responsible for subjective complaints and minor neurologic dysfunction in humans.

The majority of systemically absorbed TCE is rapidly eliminated by expiration of the unchanged compound. A small proportion, which is believed to accumulate primarily in adipose tissue, is eliminated much more slowly. A relatively minor portion of the total TCE that is absorbed into the body is metabolized and slowly excreted in the urine (Fernandez *et al.,* 1976). Ikeda (1977) calculated that the respiratory half-life in humans is approximately 65 hr, while the urinary half-life is about 144 hr. Fernandez *et al.* (1976) detected TCE in expired air of subjects for more than 2 weeks following an 8-hr, 200-ppm exposure, and Hake and Stewart (1977) measured 4 ppm TCE in expired air of a man 25 days after he was overcome by TCE fumes. Therefore, the chemical might be expected to accumulate in the body if insufficient time is allowed to elapse between subsequent exposures. Such an accumulation was reported by Stewart *et al.* (1970a) during 5 days of daily 7-hr exposures of humans to 100 ppm TCE. Ikeda (1977) stated that TCE accumulates in the body at 3 to 4 times the rate of trichloroethylene, a relatively longlived alkyl halide.

Only a small proportion of systemically absorbed TCE is metabolized. Fernandez *et al.* (1976) estimated that only approximately 1.85% of the TCE absorbed by humans during an 8-hr, 150-ppm inhalation exposure is eliminated in the urine as trichloroacetic acid (TCA). The primary metabolites of TCE in the mouse (Yllner, 1961), rat (Daniel, 1963), guinea pig (Sakamoto, 1976), and human are TCA and chloride. Some investigators have reported trichloroethanol as a secondary metabolite in human urine (Ikeda, 1977; Ikeda *et al.,* 1972), although others (Fernandez *et al.,* 1976; Hake and Stewart, 1977) did not confirm this. Still other investigators observed, but could not positively identify, in human urine (Ogata *et al.,* 1971) and guinea pig urine (Sakamoto, 1976) what may be trichloroethanol. This alcohol is undoubtedly more toxic (Mikiskova and Mikiska, 1966) than the relatively innocuous TCA (Woodard *et al.,* 1941), although only small quantities of either metabolite would be expected to be formed from TCE. Despite wide individual variability in

urinary TCA and trichloroethanol levels, Ikeda (1977) noted that the capacity of humans to metabolize TCE is quite limited, even at low-level exposures.

Stimulation of TCE metabolism may enhance its toxicity. Results of studies of this phenomenon are conflicting, in that pretreatments of rats with ethanol (Cornish and Adefuin, 1966) and phenobarbital (Cornish *et al.,* 1973; Moslen *et al.,* 1977a) fail, but polychlorinated biphenyl (PCB) (Moslen *et al.,* 1977a) succeeds in potentiating TCE hepatotoxicity. Moslen and her coworkers found that while phenobarbital enhances urinary excretion of TCE metabolites, PCB is even more effective. The initial step in TCE metabolism is believed to involve formation of TCE oxide (Ikeda, 1977). Thus, production of increased quantities of the epoxide and/or subsequent cytotoxic products might account for toxicity potentiation. Sakamoto (1976) reported that TCE oxide is relatively toxic and may be carcinogenic or mutagenic, although Greim *et al.* (1975) noted that it is relatively stable and is not mutagenic in their microsomally supplemented *Escherichia coli* screening system.

Health Aspects

Observations in Humans Although TCE is a potent depressant of the central nervous system, residual organ damage is not commonly observed in humans who have been exposed to large quantities of the compound. TCE was formerly used widely as an intestinal anthelminthic. Lambert (1933) reported that oral doses of 2.8 to 4.0 ml given for this purpose were quite effective and safe. Inebriation was the only troublesome side effect that was noted in 46,000 treated patients. Inhalation of levels of TCE sufficient to produce inebriation and unconsciousness has failed to elicit hepatic, renal, or hematological abnormalities in some individuals (Method, 1946; Patel *et al.,* 1977). However, in other cases, mild (Hake and Stewart, 1977; Saland, 1967; Stewart *et al.*. 1961a; Stewart, 1969) to severe (Meckler and Phelps, 1966) hepatotoxicity has been diagnosed. In most such instances, liver injury was not manifest until several days after exposure. Recovery was uneventful, but sometimes prolonged, particularly in the more severe cases. TCE was quite slowly eliminated, in that approximately 1 ppm TCE was measured in the breath of victims as long as 11 to 12 days after exposure (Stewart *et al.*. 1961a; Stewart 1969). Little evidence of kidney injury or damage of any other organ was noted in any of the aforementioned cases, other than the nephrotoxicity that was reported by Hake and Stewart (1977).

Experiments involving intentional exposure of humans to TCE vapor demonstrate that low levels of the chemical can cause irritation of mucous membranes and intoxication, but not permanent injury. Stewart *et al.* (1961b), like Rowe *et al.* (1952a), reported the minimum intoxicating vapor level of TCE to be approximately 100 ppm. Stewart and his coworkers found that the concentration of TCE in the blood rises gradually during a 3-hr, 194-ppm exposure to a maximum level of approximately 2.5 ppm (TCE in blood). The investigators noted that although no TCE is detectable in the blood 30 min after exposure, the compound is measurable in expired air for up to 94 hr. Such findings raise some questions as to the efficiency/sensitivity of their blood extraction and analyses. Stewart *et al.* (1961b) reported that there were no abnormalities in laboratory tests for hepatotoxicity in their test subjects. A subsequent investigation by Stewart *et al.* (1970a) confirmed findings in the previous study, namely, that 100 ppm TCE was a threshold level for induction of early depression of the central nervous system. Altered electroencephalogram patterns suggestive of drowsiness were seen in a majority of male and female subjects who breathed 100 ppm of TCE for 7.5 hr (Hake and Stewart, 1977). Extensive evaluation of subjects, including blood chemistry, urinalyses, pulmonary function testing, electrocardiogram, and visual acuity testing, failed to reveal adverse effects during a 5-day, 100-ppm exposure regimen (Stewart *et al.,* 1970a). Such experimental and epidemiological evidence led to the recommendation that 50 ppm (as a time-weighted average) be the occupational exposure standard (National Institute for Occupational Safety and Health. 1976). The National Institute for Occupational Safety and Health believed that this standard would protect against not only tissue injury but also temporary neurological dysfunction and respiratory tract irritation.

Observations in Other Species

Acute Effects Toxicological investigations with laboratory animals have confirmed observations in humans, namely, that TCE in sufficient quantities can depress the central nervous system but has quite limited ability to damage organ systems. The acute oral LD_{50} was shown to be 4,000 mg/kg in dogs and 5.000 mg/kg in rabbits (National Institute for Occupational Safety and Health. 1976). A number of investigators have demonstrated that TCE is one of the least hepatotoxic and nephrotoxic of a series of alkyl halides when administered as a single dose by

intraperitoneal injection to several species of animals (Cornish *et al.,* 1973; Klaassen and Plaa, 1966, 1967; Plaa and Larson, 1965). "Near-lethal" doses of TCE were usually required to produce significant tissue injury. Although alterations in organ function tests, serum enzyme levels, and histopathology were observed, the extent of these changes was relatively modest. The lowest reported toxic intraperitoneal doses of TCE. as indicated by increased serum enzyme levels, were approximately 0.3 to 0.5 ml/kg in rats (Cornish *et al.,* 1973) and 0.7 ml/kg in dogs (Klaassen and Plaa, 1967). Similarly, Moslen *et al.* (1977b) observed that a single oral dose of 0.75 ml/kg elicited elevated levels of serum glutamic oxaloacetic transaminase in rats. Kylin *et al.* (1963) found that 200 ppm was the lowest vapor level of TCE to produce fatty infiltration of the liver of mice following a 4-hr exposure.

Chronic Effects Investigations of chronic toxicity of TCE in animals have all involved inhalation exposure, with the exception of a recently concluded assessment of carcinogenesis, which involved oral dosing (National Cancer Institute, 1977). Male and female mice and rats that were gavaged with doses of TCE ranging from 386 to 1,072 mg/kg/day for as long as 78 weeks exhibited a very high incidence of nephrotoxicity but little evidence of hepatotoxicity (National Cancer Institute, 1977). There was a high incidence of hepatocellular carcinoma in the mice, but not in the rats. A preliminary report by Pegg *et al.* (1978) of a 6 hr/day, 5 days/week, 12-month exposure of rats to 600 ppm TCE stated that no organ toxicity was observed.

Mutagenicity The literature contains only one investigation concerning the mutagenic potential of TCE. In the presence of a microsomal activating system, TCE was unable to induce mutations in *Escherchia coli* (Greim *et al.,* 1975). In contrast, vinyl chloride was a quite potent mutagen under these conditions. The authors, assuming that both TCE and vinyl chloride are initially oxidized to epoxides, proposed that the epoxide of vinyl chloride was relatively unstable and might, therefore, more readily alkylate biological molecules and induce mutagenesis and carcinogenesis.

Carcinogenicity Results of the limited number of studies of the carcinogenic potential of TCE are conflicting. Although thorough gross and histopathological evaluations of tissues of several species were performed in subacute and chronic studies, no mention of tumor induction has been made (Kylin *et al.,* 1963; Pegg *et al.,* 1978; Rowe *et al.,* 1952a). In an assessment of the ability of TCE to produce pulmonary

adenomas in male mice. Theiss *et al.* (1977) failed to find a carcinogenic effect. However, the NCI (National Cancer Institute, 1977) has reported TCE-induced hepatocellular carcinomas in male and female mice, but not in male or female rats. In the NCI study, daily oral doses ranging from 386 to 1,072 mg/kg were administered for up to 78 weeks. A high tumor incidence was observed at both low and high dose levels. The quantities of TCE given were so large that marked, dose-dependent mortality in both species occurred throughout the study period.

Teratogenicity Schwetz *et al.* (1975) examined the teratogenic effects of TCE in rats and Swiss Webster mice. The animals were exposed at 300 ppm for 7 hr/day on days 6 through 15 of gestation. The investigators concluded that TCE had little effect on embryonic and fetal development, and that it was not teratogenic. Nevertheless, there were a number of modest but statistically significant deviations from controls, including increased weight of maternals, decreased body weight of mouse fetuses, increased fetal resorptions, and increased incidence of split sternebrae, subcutaneous edema, and delayed ossification of skull bones in mouse fetuses.

Carcinogenic Risk Estimate In a recent study by NCI (1977), rats and mice were gavaged with TCE doses ranging from 386 to 1,072 mg/kg/day for as long as 78 weeks. There was a high dose-related incidence of hepatocellular carcinoma in the mice, but none in the rats. Each set of dose-response data was used to make statistical estimates of both the lifetime risk and an upper 95% confidence bound on the lifetime risk at the low dose level. These estimates are of lifetime human risks and have been corrected for species conversion on a dose/surface area basis. The risk estimates are expressed as a probability of cancer after a lifetime consumption of 1 liter/day of water containing 1 μg/liter of the compound. For example, a risk of 1×10^{-6} implies a lifetime probability of 2×10^{-5} of cancer if 2 liters/day were consumed by a 70-kg human and the concentration of the carcinogen was 10 ppb. This means that at a concentration of 10 ppb during a lifetime exposure, this compound would be expected to produce one excess case of cancer for every 50,000 persons exposed. Assuming the population of the United States to be 220 million, this translates into 4,400 excess lifetime deaths from cancer or 62.8 per year.

For humans exposed to 1 μg/liter TCE, the estimated lifetime risk is 6.2×10^{-8}. The upper 95% confidence estimate is 1.4×10^{-7}. Both of these estimates are the averaged risks, which have been calculated from the male and female mice.

Conclusions and Recommendations

Suggested No-Adverse-Response Level (SNARL) Although acute exposure to high levels of TCE can result in marked depression of the central nervous system, the chemical apparently has quite limited capacity to cause tissue injury. The majority of the information that is relative to human and animal exposure concerns inhalation of TCE. Although duration and levels of exposure to TCE in vapor have been reported, body burdens of the chemical have generally not been determined. Therefore, it is quite difficult to relate toxicological findings in these reports to either blood levels or toxic effects that might result from ingestion of TCE.

No accounts of toxicity resulting from ingestion of TCE by humans were found in the literature. The subcommittee has only the historical perspective of Lambert (1933) on the use of TCE as an anthelminthic, when oral doses of 2.8 to 4.0 ml apparently elicited little more than inebriation. Similarly, inhibition of psychophysiological function would appear to be the most sensitive index of exposure to TCE via inhalation.

Twenty-Four-Hour Exposure In light of the lack of definitive information regarding the quantity of TCE that must be ingested to depress psychophysiological function, it seems most appropriate that calculations for a SNARL be based upon quantities of the chemical that are required to produce tissue injury. The lowest reported intraperitoneal doses of TCE that produce increased serum transaminase levels in various species range from 0.3 to 0.7 ml/kg. A single oral administration of 0.75 ml/kg results in an apparent elevation in serum transaminase activity in rats. Therefore, the 0.3 ml/kg (0.49 g/kg) dose appears to be a reasonable "minimum toxic dose" from which to calculate a 24-hr SNARL for contamination of drinking water, assuming that the sole source of TCE during this period will be from 2 liters/day of drinking water consumed by a 70-kg human. A safety factor of 100 is applied:

$$\frac{490 \text{ mg/kg} \times 70 \text{ kg}}{100 \times 2 \text{ liters}} = 172 \text{ mg/liter.}$$

The above considerations ignore the possibility that TCE may be carcinogenic.

Seven-Day Exposure Unfortunately, there have been no studies of the subacute toxicity of ingested TCE in laboratory animals or in humans. Investigations involving five consecutive daily exposures of humans to

100 ppm of TCE vapor have failed to reveal evidence of organ damage (Stewart *et al.,* 1970a). These studies did reveal some propensity of the chemical to accumulate in the body. Nevertheless, in view of TCE's relative lack of toxicity, a 7-day standard for drinking water contamination, which was obtained by dividing the 24-hr standard by 7 (172 mg/liter/7 days = 24.5 mg/liter), should protect against adverse effects by the chemical. This standard is based upon the assumption that the sole source of TCE during this period will be drinking water. These considerations ignore the possibility that TCE may be carcinogenic on short-term exposure.

Chronic Exposure From the limited number of chronic studies it is unclear whether TCE is a carcinogen. NCI (1977) reported that it induces hepatocellular carcinomas in one strain of mice, but not in rats. The findings of this study should be interpreted with caution, recognizing the limitations of the experimental design (e.g., massive doses of TCE, large volumes of oil vehicle, marked nephrotoxicity, diminished lifespan). The absence of histopathological change in organs other than the kidney in this 78-week study supports the assumption that TCE is relatively nontoxic, even when ingested repeatedly in large quantities. Unfortunately, due to the failure of the NCI study to determine a "noeffect level" and to monitor a sufficiently broad battery of sensitive toxicological indices, it is not possible to establish a "minimum-effect level" for chronic, noncarcinogenic toxicity.

Although TCE is not overtly toxic, appropriately designed studies should be conducted with several species of animals to determine the minimum toxic dose for both single ingestions and multiple ingestions over a 1-week period. Attention should be focused on the liver and kidney, but care should be taken not to overlook effects on other organs. Assuming that inhibition of psychophysiological function is the most sensitive index of TCE-exposure in humans, studies of oral doses in volunteers might be conducted to determine whether small quantities of the chemical are intoxicating. In the absence of such information, one can only guess at the maximum no-effect dose in short-term exposure situations.

Chronic, low-level feeding studies should also be conducted to establish with some degree of certainty whether and under what conditions TCE may be a toxicant, mutagen, teratogen, and/or carcinogen. Such investigations should be conducted with several species of animals, should establish maximum no-effect levels, and should examine a range of TCE concentrations that realistically approximates anticipated potential exposures. Although the majority of systemically

absorbed TCE is exhaled unchanged, with only a small proportion excreted as urinary metabolites, it appears pertinent to determine whether TCE or its metabolites can accumulate as a result of prolonged, low-level ingestion.

Thorium

(Th)

Thorium occurs naturally in a number of minerals including monacite and thorite (Anonymous, 1959). Its concentration in the water of rivers, lakes, seas, and oceans varies between 10^{-5} and 10^{-9} g/liter. About half of the amount is present as carbonate complexes (Lyarskii *et al.,* 1970). It is the parent element of a naturally occurring radioactive family. It decays through a number of isotopes such as mesothorium, radiothorium, thorium, thorium emanation bodies, thoron gas, and, eventually, lead (^{208}Pb), emitting alpha and beta particles and gamma rays. Most of the radiation is in the form of alpha particles (4.0-4.2 mev). The half-life of ^{232}Th is 1.4×10^{10} years. Thorium is used principally as a fertile material for nuclear breeder cores, in the manufacture of incandescent lamps and mantles, as a catalyst in organic syntheses, and as an alloy in welding electrodes (Anonymous, 1959).

Metabolism

The distribution of thorium compounds depends upon an interplay between the concentration of the thorium and mode of administration (Pavlovskaya, 1969). Distribution of thorium is primarily dependent upon concentration; i.e., at concentrations less than 10^{-5} g/ml, thorium is distributed primarily to the skeleton, independent of the route of administration, while at concentrations greater than 10^{-3} g/ml, distribution depends upon route of administration. At higher doses ($> 10^{-3}$ g/ml) following intravenous administration, thorium (dioxide or citrate) is distributed primarily to the reticuloendothelial system with about 60% to 90% in the liver and the remainder in the spleen and marrow (Berenbaum and Birch, 1953). Following inhalation or intratracheal administration, 70% to 80% of the thorium dose is found in the lungs. Following intramuscular, intraperitoneal, or subcutaneous injection, more than 90% of the dose remains at the site of injection. Following oral doses, the bulk of the thorium is distributed to the skeleton since the absorption coefficient is very low (not more than 0.05%) (Pavlovskaya *et al.,* 1971). Administration of lower doses of thorium (< mg/ml) always leads to accumulation in the skeleton. Thus, thorium entering via the diet or drinking water of humans tends to be distributed to the skeleton.

Health Aspects

The salts of thorium are known to be associated with chemical as well as radiological hazard (Tandon *et al.,* 1975). Some reports have stated that thorium causes pathological changes in the spleen, liver, lungs, and hematopoietic organs. Long-term effects include production of malignant neoplasms (Pavlovskaya, 1969).

Observations in Humans There have been numerous reports of clinical carcinogenicity of thorium compounds, especially thorium dioxide (Thorotrast), which was used as a radiopaque contrast medium (Schmitz *et al.,* 1970). Cancers that were produced reflect the distribution and dose of the thorium that was administered. Consequently, after intravenous administration of thorium dioxide, most cancers were tumors of the reticuloendothelial system especially involving the liver with an equal distribution of sarcomas and carcinomas. Injection of thorium dioxide into the sinus cavities results in carcinomas. The latent period for tumor development is long (approximately 15 years), and tumorigenesis arises from a-radiation.

Observations in Other Species

Acute Toxicity McClinton and Schubert (1948) reported that the intraperitoneal LD_{50} of thorium nitrate in albino, female Sprague-Dawley rats was 68 ± 12 mg/kg.

Subacute Toxicity There are few reports on subacute toxicity of thorium. Tandon *et al.* (1975) applied daily 1-ml doses of of 5%, 10%, and 20% thorium nitrate to the skin in the lateroabdominal and scrotal areas of male albino rats for 15 days. The animals were sacrificed 15 days later. None of the animals showed symptoms of morbidity or mortality during or after treatment. There was no gross abnormality in skin, liver, kidneys, or testes of any of the rats. However, upon microscopic examination, mild degenerative changes were found in the skin and testes of animals receiving the 20% thorium solution. Skin lesions consisted of mild acanthosis and thickening of epithelial lining. Testicular lesions consisted of mild edema of seminiferous tubules and interstitium and desquamation of the spermatogenic cells. A few tubules carried spermatid-type giant cells.

Chronic Toxicity No available data.

Mutagenicity No available data.
Carcinogenicity See Observations in Humans.
Teratogenicity No available data.

Conclusions and Recommendations

Because of the paucity of data on acute and chronic oral toxicity of thorium, and since thorium produces cancer in humans, estimates for exposure limits will not be calculated. However, the following standards have been set for thorium by various groups:

1. International Commission on Radiological Protection (ICRP)—0.5 g/liter soluble ^{232}Th; 0.5 to 9 g/liter for insoluble compounds (Lyarskii *etal.,* 1970).
2. Council for Mutual Economic Aid—4 mg/liter for ^{232}Th; 2.7 mg/liter for natural thorium (Lyarskii *et al.,* 1970).
3. Sanitary Regulations (USSR)—0.1 mg/liter for both ^{232}Th and natural thorium, based on consumption of 2.2 liters of water per day and the skeleton as the critical organ (Lyarskii *et al.,* 1970).
4. Desired Maximum Ambient Environmental Levels for Elemental Forms Based on Chemical Toxicity (Battelle Laboratories, 1970)—0.0005 mg/liter of potable water. This value was calculated from acute median lethal dose (LD_{50}) by intraperitoneal injection and biological half-life.

There is a lack of data concerning acute and chronic oral toxicity of thorium compounds. Although injected thorium produces a significant risk of carcinogenicity, available data do not provide evidence of risk from thorium in drinking water. Whether the carcinogenic effect is due to the radioactive properties of this element needs to be assessed.

1,1,1-Trichloroethane

(Cl_3C-CH_3)

1,1,1-Trichloroethane (TCE), or methyl chloroform, is used widely as an industrial chemical for such purposes as a cleaner and degreaser of metals, a spot remover, and a solvent of lipophilic substances. It is a clear, colorless liquid at room temperature; its solubility in water is 4,400 mg/liter at 20°C (Verschueren, 1977); its boiling point is 74°C; and its vapor is heavier than air and nonflammable. Dioxane is commonly added to promote its stability. TCE has been identified in drinking water

supplies in the United States (U.S. Environmental Protection Agency, 1978c).

Metabolism

There have been extensive studies on the uptake and distribution of TCE in humans and laboratory animals that have been exposed to the chemical by inhalation. Unfortunately, there is little information on the pharmacokinetics of ingested TCE. One would expect that this compound would be readily absorbed from the gastrointestinal tract in light of the report by Stewart and Dodd (1964) who found that it penetrated intact human skin. Astrand *et al.* (1973) reported rapid absorption of inhaled TCE and measurable levels of the chemical in the arterial blood of human subjects after inhaling 250 ppm TCE for 10 s. The arterial blood contained substantially higher TCE levels than the venous blood throughout a 2-hr exposure, indicating ready uptake of the compound from blood into tissues. Blood concentrations of 3 to 5 ppm have been measured in humans breathing 350 ppm TCE (Astrand *et al.,* 1973; Gamberale and Hultengren, 1973; Stewart *et al.,* 1961c), the current threshold limit value for occupational exposure in the United States. Apparently, there are no data on blood levels of the compound following oral exposures.

The majority of systemically absorbed TCE is eliminated via the lungs. Hake *et al.* (1960) reported that about 98.7% of a 700 mg/kg dose of radio-labeled TCE, which was injected intraperitoneally into rats, was exhaled unchanged within 25 hr. They also observed small amounts of radio-labeled carbon dioxide in expired air and of the glucuronide conjugate of 2,2,2-trichloroethanol in urine. Later studies revealed trichloroacetic acid to be a second metabolite, although the amounts that were formed in the urine of both rats (Eben and Kimmerle, 1974; Ikeda and Ohtsuji, 1972) and humans (Stewart *et al.,* 1969) were substantially less than those for trichloroethanol. No chloral hydrate was detected in the blood or tissues of rats by Eben and Kimmerle (1974). In studies by Van Dyke and Wineman (1971) and Ikeda and Ohtsuji (1972) on the metabolism of a series of alkyl halocarbons, TCE was one of the least extensively metabolized compounds. Nevertheless, it is known to exhibit type 1 binding characteristics with cytochrome P-450 (Pelkonen and Vainio, 1975) and to be capable of inducing microsomal enzyme and P-450 activity (Fuller *et al.,* 1970). A progressive increase in urinary output of trichloroethanol was observed in humans that had been subjected to five daily inhalation exposures to TCE. This indicates that TCE induces its own metabolism (Stewart *et al.,* 1969).

Upon termination of TCE exposure, the chemical is rather quickly eliminated in exhaled air. Levels in the blood and alveolar air decrease exponentially, showing an initial rapid fall, followed after several hours by a somewhat slower decline (Astrand *et al.,* 1973; Stewart *et al.,* 1969). This latter stage probably reflects slow mobilization of the agent from lipoidal tissues. Stewart *et al.* (1969) reported that a slight amount accumulated in humans who inhaled TCE at 500 ppm, 6.5 to 7 hr/day for 5 days, despite the relatively rapid loss of TCE from the body. These investigators also found TCE in breath samples from one subject 1 month after exposure. Thus, it appears that TCE can accumulate in the body if intake is frequent enough and/or of sufficient magnitude.

The relative lack of toxicity of TCE, in comparison to certain other alkyl halocarbons, can be attributed to its relatively rapid elimination and stability. This compound is much more volatile (Ikeda and Ohtsuji, 1972) and, therefore, more readily excreted via the lungs (Morgan *et al.,* 1970) than the more toxic congener 1,1,2-trichloroethane. Although neither halocarbon is metabolized to a significant degree, Carlson (1973) observed that microsomal enzyme induction with phenobarbital potentiates hepatotoxicity of both TCE and 1,1,2-trichloroethane. Thus, it appears that the metabolite(s) of each compound is responsible for cytotoxicity, although the identity and mechanism of the actual toxicant(s) remain unknown.

Health Aspects

Observations in Humans The primary toxic effects in humans who have been subjected to short-term, high-level exposure to TCE are manifestations of depression of the central nervous system. In the majority of reports of human fatalities resulting from inhalation of TCE, death is attributed to a functional depression of the central nervous system. Levels of TCE in the victims' blood vary considerably, generally ranging from 60 (Hatfield and Maykoski, 1970; Stahl *et al.,* 1969) to 720 ppm (Hall and Hine, 1966). As might be predicted, the highest concentrations of TCE are found in the brains of victims (Caplan *et al.,* 1976; Stahl *et al.,* 1969). Due to problems that are inherent in analyses of volatile toxicants in autopsies, it is difficult to establish lethal TCE concentrations in blood or tissue.

Inhalation of high concentrations of TCE can cause irritation of the respiratory tract and minimal organ damage, as well as depression of the central nervous system. Acute pulmonary congestion and edema typically found in fatalities result from inhalation of TCE (Bonventre *et*

al., 1977; Caplan *et al.,* 1976). There are also scattered reports of modest fatty vacuolation in the liver (Caplan *et al.,* 1976; Hall and Hine, 1966; Stahl *et al.,* 1969). In most such instances there probably would have been insufficient time between exposure and death for hepatotoxicity to be fully expressed. Stewart (1971) reported the case histories of four individuals who were monitored clinically after being overcome by TCE vapors. In each case, recovery from depression of the central nervous system was quite rapid and largely uneventful. However, one of the four patients exhibited elevated urinary urobilinogen but no alteration of other indices of hepatotoxicity. These studies indicate that TCE possesses a limited capacity to exert hepatic injury in cases of acute, high-level inhalation exposure.

Clinical experience and scientific investigations suggest that acute high-level inhalation of TCE can adversely affect the cardiovascular system of humans. Dornette and Jones (1960) used concentrations of 10,000 to 26,000 ppm TCE to anesthetize surgery patients. They noted that both induction of and recovery from anesthesia were quite rapid. No evidence of respiratory depression or hepatotoxicity was seen. However, there were disturbing cardiovascular effects including diminished systolic pressure, premature .ventricular contractions, and, in one patient, even cardiac arrest.

Bass (1970) reported a syndrome termed "sudden sniffing death" in persons dying abruptly while inhaling volatile solvents for self-intoxication. TCE was one of the most frequently implicated solvents in such incidents. The fatalities were tentatively attributed to cardiac arrhythmias that resulted from a combined action of the solvent and endogenous biogenic amines. Recent investigations of the phenomenon with laboratory animals are discussed below.

A single account of ingestion of TCE by a human has appeared in the literature (Stewart and Andrews, 1966). A 47-year-old male mistakenly consumed 1 oz of TCE (approximately 0.6 g/kg). He became nauseated within 30 min and developed progressively severe vomiting and diarrhea over the next few hours. Clinical evaluation following gastric lavage revealed neither drowsiness nor difficulty with coordination. Urinalysis and clinical chemistry tests revealed evidence of only minimal hepatorenal injury early in the course of hospitalization. After resolution of the vomiting and diarrhea, the patient was asymptomatic during a 2-week observation period.

Since depression of the central nervous system is the predominant effect of TCE on humans, certain manifestations of the depression should be the most sensitive indices of the physiological action of small quantities of-the solvent. Early studies with volunteers indicate that

inhalation of 500 ppm TCE for several hours has no significant effect other than transient, mild eye irritation (Stewart *et al.,* 1961a,b; Torkelson *et al.,* 1958). Stewart and his coworkers (1969) concluded in a later study that 500 ppm may be excessive for persons who are particularly susceptible to the chemicals depressant effects on the central nervous system. In an even more recent investigation, inhalation of 350 ppm TCE for 4 hr was not effective, but 450 ppm elicited subjective complaints of transient eye irritation and dizziness (Salvini *et al.,* 1971a,b). Although a battery of psychophysiological tests did not reveal a statistically significant degree of functional inhibition, lower scores resulted when tests were conducted during TCE exposure than when under control conditions. Results of an investigation by Gamberale and Hultengren (1973) indicated that inhalation of 350 ppm TCE can significantly inhibit psychophysiological functions of humans. Blood levels in these "inhibited" subjects averaged approximately 3 to 4 ppm, although the investigators noted wide intersubject differences in blood and alveolar air concentrations. Gamberale and Hultengren concluded that it would be difficult, with any degree of accuracy, to set a threshold for the vapor concentration of TCE that would not alter function of the central nervous system. Their tests of psychophysiological function are certainly more sensitive and objective than the indices used in the earlier studies of Torkelson *et al.* (1958) and Stewart *et al.* (1961, 1969). Nevertheless, the current U.S. threshold limit value for occupational exposure to TCE remains at 350 ppm. This standard is designed to protect the majority of workers from mucous membrane irritation and performance inhibition. One interesting facet of the studies by Torkelson *et al.* (1958) and Stewart *et al.* (1969) is their failure to find any evidence of organ damage in humans that were subjected to acute TCE inhalation regimens.

Short-term exposure to TCE appears to be no more harmful to humans or laboratory animals than is acute exposure. Stewart *et al.* (1969) exposed humans via inhalation to 500 ppm TCE for 6.5 hr daily for 5 consecutive days. They observed some objective and subjective signs of depression of the central nervous system, but no evidence of toxicity upon examination for neurological, respiratory, and hepatorenal function. There were also a small accumulation of TCE and an increase in urinary trichloroethanol levels.

Observations in Other Species

Acute Effects Overall results of animal experimentation confirm the previously described findings in humans-namely, that TCE is relatively

nontoxic upon short-term exposure. The acute oral LD_{50} for TCE, as determined in several species of animals, is reported by Torkelson *et al.* (1958) to range from 5.7 to 14.3 g/kg. Unfortunately, little other toxicological data involving oral dosing are available. LD_{50} values that were derived upon administration of TCE by routes other than oral illustrate the difficulty in using such data to predict consequences of ingestion of the chemical. In contrast with an oral LD_{50} value of 11 g/kg in the mouse (Torkelson *et al.,* 1958), the LD_{50} is approximately 16 g/kg for subcutaneous injection (Plaa *et al.,* 1958) and approximately 4.9 g/kg for intraperitoneal injection (Klaassen and Plaa, 1966). By administering equivalent intraperitoneal and oral doses of carbon tetrachloride to rats, Nadeau and Marchand (1973) demonstrated that significantly higher hepatic concentrations of carbon tetrachloride and more extensive hepatotoxicity are manifested in the intraperitoneally dosed animals.

Despite the problems inherent in extrapolating data from one route of chemical exposure to another, we may gain qualitative insight into the toxicity of TCE by examining information from studies in which the oral route was not used. Plaa and his colleagues found TCE to be the least hepatotoxic of a series of alkyl halocarbons that were given subcutaneously (Plaa *et al.,* 1958) and intraperitoneally (Klaassen and Plaa, 1966) to mice and intraperitoneally to dogs (Klaassen and Plaa, 1967) and rats (Klaassen and Plaa, 1969). Near-lethal quantities of TCE were generally required to produce hepatotoxicity. They observed little to no evidence of nephrotoxicity. In contrast to TCE (ED_{50} = 2.5 ml/kg for SGPT elevation in mice), its congener 1,1,2-trichloroethane was much more toxic (ED_{50} = 0.1 ml/kg), and tetrachloroethylene was of equivalent potency (ED_{50} = 2.9 ml/kg).

In laboratory animals, as well as in humans, the primary hazard of inhalation of high concentrations of TCE is excessive depression of the central nervous system. Adams *et al.* (1950) reported the 3-hr LC_{50} in rats to. be 18,000 ppm. They observed that recovery of several test species of animals from marked depression of the central nervous system was rapid and uneventful. The lowest and shortest exposure that elicited histological change in tissues of rats was 8,000 ppm for 7 hr. This produced an increase in liver weight and fatty vacuolation of hepatocytes. Disturbance of vestibular function in rabbits infused intravenously with TCE was observed by Larsby *et al.* (1978) when blood levels of TCE in the rabbits exceeded 75 ppm. Levels of TCE in the cerebrospinal fluid were approximately one-third of that in the blood. Although this vestibular disturbance is physiologically significant, it should be recalled that Gamberale and Hultengren (1973) observed inhibition of psychophysiological function in humans with blood levels of only 3 to 5 ppm TCE.

A second hazard that is associated with acute exposure to vapor containing high concentrations of TCE is cardiovascular toxicity. The aforementioned accounts of cardiotoxic effects of TCE in humans (Bass, 1970; Dornette and Jones, 1960) have been confirmed in studies of dogs. Reinhardt *et al.* (1973) found TCE to be more potent than trichloroethylene in inducing arrhythmias in dogs concomitantly dosed with epinephrine. The lowest effective concentration of TCE was 5,000 ppm. However, Egle *et al.* (1976) did not detect adverse cardiovascular effects in freely moving dogs that had been exposed to 5,000 and 10,000 ppm TCE in a Freon propellant. They attributed the disparity between their own findings and those of Reinhardt *et al.* (1973) to differences in experimental design. Herd *et al.* (1974) found TCE to exert a biphasic action on the cardiovascular system of anesthetized dogs, which was characterized by an initial decrease in blood pressure that was associated with peripheral vasodilation as well as reflex chronotropic and inotropic effects on cardiac function, and subsequent depression of cardiac function. In a study of the biochemical mechanism of TCE's cardiotoxicity, Herd and Martin (1975) observed inhibition of respiratory function and alteration of permeability characteristics in mitochondria that were isolated from rats. Herd *et al.* (1974) emphasized that studies are needed to determine whether low-level exposure to TCE may be injurious to the cardiovascular system.

In contrast to previous findings of microsomal enzyme induction in mice (Lal and Shah, 1970) and rats (Fuller *et al.,* 1970) that inhaled 3,000 ppm TCE for 24 hr, inhibition of microsomal drug metabolism was observed in rats that had been given approximately 1.4 g/kg orally (Vainio *et al.,* 1976) and in mice that had been given 1.0 ml/kg of undiluted TCE intraperitoneally (Shah and Lal, 1976). Shah and Lal (1976) further demonstrated that dilution of the TCE with olive oil reduced the inhibitory effect, while TCE that was diluted with dimethyl sulfoxide (DMSO) potentiated the effect. These investigators suggested that the olive oil inhibited the systemic absorption of TCE and that the DMSO potentiated TCE's hepatotoxicity.

Chronic Effects McNutt *et al.* (1975) exposed mice continuously to 250 and 1,000 ppm TCE for up to 14 weeks. Serial sacrifices were performed at weekly intervals to ascertain the development of any histopathological abnormalities. Hepatocytic vacuolations and significant increases in liver weight and triglyceride content were observed throughout the study in the animals exposed to 1,000 ppm. After 4 weeks of exposure to 1,000 ppm TCE a number of ultrastructural alterations were observed in centrilobular hepatocytes, including proliferation of

smooth endoplasmic reticulum. Such a structural alteration would be expected in light of the reports of microsomal enzyme induction by Fuller *et al.* (1970) and Shah and Lal (1976).

McNutt *et al.* (1975) saw a return to normal of each of the indices at 2 and 4 weeks after exposure. Quite modest ultrastructural alterations and increases in liver weight and triglyceride were occasionally observed in the animals that were exposed to 250 ppm during the 14-week study. Thus, this exposure level might be considered a threshold for a biological effect of TCE in the mouse. Platt and Cockrill (1969) studied biochemical changes in rat livers in response to a series of aliphatic halocarbons. They found seven daily oral doses of 1.65 g/kg to enhance cytoplasmic and microsomal protein content and to exert no hepatotoxicity. Savolainen *et al.* (1977) reported slight decreases in brain RNA and liver microsomal P-450 in rats inhaling 500 ppm TCE for 6 hr daily for 4 or 5 days. The significance of these latter findings is uncertain.

The only lifetime feeding study that has been reported was conducted as a part of the National Cancer Institute (NCI) Bioassay Program (National Cancer Institute, 1977). In an initial range-finding study, oral doses ranging from 1,000 to 10,000 mg/kg TCE in corn oil were given to male and female mice and rats 5 days weekly for 6 weeks. The highest "no-effect" dose for rats was 3,160 mg/kg while that for mice was 5,620 mg/kg. Indices of toxicity that were evaluated included body weight and gross evidence of organ damage. A chronic dosing study was then initiated but had to be discontinued because of undefined intoxication in rats receiving 3,000 mg/kg. In the final chronic dosing study, male and female rats received 750 or 1,500 mg/kg TCE in corn oil by gavage 5 times weekly for 78 weeks. Similarly, male and female mice were given doses that were increased during the study when it became apparent that larger quantities of the chemical could be tolerated. The time-weighted averages for the two dose levels in mice for the 78-week regimen were approximately 2,800 and 5,600 mg/kg. Diminished body weight gain and decreased survival time were manifest in both mice and rats. Surprisingly, the incidence of histopathological change was no greater for TCE-dosed than for control animals of either species. No other indices of toxicity were evaluated.

A number of long-term animal studies of the toxic potential of inhaled TCE have been conducted over the last 20 years. These studies have been directed largely toward assessing potential hazards of TCE in occupational exposure situations. Daily exposure of a variety of species to 500 ppm of TCE over a 6-month period elicited no recognizable adverse effect, but 1,000 ppm produced fatty changes and increased weight of livers of guinea pigs (Torkelson *et al.,* 1958). Rowe *et al.* (1963)

reported similar findings when testing a solvent mixture consisting of approximately 75% TCE and 25% tetrachloroethylene. However, guinea pigs in the latter study did show some decrease in body weight gain, which was attributed to reduced food consumption, as well as an increase in liver weight. In studies of responses to even lower concentrations, Prendergast *et al.* (1967) exposed rats, guinea pigs, dogs, rabbits, and monkeys to TCE vapor continuously for 90 days. They observed depressed body weight in rabbits and dogs inhaling 370 ppm, but no adverse effects in any species inhaling 135 ppm. Eben and Kimmerle (1974) detected no evidence of hepatorenal injury, hematological change, or histopathologic alteration in rats that received 200 ppm TCE for 8 hr daily, 5 days weekly, for 14 weeks.

Mutagenicity Simmon *et al.* (1978), when conducting a mutagenesis screen of 71 chemicals that had been identified in U.S. drinking water, found TCE to be very weakly mutagenic *in vitro* for *Salmonella typhimurium.* Microsomal activation appears to have little effect on its potency.

Carcinogenicity The only study of the carcinogenic potential of TCE conducted to date failed to reveal any evidence of carcinogenicity (National Cancer Institute, 1977).

Teratogenicity No available data.

Conclusions and Recommendations

Suggested No-Adverse-Response Level (SNARL)

Twenty-Four-Hour Exposure The literature indicates that TCE is one of the least toxic of the commonly used alkyl halocarbons. Since depression of the central nervous system is its predominant effect when inhaled, it appears that loss of manual dexterity, coordination, perception, etc., may be the most sensitive indices of exposure. Unfortunately, it is unclear whether significant inhibition of psychophysiological functions will occur in humans who ingest the chemical. The 0.6 g/kg of TCE reportedly ingested by the patient of Stewart and Andrews (1966) might be considered to be a minimum oral hepatorenal toxic dose. However, the nausea, vomiting, and diarrhea experienced by the patient are toxicologically significant manifestations that must be avoided, although the gastrointestinal upset may have resulted from consumption

of undiluted TCE. Moreover, the vomiting, diarrhea, and gastric lavage may have prevented systemic absorption of a portion of the TCE.

A single case history is obviously not sufficient to serve as a basis for setting an exposure level for acute ingestion of TCE. However, a similar quantity of TCE in laboratory animals appears to be what might be termed a "minimum-effect level." Vainio *et al.* (1976) found that a single oral dose of approximately 1.4 g/kg depresses some hepatic microsomal metabolic indices in rats. In light of reports of hepatic microsomal enzyme induction in mice and rats following inhalation of TCE, it is possible that oral doses lower than 1.4 g/kg might also stimulate xenobiotic metabolism. Nevertheless, it seems appropriate that calculations for a suggested 24-hr SNARL for contamination of drinking water by TCE be based upon a minimum (oral) effect level that is derived from actual experimentation, namely 1.4 g/kg. This SNARL is based upon the assumption that the sole source of TCE during this time will be drinking water and that a 70-kg human consumes 2 liters/day (an uncertainty factor of 100 is applied):

$$\frac{1.4 \text{ g/kg} \times 70 \text{ kg}}{100 \times 2 \text{ liters}} = 490 \text{ mg/liter.}$$

Seven-Day Exposure TCE appears to be no more hazardous upon short-term exposure than it does upon acute exposure. A study in which humans were subjected to 500 ppm of TCE vapor on 5 consecutive days revealed no evidence of toxicity (Stewart *et al.,* 1969). Platt and Cockrill (1969) reported seven daily oral doses of 1.65 g/kg not to be hepatotoxic in rats, but to enhance hepatic microsomal and cytoplasmic protein content. However, their use of liquid paraffin as a vehicle may have markedly retarded systemic absorption of the TCE. Thus, because of the lack of more definitive information regarding short-term minimum-or no-effect levels, the suggested 7-day SNARL for drinking water contamination is obtained by dividing the 24-hr SNARL by 7. Assuming that the sole source of TCE during this period will be drinking water:

$$\frac{490 \text{ mg/liter}}{7} = 70 \text{ mg/liter.}$$

Definitive studies in several species of animals should be undertaken using a range of oral doses of TCE to characterize dose-effect and doseresponse relationships for both single and multiple ingestions over a 1week period. A variety of tests, which are valid indices of injury to potential target organs (e.g., heart, liver, kidneys), should be monitored. As no data pertaining to the uptake, distribution, metabolism, and

excretion of TCE upon ingestion are available, pharmacokinetic studies should be undertaken using a range of oral doses in several animal species. Vehicles for administration should be carefully selected to avoid discrepancy from actual exposures via drinking water or foods.

Limited studies of ingestion of small quantities of TCE might be undertaken in humans. These studies appear warranted since the toxic end points that might serve as a basis for setting standards include subjective (e.g., nausea) as well as subtle objective (e.g., performance) indices. It would be valuable to determine the quantity of TCE that must be consumed to produce a blood level of 3 to 4 ppm, the level that Gamberale and Hultengren (1973) associated with inhibition of psychophysiological function. Other indices that might be evaluated include cardiovascular function and microsomal xenobiotic metabolism.

Chronic Exposure TCE seems to be no more toxic upon long-term exposure than it is upon acute or short-term exposure. Quite large quantities of the chemical given orally to mice and rats 5 times weekly for 78 weeks elicited little apparent histopathological change of any organ in either species (National Cancer Institute, 1977). However, decreased body weight gain, obvious ill health, and diminished survival time in certain of these animals suggest that more sensitive and/or appropriate tests may reveal adverse effects by comparable ingestion regimens. Indeed, McNutt *et al.* (1975) reported increased liver weight, liver lipid content, and ultrastructural alterations in hepatocytes of mice that had been subjected for weeks to a vapor concentration as low as 250 ppm. Unfortunately, since no information on TCE blood or tissue levels was presented by these investigators, it is difficult to extrapolate their data to oral exposure.

In the absence of more definitive information regarding the chronic toxicity of ingested TCE, the lowest dosage level that was administered to either species in the NCI (1977) study (i.e., 750 mg/kg to rats) will be used as a basis for calculating a chronic SNARL. Depression in body weight gain in males and diminished survival time in both males and females have been observed in rats that were maintained on the 750 mg/kg oral dose. The following calculation assumes that a 70-kg human consumes 2 liters of water per day and that 20% of the total TCE intake is provided by water. An uncertainty factor of 1,000 is used and the dose is multiplied by 5/7 to convert from the 5- to 7-day exposure:

$$\frac{750\ \text{mg/kg} \times 5\ \text{days} \times 0.1\ \text{liter} \times 70\ \text{kg}}{7\ \text{days} \times 1{,}000} = 3.8\ \text{mg/liter}.$$

The study from which this value was calculated did not provide a no

observed-adverse-effect level. The large uncertainty factor is used for this reason. Because of the expected high use of this compound, the subcommittee considered it important to provide some provisional guidelines.

Appropriately designed oral, long-term dosing studies using several species of animals and a range of doses of TCE should be conducted in order to establish minimum toxic dose levels with accuracy. Vehicles for administration should be selected to assure that artificial exposure conditions are not created, e.g., use of large quantities of corn oil, as in the NCI (1977) study. Sensitive indices of aliphatic halocarbon exposure should also be selected carefully. Since only one investigation of the carcinogenic potential of TCE has been reported to date, additional research should be conducted with doses of TCE that do not shorten the life-span of the subjects. Vehicles for TCE dilution/administration should not create highly artificial exposure conditions. Appropriate studies to investigate the mutagenic and teratogenic potential of TCE should also be conducted.

Trichloroethylene

($ClHC = CCl_2$)

This compound was evaluated in *Drinking Water and Health* (National Academy of Sciences, 1977, p. 777). The following material, some of which became available after that 1977 publication, updates and, in some instances, reevaluates information in the earlier report. Also included are some references that were not assessed in the original report.

Metabolism

The pharmacokinetics of trichloroethylene (TCE) have been studied extensively in humans and laboratory animals that were exposed to the chemical by inhalation. Unfortunately, there is little information concerning the uptake and distribution of ingested TCE. One would anticipate that it would be readily absorbed from the gastrointestinal tract since it is a small, uncharged lipophilic molecule. Stewart and Dodd (1964) found that TCE penetrated intact human skin. Astrand and Ovrum (1976) reported rapid absorption of TCE vapor from the lung and a retention of approximately 55% of the amount that was inhaled by men. The arterial blood of these subjects was found to contain substantially higher TCE levels than did venous blood, indicating rapid uptake of the chemical from blood into tissues. TCE would be expected to localize in tissues of the body according to their lipid content (Sato *et*

al., 1977), although the liver should accumulate an even greater proportion of ingested TCE because of its position in the portal circulation.

Although a portion of systemically absorbed TCE is exhaled unchanged, a substantial amount is metabolized. Ogata *et al.* (1971) found that 65% to 75% of the TCE that was retained by humans during an inhalation exposure was excreted eventually as urinary metabolites. The proportion of metabolized TCE versus the proportion that was exhaled unchanged would be expected to vary inversely with dose. The details of TCE metabolism will not be related here since they have been reviewed in detail by the National Institute for Occupational Safety and Health (1973), Uehleke and Poplawski-Tabarelli (1977), and Uehleke *et al.* (1977). The implications of epoxide formation and reaction/inactivation are discussed below under mutagenicity. Suffice it to say that the major, isolable TCE metabolites in humans and laboratory animals are trichloroethanol glucuronide and trichloroacetic acid (TCA) (Ikeda and Ohtsuji, 1972). TCE is metabolized quite rapidly in humans, since both trichloroethanol and TCA appear in the blood quickly upon exposure to TCE (Muller *et al.,* 1974). Trichloroethanol is readily eliminated in the urine. The peak urinary excretion occurs from 1 to 3 hr after exposure to TCE (Ogata *et al.,* 1971). In contrast, renal clearance of TCA is delayed due to its high degree of plasma protein binding. Trichloroethanol is the predominant TCE metabolite in both humans and rodents, in that 3 to 4 times more trichloroethanol than TCA is excreted in the urine (Ikeda and Ohtsuji, 1972). Negligible quantities of both metabolites are eliminated in the feces. Measurement of both urinary metabolites has been used as an index of occupational exposure to TCE, although the procedure is of limited quantitative value due to marked individual variability (Monster *et al.,* 1976).

Health Aspects

Observations in Humans There are a number of accounts of poisoning of individuals who ingested TCE. Clinical signs and symptoms in victims are principally those of gastrointestinal upset, narcosis, and occasional cardiac abnormalities. Hepatorenal involvement is uncommon, although Kleinfeld and Tabershaw (1954) recounted a fatal case in which severe hepatorenal injury resulted from accidental ingestion of an undetermined amount of TCE. Two persons who each consumed 15 to 25 ml of TCE experienced vomiting and abdominal pain, followed by inebriation and transient unconsciousness (Stephens, 1945). Less severe depression

was observed in a 15-year-old girl who swallowed approximately 15 ml of TCE just after having eaten a large meal (Naish, 1945). A 4.5-year-old boy who ingested an estimated 7.6 g of TCE became inebriated within a few minutes, but recovered after approximately 3 hr (Gibitz and Plochl, 1973). More profound depression of the central nervous system, often accompanied by cardiac abnormalities, has been experienced by persons who consume even larger quantities of TCE (Dhuner *et al.,* 1957; Todd, 1954; Tomasini, 1976). Dhuner and colleagues (1957) reported the case histories of two patients who, upon drinking 350 and 500 ml TCE, were rendered unconscious for 4 and 8 days, respectively. Hypotension and cardiac arrhythmias were delayed in onset, but were quite serious in nature. In a number of instances, cardiac arrhythmias have been observed in patients being anesthetized with TCE and in subjects who were exposed occupationally. Those reports have been reviewed by the National Institute for Occupational Safety and Health (1973).

Experiments involving intentional, acute exposure of humans to TCE vapors reveal that inhalation of low levels of the chemical can result in mucous membrane irritation and impairment of psychophysiological functions. Complaints of eye and throat irritation and fatigue are made by some persons after exposures to 100 to 200 ppm TCE. Objective measures of psychophysiological performance generally show little evidence of inhibition (Gamberale *et al.,* 1976; Nomiyama and Nomiyama, 1977; Vernon and Ferguson, 1969). In contrast, Salvini *et al.* (1971b) reported that an 8-hr, 100-ppm exposure to TCE can inhibit performance in human subjects. Stopps and McLaughlin (1967) noted a slight decline in performance of subjects inhaling 200 ppm. This became increasingly pronounced at the 300- and 500-ppm exposure levels. TCE blood levels are approximately 1 µg/ml in subjects inhaling 100 ppm (Muller *et al.,* 1974) and 2 µg/ml in subjects inhaling 200 ppm (Vesterberg and Astrand, 1976). In light of the adverse effects of high concentrations on cardiac function, it is of interest that slight depressions in pulse rate and/or blood pressure have been observed in persons inhaling levels of TCE in doses as low as 170 ppm (Ogata *et al.,* 1971) and 200 ppm (Nomiyama and Nomiyama, 1977). Vernon and Ferguson (1969) reported that inhalation of 1,000 ppm TCE for 2 hr had no effect on blood cell count, BUN, SGOT, or urinalysis.

A limited number of inhalation studies, in which subjects were exposed to TCE for 1 to 2 weeks, indicated that the chemical is apparently no more hazardous upon repeated exposure than upon acute exposure. However, TCE does have a potential for accumulation in the body. Ikeda (1977) estimated that its urinary biological half-life in humans is 41 hr, in contrast to values of 144 hr for tetrachloroethylene

and 7 hr for both toluene and xylene. Humans that had been exposed to 50, 100, or 200 ppm of TCE vapor for 6 to 7 hr daily on 5 consecutive days exhibited progressively greater blood levels of trichloroethanol (Ertle *et al.,* 1972) and urinary excretion of trichloroethanol and tricholoracetate (Stewart *et al.,* 1970b). Stewart and his colleagues could detect TCE in the exhaled air of one subject for as long as 88 hr after exposure and metabolites in urine for up to 12 days. They also observed a striking degree of individual variability in urinary excretion of TCE metabolites. There was no alteration in clinical chemistry, urinalyses, or hematological indices, nor any evidence of effects on neurological and performance tests.

Several epidemiological studies of occupational exposures to TCE have been reviewed by the National Institute for Occupational Safety and Health (1973). There was a variety of subjective complaints and objective clinical findings, the majority of which involved irritant and depressant effects of the compound. Unfortunately, the accounts of chronic effects of TCE suffer from a number of deficiencies including the researchers' failure to determine accurate exposure levels and their inability to distinguish TCE-induced effects from those caused by other factors. In the absence of more definitive chronic exposure data, a time-weighted average (TWA) limit of 100 ppm for occupational TCE exposure was recommended in 1973. Selection of this value was based primarily upon findings in acute studies of inhibition of performance in humans. Largely on the basis of the carcinogenic potential of TCE, the National Institute for Occupational Safety and Health (1978) recommended that the TWA limit be reduced to at least 25 ppm, if not lower. It noted that ongoing epidemiological studies are looking for an association between occupational TCE exposure and cancer.

Observations in Other Species

Acute Effects A number of investigations of the acute toxicity of TCE have been conducted in various species of laboratory animals. Unfortunately, none involves oral administration of the chemical, except one in which the acute oral LD_{50} in rats was 4,920 mg/kg (Registry of Toxic Effects of Chemical Substances, 1975). Klaassen and Plaa (1967) reported that the ED_{50} for elevation of SGPT levels in mice that were given TCE by intraperitoneal injection was 1.6 ml/kg, while the acute intraperitoneal LD_{50} was only 2.2 ml/kg. In a subsequent study with dogs, the intraperitoneal ED_{50} for SGPT elevation was 0.57 ml/kg, and the acute intraperitoneal LD_{50} was 1.9 ml/kg (Klaassen and Plaa, 1967). Studies with rats revealed modest elevations in SGOT and/or histo

pathological changes in the livers of animals that were given 0.3 ml/kg by intraperitoneal injection (Cornish *et al.,* 1973) or 477 mg/kg by subcutaneous injection (Wirtschafter and Cronyn, 1964). TCE did not injure the kidneys of mice (Klaassen and Plaa, 1966) or dogs (Klaassen and Plaa, 1967) at any dosage level. In each of the aforementioned investigations, TCE proved to be one of the least hepatotoxic of a series of alkyl halides.

TCE, like most other chlorinated aliphatic hydrocarbons, elicits depression of the central nervous system and inhibits cardiac function in animals upon acute exposure. Investigations of possible adverse effects of exposure to inhaled or injected TCE have been adequately reviewed by the National Institute for Occupational Safety and Health (1973). However, it may be worthwhile to summarize the findings of a limited number of acute studies in which apparent "minimum-effect levels" of TCE were delineated. Mikiskova and Mikiska (1966) observed that a single intraperitoneal injection of 0.6 ml/kg of TCE caused a loss of muscle tone, depression of reflexes, and slowing of heart rate in guinea pigs. Tricholoroethanol, the principal metabolite of TCE in humans and laboratory animals, had similar effects and was at least 3 times as potent as TCE. On the basis of their studies of the potential of TCE to sensitize a dog's heart to epinephrine, Reinhardt *et al.* (1973) stated that the minimum effective vapor concentration was about 5,000 ppm. Kylin *et al.* (1963) reported an accumulation of 1,600 to 3,200 ppm in female mice that had been subjected to TCE inhalation for 4 hr. Lower vapor levels altered performance of rats. Inhalation of as low a concentration as 200 ppm TCE for several hours produced an increase in their spontaneous activity (Grandjean, 1960; Savolainen *et al.;* 1977); 400 and 800 ppm inhibited their swimming performance; and 1,600 ppm diminished their motor activity (Grandjean, 1963).

Chronic Effects Long-term studies of the toxicity of TCE indicate that the chemical is no more harmful upon repeated exposure than it is upon acute exposure of a variety of species of laboratory animals. One of the first comprehensive investigations of the chronic effects of TCE was conducted by Adams *et al.* (1951). They exposed several animal species to various levels of TCE vapor 7 hr/day, 5 days/week, for 6 months. "Maximum no-effect" levels were 400 ppm for monkeys, 200 ppm for rabbits and rats, and 100 ppm for guinea pigs. In a later study, Prendergast *et al.* (1967) subjected rats, guinea pigs, monkeys, rabbits, and dogs to one of two inhalation regimens: 730 ppm TCE for 5 days/week, 8 hr/day, for 6 weeks, or 35 ppm continuously for 90 days. The only evidence of effects of TCE exposure in either regimen were

occasional, slight body weight loss or depressed body weight gain. Seifter (1944) reported that 7 to 8 hr/day, 6 days/week exposures of dogs to 750 ppm TCE caused liver injury after 3 weeks. Although they stated that the TCE was 98% pure, these results should be interpreted with caution. In a more recent study Kylin *et al.* (1965) used a similar dosage schedule for 8 weeks. They found that 1,600 ppm TCE produced a slight accumulation of lipid in livers of mice. Fifty-five ppm TCE, inhaled 8 hr/day, 5 days/week, for 14 weeks, caused an increase in liver weight in rats (Kimmerle and Eben, 1973). No effects were noted in other organs, in the blood, in blood glucose levels, or in liver and kidney function. A progressive increase in urinary excretion of trichloroethanol was observed in these animals, but there was no evidence of accumulation of TCE, trichloroethanol, or trichloroacetic acid. This phenomenon led Kimmerle and Eben (1973) to speculate that TCE had induced its own metabolism.

Goldberg *et al.* (1964) reported that a daily 200 ppm TCE exposure inhibited avoidance response in certain "sensitive" members of a group of rats, although no progression in severity of the inhibition in these animals was observed during a 2-week regimen of daily, 4-hr inhalation exposures. There was a rapid development of tolerance to the depressant effects of TCE in rats that had been exposed repeatedly to 4,380 ppm of the chemical.

The only long-term toxicity studies employing oral administration of TCE were conducted as a part of the NCI (1976) investigation of TCE's carcinogenic potential. In an initial range-finding study, doses varying from 562 to 5,620 mg/kg TCE were given in corn oil to male and female rats and mice 5 days weekly for 6 weeks. Indices of toxicity that were monitored included body weight gain, food consumption, general appearance and behavior, grossly apparent lesions, and mortality. Based upon these criteria, the minimum-effect level appeared to be 1,780 mg/kg/day for rats and 3,160 mg/kg/day for mice. A chronic dosing study was then undertaken in which the animals were dosed by gavage 5 times weekly for up to 78 weeks. The time-weighted average doses were as follows: male and female rats, 549 and 1,097 mg/kg; male mice, 1,169 and 2,339 mg/kg; female mice, 869 and 1,739 mg/kg. Dose-dependent reduction in body weight gain, haggard appearance, and decreased survival time were manifest in male and female rats throughout much of the study. The only evidence of adverse effect that was observed in the mice was an increase in mortality in high-dose males. Although no histopathological change was detected in the livers of mice or rats, slight to moderate degenerative alterations of proximal tubular epithelium were apparent in kidneys of low-and high-dose male and female mice

and rats. Findings pertaining to tumor induction are discussed below under carcinogenicity.

Mutagenicity Recently, several groups of investigators have observed that TCE is mutagenic in *in-vitro* screening systems. Although Shahin and Von Borstel (1977) reported technical grade TCE to be a potent mutagen for yeast, other workers found the compound to be only weakly mutagenic in bacterial test systems (Greim *et al.,* 1975; Henschler *et al.,* 1977; Simmon *et al.,* 1978). The purity of the TCE in each investigation was not established except in the study by Henschler *et al.* who demonstrated that technical grade TCE contained at least two mutagenic contaminants, epichlorohydrin and 1,2-epoxibutane. In contrast, Henschler *et al.* (1977) noted that purified TCE was very weakly mutagenic. Donahue *et al.* (1978), employing their microsomally activated *Salmonella typhimurium* mutagenicity screening system, found that high pressure liquid chromatography purification resulted in a significant decrease in the mutagenicity of 4 of 11 chemicals tested. They suggested that experiments be conducted to determine whether highly purified TCE was mutagenic in the Ames test.

A number of investigators have speculated that hepatotoxic, mutagenic, and carcinogenic effects of TCE are related to its conversion to a reactive intermediate by the microsomal mixed-function oxidase system. It is generally accepted that this reactive molecular species is 2,2,2-trichloroepoxide, an intermediate product during the metabolism of TCE to its first stable *in-vivo* metabolite, chloral hydrate. However, Bartsch *et al.* (1976) failed to detect epoxide formation from TCE by mouse liver microsomes and did not find human liver microsomes to activate TCE to a mutagen in a *S. typhimurium* test.

Greim *et al.* (1975) demonstrated that the stability/reactivity of epoxides of a series of chlorinated ethylenes was dependent upon their pattern of chlorination. Unsymmetrically substituted ethylenes (e.g., 1,1-dichloroethylene, vinyl chloride, TCE) formed more unstable epoxides than did symmetrically substituted congeners (e.g., 1,2-dichloroethylene, tetrachloroethylene). Greim *et al.* (1975) observed that the aforementioned unsymmetrically substituted ethylenes were indeed mutagenic to *Escherichia coli,* while the symmetrically substituted compounds were not. Vinyl chloride was the most potent mutagen.

Since chlorinated ethanes are not as predisposed as ethylenes to form epoxide intermediates, they are probably less likely than ethylenes to react directly with biological nucleophiles, thereby producing toxicity, mutagenicity, or carcinogenicity. The recent findings of Uehleke *et al.* (1977) support this concept. They reported the binding of microsomally

activated chlorinated ethylenes to hepatic microsomal P-450. Listed in order of decreasing magnitude of binding, they are: 1,1-dichloroethylene, TCE, 1,2-dichloroethylene, and tetrachloroethylene. There was no apparent binding in a series of chlorinated ethanes, while synthetic TCE epoxide was a quite strong binding agent.

Binding of ^{14}C-TCE to mouse and rat hepatic microsomal proteins has been demonstrated both *in vitro* and *in vivo* (Banerjee and Van Duuren, 1978; Bolt *et al.,* 1977; Uehleke and Poplawski-Tabarelli, 1977; Van Duuren and Banerjee, 1976). Uehleke and Poplawski-Tabarelli (1977) reported that lesser amounts of radio-labeled TCE were bound to mitochondrial and cytoplasmic proteins in the livers of mice. They also noted that the TCE that was used by Van Duuren and Banerjee (1976) contained substantial amounts of labeled impurities. The binding of contaminants to microsomal proteins *in vitro* without metabolic activation was shown by Uehleke and Poplawski-Tabarelli (1977). They observed that the binding of purified TCE was considerably less than that of the original sample.

Each of the foregoing reports demonstrated that TCE microsomal protein binding, binding to cytochrome P-450, and *in-vitro* mutagenicity are dependent upon activation of the TCE by a microsomal mixed-function oxidase (MFO) system. In every instance, a microsomal enzyme-inducing agent was used to stimulate the metabolic capability of the mixed-function oxidase system. Uehleke and Poplawski-Tabarelli (1977) and Van Duuren and Banerjee (1976) noted that pretreatment with phenobarbital produced an approximately twofold increase in hepatic, microsomal covalent binding of TCE in mice. SKF 525-A, a microsomal enzyme inhibitor, blocked the covalent binding. The hepatotoxicity of TCE in rats is markedly potentiated by pretreatment with various microsomal enzyme inducers (Carlson, 1974; Moslen *et al.,* 1977a,b). Moslen and her colleagues (1977b) observed a marked decrease in liver glutathione levels in phenobarbital-pretreated rats, while Van Duuren and Banerjee (1976) found that addition of glutathione to their induced *in-vitro* system inhibited covalent binding of TCE to hepatic microsomal proteins. Thus, it appears that reduced glutathione helps stabilize or convert reactive intermediate(s) to less reactive, toxic metabolite(s). Additional evidence for the formation of a reactive intermediate (epoxide) was furnished by Van Duuren and Banerjee (1976), who found that addition of 3,3,3-trichloropropene oxide, a potent inhibitor of epoxide hydrase, to their *in-vitro* system enhanced covalent binding of TCE.

Reported strain, sex, and species differences in susceptibility to injury by TCE appear to be related to inherent differences in hepatic metabolic

capability. As reported above, TCE produced liver cancers in male and female B6C3F1 mice, but not in Osborne Mendel rats in the NCI study (1976). One basis for this species difference may be the relatively low activity of epoxide hydrase in the mouse (Oesch, 1973). Simmon *et al.* (1978) reported that liver microsomes from B6C3F1 mice pretreated with polychlorinated biphenyl (PCB) were more effective than microsomes from pretreated Sprague-Dawley rats in activation of TCE to a mutagen in the Ames test. Similarly, Banerjee and Van Duuren (1978) found greater binding of TCE to microsomal proteins of B6C3F1 mice than to proteins of Osborne Mendel rats. Such binding was greater for Sprague-Dawley rats than for Osborne Mendel rats. Covalent binding of TCE to exogenous DNA and to hepatic microsomal protein was more pronounced in males than in females of the B6C3F1 hybrids. Female mice proved more resistant than males to the tumorigenic action of TCE in the NCI study (1976).

Carcinogenicity Results of the NCI study (1976) have prompted a second investigation of the carcinogenic potential of TCE. This investigation, sponsored by the Manufacturing Chemists Association, involves inhalation exposure of B6C3F1 mice and Charles River rats for 6 hr/day, 5 days/week, to 100, 300, or 600 ppm TCE for up to 2 years. A preliminary report (Manufacturing Chemists Association, 1977) of findings in the animals that were sacrificed after 2 years of TCE exposure or that died during this period indicates that there is a modest increase in incidence of both hepatocellular carcinomas and adenomas in male mice exposed to the highest vapor concentration. Scrutiny of the data reveals what appears to be a concentration-related incidence of tumor induction, although variance from controls is not statistically significant in most cases. No liver tumors have been observed in the rats.

The findings of the NCI carcinogenicity study (1976) and *in-vitro* mutagenicity studies have stirred a good deal of controversy surrounding the true carcinogenic potential of TCE. Henschler *et al.* (1977) performed gas chromatography, mass spectrometry analyses of the same industrial grade TCE that was used by the NCI. They found the sample to contain approximately 0.2% of both epichlorohydrin and 1,2-epoxibutane, as well as smaller amounts of chloroform and carbon tetrachloride. Henschler *et al.* (1977) suggested that the epichlorohydrin and 1,2-epoxibutane were responsible for the reported carcinogenic effect, in that they found each contaminant to be a potent mutagen in a *S. typhimurium* screening test. In contrast, purified TCE was a very weak mutagen in this *in-vitro* system. Donahue *et al.* (1978) pointed out that most carcinogens are active when given orally to rodents on a daily basis in the mg/kg

body weight range and that some of the more potent carcinogens are active in the μg/kg range. Although Donahue and his coworkers discounted the role of 1,2-epoxibutane, they believed that it was quite possible that contamination of TCE with epichlorohydrin could have been responsible for the carcinogenic response that was reported by the NCI (1976).

Other questions about the design of the NCI (1976) carcinogenesis bioassay of TCE have been raised. One concerns the daily administration of extraordinarily large quantities of a chemical. In such cases, distribution, metabolism, and excretion of the chemical may be quite different from that which occurs at anticipated environmental exposure levels. In addition, repeated insult to the liver by quantities of the chemical sufficient to kill some hepatocytes will result in a continuing state of cellular regeneration. Cellular proliferation is implicated in chemical carcinogenesis in that partial hepatectomy may predispose rodents to tumor induction by various chemical agents (Cayama *et al.,* 1978). Other problems with the NCI (1976) study design include the administration of large volumes of oil as a vehicle for TCE and the housing of subjects in the same room as animals receiving a variety of other volatile chemicals.

Conclusions and Recommendations

Suggested No-Adverse-Response Level (SNARL)

Twenty-Four-Hour Exposure Although acute exposure to large quantities of TCE can result in marked depression of the central nervous system, the chemical apparently has a limited capacity to cause residual tissue injury. The lowest oral dose of TCE that has been reported to produce inebriation is approximately 300 mg/kg body weight. However, in light of the significant degree of depression of the central nervous system, which was observed at this dose level, the "minimum-effect level" for inhibition of psychophysiological functions is doubtlessly lower than 300 mg/kg. Thus, it seems that the 300 mg/kg figure derived from human clinical experience is a reasonable dose on which to base calculations for a 24-hr SNARL for contamination of drinking water by TCE. A 100-fold safety factor is used recognizing that 300 mg/kg is not a "minimum-effect level" for ingestion of TCE as a single dose. It is assumed here that the sole source of TCE during the 24-hr period will be drinking water and that a 70-kg human consumes 2 liters/day. These considerations ignore the possibility that TCE may be carcinogenic on short-term exposure:

$$\frac{300 \text{ mg/liter} \times 70 \text{ kg}}{100 \times 2 \text{ liters}} = 105 \text{ mg/liter.}$$

Seven-Day Exposure TCE appears to be no more hazardous upon short-term exposure than it is upon acute exposure. Unfortunately, the only short-term studies that have been conducted with humans or with laboratory animals involve inhalation exposure to relatively low concentrations of the chemical. No adverse effects on performance, neurological function, or clinical chemistry indices are reported in humans subjected several hours daily to as much as 200 ppm TCE for 5 consecutive days. These studies demonstrate that TCE, despite rather extensive metabolism, has some propensity for accumulation in the body at the exposure levels used. Nevertheless, in view of TCE's relative lack of toxicity, a 7day SNARL for drinking water contamination, obtained by dividing the 24-hr SNARL by 7, should protect against adverse effects of the chemical. This standard is based on the assumption that the sole source of TCE during this period will be drinking water. These considerations ignore the possibility that TCE may be carcinogenic on short-term exposure:

$$\frac{105 \text{ mg/liter}}{7} = 15 \text{ mg/liter.}$$

Chronic Exposure TCE appears to be no more toxic (noncarcinogenic) on long-term exposure than it is upon acute or short-term exposure. Unfortunately, every long-term study, with the exception of the NCI (1976) carcinogenesis investigation, involves administration of TCE to laboratory animals by inhalation. The "minimum-effect level" reported in these inhalation studies, 55 ppm, produced increased liver weight in rats that were subjected to TCE vapor 8 hr/day, 5 days/week for 14 weeks. The lowest oral dose used in the NCI study (1976), 549 mg/kg/day, produced renal injury, haggard appearance, and decreased life-span in male and female rats. Thus, due to failure of the NCI study to determine a "no-effect level" and to monitor a sufficiently broad battery of sensitive toxicological indices, it is not possible to establish a "minimum-effect level" for chronic, noncarcinogenic toxicity.

Definitive oral administration studies in several species of animals should be undertaken using a range of doses of TCE to characterize dose-effect and dose-response relationships for both a single ingestion and multiple ingestions over a I-week period. A variety of valid indices of injury to potential target organs should be monitored. Particular

attention might be focused on ascertaining the "minimum-effect level" for inhibition of psychomotor performance and cardiac function, since these parameters seem to be among the most sensitive indices of human exposure to TCE. Studies involving potential interactions of TCE with other chemicals and drugs are appropriate, in view of the likelihood of exposure of persons to combinations of chemicals. As no data pertaining to the uptake, distribution, metabolism, and excretion of TCE upon ingestion are apparently available, pharmacokinetic studies should be undertaken. Vehicles for administration should be chosen carefully to avoid discrepancies between test exposures and actual exposures from drinking water or food.

Additional long-term studies of toxicity, mutagenesis, and carcinogenesis should be conducted with purified TCE in order to determine if TCE is a toxicant, mutagen, or carcinogen, and the minimum times and doses that are required to produce adverse effects. A range of doses, including anticipated, potential exposures, should be given orally to at least two species of animals.

Trichlorofluoromethane

(CCl_3F)

This compound was previously evaluated in *Drinking Water and Health* (National Academy of Sciences, 1977, p. 781). The following material, some of which became available after that 1977 publication, updates and, in some instances, reevaluates the information in the earlier report. Also included are some references that were not assessed in the original report.

Metabolism

Theoretical metabolites of trichlorofluoromethane are dichlorofluoromethane and tetrachlorodifluoroethane. No evidence of free-radical formation in rats or mice has been shown; nor is there evidence of significant metabolism of trichlorofluoromethane although it has been shown to interact with hepatic cytochrome P-450 (Cox *et al.,* 1972).

Health Aspects

Observations in Humans No available data.

Observations in Other Species

Acute Effects Unanesthetized beagle dogs inhaled various concentrations of Freon 11 for 10 min via face mask. Threshold concentrations in blood associated with cardiac sensitization were 28.6 μg/ml (arterial) and 19.7 μg/ml (venous) (Azar *et al.,* 1973).

Conclusions and Recommendations

Suggested No-Adverse-Response Level (SNARL) There are insufficient oral data from which to determine with accuracy the effects from a large oral exposure or to estimate a threshold dose that might produce adverse effects. The solubility of Freon 11 in water is negligible at 21 °C, but is as much as 1 g/liter at 25°C. Thus, a person ingesting 2 liters of water per day after a spill could consume 2 g if the water was warm (close to 27ºC). This would translate to approximately 30 mg/kg/day in a 70-kg human. In view of the data reported by Kudo *et al.* (1971), who gave oral doses to mice for 1 month at levels as high as 220 mg/kg without severe adverse effects, it seems very unlikely that the 30 mg/kg projected above would constitute an oral toxic hazard to humans in the short or long term.

Although data for calculating a 24-hr or 7-day SNARL are very limited, one can estimate the 24-hr value from the 2.5-g oral dose in rats by Slater (1965) and the 7-day value from the 220 mg/kg maximum tolerated dose for 1 month in mice by Kudo *et al.* (1971). Assuming that a 70-kg human consumes 2 liters of water daily, with 100% of exposure from water, and using a safety factor of 1,000:

Twenty-Four-Hour Exposure:

$$\frac{2{,}500 \text{ mg/kg} \times 70 \text{ kg}}{1{,}000 \times 2 \text{ liters}} = 88 \text{ mg/liter.}$$

Seven-Day Exposure:

$$\frac{220 \text{ mg/kg} \times 70 \text{ kg}}{1{,}000 \times 2 \text{ liters}} = 8.0 \text{ mg/liter.}$$

The paucity of data on this compound particularly by the oral route precludes an accurate risk assessment. The maximum dose, which theoretically could be consumed in warm water at 2 liters/day by a 70-kg

human, would be about 30 mg/kg/day. The meager data suggest that this is a nontoxic dose. Thus, the acute spill hazard would appear to be quite low. However, it seems very pertinent to compare concentrations in the blood of humans after exposure to known levels with concentrations that are known to produce cardiac sensitization in dogs, e.g., 19.7 g/ml (venous) and 28.0 μg/ml (arterial).

Toluene

($C_6H_5CH_3$)

This compound was evaluated *in Drinking Water and Health* (National Academy of Sciences, 1977, p. 770). The following material, which became available after that 1977 publication, updates and, in some instances, reevaluates the information in the earlier report. Also included are some references that were not assessed in the original report.

The biological effects of toluene and other organic solvents have been the subject of several recent reviews (Bruckner and Peterson, 1977; Dean, 1978; Hayden *et al.,* 1977; Savolainen, 1977). Except for toluene's depressant effects on the central nervous system, there are no conclusive data indicating specific target organ toxicity. However; many biological effects have been attributed to toluene in both humans and animals. Among these are neurotoxicity, kidney and liver damage, cardiac sensitization, and blood dyscrasias.

Metabolism

An extensive reservoir for inhaled toluene is provided by body fat (Sato *et al.,* 1974). This is illustrated by the fact that saturation of the liver and brain of mice is not reached despite inhalation of toluene vapor at a concentration of 4,000 ppm for 3 hr (Bruckner and Peterson, 1976). Following inhalation or oral exposure of rats to radio-labeled toluene, similar amounts of radioactivity were found in several organs and tissues, although fat generally contained levels that were 10-fold higher than any other tissue measured. Radioactivity was rapidly eliminated from all organs and tissues, and only 1% or less of the initial radioactivity was found in tissues other than fat, which contained 3.5% to 5% (Carlsson and Lindqvist, 1977; Pyykko *et al.,* 1977). In humans, the uptake of inhaled toluene was influenced by body fat. Subjects with the least adipose tissue had the smallest uptake (Carlsson and Lindqvist, 1977). Egle and Gochberg (1976)noted that retention of inhaled toluene in the respiratory tract was greater than that for benzene. In contrast the extent of percutaneous absorption of benzene and toluene appears to be similar (Wahlberg, 1976).

The measurement of urinary hippuric acid has been used to estimate occupational exposure to toluene, both qualitatively and quantitatively (Brugnone *et al.,* 1976; Caperos and Fernandez, 1977; Engstrom *et al.,* 1976; Kira, 1977; Ogata *et al.,* 1977; Szadkowski, 1975; Szadkowski *et al.,* 1976). However, these measurements may be of little quantitative value except for exceptionally high exposures because of the variable excretion of hippuric acid by unexposed individuals (Engstrom *et al.,* 1976).

Toluene exposure may alter the metabolism, disposition, and biological effects of other agents (Hayden *et al.,* 1977). Noteworthy is the recent report by Andrews *et al.* (1977) concerning the effects of toluene on benzene disposition and toxicity. They observed that toluene ameliorates benzene toxicity.

Health Aspects

Observations in Humans A variety of effects in humans include adverse mental changes, such as altered psychomotor performance, irritability, disorientation, and unconsciousness. Additionally, toluene abuse has reportedly been associated with cardiac arrhythmias and with liver and kidney dysfunction (Hayden *et al.,* 1977; Weisenberger, 1977). Female shoemakers who were exposed occupationally to toluene vapor concentrations of 60 to 100 ppm had significantly higher urinary concentrations of hippuric acid than did controls and voiced some complaints of abnormal tendon reflexes, reduced grasping power, and decreased agility of the fingers (Matsushita *et al.,* 1975). Mixtures of organic solvents that include toluene have been implicated as the cause of lens changes in car painters (Raipta *et al.,* 1976). However, the evidence is not sufficient to establish that these effects are due to toluene. In other occupational groups exposed to toluene, no chronic toxic effects could be detected (Rosensteel and Thoburn, 1975).

Observations in Other Species

Acute Effects The acute oral toxicity of toluene in 14-day-old rats is significantly greater than in young adult rats (Kimura *et al.,* 1971). In adult rats, the 4-hr LC_{50} for inhaled toluene concentrate (toluene, 45.89%; benzene, 0.06%; paraffins, 38.69%; naphthenes, 15.36%) was 8,800 ppm (35 mg/liter). Rats tolerated 6.8 mg/liter for 4 hr, and dogs, 3.0 mg/liter for 6 hr, without signs of discomfort (Carpenter *et al.,* 1976).

Short-term, high-dosage, oral administration of toluene to rats decreased slightly the apparent liver microsomal activity of some of the

enzymes associated with the mixed-function oxidase system (Mungikar and Pawar, 1976a) and inhibited liver microsomal lipid peroxidation (Mungikar and Pawar, 1976b). In contrast, the intraperitoneal administration of toluene to male mice did not alter liver microsomal *N*- and *O*-demethylase activity or the various spectral characteristics of microsomal cytochrome P-450 (Fabacher and Hodgson, 1977).

Single, large oral dosages of toluene were essentially nonhepatotoxic in guinea pigs. However, at the highest dosage, 1.2 ml/kg, some lipid accumulation in liver was noted (Divincenzo and Krasavage, 1974).

Inhalation of toluene vapor by mice and rats at concentrations of 2,600 to 12,000 ppm for up to 3 hr inhibited "performance" as assessed by a battery of animal performance tests. However, there was no evidence of organ damage, as determined by organ function tests, organ weights, and histopathology (Bruckner and Peterson, 1978).

Rats that were exposed to 1,000 to 4,000 ppm toluene vapor for 4 hr showed changes in their sleep cycle, which were detected by electroencephalogram (EEG) (Takeuchi and Hisanaga, 1977). Mabuchi *et al.* (1974) suggested that similar changes can be observed in the EEG's of patients that have been chronically exposed to organic solvents.

Using a continuous flow bioassay system, the LC_{50} for toluene in freshwater was determined for goldfish. The 24-, 48-, 72-, 96-, and 720-hr LC_{50} values were 41.6, 27.6, 25.3, 22.8, and 14.6 ppm, respectively (Brenniman *et al.,* 1976).

Chronic Effects Toluene applied daily to the skin of rats 4 hr/day for 4 months at 10 g/kg increased plasmic and lymphoid reticular cells in bone marrow and impaired leukopoiesis, especially neutrophil maturation. Toluene at 1 g/kg was without effect (Yushkevich and Malysheva, 1975).

Daily intraperitoneal (0.25 to 1.0 ml/kg for 12 days) or subcutaneous (0.25 to 1.0 ml/kg for 3 weeks) dosages of toluene administered to male rats produced a dose-related increase in the number and total area of mitochondria per unit of cytoplasmic area in liver. A dose-related decrease in nuclear volume was also observed (Ungvary *et al.,* 1976). The specificity of these effects was not determined and their significance is uncertain.

Rats that were exposed to toluene vapors at 1,000 ppm for 8 hr/day for 1 week had slightly elevated SGOT and SGPT activities and showed metabolic acidosis (Tahti *et al.,* 1977). However, male and female rats that were exposed to toluene vapor at concentrations of 0, 30, 100, 300, or 1,000 ppm for 6 hr/day, 5 days/week, for 13 weeks (64 exposures) showed no significant alteration in body weight gain or in hematological

or clinical chemistry measurements. Furthermore, no gross or histopathological alteration in any treated rat could be attributed to toluene (Rhudy *et al.,* 1978).

Exposure of mice to 4,000 ppm toluene vapor, 5 times per week for up to 8 weeks, failed to reveal evidence of injury to the lung, liver, or kidney (Bruckner and Peterson, 1976). Exposure of male and female Fischer 344 rats to 0, 30, 100, or 300 ppm doses of toluene vapor for 6 hr/day, 5 days/week, for 6 months caused only eye reddening and varying degrees of hair loss in some animals. At high doses, both male and female animals exhibited slightly, but significantly less weight gain than controls for the 6-month period. Hematological, histopathological, and clinical chemistry studies revealed no significant dose-related differences between treated and control animals (Chemical Industry Institute of Toxicology, 1978).

Rats and dogs that inhaled 3.9, 1.9, or 0.95 mg/liter of toluene concentrate (toluene, 45.89%; benzene, 0.06%; paraffins, 38.69%; naphthenes, 15.36%) for 13 weeks, 6 hr/day, were not significantly different from their air-exposed controls in any of the criteria of injury that were monitored through hematology, clinical chemistry, and micropathology (Carpenter *et al.,* 1976).

Exposure of mice to toluene vapors at concentrations of 1, 10, 100, or 1,000 ppm for 6 hr/day for 20 days induced changes in the composition of peripheral blood and decreased wheel-turning activity. Bone marrow hypoplasia was noted at 1,000 ppm toluene (Inoue, 1975). There were decreases in wheel-turning activity on the tenth day of exposure to 1 ppm toluene and earlier in the other treatment groups (Horiguchi *et al.,* 1978).

Exposure of eight rats to toluene vapor at a concentration of 4,000 ppm, 2 hr/day, for 60 days appeared to decrease learning as measured by impairment of the acquisition of a differential reinforcement on a 12-s schedule. However, there was no effect of toluene as determined by the extinction of the fixed ratio schedule, memory in the continuous reinforcement schedule, spontaneous activity, or emotionality (Ikeda and Miyake, 1978).

Mutagenicity There are no data on the mutagenicity of toluene in bacterial systems (Dean, 1978). Lyapkalo (1973) reported chromosome damage of bone marrow cells in rats that were injected with 1 g/kg of toluene (Lyapkalo, 1973). Studies of workers who had been exposed to toluene for many years failed to show a significant increase in chromosome aberrations (Forni *et al.,* 1971). Chromosome damage induced by inhalation exposure to toluene (160 mg/m^3) in bone marrow cells was still present 1 month after terminating exposure in rats

(Dobrokhotov and Enikeev, 1977) although blood cell counts reportedly had returned to normal. These studies were also cited by Walker (1976).

Carcinogenicity A skin carcinoma in one mouse and a skin papilloma in another were observed in a group of 30 mice to whose skin 16 to 20 µl of toluene was applied topically twice a week for 72 weeks (Lijinsky and Garcia, 1972). In contrast, application of toluene to the skin of mice 3 times a week for the lifetime of the mice failed to produce any carcinogenicity that could be attributable to the toluene (Poll, 1963).

The collagen content of the dorsal skin of the mice was reduced by three weekly paintings with toluene for 10 weeks. Compared with toluene as a solvent for 0.3% 20-methylcholanthrene, acetone caused a slower decrease of collagen and a longer latency of tumor development (Mazzucco, 1975).

Teratogenicity Exposure of pregnant rats to toluene vapors at a concentration of 600 mg/m^3 for an unspecified period caused embryo toxicity but no teratogenicity (Hudak *et al.,* 1977). Women that were exposed to toluene and other agents through the occupational use of "organosiliceous" varnishes showed evidence of a fall in red blood cell and thrombocyte indices. There was a high incidence of menstrual disorders and reported effects on embryonic and fetal development such as more frequent fetal asphyxia, a greater number of newborns with low weight, and belated sucking of the maternal breast (Syrovadko, 1977).

Conclusions and Recommendations

Suggested No-Adverse-Response Level (SNARL)

Twenty-Four-Hour Exposure Single, large (1.2 ml/kg), oral doses of toluene produced minimal lipid accumulation in the liver of guinea pigs (Divincenzo and Krasavage, 1974). Assuming that this dose represents the largest minimal-effect dose and using an uncertainty factor of 100, the following 24-hr SNARL may be calculated for a 70-kg human consuming 2 liters of water per day with 100% of exposure from water during this period:

$$\frac{1{,}200 \text{ mg/kg} \times 70 \text{ kg}}{100 \times 2 \text{ liters}} = 420 \text{ mg/liter.}$$

The solubility of toluene in water is 470 mg/liter at 16ºC.

Seven-Day Exposure Daily intraperitoneal 1.0 g/kg doses of toluene administered to rats produced only minor mitochondrial changes of liver cells after 12 days of exposure (Ungvary *et al.,* 1976). These data suggest that rats can tolerate a dosage of 1.0 g/kg/day for 7 days with minimal effect. Thus, the following 7-day SNARL value may be calculated for a 70-kg human consuming 2 liters of water per day assuming an uncertainty factor of 1,000 and 100% of exposure from water during this period:

$$\frac{1{,}000 \text{ mg/kg/day} \times 70 \text{ kg}}{1{,}000 \times 2 \text{ liters}} = 35 \text{ mg/liter.}$$

Chronic Exposure Exposure of male and female Fischer-344 rats to approximately 1,130 mg/m^3 (300 ppm) toluene vapor 6 hr/day, 5 days/week, for 6 months caused minimal toxicity. Based on these data, a chronic SNARL value is calculated as follows for a 70-kg human consuming 2 liters of water per day. An uncertainty factor of 1,000 is assumed. Because these data refer to inhalation exposure, it is further assumed that a 70-kg human inhales 10 m^3/day and that 30% of the inhaled toluene is absorbed from the lung into the blood. This chronic value also assumes that 20% of the toluene comes from drinking water:

$$\frac{1{,}130 \text{ mg/m}^3 \times 10 \text{ m}^3\text{/day} \times 0.3 \times 0.2}{1{,}000 \times 2 \text{ liters}} = 0.34 \text{ mg/liter.}$$

Uranium

(U)

Uranium is a silvery-white, heavy metal that occurs ubiquitously in the crust of the earth (*Merck Index,* 1976). Natural uranium consists of three isotopes ^{238}U, ^{235}U, and ^{234}U in the relative abundance of 99.27%, 0.72%, and 0.006%, respectively (Chen *et al.,* 1961; *Merck Index,* 1976). All are naturally radioactive, being α-emitters, but on a weight basis the activity of ^{234}U is 17,000-fold and that of ^{235}U is 6-fold greater than that of ^{238}U. Thus, radiotoxicity depends upon whether one is dealing with natural uranium or enriched uranium (Chen *et al.,* 1961). Uranium has valences of 6, 5, 4, and 3, and it forms salts with many anions that also influence the solubility of uranium. ^{235}U is used in atomic and hydrogen bombs. ^{234}U and ^{235} U are used as nuclear fuel in power reactors.

Metabolism

The major exposure of the general populace to uranium is through diet and drinking water. Welford and Baird (1967) reported that the dietary intake of uranium in New York, Chicago, and San Francisco is 1.3, 1.4, and 1.3 μg/day, respectively. The U.S. Geological Survey Water Supply Paper 1812 (Durfor and Becker, 1964) indicates that the concentration of uranium in drinking water for the 100 largest U.S. cities is <0.1 μg/liter. However, in some cities, e.g., Long Beach, California, the level may reach 8.6 μg/liter (Hamilton, 1972). The International Commission on Radiological Protection (1959) estimates that the body burden of uranium in a 70-kg human is 20 μg. However, Hamilton (1972) estimated that the body burden ranges from 100 to 125 μg in the United Kingdom, where intake is approximately 1 μg/day.

Disposition studies in humans were conducted by Bernard (1958) who administered 4- to 5-mg doses of hexavalent and tetravalent uranium intravenously to terminal patients suffering from brain tumors. With both chemical forms, 60% to 70% of the uranium dose was excreted within 24 hr. The hexavalent uranium tended to distribute about equally to the skeleton and kidney. Half-lives of approximately 300 days were calculated for both organs. However, tetravalent uranium was distributed to the bones and liver. Studies by Hamilton (1972) reveal that the greatest amount of uranium in the body is found in the skeleton, but kidney burden was not measured in these studies. In small animals, the principal organ for storage of hexavalent uranium is bone (85%).

Belova and Lun'kina (1967) studied the accumulation of uranium in the teeth of inhabitants from two sites in the Volga region of Russia. The amount of uranium in the drinking water at these sites varied from 5×10^{-5} g/liter to 3×10^{-7} g/liter. These authors found that accumulation of uranium in the human skeleton depends upon the daily amount ingested. In addition, they found that the accumulation of uranium increased up the biological chain, i.e., accumulations were lowest in plants and highest in humans.

In other animal studies, McClellan *et al.* (1962) studied the distribution of ^{233}U in the milk of sheep following intravenous injection of uranium. The milk-to-plasma ratio was <0.1. Sikov and Mahlum (1968) found less than 1% of the ^{233}U dose in the fetuses of rats receiving uranium between days 15 and 19 of gestation.

Health Aspects

There is general agreement that chemical toxicity to uranium is most critical in the kidney (Bernard, 1958; Chen *et al.,* 1961). However, it should be understood that chemical toxicity of uranium is not restricted to the kidneys, but also involves the cardiovascular, endocrine, hematopoietic, and immunological systems as well as hepatic dysfunction (Novikov, 1970). ^{238}Uranium could not be a radiological hazard in humans since the doses necessary to deposit enough uranium in bone to create radiation that is equal to 0.1 μCi of radium would be far in excess of uranium doses producing lethality by kidney destruction. With the more active α-emitting uranium isotopes, ^{233}U, ^{234}U, and ^{235}U, the bone becomes the critical organ of concern for radiotoxicity (Chen *et al.,* 1961).

Observations in Humans Very few recent data deal with any aspect of the toxicity of uranium compounds in humans. In early studies, Luessenhop *et al.* (1958) found that a dose of 0.1 mg/kg of uranyl nitrate administered intravenously to terminal patients suffering from glioblastoma multiforme produces nephrotoxic effects that are characterized by catalasuria, albuminuria, and the appearance of casts in the urine. Neuman (1953) recommended a safe kidney burden of 2 to 3 μg/g of uranium for chronic exposure. The nephrotoxic intravenous dose of 0.1 mg/kg would yield a kidney burden of about 6 μg/g.

Novikov and colleagues (Novikov, 1967, 1970; Novikov and Abramova, 1969; Novikov and Yudina, 1970; Novikov *et al.,* 1968) published a series of papers describing studies conducted in humans from two towns (A and B) in the Volga region of Russia. These towns were closely matched for climate, socioeconomics, demography, etc., except for the average concentration of uranium in the drinking water, which was 0.04 to 0.05 mg/liter in town A and 0.002 to 0.004 mg/liter in town B (approximately one-tenth that of town A). In general, the Russian investigators found that the relatively high concentration of uranium (0.04-0.05 mg/liter) in the drinking water of town A did not affect the health of the population. No differences were found in birthrate or death rate, deaths from malignant neoplasms, or incidence of cancer. Studies were also conducted to examine the distribution of uranium in various human tissues from these two populations. The greatest amount of uranium was found in the kidney and the bone; however, there were no significant differences between the two towns. The authors concluded that drinking water containing uranium in concentrations of 0.04 to 0.05 mg/liter did not cause an accumulation of

the element in human tissues. Additional studies were conducted examining the effect of this uranium difference on various clinical biochemical and hematological tests. The only difference found was an alteration in the ratio of serum albumin to globulin. The inhabitants of town A had a decrease in albumin but an increase in globulins in comparison with the inhabitants of town B (Novikov *et al.,* 1968).

Observations in Other Species

Acute Effects The LD_{50} of uranium (salt not specified) in male albino rats is 750 mg/kg (route not specified) (Arsen'yeva, 1972). Stefanov and Yurukova (1964) gave a single intravenous injection of uranium nitrate to rabbits of both sexes and examined various clinical biochemistry tests as well as making histological examinations on days 1, 3, 5, 7, 10, 14, and 21 after administration of uranium. Weight losses were observed by day 5. The rabbits weighed only 70% of their initial weights by day 21. Decreases in hemoglobin and erythrocytes were observed. Nonprotein nitrogen and urea were increased. Histological examination revealed nephropathology and hepatotoxicity.

Subacute Effects Arsen'yeva (1972) administered uranium in doses of 0.6, 6.0, and 60 mg/kg to rats for 3 months. He observed alterations in alkaline phosphatase, blood urea, and urinary asparagine transaminase at the two higher doses of uranium. At the 60 mg/kg dose, he also noted an inhibition of weight gain.

Chronic Effects Novikov and Yudina (1970) examined the effects of 0.02 to 2.0 mg/kg doses of uranium which were administered *per os* for 12 months to rabbits and in doses of 0.05 to 60 mg/kg in rats. They observed no changes in urea, creatinine, or serum chloride levels. At the high doses in both groups the metabolism of nucleic acids in kidney and liver were found to be altered.

Mutagenicity No available data.

Carcinogenicity Archer *et al.* (1973) studied the mortality of uranium mine workers (662) who were examined from 1950 to 1952 until the end of study in 1967. The total death rate was 104 (1950-1967), the same as the expected rate; however, there were excess deaths due to malignant diseases of lymphatic and hematopoietic tissue. Complicating the study, there were other agents present in mills, e.g., vanadium, radium, and thorium, which may have been contributing factors. Data from animal

studies suggested that excess malignancies may have resulted from irradiation of the lymph nodes by ^{232}Th.

Heuper *et al.* (1952) studied the metallotoxic reactions following the introduction of a suspension of powdered metallic uranium (25% U in lanolin; 50 mg U/0.05 cm^3) into the right femur (first study) or into the pleural cavity (second study) of Osborne Mendel male and female rats of 4 and 6 months of age, respectively. In the first study, the investigators observed renal toxicity and necrosis of muscle tissue during the first 6 months. The major histological findings during the remaining 18 months of the study were tumors (sarcoma) in the tissues surrounding the injection site at the femur. In all, 11 of the 30 surviving rats had tumors. In addition, these tumors underwent metastasis to inguinal, lung, and lymph node sites. In the second study, major pathological findings were kidney damage, local tissue necrosis, and tumors at the site of injection.

Teratogenicity No available data.

Conclusions and Recommendations

Suggested No-Adverse-Response Level (SNARL) There is a paucity of recent human data for making a recommendation of a maximal permissible concentration for uranium in drinking water. Until 1962, the International Commission on Radiological Protection (1964) based its calculations of maximum permissible concentrations (MPC) for soluble uranium compounds on their radiation effects without taking into account their chemical toxicity (Novikov, 1970). There is general agreement that calculations of the MPC should be based upon the chemical toxicity of the element and that the kidney should be regarded as the critical organ.

Therefore, the following calculations are based upon exposure to ^{238}U with nephrotoxicity as the critical effect. They do not consider potential effects of radiation.

Twenty-Four-Hour Exposure This calculation is based upon data of Luessenhop *et al.* (1958) who observed nephrotoxicity in humans after an intravenous dose of 0.1 mg/kg and assumes that a 70-kg human drinks 2 liters/day with 100% of exposure from water during this period:

$$\frac{0.1 \text{ mg/kg} \times 70 \text{ kg}}{2 \text{ liters}} = 3.5 \text{ mg/liter.}$$

Seven-Day Exposure This is based upon data from Arsen'yeva (1972) who found no toxicity in rats given uranium at a dose of 0.6 mg/kg for 3 months. This calculation, which uses an uncertainty factor of 100, assumes that a 70-kg human consumes 2 liters/day and that 100% of exposure is from water during this period:

$$\frac{0.6 \text{ mg/kg} \times 70 \text{ kg}}{100 \times 2 \text{ liters}} = 0.21 \text{ mg/liter.}$$

Chronic Exposure A chronic exposure level will not be calculated because uranium and its compounds are suspected carcinogens.

The chemical toxicity of uranium (^{238}U) has received little attention in the United States. Apparently, even less is known about its potential for toxicity in drinking water. If considerable amounts of uranium are found in drinking water, there will be need for specific studies to evaluate the potential chemical toxicity of this element.

Xylenes

$[C_6H_4(CH_3)_2]$

These compounds were evaluated in *Drinking Water and Health* (National Academy of Sciences, 1977, p. 787). The following material, which became available after that 1977 publication, updates and, in some instances, reevaluates information in the earlier report. Also included are some references that were not assessed in the original report.

The biological effects of the xylenes (*o-, m-, p*-xylene) and other organic solvents have been the subject of several recent reviews (Bruckner and Peterson, 1977; Dean, 1978; U.S. Environmental Protection Agency, 1978a).

In commercial xylene the m-isomer predominates. Xylenes are ranked 13th out of 7,000 chemicals that were surveyed for occupational exposure: more than 4 million workers are believed to be exposed. Release of xylenes into the environment each year is estimated to be nearly 410 million kg (U.S. Environmental Protection Agency, 1978a). However, no reports of damage to the environment have been attributed to xylenes.

Metabolism

Bioaccumulation has been predicted for xylenes because of their strong partitioning into the *n*-octanol phase of the *n*-octanol/water system (U.S. Environmental Protection Agency, 1978a). However, the rapid oxidation of xylenes to their corresponding polar metabolites seems to preclude bioaccumulation in higher animal systems. Other species such as the eel may show some xylene retention, possibly because of a less active metabolizing system (Ogata and Miake, 1975).

In humans who have been exposed to approximately 0.2 to 0.4 mg/liter xylene isomers (*o-, m-, p*-xylene) or a 1:1:1 mixture for up to 8 hr, Sedivec and Flek (1976a) determined that the pulmonary retention was 64%, which under the described conditions was independent of dosage or duration of exposure. After exposure, only 5% of the retained xylenes were eliminated in expired air. More than 95% of the retained xylenes were excreted by humans into the urine in the form of methylhippuric acids. Free toluic acids, toluylglucuronic acids, and hydroxytoluic acids were not detected. A small portion of the administered xylenes was excreted into urine as the corresponding xylenol.

Engstrom *et al.* (1977) found that the percutaneous absorption rate of *m*-xylene in humans was approximately 2 $\mu g/cm^2$/min through the skin of the hands. Percutaneously absorbed *m*-xylene was primarily excreted into urine as methylhippuric acid. A small amount of xylene was also detected in expired air. Measurements of urinary methylhippuric acids have been used successfully to ascertain qualitatively and quantitatively the extent of exposure to xylenes (Caperos and Fernandez, 1977; Engstrom *et al.,* 1976; Kira, 1977; Ogata *et al.,* 1977; Sedivec and Flek, 1976b).

When *p*-xylene was incubated with hepatic or pulmonary microsomes from adult female rats, Harper *et al.* (1974) observed that hydroxylation occurred primarily on its one methyl group to form *p*-methylbenzyl alcohol. In the presence of an aldehyde or alcohol dehydrogenase, *p*-methylbenzoic (toluic) acid is formed.

Health Aspects

Observations in Humans Mixtures of organic solvents, which include xylenes, have been implicated as the cause of lens change in car painters (Raipta *et al.,* 1976). However, the evidence is not sufficient to establish that these effects are due to xylenes.

Observations in Other Species

Acute Effects The acute inhalation toxicity of *p*-xylene was 4,740 ppm (LC_{50}) in adult female rats (Harper *et al.,* 1974). This compares favorably with the 4-hr LC_{50} of 6,700 ppm for mixed xylenes (Carpenter *et al.,* 1975).

Exposure of female rats to *p*-xylene vapors for 4 hr to 1,000 to 2,000 ppm caused dose-related increases in SGPT, SGOT, glucose-6-phosphate dehydrogenase, and glutathione reductase by 24 hr after cessation of exposure. This suggests impairment of liver function (Patel *et al.,* 1976). These exposures also caused depression of the central nervous system and irritation of mucous membranes.

The acute intraperitoneal LD50 of *p*-xylene in female rats was 3.8 mg/kg. Dosages of 0.1 mg/kg/day for 3 days caused moderate fatty infiltration of the liver (Harper *et al.,* 1974).

The intraperitoneal administration of *o-, m-,* or *p*-xylene to male mice did not alter liver microsomal N-and O-demethylase activity or various spectral characteristics of microsomal cytochrome P-450 (Fabacher and Hodgson, 1977).

Xylenes may cause liver injury in guinea pigs. Increases in serum ornithine-carbamyl transferase activity and liver lipids have been noted following intraperitoneal injection (Divincenzo and Krasavage, 1974).

By the use of a continuous flow bioassay system, the LC_{50} for xylene in freshwater was determined for goldfish. The 24-, 48-, 72-, 96-, and 720-hr LC_{50} values were 30.5, 25.1, 20.7, 16.9, and 14.6 ppm, respectively (Brenniman *et al.,* 1976). In a similar experiment the xylene 96-hr LC_{50} for rainbow trout was 10 mg/liter (Folmar, 1976).

Chronic Effects No new data available.

Mutagenicity No available data.

Carcinogenicity Xylenes have been selected by the National Cancer Institute for carcinogenicity testing (U.S. Environmental Protection Agency, 1978).

Teratogenicity Xylenes have been reported to cross the human placenta (Dowty *et al.,* 1976).

Conclusions and Recommendations

Suggested No-Adverse-Response Level (SNARL)

Twenty-Four-Hour Exposure Little information is available to calculate SNARL values for xylenes in drinking water. Moreover, the insolubility of xylenes in water suggests that it is probably not necessary. However, exposure data in humans can be used to construct an impression of how much exposure to xylenes may be tolerated. Sedivek and Flek (1976a) observed no toxic or untoward effects on humans during or after inhalation of up to 0.4 mg/liter of a 1:1:1 mixture of *o-, m-,* and *p*-xylene for an 8-hr period. During the exposure, 64% of the xylenes was retained. Thus, the SNARL dose in this period may be calculated for a 70-kg man breathing 3.3 m^3 of air containing xylenes at 200 mg/m^3. Because these no-adverse-response data are from humans, an uncertainty factor of 10 is applied. It is assumed that during this period 100% of exposure is from drinking water, which is consumed at the rate of 2 liters/day:

$$\frac{200\ \text{mg/m}^3 \times 3.33\ \text{m}^3 \times 0.64\ (\%\ \text{retention})}{10 \times 2\ \text{liters}} = 21\ \text{mg/liter.}$$

Seven-Day Exposure o-Xylene inhalation exposure of rats and dogs for 6 hr/day, 5 days/week for 13 weeks at a concentration of 3,500 mg/m^3 in air was tolerated with no adverse effects (Carpenter *et al.,* 1975). Using these data to calculate a SNARL value for humans inhaling a comparable dose per day, and assuming 100% of exposure from water during this period and 2 liters/day consumption:

$$\frac{3{,}500\ \text{mg/m}^3 \times 10\ \text{m}^3\text{/day} \times 0.64}{1{,}000 \times 2\ \text{liters/day}} = 11.2\ \text{mg/liter.}$$

Chronic Exposure Because there are no data on long-term exposures to xylenes, a chronic SNARL value cannot be calculated.

CHEMICALS SELECTED BY THE CHEMISTRY SUBCOMMITTEE

Bromine/Bromide/Bromate

(Br_2, Br^-, BrO_3^-)

Elemental bromine occurs naturally as a diatomic molecule, Br_2, with a molecular weight of 159.83. It exists as rhombic crystals or dark-red

liquid. Its freezing point is -7.3° C; boiling point, + 58.73° C; density of liquid, 3.12 at 20°C; density of vapor, 5.5 (air= 1.0); and vapor pressure, 175 mm Hg at 21°C. The liquid is heavy, yet mobile, volatilizing readily to a dense red vapor with a strong, disagreeable odor resembling chlorine. (The name bromine is derived from the Greek *bromos,* which means stench.) The liquid and vapor are extremely reactive and corrosive. Bromine can be produced by chlorine displacement by electrolysis of inorganic bromides from brines or seawater. It is used as a laboratory chemical and feedstock for the production of a wide variety of inorganic and organic bromides, including ethylene bromide, which is used as an antiknock additive in gasoline. The solubility of bromine in water at 25°C is 33.6 g/liter (*Handbook of Chemistry and Physics,* 1960; Sax, 1975). Nearly all of the bromine exists in water as hydrated Br_2 molecules; but in saturated solution at 25° C, there is some disproportionation yielding 0.092 g/liter bromide ion (Br^-) and 0.11 g/liter hypobromous acid (HOBr). At basic pH, disproportionation yields hypobromite ion (BrO^-), which is unstable above 0°C and undergoes further disproportionation to give the stable bromate ion (BrO_3^-). In basic solution above 50° C, the reaction

$$3Br_2 + 6OH^- \rightarrow 5Br^- + BrO_3^- + 3H_2O$$

goes to completion. Disproportionation of bromate to perbromate does not occur. Therefore, bromate ion is quite stable in solution over a wide pH range (Cotton and Wilkinson, 1966). Inorganic bromides and bromates are highly soluble in water (sodium bromide, 795 g/liter at 0°C; sodium bromate, 275 g/liter at 0°C) (*Handbook of Chemistry and Physics,* 1960). In the past, bromides have been used as drugs for chronic control of epilepsy and as mild sedatives. They still are found in over-the-counter sedative preparations (Goldstein *et al.,* 1974). The contamination of hay by bromide has resulted from breakdown of the fumigant methyl bromide in soil (Knight and Costner, 1977). Bromates are powerful oxidizing agents capable of causing spontaneous combustion when mixed with oxidizable materials (Sax, 1975). Bromates have also been used in some home-permanent, cold-wave kits as 3% solutions (Masoud *et al.,* 1973) and are added to bread in small amounts as yeast nutrients in bread (*Merck Index,* 1976).

Bromine has been used as a disinfectant in swimming pools, but its use in drinking water disinfection has not been recommended because of its cumulative neurotoxicity. Concentrations of 2 mg/liter are required for disinfection. Taste threshold in humans for bromine in water ranges

from 0.17 to 0.23 mg/liter (as bromide) at pH 5 through 9 (Bryan *et al.,* 1973).

Metabolism

Data on elemental bromine and bromate are lacking. The characteristics of bromide absorption and excretion are relatively well known because of the former extensive use of inorganic bromides in prescription and proprietary sedatives.

Pharmacokinetically, bromide ion behaves similarly to chloride. It is readily absorbed via the gastrointestinal tract and is distributed in a volume (Vd) somewhat larger than the extracellular space (Vd in humans = 15 liters). The clearance of bromide by the kidneys is approximately 0.9 liter/day in humans, accounting for most of the excretion of that element. From the clearance and distribution volume, the elimination constant may be calculated to be Ke = 0.9/15 = 0.06 day^{-1}, yielding a theoretical biological half-life of 0.693/0.06, or 12 days (Goldstein *et al.,* 1974). This agrees exactly with the half-life determined by administering ^{82}Br to humans (Soremark, 1960a) and with the result obtained by Gay (1962). Soremark (1960b) made the interesting discovery that there is a diurnal variation in bromide excretion in humans and that there is no excretion during sleep. Flinn (1941) measured peak blood levels of 500 mg/liter in humans who had been maintained on therapeutic levels of bromide daily for 4 months. One month after cessation of bromide intake, blood levels had dropped to 18.8 mg/liter. Bromide half-life can be shortened to 3 to 4 days by administration of chloride, due to competitive reabsorption in the renal tubule. In rats, half-life values could be varied from 2.5 to 25 days by altering chloride intake from 10 to 144 mg/day (Rauws and Van Logten, 1975). In dogs that were fed sodium bromide at the rate of 200 to 400 mg/kg/day, the ratio

$$\frac{[Br^-]\ \text{blood} \,/\, [\text{Halide}]\ \text{blood}}{[Br^-]\ \text{ingested} \,/\, [\text{Halide}]\ \text{ingested}}$$

was constant (1.00 to 1.19) (Rosenblum, 1958). In a 90-day feeding study in rats, Van Logten *et al.* (1974) observed that plasma bromide increased during the first 3 weeks and then reached a constant plateau. Total halide in plasma remained constant throughout, as did the ratios of bromide in brain: plasma and kidney: plasma (0.2 and 0.6, respectively) (Van Logten *et al.,* 1974). In this study, the half-life for bromide elimination was 3 to 5 days. Soremark (1960a) reported that the half-life in mice was 1.5 days. In a study by Knight and Costner (1977), cows fed

43 mg/liter bromide in the diet produced milk containing 10.4 to 20.0 mg/liter bromide. In humans, the half-time for reaching steady-state bromide levels at a constant daily intake is 12 days. The level reached will be approximately 16 times the concentration reached on the first day (Goldstein *et al.,* 1974).

Health Aspects

Observations in Humans Elemental bromine toxicity is due to its extreme chemical reactivity. The undiluted liquid produces severe burns on contact with the skin. The resulting wounds are painful and slow to heal. Bromine vapor is likewise reactive and corrosive, producing severe irritation to the eyes and upper respiratory tract. Pulmonary irritation can also develop in instances of involuntary exposure to concentrations that would not be voluntarily tolerated. The *Hygienic Guide Series* (1958) indicates that work can be performed comfortably in air having concentrations of 0.98 to 1.95 mg/m^3, but not at 3.90 mg/m^3. Bromine is a lacrimator at concentrations below 6.5 mg/m^3. Concentrations of 65 mg/m^3 are not voluntarily tolerated, 390 mg/m^3 is dangerous for short-term exposure, and 6,500 mg/m^3 is rapidly fatal. The threshold limit value (TLV) for Br_2 is 0.7 mg/m ^{3}, based on irritation to eyes and upper airways (American Conference of Governmental Industrial Hygienists, 1976).

Masoud *et al.* (1973) estimated that the acute fatal dose of inorganic bromate is approximately 57 mg/kg. Ingestion produces a caustic reaction in contacted tissues, accompanied by nausea, vomiting, and epigastric pain. Death is due to acute renal failure, which is caused by a direct nephrotoxic effect of bromate ion. Like chlorates, high doses of bromates also produce methemoglobinemia (Masoud *et al.,* 1973).

Among the inorganic substances bromine, bromates, and bromides, the bromides have been documented most thoroughly with toxicological information because of their past use in therapeutics. The effective plasma concentration of bromide ion for sedation of humans is approximately 960 mg/liter, which corresponds to a maintenance dose of about 17 mg/kg/day. However, priming doses of 40 to 70 mg/kg/day over a period of several days are commonly given, for example, to control epilepsy. Psychotic symptoms and neurological signs occur above approximately 1,600 mg/liter (Goldstein *et al.,* 1974; Green, 1961). Flinn (1941) reported no significant adverse effects in humans who were maintained on daily doses of inorganic bromide resulting in plasma levels up to 500 mg/liter for 4 months. This corresponds to a

maintenance dose of 6.4 mg/kg/day. The most common signs and symptoms of bromide toxicity (bromism) relate to the nervous system, ranging from neuroses and psychoses through severe ataxia. High doses also produce various nonspecific gastrointestinal disturbances, and approximately 25% of the patients will exhibit a greater or lesser degree of skin rash, which may be unrelated to dose (Green, 1961; Trump and Hochberg, 1976). Normal blood levels of bromide range from 1.5 to 50 mg/liter in unexposed individuals. In forensic medicine, blood levels of at least 1,000 mg/liter are regarded as significant when attempting to attribute cause of death to bromism (McBay, 1973).

Observations in Other Species

Acute Effects The rapid germicidal action of the halogens is well known. They have found popular use because of their efficacy and relative lack of toxicity to nontarget organisms (see, for example, Farkas, 1947). The oral LD_{50} of sodium bromide in rats has been reported to be 3,500 mg/kg (*Merck Index,* 1976). Sodium bromate has an approximate oral LD_{50} in rabbits of approximately 250 mg/kg (*Toxic Substances List,* 1974).

Chronic Effects Dogs that were fed sodium bromide at 100 to 400 mg/kg/day for 6 weeks developed signs of gastrointestinal and nervous system toxicity when serum bromide levels reached 1,800 to 4,000 mg/liter (Rosenblum, 1958). In a 90-day feeding study (Van Logten *et al.,* 1974), male and female rats were given doses of 0, 75, 300, 1200, 4,800, and 19,200 ppm of sodium bromide in the diet. Plasma bromide reached a plateau in each dose group within 3 weeks. In the highest-dose male group and the three highest-dose female groups, thyroid weights were significantly increased. Histopathological examination revealed hyperplasia of the thyroid in these groups. Also in the highest-dose male groups there was a significant elevation of adrenal weight and prostate weight relative to body weight. Histopathological examination of the testis revealed decreased spermatogenesis in the highest-dose group.

El'piner *et al.* (1972) reported giving sodium bromide at 0.001 to 5.0 mg/kg/day to rabbits for 6 months. They found that serum ascorbate decreased but that serum aspartate aminotransferase was unaffected except for an increase at the highest dose. Blood glucose was also unaffected. No significant pathology was observed in brain, liver, kidney, spleen, or intestine. Rats that were given 0.01 to 50 mg/kg/day for 6 months showed hair loss and a decrease in serum ascorbate. Protein-bound iodine increased in rats that received 0.01 to 0.1 mg/kg. In rats on

0.1 mg/kg, there was a shortened latent period in which a conditioned reflex responded to a stimulus. Horses that were fed hay containing 6,800 ppm bromide developed severe neurological signs, including weakness, ataxia, and paralysis. Serum levels reached 2,954 mg/liter in intoxicated animals. In normal horse serum, the level was 78 mg/liter (Knight and Costner, 1977).

Mutagenicity No available data.

Carcinogenicity No available data.

Teratogenicity No available data.

Conclusions and Recommendations

Suggested No-Adverse-Response Level (SNARL) Data on the toxicity of elemental bromine and bromate are insufficient or inappropriate for calculation of acute or chronic SNARL values for drinking water. For bromide, the human data obtained from the use of this substance in therapeutics may be used.

Twenty-Four-Hour Exposure An acute priming dose of 40 mg/kg of bromide (as Br^-) may be given in therapeutic regimens without ill effect. Assuming 100% intake from water, 2 liters/day intake of water, and 70kg body weight:

$$\frac{40 \text{ mg/kg} \times 70 \text{ kg}}{2 \text{ liters}} = 1{,}400 \text{ mg/liter.}$$

Acute overdoses of bromide may be treated specifically by administration of excess chloride.

Seven-Day Exposure A maintenance dose of 6.4 mg/kg/day yields a serum bromide concentration of 50 mg/liter, which has been found to have no effect over a 4-month period in humans. Using this value, and assuming 100% of the bromide is from water:

$$\frac{6.4 \text{ mg/kg} \times 70 \text{ kg}}{2 \text{ liters}} = 224 \text{ mg/liter.}$$

This value is quite close to one-seventh of the 24-hr limit.

Chronic Exposure The most appropriate data for chronic exposure appear to be the concentrations of bromide found in the serum of normal individuals (1.5-50 mg/liter). The midrange figure, 26 mg/liter, corresponds to a daily intake of 26 mg/liter x 0.06 x 15 liter = 23.4 mg, or a daily dose of 0.334 mg/kg. Assuming a 20% intake from water in a chronic situation:

$$\frac{0.334 \text{ mg/kg} \times 70 \text{ kg} \times 0.20}{2 \text{ liters}} = 2.3 \text{ mg/liter.}$$

This value corresponds favorably with the 2 mg/liter required for disinfection. In 1969, the World Health Organization recommended an acceptable daily intake (ADI) of 1.0 mg/kg for total inorganic bromide intake. Using this figure and assuming a 20% intake from water, one can calculate a permissible bromide concentration of 7 mg/liter. The Food and Drug Administration (U.S. Department of Health, Education, and Welfare, 1975) lists tolerances for inorganic bromide in various foodstuffs as a range from 12 to 400 ppm and for fermented malt beverages as 25 ppm. The Russian investigators cited above (El'piner *et al.,* 1972) recommended a maximum bromide concentration of 0.2 mg/liter in drinking water. Their toxic end point was based on presumed neurological effects in rats as measured by changes in response to a conditional stimulus. The significance of these results is difficult to assess.

Bromodichloromethane

($CHCl_2Br$)

Although bromodichloromethane is generally considered to be insoluble in water, Dowty *et al.* (1975) have identified it as a component of New Orleans drinking water. They reported the highest concentration in raw water (source unidentified) to be 11 µg/liter and in finished water, 116 µg/liter.

Metabolism

Smith *et al.* (1977b) reported that a single oral dose of 20 mg/kg (as ^{14}C) in rats is cleared very rapidly. Only 32% is recovered from the gastrointestinal tract and the carcass after 3 hr, and 41% after 6 hr. Most of the compound was recovered from the stomach. Fat contained more than any other tissue. Less than 1% appeared in the urine. The balance of the dose is probably exhaled either as the parent compound or as a metabolite. In monkeys, Smith *et al.* (1977b) observed that the half-life of a similar dose was 4 to 6 hr and that the peak concentration in blood varied from 1 to 2 mg/liter (as ^{14}C).

Health Aspects

Observations in Humans There are no data on the toxicity of bromodichloromethane in humans. Dowty *et al.* (1975) identified trace amounts in pooled plasma samples from eight persons who drank New Orleans drinking water.

Observations in Other Species

Acute Effects Bowman *et al.* (1978) reported that the oral LD_{50} values for adult female and male Swiss ICR mice are 900 (range, 811-999) and 450 (range, 326-621) mg/kg, respectively. The difference between males and females is statistically significant ($P < 0.05$). Deaths occurred over a period of 1 to 6 days. Administration of 500 mg/kg produced sedation and anesthesia that lasted approximately 4 hr. Postmortem examination indicated fatty infiltration of the liver, pale kidneys, and hemorrhaging of the adrenals. The investigators observed no gross changes in other tissues.

Chronic Effects No available data.

Mutagenicity Bromodichloromethane was mutagenic in the *Salmonella typhimurium* (TA-100 without S-9 mix) assay (Simmon *et al.,* 1978).

Carcinogenicity Intraperitoneal injections given over 8 weeks in a cumulative dose of 2,400 mg/kg did not produce pulmonary tumors in strain A/St mice, which were examined 24 weeks after the first injection under conditions in which bromoform was found to be positive (Thiess *et al.,* 1977).

Conclusions and Recommendations

Since the only acute oral exposure data fall within the LD_{50} range for male mice, the suggested 24-hr no-adverse-response level (SNARL) cannot be calculated.

In view of the lack of data on the sublethal and chronic oral toxicity of bromodichloromethane, SNARL values for 7-day and lifetime exposures cannot be calculated.

Bromoform

($CHBr_3$)

Bromoform was evaluated in *Drinking Water and Health* (National Academy of Sciences, 1977, p. 695). The following material became available since that 1977 publication. In some instances, the new information necessitated reevaluation of the information in the earlier report. Also included are some earlier references that were not assessed in the original report.

Health Aspects

Observations in Species Other Than Humans The oral LD_{50} values for adult female and male Swiss ICR mice are 1,550 (range, 1,165-2,062) mg/kg and 1,400 (range, 1,205-1,595) mg/kg, respectively. Deaths occurred 1 to 9 days following exposure (Bowman *et al.,* 1978).

Mutagenicity Simmon *et al.* (1978) reported that bromoform was mutagenic in the *Salmonella typhimurium T*A-100 microsome assay under conditions in which chloroform was negative.

Carcinogenicity Theiss *et al.* (1977) demonstrated that strain A/St male mice given 23 intraperitoneal injections (3/week) of 48 mg/kg bromoform (a cumulative dose of 1,100 mg/kg) developed a statistically significant increase in the number of pulmonary tumors when examined 24 weeks after the first injection. There was a 75% survival rate in the bromoform-treated group compared with a survival rate of 94% in the control group. Mice that were similarly treated with 100 mg/kg (a cumulative dose of 2,400 mg/kg) did not have an increase in the number of pulmonary tumors. Moreover, all other compounds tested, including chloroform, were negative in this particular bioassay. The data are difficult to interpret because of the lack of dose-response in this study.

Conclusions and Recommendations

In view of the lack of data on sublethal oral toxicity of bromoform, suggested no-adverse-response-level (SNARL) values for 24-hr and 7day exposures cannot be calculated. Because the chronic exposure data are considered to be equivocal, they are inadequate for calculating a chronic SNARL.

Catechol

$[C_6H_4(OH)_2]$

Catechol is used industrially as a polymerization inhibitor, antioxidant, component in electrosensitive copy paper, as a photographic developing agent, as an oxidation base in certain hair dye preparations, in the manufacture of rubber, and in the synthesis of pharmaceuticals and pesticides. Catechol also occurs in natural products, such as onions, crude beet sugar, wood tar, and in coal and cigarette smoke (Raff and Ettling, 1966). In 1970, the consumption of catechol in the United States was approximately 545,000 kg (Anonymous, 1973).

The acute toxicity of catechol has been compared to that of phenol (Deichmann, 1971; Deichmann and Keplinger, 1963). Recent reviews concerning the toxicity of catechol have also considered the similarity of this compound's response to those produced by closely related agents such as resorcinol and hydroquinone (Flickinger, 1976; International Agency for Research on Cancer, 1977).

Metabolism

Catechol is readily absorbed from the gastrointestinal tract as well as through the skin (Flickinger, 1976). It oxidizes *in vivo* to form the corresponding quinone (Deichmann and Keplinger, 1963) which may be more reactive than the parent compound. Semiquinone radicals may be formed which, in turn, produce superoxide anion and hydroperoxides (Timbrell and Mitchell, 1977). Catechol and its conjugation products, as well as hexuronic, sulfuric, and other acids, are found in urine (Deichmann and Keplinger, 1963). Glucuronide and sulfate conjugates have also been detected in the urine of chickens and dogs following an injection of [^{3}H]-catechol into the renal artery (Rennick and Quebbemann, 1970). In humans and rats the biological half-life of catechol is comparable to that of phenol (Hirosawa *et al.,* 1976).

Health Aspects

Observations in Humans Occupational exposures to catechol resulting in serious health effects have not been reported. However, Hirosawa *et al.* (1976) observed that exposure of workers to a combination of catechol and phenol resulted in irritant effects on the upper respiratory tract. The average exposures were 1.8 ppb in air for catechol and 55.6 ppb for phenol. The investigators reported excursion peaks up to 70 ppb and 260 ppb for catechol and phenol, respectively. The observed effects

that resulted from the combination of catechol and phenol could not be attributed to catechol alone (Hirosawa *et al.,* 1976). Deichmann and Keplinger (1963) reported eczematous dermatitis in humans following skin contact with catechol. They also observed central nervous system convulsions in humans following inhalation exposure to catechol.

Observations in Other Species

Acute Effects The acute oral LD_{50} of catechol ranges from 0.1 to 0.3 g/kg body weight in various animal species (Deichmann and Keplinger, 1963; Flickinger, 1976). The lethal intravenous dose for dogs is approximately 0.04 g/kg (Deichmann and Keplinger, 1963). Flickinger (1976) observed that the single dose LD_{50} for catechol, when applied to the skin of albino rabbits for 24 hr, is approximately 0.8 g/kg. Catechol is also an eye irritant and a primary irritant of the skin in rabbits (Flickinger, 1976).

The acute toxic effects in rats that had inhaled catechol-water aerosols during single 1- or 8-hr periods were limited to irritation of extremities and tremors on the first day following exposure to concentrations between 2,000 and 2,800 mg/m^3 in chamber air (Flickinger, 1976). Flickinger observed no effects following 8 hr of exposure to 1,500 mg/m^3.

Angel and Rogers (1972) anesthetized rats to prevent the reflex withdrawal of their hind legs. When these rats were exposed to 0.38 mmol/kg catechol, they responded to a strong pinch by developing myoclonic convulsions.

Chronic Effects Other than the effects noted below for catechol carcinogenicity, no chronic effects are known.

Mutagenicity There are no data on the mutagenicity of catechol in bacterial systems (Dean, 1978). However, spindle effects, anaphase fragments, and a variety of metaphase aberrations have been observed in chromosomes of onions (*Allium cepa).*

Carcinogenicity Catechol demonstrates potent cocarcinogenicity when applied to the skin of mice 3 times a week in combination with benzo[α]pyrene (Van Duuren and Goldschmidt, 1976; Van Duuren *et al.,* 1973). Van Duuren and Goldschmidt (1976) gave mice 40 µ*g* of catechol simultaneously with 5 µg of benzo[α]pyrene. Catechol more than doubled the number of mice that developed papillomas and

carcinomas as compared with the number of control mice that received only benzo[α]pyrene. When they tested for tumor-promoting activity of catechol, no tumors developed (Van Duuren and Goldschmidt, 1976). Cholesterol pellets containing 20% catechol, which were implanted into the bladders of mice, may have increased the incidence of bladder carcinomas after 25 weeks (Boyland *et al.,* 1964).

Teratogenicity No available data.

Conclusions and Recommendations

Suggested No-Adverse-Response Level (SNARL)

Twenty-Four-Hour Exposure The data on the acute oral toxicity of catechol are insufficient to allow for the calculation of reliable 24-hr, 7day, or chronic SNARL values. However, inhalation by rats of 1,500 mg/m^3 of a catechol-water aerosol was tolerated without adverse effect (Flickinger, 1976). If these data are used to calculate a SNARL for acute catechol exposure, the following calculation may be made for a 70-kg human who consumes 2 liters of water in 24 hr, assuming that water contributed 100% of exposure during this period and an uncertainty factor of 1,000:

$$\frac{1{,}500\ \text{mg/m}^3 \times 10\ \text{m}^3\text{/day} \times 0.3\ (\%\ \text{retention})}{1{,}000 \times 2\ \text{liters}} = 2.2\ \text{mg/liter}.$$

No comparable additional data are available to provide for the calculation of 7-day or chronic SNARL values.

Other than the cocarcinogenic potential of catechol, no serious toxicity has been reported. Given the long-term experience with catechol in industry, the toxic properties are minimal in comparison with many other agents. High acute doses of catechol may produce effects on the central nervous system (convulsions), and contact with the skin may produce dermatitis. Catechol and its metabolites are readily excreted in urine, and bioaccumulation is not expected.

In view of the dearth of data concerning the potential chronic effects of catechol, further experimentation on long-term oral toxicity is recommended. Adequate teratogenicity and mutagenicity studies are also required. In the absence of essential information, no chronic SNARL values for catechol in drinking water can be recommended.

Chlorine Dioxide, Chlorite, Chlorate, Chloramines

(ClO_2, ClO_2-, ClO_3-, NH_2Cl)

The chlorine oxides and the oxygen acids of chlorine and their salts are widely used as oxidizing agents in explosives and for bleaching and sterilizing. The following compounds are of interest as constituents of drinking water as a result of disinfection treatments that are alternatives to or combined with the use of free chlorine: chlorine dioxide; chloric acid, chlorate ion; chlorous acid, chlorite ion; hypochlorous acid, hypochlorite ion; monochloramine; and dichloramine.

As disinfecting agents, two classes may be distinguished in order of relative activity (Symons *et al.,* 1977): (1) powerful oxidants (chlorine dioxide > hypochlorous acid) and (2) weaker compounds (hypochlorite ion > dichloramine > monochloramine).

Chlorite is a reduction end product from reactions of chlorine dioxide with materials in water. Chlorite may also persist in water as an excess reagent when chlorine dioxide is generated in the aqueous solution. Alternatives to chlorine disinfection, which are under consideration in order to reduce concentrations of trihalomethane, include chlorine dioxide, ozone plus chlorine, which is followed after a time interval by ammonia to reduce trihalomethane accumulation by the formation of chloramines, and less-than-adequate chlorine, which is followed by chlorine dioxide or chloramines to maintain a residual (Symons *et al.,* 1977).

Chlorine Dioxide

Chlorine dioxide is a greenish-yellow gas with an irritating odor, which decomposes readily and with explosive force to chlorine and oxygen. Mixtures of chlorine dioxide in air are potentially explosive at > 10%, and react violently with organic materials at even lower concentrations (Haller and Northgraves, 1955; Paulet and Desbrousses, 1970). For these reasons, it is usually manufactured at the point of use rather than transported. It is soluble in water to 2.9 g/liter. Although volatile and photosensitive, it is quite stable and solutions in pure water can be maintained for months in closed containers (White, 1972). It is used primarily as a bleach for textiles and for pulp in the manufacture of paper. This capacity as an oxidant also led to its application in drinking water treatment to improve quality of taste and odor at Niagara Falls. New York. in 1944 (Symons *et al.,* 1977). It is now used in drinking water treatment for control of phenols, for oxidation of iron and manganese, and for final disinfection prior to distribution. In Europe. it is used more

widely, but at lower levels than in the United States (Symons *et al.,* 1977).

1. Chlorine

$$Cl_2 + H_2O \rightarrow HOCl + HCl$$

$$HOCl + HCl + 2NaClO_2 \rightarrow 2ClO_2 + 2NaCl + H_2O$$

2. Sodium hypochlorite

$$NaOCl + HCl \rightarrow NaCl + HOCl$$

$$HOCl + HCl + 2NaClO_2 \rightarrow 2ClO_2 + 2NaCl + H_2O$$

Chlorine dioxide is formed from chlorite at low pH. Inattention to input ratios or failure to maintain a pH < 3.5 can result in dosing the water with chlorite (White, 1972). The chemistry of chlorine dioxide in water treatment is not well understood since a balance sheet determination of the various ions of chlorine is complicated and since the nature and amount of by-products and end products can vary with the amount of chlorine and the amount and type of organic material present (Miltner, 1977). Chlorate is generated by the oxidation of chlorine dioxide in the presence of hypochlorous acid and is produced during the generation of chlorine dioxide (Miltner, 1977):

$$HOCl + ClO_2 \rightarrow ClO_3 + HCl.$$

Chlorine dioxide does not cause formation of trihalomethanes. does not react with ammonia, and does not cause formation of chloramines (Stevens *et al.,* 1976). It can be forced to disproportionate to chlorate and chlorite but only by raising pH to 11 or 12 (White. 1972). This is not believed to be an important reaction in the water undergoing treatment (Benarde *et al.,* 1965):

$$2ClO_2 + 2NaOH \rightarrow NaClO_3 + NaClO_2 + H_2O.$$

It is generally accepted that the predominant reaction product of chlorine dioxide in water treatment is chlorite and that chlorate and other ions are produced in minor amounts (White, 1972). An approximately 50% conversion of chlorine dioxide to chlorite was reported by Miltner (1977) and Noack (1978) who used water containing natural humic acids. Oxidation of manganese and of iron with chlorine dioxide to remove them from drinking water using chlorine dioxide also yields chlorites as the reduction product.

Health Aspects

Observations in Humans Proposed limits of use of chlorine dioxide were based primarily upon assessment of the hazards of residual chlorite. Concerned with possible *in-vivo* methemoglobin production by chlorite, Musil *et al.* (1964) recommended that no chlorite reach the distribution point. That recommendation is endorsed by the Norwegian Health Authority (Symons *et al.,* 1977). In the USSR, Fridlyand and Kagan (1971) established a threshold concentration for chlorine dioxide of 0.45 to 0.40 mg/liter, based upon organoleptic properties in water. Van Haaren (cited in Atkinson and Palin, 1973) noted that in West Germany, the maximum applied dose is limited to 0.3 mg/liter. Others practices suggest a residual of 0.5 mg/liter leaving the treatment plant (Atkinson and Palin, 1973). The U.S. Environmental Protection Agency has proposed that the amount used in the treatment process not exceed 1 mg/liter of water.

Observations in Other Species

Chronic Effects A 90-day study examined effects of 20 ppm (2 mg/kg/day) and 200 ppm (9 mg/kg/day) of chlorine dioxide in the drinking water of African green monkeys. Minimal local irritation of the oral mucosa was observed in the highest exposure group of monkeys, which had a lower water consumption than the controls, or in the lowest exposure group. Preliminary reports state that no measurable toxicity to the hematopoietic system or to other systems was observed (U.S. Environmental Protection Agency, 1977b,c). In a 2-year study, rats exposed to chlorine dioxide in their drinking water showed no adverse effects after consuming 1.1 mg/kg/day, but those drinking 11 mg/kg/day showed a higher mortality by the end of the study (Haag, 1949).

Mutagenicity No available data.
Teratogenicity No available data.
Carcinogenicity No available data.

Conclusions and Recommendations

Suggested No-Adverse-Response Level (SNARL)

Chronic Exposure Details of the African green monkey experiment were not available at the time of this writing, but the preliminary report stated that maintenance for 90 days on 200 ppm, 9 mg/kg/day, gave no adverse effect other than irritation of oral mucosa. For rats, the highest no-adverse-effect level in the 2-year study of chronic toxicity was 1.1 mg/kg/day. Calculating a SNARL for a 70-kg human, assuming the uncertainty factor = 100 and that 100% of daily exposure during this period is from 2 liters of drinking water:

$$\frac{1.1 \text{ mg/kg/day} \times 70 \text{ kg}}{100 \times 2 \text{ liters}} = 0.38 \text{ mg/liter.}$$

As a disinfectant, the residual level of this oxidant depends upon the reducing capacity of organic matter in the water. Although a better understanding of chlorine dioxide and its products in drinking water is necessary, the typical result under desired conditions would appear to be a minimal residual of chlorine dioxide, with approximately 50% of the initial oxide persisting as chlorite ion. The current recommendation that no more than 1 mg/liter of chlorine dioxide be added during treatment of drinking water is consistent with the toxicity data available for chlorine dioxide.

Short-Term Exposure to Higher Levels of Chlorine Dioxide Exposure to appreciably higher levels would be self-limiting in almost all cases, since chlorine dioxide is typically not transported but is generated at the point of use, is volatile in water, and, apparently, has a taste and odor threshold of 0.4 to 0.45 mg/liter, which is only slightly above the calculated SNARL for chronic exposure.

Chlorite

Health Aspects

Observations in Humans No available data.

Observations in Other Species

Acute Effects Most studies of acute toxicity have used commercial sodium chlorite at 78% to 83% in aqueous solution. Oral LD_{50} values reported for rats were 182 mg/kg (Sperling, 1959) and 140 mg/kg (Musil *et al.,* 1964). Musil *et al.* (1964) found that the pattern of death of rats in the acute toxicity study was characterized by slowed breathing, feebleness, and death without spasms. On autopsy, the lungs were filled with hemorrhages, the heart was filled with blood, and the liver and kidneys were normal. In seven rats, which were treated *per os* with 320 mg/kg (over twofold the LD_{50}) and then killed and exsanguinated within 10 min of treatment, an average of 57.3% of their total hemoglobin was in the form of methemoglobin (Musil *et al.,* 1964).

Chronic Effects Oxidative damage of red blood cells is abundantly evident from studies using lower exposure levels. The cat is recognized as being highly susceptible to the induction of methemoglobin formation by aromatic amines and by nitrite (Spicer, 1950). This effect of chlorite on hemoglobin was examined in a series of *in-vitro* and *in-vivo* studies by Heffernan and Guion (1978a,b). Washed red blood cells of cats and of other mammals, including humans, became oxidized to contain methemoglobin in a dose-dependent response to chlorite. The most sensitive adverse response from chlorite in drinking water was a 20% decrease in the half-life of red blood cells of cats that had received 3 mg/kg/day for 30 days.

Mutagenicity No relevant data were available.

Tetratogenicity No available data.

Carcinogenicity No available data.

Conclusions and Recommendations

Suggested No-Adverse-Response Level (SNARL)

Twenty-Four-Hour Exposure No acute toxicity data other than an LD_{50} were available.

Seven-Day Exposure The generation of chlorite is believed to occur in approximately 50% yield following treatment of water with chlorine dioxide. With inefficient utilization of sodium chlorite in the initial generation of chlorine dioxide, a chlorine dioxide treatment that is intended to be 1 mg/liter could result in chlorite concentrations of nearly 1 mg/liter. The most sensitive adverse response reported for chlorite in drinking water was a decrease in half-life of red blood cells in the cat, for which 0.6 mg/kg/day was the highest no-adverse-effect dose.

For a 70-kg human, assuming an uncertainty factor of 100 and 100% of the exposure coming from drinking 2 liters of water daily during this period, a calculated no-adverse-effect dose is:

$$\frac{0.6 \text{ mg/kg} \times 70 \text{ kg}}{100 \times 2 \text{ liters}} = 0.21 \text{ mg/liter.}$$

Implications of this effect of chlorite and this calculated level are discussed in the Recommendations section below.

Chlorate

Chlorate ion is a strong oxidizing agent. It can be formed readily from hypochlorite ion in weakly alkaline or acid conditions by the reaction:

$$3\,HClO \rightarrow 2\,HCl + HClO_3.$$

As sodium chlorate, it has been used widely as a nonselective herbicide and in leather, paper, and textile processing. Potassium chlorate was recommended for ulcerative stomatitis in humans for over 100 years, but it was dropped from the National Formulary in 1960 because of its lack of efficacy and potential toxicity.

Metabolism

Chlorate ion *per os* in aqueous solution is known to be rapidly eliminated in the urine. In a study of seven female dogs, Ross (1925) gave 500 mg/kg doses of chlorate in 500 ml of water. Excretion was at its highest rate in the second 2-hr period. From 55% to 70% was excreted in the first

6 hr. By 24 to 48 hr, 76% to 99% had been excreted unchanged in the urine. The amount of chlorate in the blood peaked at 2 hr. It ranged from 5 to 81 mg/100 ml in five dogs, and it decreased to little or none by 24 hr. None of the dogs showed adverse effects.

Health Aspects

Observations in Humans There are clinical descriptions of poisoning from accidental or suicidal ingestion of sodium chlorate weed killer, overdose of potassium chlorate, or consumption of potassium or sodium chlorate when the respective chloride was intended (Lee *et al.,* 1970). Common poisonous effects are the rapid oxidative destruction of red blood cells, possibly followed or preceded by increased methemoglobin, and eventual cyanosis and progressive kidney failure. From these cases, the lethal dose in adults is estimated to be 20 to 35 g for sodium chlorate (Jackson *et al.,* 1961) and 5 to 30 g for potassium chlorate (Rosenblatt *et al.,* 1975). For a 70-kg human, the oral lethal dose for these salts is 71 to 500 mg/kg.

Human blood containing 0.25% potassium chlorate and standing at room temperature showed no evidence of methemoglobin after 8 hr and showed complete change to methemoglobin with hemolysis in 24 hr. Blood with the same chlorate concentration was converted to methemoglobin with hemolysis if left standing 5 hr at 39°C. Methemoglobin formation was also accelerated if blood cells were first hemolyzed in distilled water or if the blood was stored saturated with carbon dioxide (Richardson, 1937).

The taste threshold for chlorates, 20 ppm, is the recommended permissible level for reservoir waters in the USSR (Rosenblatt *et al.,* 1975).

Observations in Other Species

Acute Effects Lipschitz (1932) studied acute toxicity of sodium chlorate in water given gastrically to cats. Single doses of 1.35 g/kg or greater administered in 60- to 80-ml solutions resulted in typical poisoning symptoms (cyanosis, dyspnea, circulatory depression, methemoglobinemia and death) in 5 hr and in less time with increasing dose. Doses of 450 mg/kg or less were without effect.

Chronic Effects Richardson (1937) gave seven cats intramuscular injections of potassium or sodium chlorate in 5% or 10% solution. Daily

doses of 0.05 to 0.25 g/kg were administered for 25 to 32 days. None of these cats had demonstrable methemoglobin. But, upon histological examination, all cats receiving greater than 0.05 g/kg showed fibrosis and atrophy of distal renal tubules.

Rats maintained for 6 months on oral doses of magnesium chlorate at 3 g/kg/day produced 61 offspring which were examined 1 day after birth (Rosenblatt *et al.,* 1975). The progeny showed pulmonary and cerebral edema and focal hemorrhage in lungs.

Mutagenicity No available data.

Teratogenicty No available data.

Carcinogenicity No available data.

Conclusions and Recommendations

Under usual circumstances of chlorine dioxide treatment of drinking water, chlorate is expected to be a minor end product, and the risk of exposure to chlorite in the drinking water would be expected to be greater than the risk that is related to the chlorate generated as an end product from chlorine dioxide treatment.

There are insufficient data concerning the effect of dilution on the toxic effects of high doses of chlorate. The lowest published lethal concentration (LDLo) of concentrated solutions in humans is 71 mg/kg.

Chloramines

Chloramines are generated by the combination of ammonia and hypochlorous acid. The reaction:

$$HOCl + NH_3 \rightarrow NH_2Cl + H_2O$$

yields monochloramine. Further substitution generates dichloramine and nitrogen trichloride. For water treatment, monochloramine (plus some dichloramine) is generated on site by combining chlorine to ammonia at a weight ratio of 3:1 (White, 1972). Nitrogen trichloride is avoided since its presence may contribute to taste and odor problems in the water. The presence of chloramines in drinking water is a result of (a) chlorination of water with reaction of the natural free ammonia; (b) addition of ammonia followed by chlorine to decrease the production of trichloro

201 phenol and other substances with objectionable taste and odor; (c) chlorination followed after a time by ammonia; and (d) generation of chloramine in finished water by the addition of chlorine and ammonia. Chloramines are termed "combined chlorine." In cases (c) and (d) they are generated to provide a residual disinfectant during distribution of drinking water. Use of combined chlorine (NH_2Cl) rather than "free chlorine" (HOCI) limits the further production of trihalomethanes in the water distribution system (Hubbs *et al.,* 1978; Symons *et al.,* 1977). According to White (1972), chloramines are most widely used in water treatment for the purpose of maintaining a chlorine residual, although they have also been used for primary disinfection. Chloramines are weak disinfectants in comparison to ozone, chlorine dioxide, and chlorine (Symons *et al.,* 1977). Some of the disinfectant capacity of chloramines may be due to the hypochlorous acid that is formed at equilibrium by the reversible synthetic reaction (White, 1972).

Health Aspects

Observations in Humans Eaton *et al.* (1973) demonstrated that chloramine in dialyzing fluid causes acute hemolytic anemia, which is characterized by oxidation of red blood cells to form Heinz bodies and increased concentration of methemoglobin in uremic patients undergoing hemodialysis. In the characterization study, red blood cells (RBC) showed a dose-dependent increase in relative methemoglobin content upon incubation in water containing chloramines. The RBC also showed an inhibition of hexose monophosphate metabolism (HMP shunt). Gubar and Kozlova (1966) proposed a permissible residual concentration of chloramine in drinking water to be 0.5 to 1.0 mg/liter, on the basis of organoleptic properties.

Mutagenicity In studies by Shih and Lederberg (1976) monochloramine was a weak mutagen for converting a tryptophan auxotroph *trpC* to tryptophan independence in addition to being a potent bactericidal agent for *Bacillus subtilis.* Wlodkowski and Rosenkranz (1975) showed that sodium hypochlorite was a weak base exchange mutagen in *Salmonella typhimurium* strains TA-1530, TA-1536, and TA-1535. Whether these bactericidal or mutagenic effects are due to a common ionic or nonionic form of these test agents or of their reaction products in the culture media was not investigated. (Chloramines of water, mono-, and dichloramine are not to be confused with "chloramine" which is nitrogen mustard, 2.2'-dichloro-N-methyl diethylamine.)

Carcinogenicity No available data.
Teratogenicity No available data.

Conclusions and Recommendations

Suggested No-Adverse-Response Level (SNARL) There are insufficient data for an estimate of a SNARL in humans for either acute or chronic exposure.

Chloramino Acids

In addition to reacting with natural ammonia in the water, hypochlorous acid reacts with amino acids and other amines to form organic haloamines. Oxidative deamination of organic amino compounds is also expected to occur as a result of chlorination (Helz *et al.,* 1978).

Health Aspects

No available data for humans or other species.

Conclusions and Recommendations

Suggested No-Adverse-Response Level (SNARL) There are insufficient data for an estimate of a SNARL in humans for either acute or chronic exposure.

Recommendations

Additional studies are needed on the chemistry of chlorine dioxide and chloramines in reactions with organic material of drinking water.

Maintenance of the integrity of the red blood cell and of its hemoglobin is promoted by several enzyme systems in the cell. The tendency of either chlorite, chlorate, or chloramines to cause oxidation of hemoglobin to Heinz bodies or to methemoglobin would be opposed by the reductive capacity of glutathione and by increased production of NADPH from glucose utilization via the hexose monophosphate shunt. The first step in this latter pathway requires glucose-6-phosphate dehydrogenase (G-6-PDH). Humans deficient in or possessing variant forms of this enzyme are known to be at greater risk to drug-induced methemoglobinemia.

Considerable data are available on the sensitivity of the very young to oxidative damage of red blood cell constituents and on the genetic types and distribution of glucose-6-phosphate dehydrogenase deficiency among humans of different racial and geographic origin (Goldstein *et al.,* 1974). Other genetic traits, such as idiopathic methemoglobinemia, are also known as is the possibility of synergistic responses from various drugs leading to oxidative changes in the red blood cells. Studies of the susceptibility of these human populations to damage of the hematopoietic system by chlorate and, particularly, by chlorite and chloramines are needed.

Chloroform

($CHCl_3$)

This compound was evaluated in *Drinking Water and Health* (National Academy of Sciences, 1977, p. 713). The following material, which became available after that 1977 publication, updates and, in some instances, reevaluates the information in the earlier report. Also included are some references that were not assessed in the original report.

Health Aspects

Observations in Humans Oral doses of 30 ml (44.6 g) and 100 ml (148.3 g) produce severe nonfatal poisonings in humans. Ingestion of 200 ml (296.6 g) was fatal to human adults (Van Oettingen, 1964). Dowty *et al.* (1975) identified chloroform in pooled plasma samples from eight persons who drank New Orleans drinking water.

Observations in Other Species Bowman *et al.* (1978) reported that the oral LD_{50}'s for adult female and male Swiss ICR mice are 1,400 mg/kg (range, 1,120-1,680) and 1,120 mg/kg (range, 789-1,590) of body weight, respectively. Death occurred 1 to 9 days following exposure. Hill (1977) provided some evidence that genetic factors can influence the sensitivity of mice to the lethal effects of chloroform. However, there were no genotypic differences in the hepatotoxic effects although renal toxicity appeared to be related to gene type.

Chronic Effects Miklashevskii *et al.* (1966) exposed male guinea pigs and male albino rats to 0.4 mg/kg oral doses and other groups of the same two species to 2% of their respective oral LD_{50}'s, which amounted to 35 mg/kg for guinea pigs and 125 mg/kg for rats. The experiment ran for 5 months. Daily administration was implied but not specified.

Neither species exposed to 0.4 mg/kg doses showed any changes in conditioned reflexes, autonomic or cardiac activity, blood protein ratios, catalase concentrations, or phagocytic capacity. In the guinea pigs given 35 mg/kg doses, the blood albumin-globulin ratio decreased and the blood catalase activity decreased by the second month of exposure. Guinea pigs that died at this exposure had fatty infiltration, necrosis, cirrhosis of the liver parenchyma, lipoid degeneration, and proliferation of interstitial cells in the myocardium. The rats that received the 125 mg/kg doses had an impaired ability to develop new conditioned reflexes during the fourth and fifth months.

Mutagenicity Chloroform was negative in the *Salmonella typhimurium* TA-100 microsome assay under conditions in which other trihalomethanes were positive (Simmon *et al.,* 1978).

Carcinogenicity Male strain A/St mice given 24 intraperitoneal injections of 200 mg/kg over 8 weeks (a cumulative dose of 4,800 mg/kg) did not develop pulmonary tumors under conditions in which bromoform was positive (Theiss *et al.,* 1977).

Conclusions and Recommendations

Suggested No-Adverse-Response Level (SNARL) The following calculations are for *noncarcinogenic* effects only.

Twenty-Four-Hour Exposure Human data indicate that the lowest dose producing toxic symptoms is 30 ml (44.5 g). Assuming a 1,000-fold uncertainty factor, a 2 liters/day water intake, and that 100% of the exposure is from drinking water, the SNARL is calculated as follows:

$$\frac{44.5\ \text{g} \times 1}{1{,}000 \times 2\ \text{liters}} = 22\ \text{mg/liter}.$$

Seven-Day Exposure Assuming that repeated daily intake of chloroform produces cumulative effects, the SNARL value for 7-day exposures should be one-seventh of the 24-hr exposure limit. This is 3.2 mg/liter.

Lifetime Exposure This value cannot be calculated because chloroform is a carcinogen in animals.

Dibromochloromethane

($CHBr_2Cl$)

Although it is generally considered to be insoluble in water, dibromochloromethane has been identified as a component of drinking water in New Orleans (Dowty *et al.,* 1975).

Health Aspects

Observations in Humans There are no data on the toxicity of dibromochloromethane in humans. Trace amounts have been identified in pooled plasma samples that were taken from eight persons who drank water from New Orleans supplies (Dowty *et al.,* 1975).

Observations in Other Species

Acute Effects The oral LD_{50}'s for female and male Swiss ICR mice are 1,200 (945-1524) mg/kg and 800 (667-960) mg/kg, respectively. The difference between the two groups is statistically significant ($P < 0.05$). Deaths occurred from 1 to 5 days after exposure. Oral administration of 500 mg/kg produced sedation and anesthesia that lasted approximately 4 hr. Upon postmortem examination, Bowman *et al.* (1978) found fatty infiltration of the liver, pale kidneys, and hemorrhaging of the adrenals, but they observed no gross changes in other tissues.

Chronic Effects No available data.

Mutagenicity Dibromochloromethane is mutagenic in the *Salmonella typhimurium* TA-100 strain assay (Simmon *et al.,* 1978).

Teratogenicity No available data.

Carcinogenicity No available data.

Conclusions and Recommendations

Suggested No-Adverse-Response Level (SNARL)

Twenty-Four-Hour Exposure Animal data indicate that 500 mg/kg produces sedation and anesthesia. Using this dose and an uncertainty factor of 1,000, and assuming a 70-kg body weight and a daily water consumption of 2 liters/day, the following SNARL can be calculated:

$$\frac{500 \text{ mg/kg} \times 70 \text{ kg}}{1{,}000 \times 2 \text{ liters}} = 18 \text{ mg/liter.}$$

In view of the lack of data on the sublethal oral toxicity of dibromochloromethane, SNARL values for 7-day and lifetime exposures cannot be calculated.

2,4-Dichlorophenol

($C_6H_3OHCl_2$)

This compound was evaluated in *Drinking Water and Health* (National Academy of Sciences, 1977, p. 725). The following material, which became available after that 1977 publication, updates, and, in some instances, reevaluates the information in the earlier report. Also included are some references that were not assessed in the original report.

Health Aspects

Acute oral LD_{50} values for 2,4-dichlorophenol have been reported for rats at 580 mg/kg (Christensen *et al.,* 1976), the male rat at 2,830 mg/kg (Vernot *et al.,* 1977), and the mouse at 1,600 mg/kg (Christensen *et al.,* 1976) and 1,630 mg/kg (Vernot *et al.,* 1977). Acute intraperitoneal and subcutaneous LD_{50} values for the rat have been reported to be 430 and 1,730 mg/kg (Christensen, 1976).

Extensive toxicity studies on 2,4-dichlorophenol have been undertaken by Kobayashi *et al.* (1972). Acute oral LD_{50} values were 1,600 mg/kg in ICR (Institute for Cancer Research) mice and approximately 4,000 mg/kg in Sprague-Dawley rats. The investigators found no appreciable differences between males and females. Moreover, they observed no remarkable symptoms of intoxication except for depression of motor activity.

In a 6-month feeding study in which male mice were fed diets containing 2,4-dichlorophenol *ad libitum* (0, 45, 100, and 230 mg/kg/day), Kobayashi *et al.* (1972) observed no adverse changes in growth rate, hematology, serum SGOT and SGPT, or behavior up to the maximum dosage level of 230 mg/kg/day. They did find slight abnormalities in liver histopathology in animals receiving the highest dosage. They regarded 100 mg/kg/day as the maximum no-effect level. The authors concluded that 2,4-dichlorophenol was a relatively safe substance.

Conclusions and Recommendations

Suggested No-Adverse-Response Level (SNARL)

Chronic Exposure Little information is available on the toxicity of 2,4-dichlorophenol. In the absence of other, more definitive data, lifetime no-effect levels may be estimated from the study of Kobayashi *et al.* (1972). The highest no-adverse-effect dose in this study with mice was 100 mg/kg/day. Using an uncertainty factor of 1,000, the following calculations may be made, assuming that 20% of this compound will be due to drinking water and that a 70-kg human consumes 2 liters of water per day:

$$\frac{100 \text{ mg/kg/day} \times 70 \text{ kg} \times 0.2}{1{,}000 \times 2 \text{ liters}} = 0.7 \text{ mg/liter.}$$

This yields a SNARL in drinking water of 0.7 mg/liter for a lifetime exposure to 2,4-dichlorophenol. This value should be considered as tentative pending completion of longer term animal feeding experiments.

There are insufficient data for estimating a 1- or 7-day SNARL.

Long-term chronic toxicity and carcinogenicity studies are needed for 2,4-dichlorophenol in at least two species along with studies on the mutagenic, teratogenic, and carcinogenic properties of the compound in order to verify the current SNARL for drinking water.

Glyoxylic Acid, Glyoxal, and Methyl Glyoxal

(OHCCOOH, OHCCHO, CH_3COCHO)

Glyoxylic acid, glyoxal, and methyl glyoxal are of interest as potential contaminants of drinking water because of their apparent, or possible, formation from a variety of organic precursors during ozonization of drinking water. There is no information indicating potential point sources for contamination of drinking water supplies by these substances. Therefore, the potential for accidental contamination at high concentrations is extremely remote.

Metabolism

The mammalian metabolism of glyoxylic acid has received considerable attention, largely in relation to the formation of oxalic acid from endogenous or exogenous precursors. The 24-hr urinary excretion of glyoxylate by 12 normal human adults averaged 3.9 mg (2.2-6.0 mg).

Since studies of the metabolic fate of infused glyoxylate had indicated that only a small proportion was excreted unchanged, Hockaday *et al.* (1964) estimated that humans normally synthesized from 150 to 600 mg of glyoxylate daily. The source of endogenous glyoxylate is uncertain, but oxidative deamination or transamination of glycine was thought to be the major source (Hockaday *et al.,* 1964; Williams and Smith, 1968).

Exogenous precursors of glyoxylate in mammals include ethylene glycol. McChesney *et al.* (1972) summarized the central role of glyoxylate in the metabolism of ethylene glycol, which is converted first to glycol aldehyde, then to glycolic acid and, finally, to glyoxylic acid. These investigators observed that female rhesus monkeys excreted largely unchanged glyoxylate following a large oral dose of 500 mg/kg, but after a single dose of 60 mg/kg, oxalate was the major urinary metabolite (14% of the dose).

In addition to direct oxidation of glyoxylic acid to oxalic acid, at least seven other metabolic pathways of glyoxylate have been identified in mammalian systems (Williams and Smith, 1968). These include transaminations with L-glutamate, L-ornithine, or other amino acids to yield glycine and the corresponding keto acids; reduction to glycolate; reaction with α-ketoglutarate or with pyruvate to form α-hydroxy-*β*-ketoadipate or 2-keto-4-hydroxyglutarate; and FMN (flavin mononucleotide) catalyzed condensation with acetyl-CoA (coenzyme A) to form carbon dioxide and formyl-S-CoA.

Frederick *et al.* (1963) gave normal patients a 1 μmol dose of ^{14}C-labeled glyoxylic acid intravenously. The subjects excreted approximately 15% of the administered ^{14}C as $^{14}CO_2$ during the first 3 hr after administration. Patients with primary hyperoxaluria, a genetic disorder of oxalate metabolism, excreted only 2% to 5% of the dose as carbon dioxide. However, the hyperoxaluric patients excreted 25% of the administered dose as oxalate and 15% as glycolate in the first 24-hr urine sample, in contrast to approximately 12% and 4%, respectively, in control subjects.

No information on metabolism of glyoxal or methyl glyoxal was available for review.

Health Aspects

Observations in Humans Other than the metabolic studies cited above, no data concerning effects of glyoxylic acid in humans were available for review. It appears from the report of Frederick *et al.* (1963) that a single

dose of at least 1 μmol of glyoxylic acid can be tolerated without serious injury.

Glyoxal was rated as a strong sensitizer when applied as a 10% solution to human skin in tests to evaluate contact sensitizers (Kligman, 1966).

There were no data concerning effects of methyl glyoxal on human health.

Observations in Other Species

Acute Effects Bove (1966) conducted limited studies of the acute toxicity of glyoxylic acid in rats in connection with studies of the toxicity of ethylene glycol. Rats that were given oral doses of glyoxylate at 3 or 6 g/kg body weight all died within 1 hr. They tolerated 1 g/kg for at least 24 hr prior to sacrifice; however, all of the rats had renal tubular oxalosis. In these experiments, glyoxylate was more acutely toxic than ethylene glycol, glycoaldehyde, or glycolic acid. Richardson (1973) confirmed that glyoxylate had greater toxicity than ethylene glycol and glycolate and found that partial hepatectomy increased the susceptibility of rats to oral 0.5-g doses of sodium glyoxylate. Three of 15 intact rats died; 6 of 15 died in a group that had one-third hepatectomies 24 hr previously; and 11 of 15 died in a group that had two-thirds hepatectomies. The author concluded that the acute toxicity of glyoxylate was not due to the oxalate that was formed but, rather, to an effect of glyoxylate itself. However, this conclusion is tenuous because hepatectomy also resulted in increased excretion of urinary oxalate.

Laborit *et al.* (1971) found that 200 mg/kg of glyoxylate given intraperitoneally was an LD_{100} in rats. Mice were less susceptible with an approximate intraperitoneal LD_{50} of 325 mg/kg. Glyoxylate was twice as toxic as glycolaldehyde and approximately 5 times as toxic as glycolate for mice. Deaths occurred rapidly and did not appear to involve either a direct mechanism of the central nervous system or to depend on the formation of oxalate.

McChesney *et al.* (1972) reported that an oral dose of 500 mg/kg of glyoxylic acid "appeared to be well tolerated" by female rhesus monkeys. *In-vitro* and *in-vivo* studies have demonstrated that glyoxylic acid inhibits oxidative metabolism, and it seems probable that a direct metabolic effect of glyoxylate is responsible for its acute toxicity (Kleinzeller, 1943; Lamothe *et al.,* 1971).

Smyth *et al.* (1962) reported that a solution of 29% glyoxal was acutely toxic when fed to rats in doses of approximately 7.46 ml/kg body weight.

Dermal LD_{50} was greater than 20 ml/kg for this solution which was mildly rated or moderately irritating to rabbit skin and eyes.

Chronic Effects There were no data concerning the effects of chronic exposure to glyoxylic acid, glyoxal, or methyl glyoxal.

Carcinogenicity No available data.

Mutagenicity No available data.

Teratogenicity No available data.

Conclusions and Recommendations

Suggested No-Adverse-Response Level (SNARL) The only available data concern acute (LD_{50}) exposures and are inadequate for estimates of a SNARL for either acute or chronic exposure.

The chemistry of ozonization of water should be reviewed and further research conducted to determine if the treatment of drinking water with ozone is likely to result in the formation of glyoxylate or related compounds that would deliver a dose to humans at approximately 100 mg/day. If so, extensive research on the chronic effects of this substance, especially in relation to hyperoxaluric conditions, should be conducted. The 100 mg/day dose is suggested as a guideline because this dose appears to be the approximate lower limit of daily endogenous production of glyoxylate (Hockaday *et al.,* 1964).

Hexachlorobenzene

(C_6Cl_6)

This compound was evaluated in *Drinking Water and Health* (National Academy of Sciences, 1977, p. 667). The following material, which became available after that 1977 publication, updates and, in some instances, reevaluates information in the earlier report. Also included are some references that were not assessed in the original report.

Metabolism

Absorption of hexachlorobenzene (HCB) is extremely poor when administered to rats in aqueous suspension but is quite good (average 80%) when the chemical is given in an oil solution (Koss, 1976). Peak

tissue radioactivities were reached 2 to 5 days following administration of ^{14}C-labeled HCB.

A urinary metabolite, 2,4,5-trichlorophenol, was identified in the urine of rats that had been fed HCB (Renner and Schuster, 1977). HCB is degraded in the rat primarily to pentachlorophenol and then to 2,3,4,5-tetrachlorophenol (Engst *et al.,* 1976). Minor metabolites of HCB in the rat include pentachlorobenzene, 2,3,4,6-tetrachlorophenol, 2,3,4-trichlorophenol, and 2,4,6-trichlorophenol.

Health Aspects

Observations in Humans During 1955-1959, an outbreak of human poisoning occurred in Turkey as a result of the consumption of HCB-treated wheat (Cam and Nigogosyan, 1963; DeMatteis *et al.,* 1961; Schmid, 1960). Some deaths resulted, but the major syndrome was cutaneous porphyria with skin lesions, porphyrinuria, and photosensitization. The estimated dosage was approximately 50 to 200 mg/day (0.71 to 2.9 mg/kg/day) for long periods before toxic manifestations became apparent (Cam and Nigogosyan, 1963; Schmid, 1960).

Peters (1976) described the Turkish poisoning incident and the successful use of ethylenediaminetetraacetic acid (EDTA) in the treatment of poisoned individuals.

Observations in Other Species

Subchronic and Chronic Effects HCB was administered daily by gavage to adult female rhesus monkeys for 60 days at doses ranging between 8 and 128 mg/kg (Iatropoulos *et al.,* 1976). Cloudy swelling and centrilobular hepatocellular hypertrophy were seen in the liver of the HCB-treated monkeys including the one animal that was dosed at 8 mg/kg/day.

In a 90-day study, male pigs received 0.05, 0.5, 5.0, and 50 mg/kg/day of 100% pure HCB mixed in a diet (den Tonkelaar *et al.,* 1978). Animals that consumed the highest dosage level died during the experiment. Increased excretion of coproporphyrin was observed in the 5.0 and 0.5 mg/kg/day groups, and induction of liver microsomal enzymes and characteristic histopathological changes in the liver occurred at 5.0 mg/kg/day. The investigators determined the no-effect level to be 0.05 mg/kg/day under the conditions of this experiment.

HCB treatment results in a suppression in the immune response of mice that are fed a diet containing 167 ppm HCB for 6 weeks (Loose *et*

al., 1977) and in rats fed a diet with 250 ppm HCB for 8 to 10 weeks (Zprin and Fowler, 1977).

Groups of six male and six female beagle dogs were given daily doses of 1,000, 100, 10, and 1 mg of 99.9% pure HCB per dog for 12 months (Gralla *et al.,* 1977). Mortality, anorexia, and weight loss occurred at the highest and, to a lesser degree, at the next highest dosage level (approximately equivalent to 108 to 176 mg/kg/day and 10.1 to 16.5 mg/kg/day, respectively). A dose-related neutrophilia appeared in animals receiving the two highest dosages. While the dogs were free of porphyria, nodular hyperplasia of gastric lymphoid tissues was found in all treated dogs including those given 1 mg/day (equivalent to 0.01 to 0.15 mg/kg/day).

Livers of male and female Sprague-Dawley rats that had been fed diets containing 1, 5, 10, and 25 ppm pure HCB for 3 to 12 months were examined by electron microscopy (Mollenhauer *et al.,* 1975). There were significant changes in the cellular regions, which contained smooth endoplasmic reticulum as early as 3 months at dietary levels of HCB as low as 5 ppm (0.25 mg/kg/day). Mitochondria became elongated or swollen under similar conditions. In a second report, Mollenhauer *et al.* (1976) observed a unique degeneration of lipid vesicles into an autophagic vacuole or storage vesicle in rats that had been fed 5 ppm HCB (0.25 mg/kg/day) for 3 to 12 months.

Rats receiving diets containing 50 ppm HCB for 2 weeks incurred increases in hepatic microsomal mixed-function oxidase activities (den Tonkelaar and van Esch, 1974), but no inductive effect was seen at 20 ppm (1.0 mg/kg/day). Food deprivation stimulated the induction of microsomal enzyme activity in male and female rats that had consumed 40 ppm HCB (2.0 mg/kg/day) and also resulted in higher tissue HCB concentrations (Villeneuve *et al.,* 1977).

Stonard and Greig (1976) fed female rats diets containing 0.01% HCB (mean daily intake of 1.89 mg/kg/day) for 90 days. There was a mixed pattern of hepatic microsomal enzyme induction, with some characteristics of both the 3-methylcholanthrene and phenobarbital type of inducers. They observed similar but less dramatic effects after a 14-day feeding at the same level. Daily oral administration of 10 mg/kg HCB to adult male rats for 14 days caused significant increases in hepatic cytochrome P-450 levels (Carlson and Tardiff, 1976).

HCB-induced porphyria and accumulation of uroporphyrinogen III in chick cells require the presence of endogenous iron (Sinclair and Granick, 1974) and are also markedly enhanced in siderotic rats (Louw *et al.,* 1977). HCB treatment has no effect on erythrocyte porphyrin content but causes an increase in the porphyrin content of the kidney

and spleen and a marked increase in the liver (San Martin de Viale *et al.,* 1977).

Rajamanickam *et al.* (1972) reported that female rats fed 0.2% HCB in their diet for several days developed a large increase in hepatic cytochrome P-450 levels, under conditions where total heme synthesis and δ-aminolevulinic acid (ALA) synthetase activity were unchanged. After a week or more of the HCB diet, the rats exhibited a twofold increase in ALA synthetase activity and severe porphyria. Experimental HCB porphyria in rats is characterized by an increased excretion of porphyrins belonging essentially to isomeric type III and a massive accumulation of uroporphyrinogen III and 7-COOH porphyrin in the kidney, spleen, and liver (San Martin de Viale *et al.,* 1970; 1977). The high excretion of uroporphyrinogen III and its accumulation in the liver and other tissues of HCB-dosed animals are apparently the combined result of an increase in δ-aminolevulinic acid synthetase activity and inhibition of the enzyme uroporphyrinogen III decarboxylase (Elder *et al.,* 1976; Louw *et al.,* 1977).

Carcinogenicity A recent report by Cabral *et al.* (1977) indicates that HCB is carcinogenic in the Syrian golden hamster. Animals were fed lifetime diets containing 50, 100, and 200 ppm 99.5% pure HCB *ad libitum.* After 70 weeks there was a significantly higher dose-related incidence of tumors in the treated animals, particularly in males. The percentage of male control animals with tumors was 7.5% while the incidence in males receiving the 50 ppm (4 mg/kg/day), 100 ppm (8 mg/kg/day), and 200 ppm (16 mg/kg/day) doses was 60.0%, 90.0%, and 98.2%, respectively. Major tumor types included alveolar adenomas of the thyroid, hepatomas, liver hemangioendotheliomas, and adrenal tumors.

Another, yet to be confirmed study in mice, in which only lung neoplasia was observed, failed to show any HCB-induced pulmonary neoplasia (Theiss *et al.,* 1977). Strain A/S+ male mice were given intraperitoneal injections of HCB at 8, 20, and 40 mg/kg 3 times a week for a total of 24 injections. Twenty-four weeks after the first injection, the animals were sacrificed. The number of pulmonary adenomas in the treated mice was not significantly different from that of the controls.

Carcinogenic Risk Estimate In a recent study by Cabral *et al.* (1977), Syrian golden hamsters were fed *ad libitum* for life with diets containing 50 to 200 ppm of 99.5% pure HCB. There was a significantly higher doserelated incidence of alveolar adenomas of the thyroid, hepatomas, and adrenal tumors in the HCB-exposed animals when compared with the

controls. The available dose-response data were used to make statistical estimates of both the lifetime risk and an upper 95% confidence bound on the lifetime risk at the low dose level. These estimates are of lifetime human risks and have been corrected for species conversion on a dose/surface area basis. The risk estimates are expressed as a probability of cancer after a lifetime consumption of 1 liter/day of water containing 1 µg/liter of the compound. For example, a risk of 1 x 10^{-6} implies a lifetime probability of 2 x 10^{-5} of cancer if 2 liters/day were consumed; and if the concentration of the carcinogen was 10 µg/liter during a lifetime exposure, this compound would be expected to produce one excess case of cancer for every 50,000 persons exposed. Using 220 million as the population of the United States, there would be 4,400 excess deaths from cancer, or 62.8 per year.

For hexachlorobenzene at 1 µg/liter, the estimated lifetime risk for humans is 1.9 x 10^{-5}. The upper 95% confidence estimate is 2.9 x 10^{-5}. Both of these estimates are the averaged risks which have been calculated from data on the male and female hamsters.

Conclusions and Recommendations

The acute toxicity of HCB is relatively low, but subchronic or chronic exposure of laboratory animals or humans to the compound induces a variety of toxic effects, including the development of severe porphyria in females and, to a lesser degree, in males.

Recently, Cabral *et al.* (1977) obtained convincing evidence that HCB is carcinogenic in the Syrian golden hamster. Lifetime feeding of diets containing as little as 50 ppm of 99.5% pure HCB (4 mg/kg/day) resulted in a 60.0% incidence of tumors as compared to 7.5% in the control animals. Since the compound appears to be a carcinogen, lifetime exposure concentrations were not estimated.

Suggested No-Adverse-Response Level (SNARL)

Seven-Day Exposure Data that are suitable for estimating a 1- or 7day SNARL for HCB are very limited. However, some estimations may be made. Weil *et al.* (1969) have given a formula for predicting minimal effect dosage levels for a 1-week exposure from the results of a 90-day feeding study. Mollenhauer *et al.* (1975, 1976) observed abnormal liver ultrastructure in rats fed 5 ppm of HCB in the diet (0.25 mg/kg/day) for 3 to 12 months. Multiplying this value by 3.0 (Weil *et al.,* 1969), a 7-day minimal-effect dosage level of 0.75 mg/kg/day may be obtained. Using an uncertainty factor of 1,000, and assuming that all of the HCB is

ingested via drinking water during this period and that a 70-kg human consumes 2 liters of water per day, the following calculation can be made to yield a SNARL in drinking water of 0.03 mg/liter for a 7-day exposure to HCB:

$$\frac{0.75 \text{ mg/kg/day} \times 70 \text{ kg}}{1{,}000 \times 2 \text{ liters}} = 0.03 \text{ mg/liter.}$$

The data are insufficient to make any realistic estimate based on a 1 day exposure to HCB.

Recently, Cabral *et al.* (1977) obtained convincing evidence that HCB is carcinogenic in the Syrian golden hamster. Lifetime feeding of diets containing as little as 50 ppm of 99.5% pure HCB (4 mg/kg/day) resulted in a 60.0% incidence of tumors as compared to 7.5% in the control animals. Since the compound appears to be a carcinogen, lifetime exposure concentrations were not estimated.

Additional studies are needed to confirm the carcinogenicity of HCB and also to determine whether the compound is mutagenic.

Iodine/Iodide/Iodate

(I_2, I^-, IO_3^-)

Elemental iodine occurs in the diatomic state, I_2, and has a molecular weight of 253.8. In solid form, it has a metallic luster, forming blue-black scales or plates. Iodine sublimes readily to a deep blue vapor, which becomes violet when mixed with air. The density of the solid is 4.93 at 20 C; its melting point is + 113.5 C; its boiling point is + 184.4 C; and its vapor pressure is 0.31 mm of mercury at 25°C. At 25°C and 1 atm, saturated air contains 408 ppm, or 4,243 mg/m^3 (1 ppm in vapor = 0.0104 mg/liter, or 10.4 mg/m^3). Its solubility in water at 25°C is 0.34 g/liter. The solubility of elemental iodine is greatly enhanced in the presence of iodine salts due to the reaction:

$$I_2 + I^- \rightarrow I_3^-.$$

The iodine and triiodide ions are colorless *(Hygienic Guide Series, 1965).*

The element is prepared by reduction of iodates and periodates that occur with natural deposits of saltpeter ($NaNO_3$). Iodide ion is oxidized by other halogens, e.g., bromine:

$$2I^- + Br_2 \rightarrow I_2 + 2Br^-.$$

This reaction involves nucleophilic displacement of bromide (Br^-) by iodide (I^-), with intermediate formation of the interhalogen compound,

iodine bromide (IBr). In a similar manner, thiosulfate is oxidized to tetrathionate by iodine by stepwise nucleophilic displacement of iodide by thiosulfate:

$$2S_2O_3^{2-} + I_2 \rightarrow S_4O_6^{2-} + 2I^-.$$

Thiosulfate can be administered as an antidote against the irritant properties of elemental iodine. The solution chemistry of iodine is analogous to bromine, undergoing basic hydrolysis to the hypohalous acid, which is followed by disproportionation to the halate and halide ions:

$$HO^0 + I_2 \rightarrow HOI + I^-$$

$$3I_2 + 6OH^- \rightarrow IO_3^- + 5I^- + 3H_2O$$

Periodates (IO_4^-) are powerful oxidizing agents (Gould, 1962).

Chemical uses of elemental iodine or iodine compounds include pharmaceuticals, antiseptics, photographic materials, catalysts, analytical reagents, and agents in the purification of zirconium and hafnium (*Hygienic Guide Series,* 1965). Iodine was first used as an antiseptic by a French surgeon in 1839. It is still widely used for this purpose because of its proven efficacy, economy, and low toxicity in tissue. It is bactericidal, virucidal, and amebicidal at dilutions of 5×10^{-2} to 5×10^{-3} g/liter. Its germicidal properties are present in either aqueous or alcoholic solution (Esplin, 1970).

Iodine is an essential trace element, which is required for synthesis of thyroid hormone. Estimates for the iodine requirement of adult humans may range from 80 to 150 µg/day, which are similar to those for iodide (National Academy of Sciences. 1974). Iodine deficiency results in goiter, a compensatory hyperplasia of the thyroid. In most areas of the world, the proportion of total intake of iodine from water is negligible or very low. The source of most of the intake is food. However, iodide levels in water often serve as an indicator of high or low iodide intake in a region and correlate inversely with high or low prevalence of goiter (Underwood, 1977).

Iodide can be used therapeutically to treat hyperthyroidism and goiter. The primary source of dietary iodide is seafood, which may contain from 200 to 1,000 µg/kg of iodide. Kelp and other seaweeds may contain as much as 0.1% to 0.2% iodide by weight. In Japan, where seaweed is regularly consumed as a national delicacy, goiter is virtually unknown.

To provide the approximate daily requirement of 100 µg/day, table salt in the United States is supplemented with 100 µg of potassium iodide per 1 g of sodium chloride (Astwood, 1970). In addition, bread made with iodine-containing dough conditioners may also supply substantial dietary iodine (Kidd *et al.,* 1974). A recent study by Oddie *et al.* (1970) determined geographic distribution of iodide intake in the United States by measuring thyroid ^{131}I uptake in approximately 30,000 euthyroid subjects throughout the country. Iodide intake is inversely correlated with the uptake of radioiodine in the thyroid. Regions of lowest iodide intake coincided approximately with regions previously (1920-1940) having a high prevalence of endemic goiter. The present range of intake was found to be 240 to 740 µg/day, considerably above current estimates of the iodide requirement. Major sources are thought to be iodized salt and iodine compounds in bread. Underwood (1977) cites an upward trend in iodine consumption in the United States, mainly due to cumulative addition from adventitious sources including iodates in bread, iodized salt. vitamin preparations, iodine-containing medications and antiseptics, and coloring agents. Iodoform antiseptics, which are used in various stages of milk production, processing, or transportation, have increased iodide levels in milk from 13 to 23 µg/liter to 113 to 346 µg/liter.

Iodine has been used to disinfect both drinking water supplies (Black *et al.,* 1968: Morgan and Karpen, 1953) and swimming pools (Byrd *et al.,* 1963) with excellent results. A panel determined the taste threshold of iodine to be 0.147 to 0.204 mg/liter. This was expressed as I^-, but it includes all chemical forms that are present from pH 5 to 9. The iodide residual for effective disinfection in either swimming pools or drinking water is approximately 1.0 mg/liter (Bryan *et al.,* 1973).

Data in *Drinking Water and Health* (National Academy of Sciences, 1977) indicate that bromination and iodination are probably efficacious methods of water disinfection, but there are many unknowns surrounding toxicity and other considerations. The same document contains a brief discussion of radioactive isotopes of iodine in the section on Radioactivity in Drinking Water. Radioiodine as a potential hazard will not be discussed in this report.

Metabolism

Iodine is converted rapidly to iodide ion and is absorbed efficiently as such throughout the gastrointestinal tract (Welt and Blythe, 1970). Small amounts of the element may be absorbed through the skin. Iodine vapor reaching the lung is converted to iodide and is absorbed (*Hygienic Guide*

Series, 1965). The volume (V) of distribution for iodide ion is larger than the total extracellular space (Welt and Blythe, 1970). Compartmental analysis of iodide distribution in four normal men gave V = 18.2 liters, which was adjusted to the 70-kg mass (Hickey and Brownell, 1954). Iodide accumulates in the thyroid to an extraordinary degree. Total iodine content of a healthy human adult is 15 to 20 mg. Between 70% and 80% of this is found in the thyroid. The normal thyroid weighs 15 to 25 g, which is 0.03% of the body weight. Much of the total iodine is found in iodoamino acids, which are incorporated into a storage protein, thyroglobulin. Even so, the free iodide ion concentration in the thyroid is normally about 50 times the plasma concentration. Normal plasma iodide occurs in the range of 0.8 to 6.0 μg/liter. Saliva iodide is proportional to plasma iodide from physiological levels up to about 1,000 μg/liter. Muscle iodide concentration is less than 0.001 times the thyroid concentration; however, due to the large total mass of muscle, the next largest proportion of total iodide is contained in this tissue. The eye contains a relatively high concentration, particularly in the orbitary fat and orbicular muscle (Underwood, 1977; Welt and Blythe, 1970).

Excretion of iodide is largely renal. It appears to be filtered and partially reabsorbed, largely by passive diffusion (Welt and Blythe, 1970). In four human subjects with average body weights (92 kg) and serum iodide (4.1 μg/liter), urinary excretion of iodide was 221 μg/day (Hickey and Brownell, 1954). Rats excreted 50% of an administered iodide dose in 6 to 7 hr. Metabolic equilibrium of retained iodide is attained in 4 days. The half-life of this second phase is stable at about 9.5 days, representing a turnover of organic iodine (Underwood, 1977). Iodide is secreted in milk. Cow's milk normally contains 20 to 70 μg/liter. This can be increased to 510 to 1,070 μg/liter by feeding potassium iodide at 100 mg/day (Underwood, 1977).

Health Aspects

Observations in Humans Direct toxicity of iodine is due to its irritant properties. The *Hygienic Guide Series* (1965) recommended a maximum allowable concentration for iodine vapor of 0.1 ppm (1.04 mg/m^3) based on human experience and analogy to chlorine. This value may be too high to prevent eye irritation. Short exposure tolerance studies in humans indicate that 0.1 ppm (1.04 mg/m^3) is tolerable and that 0.3 ppm (3.12 mg/m^3) is not (time limit not stated). In another study, four humans tolerated 0.57 ppm (5.93 mg/m^3) of iodine vapor for 5 min without eye irritation, but all suffered eye irritation within 2 min at 1.63

ppm (17.0 mg/m^3). The atmospheric level that is immediately hazardous to human life is not known. Pulmonary edema is produced rapidly following exposure to high concentrations. In humans and animals, cough and excessive tearing are characteristic signs. Skin irritation is possible. Saturated vapor produces brown staining and loss of the corneal epithelium upon exposure of the human eye. Complete healing occurs in 2 to 3 days. The fatal ingested dose of I_2 in humans is estimated to be 2 to 3 g (29 to 43 mg/kg), but recovery has been reported after ingestion of 10 g (*Hygienic Guide Series,* 1965). For tincture of iodine (2% I_2 + 2% NaI in alcohol), the fatal dose is 30 to 250 ml (9 to 70 mg/kg of I_2). Acute toxicity is due largely to irritation of the gastrointestinal tract. Little I_2 is absorbed, although I^- is absorbed. I_2 is bound to starches and proteins in the gastrointestinal tract. Severe irritation produces fluid loss and consequent shock. Rarely are individuals hypersensitive to iodine. But those that do may react markedly, even to skin application, exhibiting skin eruptions and fever (Esplin, 1970).

Acute poisoning in humans from an initial dose of iodide is rare. Oral doses of approximately 3.3 mg/kg iodide (as KI) are commonly given every few hours as an expectorant without untoward effects, with the exception of occasional hypersensitivity with angioneurotic edema, which sometimes progresses to swelling of the larynx, resulting in suffocation (Welt and Blythe, 1970). Occasionally, other toxic responses have been seen within hours after administration of iodine. These include fever, arthralgia, lymphoadenopathy, eosinophilia, and, more rarely, multiple petechiae of the skin and mucous membranes (Bianco *et al.,* 1971). In a report of seven case histories of adverse reactions to iodides, most of the reactions occurred in response to simple inorganic iodides, but some were observed after exposure to organic compounds that release iodide. The most frequent reaction was swelling of the salivary glands ("iodide mumps"), although a reaction resembling severe serum sickness was also observed, as were erythema and a pounding headache. Underwood (1977) and Cheftel and Truffert (1971) pointed out that iodide toxicity is relatively low and that there is a large safety margin due to prompt excretion of excess iodide. However, they noted the occasional hypersensitivity reaction. Even so, signs of toxicity generally disappear upon removal of exposure to excess iodide.

Chronic iodide poisoning (iodism) is more common than acute reactions. Toxicity to iodide occurs eventually in all individuals, when they are given a sufficiently high dose (Welt and Blythe, 1970). Symptoms often resemble a "sinus cold" and might also include acneiform skin lesions and irritation of the gastrointestinal tract.

Symptoms disappear spontaneously after administration of iodide is stopped. Bianco *et al.* (1971) described a 54-year-old man who reacted adversely to a vitamin preparation containing 0.15 mg of iodide (as KI) for 10 days. Symptoms of chronic iodism were present, including a marked swelling of the salivary glands. The patient returned to normal after 8 days. Other signs and symptoms that have been cited in reports of chronic iodism are a metallic or brassy taste, gingivitis, burning sensation of oral mucosa, increased salivation, coryza, sneezing, irritation of conjunctiva, edema of eyelids, and severe headache. The mechanism of the syndrome is unknown, and treatment is mainly supportive and empirical, removal of iodide being the principal remedy (Bianco *et al.,* 1971).

Underwood (1977) suggested that chronic intake of 2,000 µg/day of iodide should be regarded as excessive and potentially harmful, although daily intake in some regions of Japan may reach 50,000 to 80,000 µg without apparent ill effect. Special segments of the population may be at risk to increasing iodide intake due to adventitious sources. For example, in Tasmania, where potassium iodide is added to bread and iodoform antiseptics are used in the production of milk, an increased incidence of thyrotoxicosis was observed in 40- to 80-year-old women who had preexisting goiter (Underwood, 1977).

Iodination has been proposed as a disinfection procedure in lieu of chlorination. To investigate this possibility, the chronic toxicity of iodide was evaluated by adding iodide to chlorinated water at a military field station in the tropics. The dose was 12 mg/man/day for the first 16 weeks. It was then increased to 19.2 mg/man/day for the following 10 weeks. Men were given periodic physical examinations and routine checks of body weight, skin, heart, thyroid, renal function, and hematological indices (Morgan and Karpen, 1953). No ill effects were observed over a period of months.

Black *et al.* (1968) studied the effectiveness of iodine in disinfection of drinking supplies and swimming pools. They evaluated the toxicity in humans at levels used in disinfection. Two water systems, which serve three prisons in Lowell, Florida, were used in the study. The total inmate population was composed of 750 men, women, and 13- to 16-year-old girls when the study began in October 1963. For 31 of the 43 months of the study, 1 ppm of I_2 was added to the water at the women's prison and girls' school. This level was added to the water in the men's prison for 41 of the 43 months. For 2 months, water was supplemented with 4 ppm of iodide ion. Then, in the women's prison and girls' school, total iodide was reduced in gradual increments to 0.3 ppm for 10 months in order to evaluate bactericidal effectiveness. General health and thyroid function

of a test group of 133 inmates were evaluated twice before iodine exposure began, 4 times during the first 10 months of exposure, and a fifth time after 37 months. At iodine doses of 1 ppm, radioiodine uptake by the thyroid was depressed, but protein-bound iodine changed relatively little until iodide was increased to 5 ppm. Serum thyroxine levels remained unchanged throughout the exposure. No adverse effects or hypersensitivity reactions were detected. By 37 months, the test group had declined to 29 of the original 133, but the results were the same. Serum iodide increased from a normal range of 10 to 20 µg/liter to 60 µg/liter as measured at 1 month and at 37 months. In an ancillary study of nonprison personnel, who swam in a pool containing water at 5 ppm iodide, no changes in thyroid function were observed.

Byrd *et al.* (1963) evaluated eye irritation, measured protein-bound iodine and urine iodide, and enumerated bacteria count in water after a single exposure and after 1 week and 1 month of repeated daily exposure of members of a college swim team and swim class, who swam in pools containing iodine residuals of 0.4 ppm. Disinfection efficacy was rated as good, and eye irritation was notably absent. There were no significant changes in protein-bound iodine or urinary iodide.

Observations in Other Species

Acute Effects The *Hygienic Guide Series* (1965) reported a limited study in which four rats survived exposure to 370 ppm (3,848 mg/m ^{3}) of iodine vapor. Dogs that had been exposed to high concentrations of iodine vapor rapidly developed pulmonary edema.

The approximate LD_{50} for potassium iodide given intravenously to rats is 285 mg/kg; sodium iodide has an approximate intravenous LD_{50} in the rat of 1.300 mg/kg; and sodium iodate has an approximate intravenous LD_{50} in the rabbit of 75 mg/kg (*Toxic Substances List,* 1974).

Bock and Wright (1964) presented evidence suggesting that resistance to acute lethality from sodium iodide may be subject to control by a single dominant gene. They found considerable strain difference in 14-day LD_{50} values which were obtained following intraperitoneal injection of sodium iodide in mice. Fourteen strains of mice were used, giving LD_{50} values ranging from 430 to 2.030 mg/kg.

Clifton and Makous (1973) reported a disturbing acute effect of sodium iodate. A single intravenous dose of approximately 30 mg/kg in rabbits caused eventual retinal degeneration and blindness. This effect of iodate is rather specific. It is attributable to effects on the retinal epithelium occurring within 1 hr of dosing (Clifton and Makous, 1973).

Chronic Effects Underwood (1977) identified four stages of response to excess levels of iodide intake, all pertaining to thyroid function: at low levels, a temporary increase in the absolute uptake of iodine by the thyroid and incorporation into organic iodine compounds; inhibition of release of iodine from thyrotoxic thyroid; and inhibition of organic iodide formation, leading to iodide goiter (Wolff-Chaikoff effect); and, at very high levels, saturation of action transport for iodide and, usually, intervention of acute toxic effects of iodide. There is considerable species difference in susceptibility to toxic effects of iodide. Also, there appears to be a high tolerance to normal nutritional levels of iodide, pointing to a large margin of safety.

Domestic livestock are frequently given dietary supplements of iodine as prophylaxis or treatment for various diseases. McCauley *et al.* (1972) reported four field cases in which organic iodine supplements seemed to interfere with the ability of cattle to cope with infectious or noninfectious insult under situations of added stress (e.g., transportation, weather, high milk production, or parturition). In these situations, cattle on ethylenediamine dihydroiodide, which is used to prevent foot rot, developed coughing and increased bronchial, nasal, and lacrimal secretion. Serum iodide levels were 1,030 to 2,750 μg/liter. The normal range in cattle is 60 to 80 μg/liter.

Newton *et al.* (1974) fed 0 to 200 ppm iodide to Holstein bull calves as calcium iodate for approximately 100 days. The normal dietary requirement of cattle for iodide is about 0.1 ppm in the feed. Animals fed 100 to 200 ppm developed coughing and profuse nasal discharge. Effect on thyroid weights was variable. All levels of iodate in feed increased serum iodide concentrations. At 200 ppm there was decreased hemoglobin and serum calcium and increased serum butanol-extractable iodine. In two of the trials, in which iodide was fed at 25 to 100 ppm, all levels increased adrenal weights. Above 50 ppm there was decreased body weight gain and feed intake. Minimum toxic dose was thought to be near 50 ppm, although a portion of the animals had adverse signs at lower levels. At 25 ppm, the daily dose of iodide would have been 1.42 mg/kg at the start of feeding and 0.54 mg/kg at the end (due to body weight gain), based on figures given for feed consumption (Newton *et al.,* 1974).

In similar experiments, Newton and Clawson (1974) found swine to be more resistant to iodine in the diet than were cattle. An iodide concentration of 0.2 ppm is sufficient to meet the dietary requirement for this element in swine. In these experiments, one group of swine received 0, 10, 20, 40, and 80 ppm (as I^-) in the form of calcium iodate. A second group of swine received 25, 50, 100, 200, 400, 800, and 1,600 ppm in the same manner. Body weight, feed intake, thyroid weight, liver iron, total

serum iodine, butanol-extractable iodine, and hemoglobin were measured. All levels of iodine supplementation increased serum iodine levels and thyroid weights, but no differences in performance were measured at or below 400 ppm in the feed. At 400 and 1,600 ppm, liver iron was significantly decreased. Growth rate, feed intake, and hemoglobin were decreased at 800 ppm. Dietary or intramuscular iron supplements offset the effects of iodide on growth rate and feed intake, but had no effect on serum iodide level. For the first 28 days of an 84-day exposure to 0 to 80 ppm, there was salivary gland swelling, which then subsided. This swelling was not noted in high-dose experiments. At 800 and 1,600 ppm, there was encrustation around the eyes due to increased lacrimal secretion. One pig in each of the 800- and 1,600-ppm groups developed corneal opacity and hyperplasia, which was confined to the cornea. For overt signs of toxicity that did not reverse spontaneously on continued feeding, the minimum toxic dose was considered to be 400 ppm (corresponding to about 11 mg/kg iodide at the end of the feeding period) (Newton and Clawson, 1974).

Braverman and Ingbar (1963) reported that rats that received 0.01% iodide as sodium iodide in drinking water for 5 days and then 500 µg parenterally 3 hr before sacrifice adapted to the acute inhibitory effect of iodide on incorporation of iodide into organic iodine compounds. The adaptation appears to be at the level of active transport of iodide into the thyroid (Braverman and Ingbar, 1963).

Cantin (1967) demonstrated that erythema in rats produced by repeated daily doses of various inorganic iodide salts (0.125-1.00 mmol/kg) could be prevented by prior chronic treatment (20 days at 0.5 mmol/kg). An injection of Evans blue dye showed increased permeability of skin capillaries that was associated with erythema. Cantin also observed that damage to mast cells was dose-related.

Ammerman *et al.* (1964) fed adult female rats 0 to 2,500 ppm iodide from 0 to 35 days post partum. Some were allowed to mate, and the survival of their offspring was noted. Others were killed at 17 to 19 days of gestation or 24 to 48 hr post partum. The ovulation rate, implantation rate, fetal development, and histology of mammary tissue were examined. Offspring showed increasing mortality with increasing dose. Most deaths of the young rats occurred within 24 hr. Rats surviving 48 to 72 hr post partum usually survived until weaning. Dead pups had virtually no milk in their stomachs. Secretion of milk in mammary glands of mothers was absent or markedly diminished. No other significant effects were noted. In an ancillary experiment, Ammerman and coworkers fed iodide at 2,500 ppm to male rats for 200 days. This resulted in no impairment in reproductive performance.

In the same study, they found no adverse effects on reproduction in males or females other than pup survival due to decreased lactation in the mothers, which resulted from high dietary levels of iodide.

Mutagenicity No available data.

Carcinogenicity No available data.

Teratogenicity No available data.

Conclusions and Recommendations

The toxicity of iodine and iodide appears to be relatively low, although hypersensitivity does occur. Levels of approximately 100 μg/day must be consumed to maintain suitable steady-state concentrations of this essential element. Because of the essentiality of iodine, Pechenkina (1964) has recommended that levels of iodine in drinking water be no less than 0.002 ppm. Dawson (1974) has recommended 10 mg/liter of iodide as a safe level for potable water, but this would result in a daily intake of at least 20 mg from water alone, which is excessively high. Since hypersensitivity apparently does not occur at levels of iodide that provide the dietary requirement, similar levels in water should be acceptable.

Data for calculating a SNARL value for elemental iodine or for iodate are lacking although there are some data for total iodine in drinking water. Care should be taken when substituting iodates for iodides in dietary supplements in view of the specific retinal toxicity of iodate. This latter aspect should be investigated further.

Suggested No-Adverse-Response Level (SNARL) For iodide, estimates of acceptable levels can be based on clinical experience with iodides and the above-mentioned test exposures of humans to iodide in drinking water.

Twenty-Four-Hour Exposure Using the typical dose of 3.3 mg/kg iodide (as an expectorant), and assuming 100% intake from 2 liters/day of drinking water by a 70-kg human during this period:

$$\frac{3.3 \text{ mg/kg} \times 70 \text{ kg}}{2 \text{ liters}} = 115.5 \text{ mg/liter.}$$

Seven-Day Exposure Since a dosing regimen of iodide expectorant could be expected to continue over several days, it would not be unreasonable to use the single dose for the 24-hr exposure and simply divide it over a 7-day period:

$$\frac{115.5 \text{ mg/liter}}{7} = 16.5 \text{ mg/liter.}$$

Over 7 days at this level, some hypersensitivity reactions may be encountered.

Chronic Exposure Human studies involving 16 weeks of exposure to approximately 0.17 mg/kg/day iodide in drinking water followed by 10 additional weeks at 0.27 mg/kg/day resulted in no ill effects.

This SNARL assumes a 20% intake from drinking water:

$$\frac{0.17 \text{ mg/kg} \times 70 \text{ kg} \times 0.20}{2 \text{ liters}} = 1.19 \text{ mg/liter.}$$

This calculation corresponds to the finding of no ill effects in the prison and girls' school study, which used iodine disinfection at a level of 1 ppm I_2 for 31 months.

Nonanal

$[CH_3(CH_2)_7CHO]$

Nonanal, which has a molecular weight of 142.24, is found in at least 20 essential oils, such as rose, citrus, and pine. In the United States, less than 4.550 kg/year is used as an ingredient in various fragrances (Opdyke, 1973). It is approved by the Food and Drug Administration for use in food (21 CFR 121.1164) as a result of a GRAS (generally recognized as safe) review in 1965. The Council of Europe recommends an ADI of 1.0 mg/kg. The Food and Agricultural Organization/World Health Organization (1969) recommends an ADI of 0.1 mg/kg.

Metabolism

No data are available on this aldehyde specifically, but the C-8 and C-10 are readily oxidized by animals to fatty acids and then to carbon dioxide and water.

Health Aspects

Observations in Humans Maximization tests in 25 volunteers using 1% concentration resulted in no sensitization (Kligman, 1966).

Observations in Other Species

Acute Effects Acute oral LD_{50} in rats and dermal LD_{50} in rabbits were >5.0 g/kg (Shelanski, 1971). No symptomatology was given.

Chronic Effects No available data.

Mutagenicity No available data.

Carcinogenicity No available data.

Teratogenicity No available data.

Conclusions and Recommendations

The risk of acute hazard would appear to be very low in view of the high (>5 g/kg) oral and topical LD_{50} in animal tests. These very high acute LD_{50} results suggest that the ADI established by the Council of Europe (1 mg/kg) is providing a high, though not quantifiable, safety factor.

No estimate of acute or chronic risk can be made due to lack of data.

Octanal

$[CH_3(CH_2)_6CHO]$

Octanal, which has a molecular weight of 128.22, is found in essential oils such as citrus oils. In the United States less than 4,550 kg/year is used as an ingredient in various fragrances. It is approved by the Food and Drug Administration for use in food (21 CFR 121.1164) as a result of a GRAS (generally recognized as safe) review in 1965. The Council of Europe recommends an ADI of 1 mg/kg.

Metabolism

According to Williams (1959) octanal is readily oxidized in animals to the corresponding fatty acid which is then normally oxidized to carbon dioxide and water.

Health Aspects

Observations in Humans A standard repeat-insult patch test in 40 subjects, using 0.25% concentration in alcohol, resulted in no sensitization (Majors, 1972).

Observations in Other Species

Acute Effects Smyth *et al.* (1962) found that the single dose oral LD_{50} in rats is 5.63 ml/kg; the single skin penetration LD_{50} in rabbits is 6.35 ml/kg; 8-hr inhalation of "concentrated" vapor by rats results in no deaths; and skin and eye irritations in rabbits are mild. They reported no symptomatology. Penetration through intact mouse skin was reported by Meyer *et al.* (1959).

Chronic Effects No available data.

Mutagenicity No available data.

Carcinogenicity No available data.

Teratogenicity No available data.

Conclusions and Recommendations

The acute data in animals, the one topical study in humans, and the brief, although apparently straightforward, metabolic conversion of octanal seem to characterize a compound of low hazard if orally ingested.

To consume the Council of Europe's recommended ADI of 1 mg/kg would require a concentration of 35 mg/liter of water (assuming consumption of 2 liters/day in a 70-kg human). An ADI of even this magnitude would still appear to allow for a large safety factor based on the acute oral data in rats.

The lack of subacute or chronic toxicity data in humans or laboratory animals precludes assessment of acute or chronic risk.

Resorcinol

$[C_6H_4(OH)_2]$

Resorcinol is used industrially in the production of dyes, plasticizers, textiles, resins, pharmaceuticals, and adhesives for wood, plastics, and

rubber products (Flickinger, 1976). In 1974, the U.S. production of resorcinol was 16 million kg (U.S. International Trade Commission, 1976), 4,500 kg was imported, and 3.6 million kg was exported. Typically, industrially produced resorcinol has a purity greater than 99.5% (Flickinger, 1976).

The acute toxicity of resorcinol has been compared to that of phenol and catechol (Deichmann, 1971; Deichmann and Keplinger, 1963). Recent reviews concerning the toxicity of resorcinol have also considered the similarity of its response to that produced by closely related agents such as hydroquinone and catechol (Flickinger, 1976; International Agency for Research on Cancer, 1977). The threshold limit value for occupational exposures to resorcinol, based on a time-weighted average value (8-hr workday, 40-hr workweek), was set in 1976 as 45 mg/m^3 (10 ppm) (American Conference of Governmental Industrial Hygienists, 1976).

Metabolism

Resorcinol is readily absorbed from the gastrointestinal tract. However, compared to catechol or phenol, resorcinol is less readily absorbed through the skin of rabbits (Flickinger, 1976). Resorcinol and its conjugation products with hexuronic, sulfuric, and other acids are found in urine (Deichmann and Keplinger, 1963).

Health Aspects

Observations in Humans The application of resorcinol to skin may cause redness, itching, dermatitis, edema, or corrosion (Deichmann and Keplinger, 1963). Additionally, ingestion may produce hypothermia, hypotension, decreased respiratory rate, tremors, and hemoglobinuria (Deichmann and Keplinger, 1963).

Observation of production and maintenance workers exposed to resorcinol (analytical surveys showed airborne concentrations of resorcinol up to 9.6 ppm) revealed no lost time at work and no compensation claims due to occupational disease (Flickinger, 1976).

DoPico *et al.* (1975) reported inflammatory diseases involving the upper and lower respiratory tract of workers in the synthetic rubber tire industry who had been exposed to a thermosetting resin containing resorcinol and methylene aminoacetonitrile. However, they did not establish the relationship between occupational disease and resorcinol.

Observations in Other Species

Acute Effects The acute oral LD_{50} of resorcinol ranges from 0.37 to 0.98 g/kg of body weight in various animal species (Deichmann and Keplinger, 1963; Flickinger, 1976). The lethal intravenous dose for dogs is approximately 0.7 to 1.0 g/kg (Deichmann and Keplinger, 1963). The single dose LD_{50} for resorcinol applied to the skin of albino rabbits for 24 hr is 3.36 g/kg (Flickinger, 1976). Resorcinol is also an eye irritant but not a primary irritant of the skin in rabbits (Flickinger, 1976).

Acute toxic effects in rats that were exposed to single 1-hr or 8-hr inhalation exposures to resorcinol-water aerosols were not detected. In rats exposed to resorcinol at 2,000 to 7,800 mg/m^3 for 8 hr, there was no effect on weight gain after 14 days. No gross lesions attributable to inhalation of the aerosols were noted at autopsy (Flickinger, 1976).

Flickinger (1976) also reported no evidence of gross respiratory damage in rats or guinea pigs whose throats had been sprayed 3 times a day for 2 weeks with a 1% water solution of resorcinol. Histopathological examination of lungs showed slight tracheobronchitis, but the incidence was the same in controls and resorcinol-treated animals (Flickinger, 1976). No evidence of toxic effects was detected in rats, rabbits, or guinea pigs that were sacrificed periodically over several months following inhalation exposure to water aerosols of 34 mg/m^3 resorcinol 6 hr per day for 2 weeks (Flickinger, 1976).

Chronic Effects No available data other than that noted below under carcinogenicity.

Mutagenicity No mutations were induced by resorcinol in *Salmonella typhimurium* in the presence or absence of rat liver activating systems (Ames *et al.,* 1975; Dean, 1978; McCann *et al.,* 1975a). Furthermore, resorcinol had no effect on the incorporation of tritiated thymidine into testicular DNA of mice (Seiler, 1977). However, Dean (1978) showed that resorcinol induced chromosome breakage in plant cells.

Carcinogenicity Resorcinol was not cocarcinogenic in mice in combination with benzo[α]pyrene (Van Duuren and Goldschmidt, 1976). Moreover, no tumor-promoting activity of resorcinol has been detected (Van Duuren and Goldschmidt, 1976).

The repeated application of resorcinol to the skin of female mice for their lifetime produced no statistically significant increases in tumor incidence (Stenback and Shubik, 1974). In addition, application of 0.02 ml of a 5%, 10%, or 50% solution of resorcinol to the dorsal skin of

rabbits produced no chemically related toxicity or tumorigenesis following lifetime observation (Stenback, 1977). Hair dye formulations containing resorcinol as components also failed to produce evidence of toxicity, aplastic anemia, or carcinogenicity when tested for 18 months by topical application to mice (Burnett *et al.,* 1975, 1976, 1977-78).

Teratogenicity Topical applications of hair dye formulations containing resorcinol to pregnant rats on gestational days 1, 4, 7, 10, 13, 16, and 19 failed to elicit any teratogenic or embryotoxic effects (Burnett *et al.,* 1976). Teratological studies using resorcinol alone have not been reported.

Conclusions and Recommendations

Suggested No-Adverse-Response-Level (SNARL)

Twenty-Four-Hour Exposure The data on the acute oral toxicity of resorcinol are inadequate; however, no-adverse-response dosages have been determined for resorcinol in rats following exposure to resorcinol in air at 7,800 mg/m^3 for 8 hr (Flickinger, 1976). Assuming that this dose represents the largest minimal-effect dose, and an uncertainty factor of 1,000, the following 24-hr SNARL value may be calculated for a 70-kg human consuming 2 liters of water daily with 100% of exposure coming from water during this period. Both of the acute SNARL's are based on data derived from inhalation studies and a 30% retention.

$$\frac{7{,}800\ \text{mg/m}^3 \times 10\ \text{m}^3\text{/day} \times 0.3\ (\%\ \text{retention})}{1{,}000 \times 2\ \text{liters}} = 11.7\ \text{mg/liter}$$

Seven-Day Exposure Inhalation of water aerosols of resorcinol (34 mg/m^3) 6 hr/day for 2 weeks was tolerated without adverse effects by rats, rabbits, and guinea pigs (Flickinger, 1976). Using these data, an uncertainty factor of 100, and the same assumptions as above, the following calculation may be made for a 7-day SNARL:

$$\frac{34\ \text{mg/m}^3 \times 10\ \text{m}^3\text{/day} \times 0.3\ (\%\ \text{retention})}{100 \times 2\ \text{liters}} = 0.5\ \text{mg/liter.}$$

This value may be far lower than necessary since lifetime topical application of resorcinol to the skin of female mice produced no carcinogenicity or toxicity (Stenback and Shubik, 1974). However, there are no data allowing for the calculation of a chronic SNARL based upon oral resorcinol exposure.

The acute toxic effects of resorcinol are similar to those of catechol, hydroquinone, and phenol. However, resorcinol is less toxic following dermal exposure than either catechol or phenol. No long-term toxic effects due to resorcinol have been observed in humans or animals. Short-term mutagenesis tests do not indicate any mutagenic properties for resorcinol. Furthermore, topical applications of resorcinol to the skin of animals over a lifetime have not indicated any increases in tumor incidence.

However, studies to determine chronic effects of resorcinol following long-term oral or inhalation exposure are required to assess fully the potential toxicity of resorcinol. Adequate teratogenicity and mutagenicity studies are also required. In the absence of essential information, no limit values for resorcinol in drinking water can be recommended.

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V

The Contribution of Drinking Water to Mineral Nutrition in Humans

GENERAL CONSIDERATIONS OF MINERAL INTAKE FROM WATER

The initial undertaking of the first Safe Drinking Water Committee (SDWC) was the identification of substances and their concentrations in the nation's water supply that might pose risks to the public health and, therefore, require the setting of limits. The committee's report, *Drinking Water and Health* (National Academy of Sciences, 1977) contained gaps for which data were not available or were just emerging at the time the report was written. In other cases, the data were not reviewed in depth because the specific substances were not considered pertinent to the initial charge of the committee, i.e., identification of adverse consequences of various substances in water.

One such area was that of nutrients, known to be essential or strongly suspected as being necessary for optimal health of humans and animals. While a few of the nutrients, notably the trace elements, were reviewed in the first report, the coverage was generally toxicological. The committee examined them as sources of potential risk to human populations.

In view of these considerations, the second Safe Drinking Water Committee established a Subcommittee on Nutrition and charged it with the responsibility of reviewing this area by selecting elements of interest and evaluating the effects of their presence in water. In this report, the subcommittee has examined the concentrations of nutrients in drinking water and the contribution of these concentrations to the observed

intake and optimal nutrient requirements of human populations. It studied the benefits of the presence of an element in water and, in cases in which symptoms of both deficiency and toxicity are known to occur, adverse effects. This is a departure from most of the studies of the SDWC conducted previously or in progress, which were or are limited to adverse effects. The subcommittee chose to title this review *The Contribution of Drinking Water to Mineral Nutrition in Humans,* focusing on the positive effects of suites of elements that are known or assumed to interact in the environment or in biological systems.

In *Drinking Water and Health* (National Academy of Sciences, 1977), the committee reviewed eight metals (chromium, cobalt, copper, magnesium. manganese, molybdenum, tin, and zinc) that are essential to human nutrition. The nutritional aspects of others, such as nickel, selenium, arsenic, and vanadium, were not considered. Rather, their toxicity was reviewed. In this study the subcommittee has reviewed potassium, chloride, iron, calcium, phosphorus, and silicon, and has extended the original review only where there was a need for updating or for examining a particular element as a nutrient as opposed to a potentially toxic substance. In the section on fluoride, the subcommittee decided against including an in-depth review because of its uncertainty concerning fluoride's essentiality to nutrition. However, in view of the contribution of fluoride to overall dental health and, through this, its effect on total health, some discussion of fluoride has been included.

Chromium has not been dealt with at great length because it is not certain that the nutritionally useful form of the element occurs in water. It is generally thought that cobalt has nutritional value only as a component of vitamin B_{12}. Although some preliminary studies suggest that inorganic cobalt may have a physiological role independent of its functon in vitamin B_{12} (Roginski and Mertz, 1977), cobalt has not been discussed in this chapter.

The subcommittee also examined the difference in water intake between young and adult humans. Infants (7 kg) consume approximately one-third as much water on the average as an adult, but their body weight is only approximately one-tenth of adult weight and their food intake is also obviously lower. For this reason, the water intake of an infant may contribute a significant quantity of a given element (National Academy of Sciences. 1974).

When people consume unusual diets, e.g., the diets of vegans. who consume no animal foods or dairy products, the intake of certain elements may be significantly different from the average. Athletes or people engaged in heavy labor and those living in a hot climate consume larger than normal amounts of water. In these instances, the contribution

of water to the overall nutrient intake may be significantly different from the average.

The contributions from air have been considered only when amounts of possible significance were suspected. Where such contributions were negligible, no comment has been made. Only in rare instances, such as unusually high airborne levels, might air contribute to the nutrient needs of individuals. It is not always known whether elements taken in from such exposures are used for nutritional (metabolic) purposes.

Requirements for nutrients are generally discussed in terms of the recommended dietary allowances (RDA's) (National Academy of Sciences, 1974) or those intakes that have been judged adequate and safe (National Academy of Sciences. 1980)—not minimal intakes necessary for survival.

The subcommittee examined the new literature on water hardness because it involves nutritionally essential elements. However, it found no significant conclusive data concerning the relationship of water hardness and the incidence of cardiovascular disease since *Drinking Water and Health* (National Academy of Sciences, 1977) was published. An extensive evaluation of the literature in this area has recently been published (National Academy of Sciences, 1979). Therefore, this topic is not covered in this report.

Clearly, some elements that have been reviewed are subject to changes in concentration in water because of the activities of humans. Elements in this category are zinc, copper, molybdenum, tin, manganese, nickel, and vanadium. These may require somewhat closer surveillance than elements such as magnesium whose concentrations in water appear to be little affected by human activity.

The subcommittee believes that a study of the contribution of drinking water to mineral nutrition in humans is essential in a balanced appraisal of drinking water. It also believes that the data in this review are up-to-date and accurate and that they should help those charged with evaluating the nutritional value of drinking water in the United States.

Most information on the mineral composition of water has been gathered from large water-supply systems. In 1975, approximately 35.7 million water consumers (16.7% of the population) were served by systems supplying less than 25 persons. The minerals in water from smaller systems and individual supplies, e.g., wells, may exceed the concentrations in large water supplies, which form the basis for most levels cited in this report. Therefore, the potential contributions of water to nutrient intake that are given below must not be taken as the absolute limits.

The interplay between mineral elements and nutrition is exceedingly

complex. In this report, it has been considered in light of the best available knowledge, but it should be remembered that this knowledge is still incomplete.

CALCIUM

Presence in Food and Water

Dairy products provide the largest source of calcium in the American diet. Table V-1 lists calcium concentrations for some of these products and other foods (Davidson *et al.,* 1975).

In a survey of U.S. surface waters from 1957 to 1969, calcium levels ranged from 11.0 to 173.0 mg/liter (mean, 57.1 mg/liter) for 510 determinations (National Academy of Sciences, 1977). Finished water that was sampled in public water supplies for the 100 largest cities in the United States contained almost as much calcium (range, 1-145 mg/liter). The calcium concentrations in 93% of the city supplies were less than 50 mg/liter (Durfor and Becker, 1964). Similar results were reported in a Canadian study (Neri *et al.,* 1977). Zoeteman and Brinkmann (1977) reported that the public water supplies for 21 large European cities contained between 7 and 140 mg/liter (mean, 85 mg/liter).

The daily intake of calcium for most western adult populations averages between 500 and 1,000 mg (Walker, 1972). The U.S. Health and Nutrition Examination Survey estimated calcium intakes for 20,749 people from 1 to 74 years old, and concluded that the only population segment with an intake appreciably (30%-40%) below the recommended daily allowance was the adult black female. The allowance values used in this survey were 450 mg for children aged 1 to 9 years, 650 mg for ages 10 to 16 years, 550 mg for ages 17 to 19 years, 400 mg for men 20 years and older. 600 mg for women 20 years or older, 800 mg for pregnant women, and 1.100 mg for lactating women (Abraham *et al.,* 1977).

Requirements

The amount of calcium required by the body daily and the level of dietary calcium needed to meet this requirement are controversial issues. Healthy individuals accustomed to low-calcium diets appear to do as well as similar individuals accustomed to high calcium intakes. To some extent, the daily calcium allowances recommended by various international agencies reflect the calcium levels of normal local diets. In the United States, the Food and Nutrition Board of the National Research

Council (National Academy of Sciences, 1974) has recommended daily calcium intakes of 800 mg/day for adults on the basis that the daily excretion of calcium is 320 mg and that only 40% of dietary calcium is absorbed by the average American. However, the excretion rate and absorption percentage can vary with age and physiological state. The recommended dietary allowances (RDA) of calcium for Americans, then, are 360 mg for infants less than 6 months old, 540 mg for 6to 12-month-old infants, 800 mg for children aged 1 to 10 years, 1,200 mg for 11- to 18-year-old children, and 800 mg for individuals 19 years and older. During pregnancy and lactation the RDA is increased to 1,200 mg/day.

TABLE V-1 Calcium Concentrations in Foods and Foodstuffs[a]

Food Item	Calcium Concentration, mg/100 g or mg/100 ml
Cheese, hard	500-1,200
Cheese, soft	80-725
Milk, cow	120
Milk, human	20-40
Nutmeats	13-250
Legumes, dried	40-200
Vegetables, leafy	25-250
Vegetables, roots	20-100
Grains, whole	4-60
Eggs	50-60
Fish	17-100
Sardines, whole	400
Meats	3-24
Fruits	3-60

[a] Data from Davidson *et al.,* 1975.

Toxicity Versus Essential Levels

Deficiency

There is no clearly defined calcium deficiency syndrome in humans. This may be due, in part, to an adaptation in calcium absorption and utilization which varies with calcium intake. In a study of 26 male prisoners ranging in age from 20 to 69 years, Malm (1958) observed that 23 of them achieved calcium balance immediately or within several

months after restricting their calcium intakes from 650 or 930 mg/day to approximately 450 mg/day.

The etiology of osteoporosis, a degenerative disease involving loss of bone calcium, is not clear, but prolonged inadequate intakes of calcium may play an integral role. Diets that were deficient in both calcium and vitamin D caused rickets and osteoporosis to develop in rats 6 weeks after they had been started on the diet at weaning. Osteoporosis was reversed when the rats were given a high-calcium diet that still lacked vitamin D (Gershon-Cohen and Jowsey, 1964). When the animals were 2 months old before receiving the low calcium, vitamin-D-deficient diet, osteoporosis resulted without rickets.

Osteoporosis affects a large portion of older people and is most prevalent in older women. Calcium supplements that were given to osteoporosis patients for 2 years did not appear to reverse the calcium loss from bone (Shapiro *et al.,* 1975).

Hypocalcemia due to impaired alimentary adsorption of calcium in newborn children can result in tetany, consisting of twitches and spasms (Davidson *et al.,* 1975, p. 645).

Toxicity

Calcium is relatively nontoxic when administered orally. There have been no reports of acute toxicity from the consumption of calcium contained in various foods. Peach (1975a) indicated that calcium intakes in excess of 1,000 mg/day when coupled with high vitamin D intakes can raise blood levels of calcium. An excess of 1,000 mg/day (2.5 times the RDA) for long periods can depress serum magnesium levels. Diets that are high in calcium have also produced symptoms of zinc deficiency in rats, chickens, and pigs after prolonged feeding. Kidney stones in humans have been associated with high calcium intakes (Hegsted, 1957).

Interactions

Low calcium intakes increase the rat's susceptibility to lead poisoning (Snowdon and Sanderson, 1974), while high intakes of calcium decrease lead absorption from the intestine (Kostial *et al.,* 1971). Recent studies in young children have associated high blood levels of lead with low dietary intakes of calcium. Mahaffey and coworkers (1976) observed that 12- to 47-month-old children with normal concentrations of lead (<0.03 mg/100 ml) in their blood had higher levels of dietary calcium (and

phosphorus) than did matched children with elevated (>0.04 mg/100 ml) lead levels in their blood. Dietary calcium intake was not reported. Sorrel and coworkers (1977) found concentrations of lead and calcium in blood inversely correlated in control and lead-burdened children aged 1 to 6 years. For children with high concentrations of lead (≤0.06 mg/100 ml blood), average daily calcium intakes were 610 ± 20 mg, while children with blood lead concentrations <0.03 mg/100 ml had average daily calcium intakes of 770 ± 20 mg. Itokawa *et al.* (1974) suggested that the bone pain in itai-itai disease in Japan was causally related to diets low in calcium and protein coupled with cadmium poisoning. Low calcium intakes increase the intestinal absorption of cadmium and the deposition of cadmium in bone and soft tissue (Pond and Walter, 1975). Furthermore, cadmium inhibits the synthesis of 1,25-dihydroxycholecalciferol by renal tubules (Suda *et al.,* 1973). This hormone facilitates intestinal absorption of calcium (Suda *et al.,* 1974), an especially important function when calcium intake is low. The same or highly similar mechanisms may control the absorption of calcium and magnesium into the bloodstream and their deposition into tissues.

Contribution of Drinking Water to Calcium Nutrition

Using an average calcium concentration in public water supplies of 26 mg/liter and a maximum of 145 mg/liter (Durfor and Becker, 1964) and assuming that the average adult drinks 2 liters of this water daily, then the drinking water could contribute an average of 52 mg/day and a maximum of 290 mg/day. On an average basis this would represent 5% to 10% of the usual daily intake or approximately 6.5% of the adult RDA. For hard waters with high calcium levels, the water would contribute approximately 29% to 58% of the usual daily intake or approximately 36% of the adult RDA. Thus, public drinking water generally contributes a small amount to total calcium intake, but in some instances it can be a major contributor.

Conclusions

Current levels of calcium in U.S. drinking water are well below levels that pose known risks to human health. No upper limit for calcium need be set to protect public health. In cases of dietary calcium deficiencies. the presence of this element in drinking water may provide nutritional benefit.

MAGNESIUM

Presence in Food and Water

Schroeder and coworkers (1969) measured the magnesium contents of a variety of foods and foodstuffs using atomic absorption spectrophotometry. On a wet weight basis, spices, nuts, and whole grains had the highest magnesium contents, and refined sugars, human milk, oils, and fats had the lowest. The food data are summarized in Table V-2.

Magnesium and calcium are responsible for most of the hardness of drinking water. In a nationwide study in Canada, the mean concentration of magnesium in finished water before it entered the distribution systems was 10.99 mg/liter. This concentration changed little during distribution (Neri *et al.,* 1977). In the United States, the mean concentration of magnesium in public water supplies in 100 cities was 6.25 mg/liter (range, 0-120 mg/liter). The concentration of magnesium in 96% of the water supplies was <20 mg/liter (Durfor and Becker, 1964). From 1957 to 1969, the average magnesium concentration in U.S. surface waters was 14.3 mg/liter (range, 8.5-137 mg/liter) for 1,143 determinations (National Academy of Sciences, 1977).

In the United States, the average adult ingests between 240 and 480 mg of magnesium daily (Wacker *et al.,* 1977). Approximately 60% to 70% of this is excreted in the feces. The British diet is reported to provide 200 to 400 mg of magnesium daily (Davidson *et al.,* 1975).

Requirements

The daily need for dietary magnesium is a function of the amounts of calcium, potassium, phosphate, lactose, and protein consumed. For the average healthy American on an average diet, the daily magnesium intake recommended by the Food and Nutrition Board of the National Research Council (National Academy of Sciences, 1974) is 60 mg for infants less that 6 months old, 70 mg for 6- to 12-month-old infants, 150 mg for 1- to 3-year-old children, 200 mg for 4- to 6-year-old children, 250 mg for 7- to 10-year-old children, and 300 mg for females 11 years and older. For adolescent and adult males the recommended dietary allowances (RDA's) are 350 mg for ages 11 to 14, 400 mg for ages 15 to 18 years, and 350 mg for those 19 years of age and older. The RDA for pregnant and lactating women is 450 mg.

TABLE V-2 Magnesium Concentrations in Foods and Foodstuffs[a]

	Magnesium Concentration, μg/g	
Food Item	Single Values or Range	Mean
Condiments, spices	230-4,225	2,598
Nuts	1,078-3,175	1,970
Grains and cereal products	18-2,526	805
Fish and seafood	154-532	348
Meats	195-402	267
Vegetables, fresh legumes	185-297	241
Fresh roots	75-478	226
Fresh fleshy	66-487	174
Fresh leafy	85-321	170
Dairy products, eggs	102-270	183
Fruits and juices	102-270	78
Sugars and syrups	0.1-108	59
Milk, human	28-29	29
Oils and fats	1-27	7
Beverages		
Coffee	48	NR[b]
Tea	3-11	NR
Whisky, gin	0.3-4.5	NR
Wine, white	98	NR
Beer	100	NR
Vermouth, Italian	135	NR

[a] Data from Schroeder *et al.,* 1969.
[b] NR, not reported.

Toxicity Versus Essential Levels

Deficiency

Despite several studies. magnesium deficiency in humans is still not well defined, primarily because it has been studied in individuals also

suffering from other metabolic and physiological disorders. Electrolyte imbalance, especially for calcium and potassium, is characteristic of magnesium deficiency.

Magnesium deficiency is most often observed in patients with gastrointestinal diseases that lead to malabsorption and in those with hyperparathyroidism, bone cancer, aldosteronism, diabetes mellitus, and

thyrotoxicosis (Wacker and Parisi, 1968). Alcohol can deplete magnesium levels in heavy drinkers by apparently increasing renal loss. These heavy drinkers show extensive neuromuscular dysfunction such as tetany, generalized tonic-colonic and focal seizures, ataxia, vertigo, muscular weakness, tremors, depression, irritability, and psychotic behavior. By giving them magnesium, these dysfunctions can be reversed (Wacker and Parisi. 1968).

In the rat, prolonged magnesium deficiency retards growth and results in loss of hair, skin lesions, edema, and degeneration of the kidney (Kruse *et al.,* 1932).

Toxicity

Because magnesium is rapidly excreted by the kidney, it is unlikely that magnesium in food and water is absorbed and accumulated in tissues in sufficient quantities to induce toxicity. Magnesium salts are used therapeutically as cathartics, e.g., magnesium sulfate ($MgSO_4$), hydroxide [$Mg(OH)_{2]}$, and citrate [Mg_3][$OOCCH_2COH(COO)CH_2COO$]; as antacids, e.g., magnesium hydroxide, carbonate [$Mg(CO_3)$], and trisilicate ($Mg_2O_8Si_3$); and as anticonvulsants to control seizures associated with acute nephritis and with eclampsia of pregnancy (magnesium sulfate). In patients with renal disease and impaired magnesium excretion, large excesses of magnesium can lead to severe toxicity resulting in muscle weakness, hypotension, sedation, confusion, decreased deep tendon reflexes, respiratory paralysis, coma, and death. At plasma concentrations exceeding 9.6 mg/100 ml (8 mEq/liter) central nervous system depression is evident. Anesthesia is reached near 12 mg/100 ml (10 mEq/liter), and paralysis of skeletal muscle can be produced at plasma concentrations of approximately 18 mg/100 ml (15 mEq/liter) (Peach, 1975a). Normal values are 1 to 3 mg/100 ml (0.8 to 2.5 mEq/liter). Calcium ameliorates magnesium toxicity.

Interactions

The interactions of trace elements in nutrition were reviewed in *Drinking Water and Health* (National Academy of Sciences, 1977). The metabolism of magnesium is tied closely to that of calcium and potassium. Magnesium deficiency results in potassium loss, probably due to the interaction of magnesium and phosphate in the active transport of potassium and sodium across cell membranes. The release of parathyroid hormone, calcitonin, and 1,25-dihydroxycholecalciferol, which are hormones that govern calcium and phosphorus metabolism, is reduced

by lowered magnesium intakes. The mechanism for this reduction is not understood.

Contribution of Drinking Water to Magnesium Nutrition

Using the magnesium concentrations reported by Durfor and Becker (1964) for U.S. drinking waters (median, 6.25 mg/liter; maximum, 120 mg/liter), a daily intake of 2 liters of drinking water would supply an average of approximately 12 mg of magnesium and a maximum of up to 240 mg. For Canadian (Neri *et al.,* 1977) and Western European (Zoeteman and Brinkmann, 1977) drinking waters the daily contribution would be approximately 20 and 24 mg of magnesium, respectively. Therefore, typical drinking water in the United States, Canada, or Europe provides approximately 3% to 7% of the RDA for magnesium intake by a healthy human. In areas where the magnesium concentration is high, over 50% of the RDA could come from 2 liters of water (see Table V-32). Thus, drinking water could provide a nutritionally significant amount of magnesium for individuals consuming a diet that is marginally deficient in magnesium, especially in areas where the magnesium concentration in water is high.

Conclusions

Current levels of magnesium in U.S. drinking water supplies appear to offer no threat to human health, and no upper limit for magnesium concentrations needs to be set to protect public health. For individuals consuming a magnesium-deficient diet, the presence of this element in drinking water may provide nutritional benefit.

PHOSPHORUS

Presence in Food and Water

Phosphorus, in the form of phosphate, is common to most foods and foodstuffs. In foods of plant origin, phosphorus concentrates in seeds. Nuts, beans, and whole grains contain high levels of phosphorus, whereas leafy vegetables contain low levels. Fruits contain little phosphorus, but meat and fish are relatively rich in the mineral. Table V-3 summarizes the phosphorus contents of some of the foods listed by Sherman (1952).

Data collected on the average daily consumption of soft drinks in the

United States are summarized in Table V-4. The estimates for phosphorus intakes from soft drinks indicate that such products contribute little to the phosphorus intakes for the general public. Bell and coworkers (1977) indicated that high phosphorus diets might include as much as 100 mg of phosphorus per day from soft drinks for adults.

The average daily intake of phosphorus in the United States and the United Kingdom is approximately 1,500 mg (Davidson *et al.,* 1975, p. 645; National Academy of Sciences, 1974). Approximately 70% of the ingested mineral is absorbed as the free phosphate (Hegsted, 1973).

Most municipal drinking waters contain little phosphorus. Using spectrographic analysis, Durfor and Becker (1964) determined that 92% of the public water supplies of the 100 largest U.S. cities had undetectable levels of phosphorus. Zoeteman and Brinkmann (1977) reported that the public water supplies of 12 large cities in Europe had a mean phosphate concentration of 0.32 mg/liter (0.10 mg of phosphorus) and a maximum of 3.0 mg/liter (1.0 mg of phosphorus).

A survey of U.S. rivers and lakes from 1962 to 1967 indicated that 747 of the 1,577 water samples that were analyzed contained phosphorus. The mean concentration of phosphorus was 0.12 mg/liter, and the maximum was 5.04 mg/liter (Kopp and Kroner, 1967).

TABLE V-3 Phosphorus Concentrations in Foods and Foodstuffs [a]

Food Item	Phosphorus Concentration, mg/100 g
Cocoa	709
Cheese, hard	610
Nuts	324-625
Beans, dry	128-586
Grains, whole	303-405
Corn	302
Fish	112-320
Meats	198-221
Eggs	210
Milk	93
Vegetables	21-92
Fruits	4-20

[a] Data from Sherman, 1952.

TABLE V-4 Estimates of Phosphorus Intakes from Soft Drink Consumption by Age Group

Age, yr	Average Soft Drink Consumption, ml/day[a]	Intake from Consumption, ml/day[b]	Average Cola Cola Drinks, mg/day[c]
1-2	68	42	3
3-5	111	69	5
6-8	144	89	7
9-11	165/167	102/104	8
12-14	187/229	116/142	9/11
15-17	240/285	149/177	11/13
18-19	246/314	153/195	12/15
20-34	191/229	118/142	9/11
35-54	99/115	61/71	5
55-64	65/75	40/47	3/4
65-74	41/46	25/29	2
75+	27/38	17/24	1/2

[a] Values for ages over 9 years are average values for females (lower figures) and males (higher figures). Data from U.S. Department of Agriculture, 1965.

[b] Assuming that approximately 62% of all soft drinks were colas. Based on a report by the National Soft Drink Association, 1978.

[c] Assuming the average concentration of phosphorus in colas is 0.076 mg/g drink. Based on a report by Houston and Levy, 1975.

Requirements

Except for the infant, the daily allowance of phosphorus recommended by the National Research Council (National Academy of Sciences, 1974) is the same as that for calcium. As long as the diet contains sufficient vitamin D, the ratio of calcium to phosphorus can vary considerably. However, the ratio for the infant should be close to 1.5 : 1 to guard against the possible occurrence of hypocalcemic tetany during the first weeks of life (Mizrahi *et al.*. 1968). The recommended dietary allowance (RDA) is 240 mg of phosphorus for infants less than 6 months old and 400 mg for infants between 6 and 12 months old: for children aged 1 to 10 and adults 19 years or more, the RDA is 800 mg. Children between the ages of 11 and 18 years and pregnant and lactating women should consume 1,200 mg phosphorus daily (National Academy of Sciences, 1974).

Toxicity Versus Essential Levels

Deficiency

Dietary deficiency of phosphorus is not known to occur in humans because of the widespread presence of the mineral in foods. Excessive use of nonabsorbable antacids can induce phosphorus depletion, which causes weakness, anorexia, and bone pain. Familial hypophosphatemia is attributed to defective absorption of the phosphate ion (PO_4) from the intestine or to defective reabsorption from the renal tubules. It is characterized by rickets and dwarfism (Glorieux *et al.,* 1972; Short *et al.,* 1973). There may also be decreased concentrations of erythrocyte adenosine triphosphate (ATP) and 2,3-diphosphoglycerate. In severe hypophosphatemia, acute hemolytic anemia can also occur (Jacob and Amsden, 1971: Lichtman *etal.,* 1969).

Toxicity

Sodium orthophosphate (Na_3PO_4) is poorly absorbed and relatively nontoxic. Acute iatrogenic poisoning with inorganic pyro-($Na_4P_20_2$) or meta-($Na_4P_4O_{12}$) phosphate salts can inhibit calcium utilization and produce nausea, diarrhea. gastrointestinal hemorrhages and ulcerations, and cellular damage in the kidney and the liver. Mazess and Mather (1974) suggested that the high phosphate content of the diet of Eskimos may contribute to the development of osteoporosis, but this has not been confirmed. Rats fed a 5% phosphorus (as NaH_2PO_4) diet for 20 to 30 days develop renal damage. This level is 10 times the level of dietary phosphorus thought to be necessary for adequate nutrition for the rat (Duguid, 1938).

Interactions

Cations that form insoluble phosphates interfere with the absorption of phosphorus. For example, high intakes of aluminum decrease absorption of phosphorus (as phosphate) by forming insoluble aluminum phosphate ($AIPO_4$) and increasing the excretory loss of phosphorus (Ondriecka *et al.,* 1971).

Contribution of Drinking Water to Phosphorus Nutrition

Because public drinking waters contain little phosphorus (μg/liter concentrations) and because foods provide more than 1 g of phosphorus

per day, it can be concluded that phosphorus levels in drinking water contribute only negligibly to human requirements for this mineral.

Conclusions

There is no nutritional basis for the regulation of phosphorus levels in U.S. drinking water supplies.

FLUORIDE

Scientific issues relating to fluoride in drinking water have been adequately defined in *Drinking Water and Health* (National Academy of Sciences, 1977). Fluoride is included in the present report only to provide complete coverage of the nutritional aspects of drinking water.

Presence in Food and Water

Fish (especially bones) and fish products are often high in fluoride. Tea is high in fluoride (a few hundred mg/kg) and two-thirds of the mineral is extracted into the infusion (Harrison, 1949). Cholak (1959) reported the fluoride concentrations in fresh foods (Table V-5).

In 1962, most public water supplies of the 100 largest U.S. cities contained fluoride, according to a survey reported by Durfor and Becker (1964). Ninety-two percent of these supplies contained less than 1 mg/liter (median, 0.4 mg/liter: maximum, 7.0 mg/liter). Of the 969 water supplies sampled in the Community Water Supply Survey of the Public Health Service (U.S. Department of Health, Education, and Welfare, 1969), the fluoride contents ranged from 0.2 to 4.40 mg/liter. Fleischer and colleagues (1974) reported the fluoride contents of a variety of groundwaters: rivers contained 0.0 to 6.5 mg/liter; lakes contained up to 1,627 mg/liter; various groundwaters contained 0.0 to 35.1 mg/liter; and seawater had an average concentration of 1.2 mg/liter.

Osis *et al.* (1974) determined fluoride intakes for a variety of diets in Chicago with and without fluoridation of the drinking water supply. They reported that the average daily intake of fluoride was 1.6 to 1.9 mg when the drinking water was fluoridated and approximately half this when the water was not. These values do not include the contribution made by the consumption of drinking water directly but do include that added by water used for cooking. In other areas in the United States dietary intakes of fluoride ranged from 1.73 to 3.44 mg/day, and intakes of fluoride from water ranged from 0.53 to 1.27 mg/day. In four

unfluoridated areas, the diet contributed 0.78 to 1.03 mg of fluoride per day and the drinking water added 0.08 to 0.44 mg/day (Kramer *et al.,* 1974).

TABLE V-5 Fluoride Concentrations in Foods and Foodstuffs[a]

Food Item	Fluoride Concentration, mg/kg
Fish	0.1-24
Cereals/cereal products	0.1-20
Meats	0.01-7.7
Wine	0.0-6.34
Vegetables	0.1-3.0
Eggs	0.00-2.05
Cheese	0.13-1.62
Coffee	0.2-1.6
Fruits	0.02-1.32
Milk	0.04-0.55
Sugar	0.10-0.32

[a] Data from Cholak, 1959.

Wiatrowski *et al.* (1975) reported that the total daily fluoride intake was 0.32 mg for infants aged 1 to 4 weeks, 0.47 mg for ages 4 to 6 weeks, 0.57 mg for ages 6 to 8 weeks, 0.71 mg for ages 2 to 3 months, 1.02 mg for ages 3 to 4 months, and 1.23 mg for infants between the ages of 4 and 6 months.

Requirements

The Food and Nutrition Board of the National Research Council has not previously recommended a daily intake of fluoride (National Academy of Sciences, 1974), but has recently estimated adequate and safe intakes of 0.1 to 0.5 mg fluoride for infants less than 6 months of age, 0.2 to 1.0 mg for infants between 6 and 12 months, 0.5 to 1.0 mg for children between the ages of 1 and 3 years, 1.0 to 2.5 mg for 4- to 6-year-old children, 1.5 to 2.5 mg for children from 7 years to adulthood, and 1.5 to 4.0 mg for adults (National Academy of Sciences, 1980). These levels are considered to be protective against dental caries and osteoporosis (Mertz, 1972).

Toxicity Versus Essential Levels

Deficiency

Fluoride has not been shown unequivocally to be an essential element for human nutrition, except for its effectiveness in reducing the incidence of dental caries. Reports of the depression of growth in rats (Schwarz and Milne, 1972) and progressive infertility in mice (Messer *et al.,* 1972, 1973) as consistent responses to fluoride-deficient diets have not been confirmed (Tao and Suttie, 1976; Wegner *et al.,* 1976). The role of fluoride in dental health has been demonstrated in humans (Dean *et al.,* 1941; Hodge, 1950). The incidence of dental caries was associated with low-fluoride diets, and inhibition of caries was observed in subjects who drank water containing $\leq$ 1.3 mg/liter of fluoride. As water intake varies with ambient temperature, so does the ingestion of fluoride. Thus, in warm climates a lower concentration of fluoride in the drinking water may be sufficient to reduce caries.

The fluoride concentration in drinking water is not critical for caries protection. Rather, it is the amount of fluoride consumed during the tooth-forming years. As the uptake and deposition of fluoride are greatest before eruption and calcification of the teeth, its anticariogenic effect is greatest with children, especially those less than 8 years old.

Toxicity

The acute and chronic toxicity of fluoride in humans was reviewed in *Drinking Water and Health* (National Academy of Sciences, 1977).

Acute poisoning by fluoride is rare in humans. Peach (1975b) estimated that a lethal dose for an adult human is approximately 5 g as sodium fluoride (NaF). The response to ingested fluoride is swift. It acts directly on the gastrointestinal mucosa causing vomiting, abdominal pain, diarrhea, convulsions, excessive salivation, and paresthesia. It also disrupts calcium-dependent functions.

Ingestion of drinking water containing excessive fluoride can result in mottling of the teeth and dental fluorosis in children. Increased density and calcification of bone (osteosclerosis) has been associated with chronic ingestion of high-fluoride water (Hodge and Smith, 1965). At unusually high levels, chronic fluoride ingestion can result in crippling skeletal fluorosis. Several studies have been conducted to determine the exact levels of fluoride at which these adverse effects occur, but the results often conflict due to lack of control or failure to account for various parameters in the study populations. Dental mottling and

changes in tooth structure may develop in a few children when fluoride levels in water exceed approximately 0.7 to 1.3 mg/liter, depending on ambient temperature (Richards *et al.,* 1967) and diet. Roholm (1937) estimated that a 10- to 20-year daily ingestion of 20 to 80 mg fluoride could result in crippling skeletal fluorosis.

Interactions

Calcium and aluminum salts decrease the absorption of fluoride from the intestinal tract. In sheep and rats magnesium salts are somewhat less effective (Underwood, 1977; Weddle and Muhler, 1954). In studies of humans, Spencer and coworkers (1977) demonstrated that ingestion of antacids containing aluminum hydroxide [$AI(OH)_3$] increased fecal excretion of fluoride by as much as 12 times, resulting in decreased absorption and lowered plasma levels of fluoride. On the other hand, increasing calcium and phosphorus intake did not affect fluoride balance, although these latter minerals, as well as magnesium, did increase fecal excretion of fluoride.

Contribution of Drinking Water to Fluoride Nutrition

Kramer and colleagues (1974) measured the fluoride content of meals and water samples from 12 cities with fluoridated drinking water and four cities without fluoridation. From these data (Table V-6), the contribution of drinking water to individual diets can be estimated if 2 liters/day consumption is assumed. These data demonstrate that drinking water, whether artificially fluoridated or not, can make an important contribution to the total daily fluoride intake. In fluoridated areas, the contribution ranges from 25.9% to 53.5% of the total intake. In unfluoridated areas, it ranges from 13.5% to 48.1%.

Recommendations and Conclusions

Human populations should be studied in detail to determine more precisely the levels of fluoride intake (total and from drinking water) that may be causally related to dental fluorosis and osteosclerosis.

Concentrations of fluoride in drinking water that are recommended for anticariogenic effects appear to be below levels that have been associated with adverse effects in the general U.S. population. Until more precise measures of the margin of safety for the use of fluoride are available, the levels of fluoride in drinking water should not exceed the optimal levels for anticariogenic benefits.

TABLE V-6 Contribution of Drinking Water to Total Daily Fluoride Intake[a]

City	Dietary, mg/day	Fluoride in Water, mg/2 liters daily	Contribution of Fluoride in Water to Total Fluoride Intake, %
Fluoridated Areas:			
Martinez, Calif.	1.73	1.62	48.4
Chicago, Ill.	1.97	1.90	49.1
Louisville, Ky.	1.98	2.28	53.5
St. Louis, Mo.	2.10	1.82	46.4
New York, N.Y.	2.55	1.76	40.8
Durham, N.C.	2.62	1.06	28.8
Lexington, Ky.	2.84	2.30	44.8
Madison, Wis.	2.88	2.22	43.5
Tuscaloosa, Ala.	2.94	1.52	34.1
Cleveland, Ohio	3.05	2.54	45.4
Milwaukee, Wis.	3.41	1.70	33.3
Corvallis, Ore.	3.44	1.20	25.9
Unfluoridated Areas:			
Birmingham, Ala.	0.78	0.16	17.0
Chicago, Ill.	0.86	0.66	43.4
Houston, Tex.	0.95	0.88	48.1
Iron Mountain, Mich.	1.03	0.16	13.5

[a] Derived from Kramer *et al.,* 1974.

SODIUM

Sodium is the most abundant cation of those found in the extracellular fluid. The sodium ion is essential to the regulation of the acid-base balance and is a very important contributor to extracellular osmolarity. It functions in the electrophysiology of cells and is required for the propagation of impulses in excitable tissues. Furthermore, sodium is essential for active nutrient transport including the active transport of glucose across the intestinal mucosa (Harper *et al.,* 1977).

Presence in Food and Water

The total intake of sodium is influenced mainly by the extent that salt (sodium chloride) is used as an additive to food, the inherent salt content of the foods consumed, and the intake of other sodium salts in the diet

TABLE V-7 Average Sodium Content by Commodity Groups in Adult Market Baskets[a]

	1977 (25 Baskets)			1978 (8 Baskets)		
Commodity Groups	mg/day	SE[b]	% of Total	mg/day	SE	% of Total
Dairy products	704	(± 15.3)	10.5	792	(± 19.8)	11.4
Meat, fish, and poultry	952	(± 41.2)	14.2	921	(± 79.2)	13.3
Grain and cereal products	2,005	(± 84.5)	29.9	2,002	(± 123.1)	28.9
Potatoes	75	(± 6.2)	1.1	82	(± 10.6)	1.2
Vegetables, leafy	22	(± 1.8)	—	22	(± 2.3)	—
Vegetables, legume	243	(± 8.5)	3.6	258	(± 13.8)	3.7
Vegetables, roots	18	(± 1.0)	—	17	(± 3.0)	—
Vegetables, miscellaneous, and vegetable products	285	(± 12.8)	4.2	284	(± 42.2)	4.1
Fruits	66	(± 5.8)	1.0	75	(± 14.7)	1.1
Oils, fats, and shortenings	387	(± 17.1)	5.8	406	(± 30.9)	5.9
Sugar and adjuncts	1,923	(± 80.4)	28.7	2,042	(± 123.8)	29.5
Beverages, including drinking water	17	(± 2.4)	—	27	(± 6.6)	—
TOTAL	6,697	(± 130.6)		6,928	(± 206.8)	

[a] Data from Shank, 1978.
[b] SE, standard error.

and in medications. Sodium is a natural constituent of both vegetable and animal products in varying concentrations.

In addition to salt, rich dietary sources of sodium are sodium-containing condiments such as monosodium glutamate, sauces, relishes, sweet and sour pickles, gherkins, olives, tomato ketchup, and a number of other foods including ham, bacon, sausages, dried beef, cold cuts, frankfurters, anchovies, canned crab, canned tuna, other canned fish, cheese, canned vegetables, sauerkraut, potato chips, other salted snack foods such as pretzels, saltines, soda crackers, breakfast cereals, and breads such as cornbread or biscuits. The average sodium content of foods analyzed in the Food and Drug Administration's Total Diet Study (market-basket survey) for 1977 and 1978 is shown in Table V-7 for typical adult intakes.

During the preservation and processing of foods, sodium and sodium chloride (NaCl) are added as are numerous other chemical additives including sodium saccharin ($C_7H_4NO_3SNa$), monosodium glutamate [$HOOCCH(NH_2)CH_2CH_2COONa$], sodium nitrite ($NaNO_2$), sodium nitrate ($NaNO_3$). sodium benzoate ($C_6H_5COONa$), sodium ascorbate ($C_6H_7NaO_6$), sodium proprionate ($CH_3CH_2COONa$), sodium caseinate, etc. Sodium chloride or monosodium glutamate may be added to foods to suit individual tastes—not only during the commercial preparation of food but also in the home either in the kitchen or at the table. Other sources of sodium are medications, drinking water, cooking water, soft drinks, and alcoholic beverages (Newborg, 1969; Weickart, 1976). Sodium intake from carbonated beverages may be more than 200 mg/day (Table V-8).

Intake of sodium chloride by American males averages an estimated 10 g/day (range, 4-24 g) (Dahl, 1960). On the basis of these estimates, which were obtained from excretion data, sodium intake would range from 1,600 to 9,600 mg/day. The average sodium intake per capita per day, which was estimated from analysis of hospital diets, has been given as 3,625 mg ± 971 (SD). This figure is for diets "as selected" and not "as eaten" and does not include salt that may have been added at the table (California State Department of Public Health, 1970). Sodium intake by infants depends on milk source, formula composition, and the amount of salt added as seasoning by the preparer.

The sodium content of drinking water is extremely variable. Greathouse and Craun (1979) reported that levels of sodium in household tap waters were above detection limits in 99.79% of the areas sampled. The maximum sodium concentration was approximately 80 mg/liter, the minimum was approximately 4.0 mg/liter, and the mean concentration was approximately 28 mg/liter (Craun *et al.,* 1977).

Raw water samples were analyzed by the U.S. Environmental Protection Agency Region V Laboratory and Indiana State University (U.S. Environmental Protection Agency, 1975) during 1971-1975. The sodium content was between 1.1 and 77.0 mg/liter. Finished drinking water samples, which were also analyzed by these same groups, had sodium contents of 1.0 to 91.0 mg/liter.

In a survey of community water systems undertaken as a cooperative study by the New York State Department of Health (1977) and the U.S. Department of Interior, Geological Survey, approximately 12% of the water samples analyzed contained sodium concentrations in excess of 20 mg/liter. The highest concentration of sodium in drinking water recorded in this survey was 220 mg/liter in two systems—one in Chemung County and one in Wayne County. The report of this study pointed out that sodium is added during the treatment of public water supplies as follows:

1. Ion exchange softening—sodium ion in an exchange medium. Sodium, in the forms of sodium chloride, sodium carbonate (Na_2CO_3), and sodium hexametaphosphate [$(NaPO_3)_x$], is exchanged for magnesium and calcium.
2. Chemical precipitation softening—sodium carbonate (soda ash).
3. Disinfection (chlorination)—sodium hypochlorite (NaOCl).
4. Fluoridation—sodium fluoride (NaF) or sodium silicofluoride (Na_2SiF_6).
5. Corrosion control—sodium carbonate or sodium hexametaphosphate.
6. pH Adjustment—sodium carbonate or sodium hydroxide (NaOH).
7. Coagulation—sodium aluminate ($NaAlO_2$) or sodium silicate ($NaSiO_3$).
8. Dechlorination—sodium bisulfite ($NaHSO_3$).

Many residences with public water or individual well water supplies also have water-softening units that can add sodium to the water. In most home water-softening units, the ion-exchange process is used. This process increases the sodium concentration in the finished water since it adds two atoms of sodium for every atom of calcium removed.

To summarize the distribution of sodium in New York State water supplies, 265 community water systems had a sodium content between 5.01 and 20.00 mg/liter, 57 such systems contained between 20.01 and 50.00 mg/liter, and 32 systems, which served a total of 22,080 people, contained more than 50.01 mg/liter (New York State Department of Health, 1977).

TABLE V-8 Sodium Content of Soft Drinks[a, b]

	Sodium, mg			
Proprietary Name and Type	Na+/ml	Na+/240ml Serving	Na+/360ml Can	Calculated daily Na+ Intake per Capita from Soft Drinks[c]
7-Up, regular	0.09	22	32	31
7-Up, sugar-free	0.14	32	48	47
Coca-Cola	0.08	20	30	29
Sprite, regular	0.18	42	62	61
Sprite, without sugar	0.18	42	62	61
Mr. PiBB, regular	0.10	23	35	34
Mr. PiBB, without sugar	0.16	37	55	54
Fanta, orange	0.09	21	31	30
Fanta, grape	0.09	21	31	30
Fanta, root beer	0.10	23	35	34
Fanta, ginger ale	0.13	30	46	45
Fresca	0.24	57	85	83
Tab	0.13	30	46	45
Tab, black cherry	0.20	48	72	71
Tab, root beer	0.18	42	62	61
Tab, ginger ale	0.20	47	71	70
Tab, orange	0.18	43	65	64
Tab, grape	0.19	44	65	65
Tab, lemon-lime	0.18	42	62	61
Tab, strawberry	0.17	39	59	58

[a] Values for sodium content of soft drinks vary with sodium content of the water that is used during manufacture.

[b] Information derived from the Consumer Information Center, Coca Cola Company, Atlanta, Georgia, and the Seven-Up Company.

[c] Based on 1977 estimate of per capita annual consumption of soft drinks at 359 12-oz units (98% of a 12-oz can/day). Personal communication from the National Soft Drink Association, Washington, D.C. Sales Survey of the Soft Drink Industry.

Requirements

The estimated adequate and safe intakes for sodium range from 1,100 to 3,300 mg/day for normal adults or 1 g of sodium per kilogram of fluid and food intake (Meneely and Battarbee, 1976; National Academy of

Sciences, 1980). In infants, estimated adequate and safe intakes are approximately 115 to 750 mg/day (National Academy of Sciences, 1980).

Toxicity Versus Essential Levels

Acute Toxicity

Salt poisoning or acute sodium toxicity is produced by massive overload, particularly in the very young. In Broome County, New York, 6 out of 14 infants exposed to a sodium concentration of 21,140 mg/liter died when salt was mistakenly used in place of sugar in their formulas (Finberg *et al.,* 1963). Acute toxicity from sodium chloride in healthy adult males accompanied by visible edema may occur with an intake of 35 to 40 g of salt per day (Meneely and Battarbee, 1976).

Chronic Toxicity

Evidence suggests that chronic excessive intake of sodium may be associated with hypertension, which is defined on the basis of blood pressure readings, i.e., mild hypertension implies diastolic blood pressure above 90 mm Hg and systolic blood pressure above 140 mm Hg (Reader, 1978). In populations residing in different geographic areas, a positive correlation has been found between salt intake and the incidence of hypertension (Dahl, 1972; Sasaki, 1962).

Sodium toxicity leading to hypertension has been associated with intakes of salt (NaCl) greater than 30 g/day, but may occur at lower intakes in persons predisposed to hypertension or suffering from hypertension, congestive heart failure, cirrhosis, or renal disease. Toxicity of sodium salts may be influenced by the anion with which sodium is paired (Venugopal and Luckey, 1978). Until recently, the failure to demonstrate a causal relationship between salt intake and the development of hypertension has discouraged scientists from recommending limitation of salt intake according to the Advisory Panel of the British Committee on Medical Aspects of Food Policy (Nutrition) on Diet in Relation to Cardiovascular and Cerebral Vascular Disease (1975).

The level of sodium in drinking water may influence blood pressure. The blood pressure distribution patterns for systolic and diastolic pressures of high school students living in a community with elevated levels of sodium in the drinking water (100 mg/liter) showed a significant upward shift as compared with the patterns for matched students in a

neighboring community with low sodium levels in the drinking water (8 mg/liter) (Calabrase and Tuthill, 1977).

Twenty percent of the adult U.S. population has hypertension (Intersociety Commission for Heart Disease Resources, 1971). In the treatment of essential hypertension, restriction of dietary sodium leads to a fall in blood pressure (Allen and Sherrill, 1922). Dahl *et al.* (1958) reported that weight reduction in obese individuals leads to a decrease in blood pressure only when sodium intake is restricted. On the other hand, sodium restriction in the obese significantly reduces blood pressure even when calories are not restricted.

In hypertensive subjects, a lowering of blood pressure may be effected either by reducing total sodium intake or by the use of thiazide diuretics, which promote sodium excretion. In 1960, the American Heart Association (AHA) reported that diuretics could reduce the need for a very restricted sodium diet and that they produced quick results, a desirable factor when there is an acute need for lowering blood pressure. The AHA advocated a sodium-restricted diet for the long-term management of hypertension (Pollack, 1960).

A maximum level of sodium in drinking water of 20 mg/liter has been suggested by the American Heart Association (1957).

Currently, antihypertensive medication, including diuretics, is considered a requirement in the management of established hypertension. Sodium restriction alone can control borderline hypertension thereby reducing the need for diuretics (American Medical Association, 1973; Mayer, 1971).

According to Freis (1976), the consumption of less than 5 g of salt a day might reduce the incidence of hypertension and its related diseases by 80% but the evidence available to support such specific figures is limited. Blackburn (1978) suggested a public health intervention trial "to test . . . reduced sodium consumption and culture-wide changes in salt-eating habits as a long-term public health approach to primary prevention of hypertension."

Sodium-restricted diets are required in the treatment of congestive cardiac failure, renal disease, cirrhosis of the liver, toxemia of pregnancy, and Meniere's disease. Sodium-restricted diets may also be required for patients on prolonged corticosteroid therapy. Moderate sodium restriction has been advocated for the management of premenstrual fluid retention (Wintrobe *et al.,* 1970).

Clinical experience has shown that patients often do not adhere to prescribed sodium-restricted diets. However, it is difficult to check compliance from dietary records since patients either forget that certain foods contain appreciable amounts of sodium or are unaware of sodium

sources in foods, drugs, or water. Pietinen *et al.* (1976) proposed the use of mean urinary sodium values (mean of three 9-hr overnight urinary sodium estimations) to check compliance with dietary suggestions.

In Australia, Morgan *et al.* (1978) treated patients with mild hypertension by moderately restricting their salt intake, which was monitored by checking urinary sodium values. Results were compared with those of a control group and a group maintained on antihypertensive medication. Salt restriction reduced the diastolic blood pressure by 7.3 ± 1.6 mm Hg, a result similar to that of patients treated with the antihypertensive drugs. In the untreated group, the diastolic blood pressure rose by 1.8 ± 1.1 mm Hg. The authors pointed out that most patients did not achieve the amount of salt restriction their physicians desired and inferred that stricter adherence to the diet could have caused further reductions in blood pressure. To effect restriction of sodium intake, they recommended the avoidance of both salted foods and addition of salt to food at the table. However, they acknowledged that it would be difficult to reduce the intake of sodium below approximately 2,300 mg/day in Australia because of the widespread use of sodium salts in prepared foods (Morgan *et al.,* 1978). This is also probably true in the United States. (See Table V-7.)

Elliott and Alexander (1961) reported adverse health effects in persons on sodium-restricted diets when they consumed water with a high sodium content. The authors observed recurrent episodes of heart failure which ceased when water with a low sodium content was substituted.

Deficiency

Sodium deficiency may result from renal disease, diuretic therapy, osmotic diuresis, adrenal insufficiency, vomiting, diarrhea, wound drainage, excessive sweating, burns, mucoviscidosis (cystic fibrosis), peritoneal drainage, and pleural, pancreatic, and biliary fistulae drainage. Salt restriction leads to sodium deficiency under conditions of renal impairment.

Sodium depletion can be either iatrogenic or noniatrogenic. Iatrogenic causes include administration of excessive amounts of free water, thiazides, other diuretics, such as furosemide and ethacrynic acid, barbiturates, and oral hypoglycemic drugs (de Bodo and Prescott, 1945; Fichman *et al.,* 1971; Fuisz, 1963; Stormont and Waterhouse, 1961).

Noniatrogenic causes of hyponatremia are commonly related to the inability to excrete free water which is either administered or generated from endogenous sources. Hyponatremia can also be induced by poor

secretion of antidiuretic hormones, alcoholic cirrhosis, adrenocortical insufficiency, congestive heart failure, and cachexia (Bartter and Schwartz, 1967; Birkenfeld *et al.,* 1958; Kleeman, 1971; Matter *et al.,* 1964).

Drinkers of large quantities of beer who eat very little food can experience fatigue, dizziness, and muscle weakness. These patients are both hyponatremic and hypokalemic. The syndrome is rapidly resolved by abstaining from beer consumption and eating a normal diet. Hilden and Svendsen (1975) stated that beer is often low in sodium (20-50 mg/liter), that the patients did not obtain adequate sodium or potassium from their diets, and that water diuresis is inhibited and hyponatremia develops when animals or humans are kept on a sodium-deficient regimen.

Arieff *et al.* (1976) discussed the neurological symptoms of hyponatremia. In addition to nonneurological symptoms (anorexia, nausea, vomiting, and muscle weakness), 14 patients with acute hyponatremia had some depression of the sensorium and four of them had grand mal seizures. Seven of these patients were treated with hypertonic saline while four were treated only with fluid restriction. Of the seven patients that were treated with hypertonic saline, five survived. Three of the four patients treated with fluid restriction died. The authors emphasized that edema of the brain that may occur with hyponatremia may go undiagnosed.

Sweat is a major route for sodium losses. Consolazio *et al.* (1963) studied three normal young men who were made to sweat by daily exercise on a stationary bicycle at temperature of 24°C or 37.8ºC. Sweat was collected in arm bags made of polyethylene. The percentage of total sodium excretion that was excreted in sweat was 62.8% in 7.5 hr and 88.7% in 16.5 hr. The sodium content of sweat varies from approximately 500 to 1,200 mg/liter. Lower values result if subjects have been acclimatized to heat. Lee (1964) reported that excessive sweating, which can be brought on by exercise, heat, or fever, can result in sodium losses from the skin as high as 7 g/day. He suggested that sodium chloride be administered to prevent hyponatremia whenever more than a 4-liter intake of water is required to replace sweat losses. Furthermore, he recommended that 2 g of sodium chloride be given for each additional liter of water lost. This would amount to approximately 7 g/day for people doing heavy work in extreme heat (Lee, 1964). People who are exposed to high temperatures occupationally or during leisure and those performing heavy physical work may find it convenient to take salt pills or to add sodium chloride to their drinking water.

Interactions

Potassium chloride (KCl) counteracts the hypertensive effects of chronic excess of sodium chloride. Lithium from lithium carbonate (Li_2CO_3) accumulates in the body of sodium-depleted persons (Meneely and Battarbee, 1976).

Contribution of Water to Sodium Nutrition

Given an intake of 2 liters of drinking water per day with a mean sodium concentration of 28 mg/liter, a contribution of sodium in water to the estimated adequate and safe intake would be approximately 1.7% to 5.0%. The contribution of sodium at 28 mg/liter in water to the observed current dietary sodium intake is between 0.6% and 3.4%. (These figures do not take into account sodium sources from medication, and they refer to adults only.)

Conclusions

Table V-10 (see page 308) summarizes information on sodium in water and the diet.

Data suggest that whereas health benefits could accrue to certain segments of the population from reduction in sodium intakes, the amount of sodium contributed to the intake from drinking water is small except for persons on sodium-restricted diets (<2,000 mg/day). According to the National Center for Health Statistics, approximately 2.8% of Americans are on low sodium diets (National Academy of Sciences, 1977). The size of the population that is predisposed toward hypertension when exposed to elevated sodium intake is not known with any certainty.

Options available to reduce sodium intake are (in order of decreasing potential) reducing salt added to food as seasoning when eating or cooking, consuming foods with lower sodium levels, reducing sodium in drugs and additives, and reducing sodium levels in water.

Recommendations

Research is needed to define more exactly the contributions of sodium intake, sodium-potassium intake ratios, and other physiological factors to the development of hypertension.

The relationship of the level of sodium and the sodium: potassium ratio in drinking water to blood pressure should be investigated further.

The level of sodium in drinking water should be monitored and physicians informed of the level via local public health departments.

The sodium content of drinking water should not be increased purposefully. Safeguards should be taken against accidental increases, e.g., salting of roads in winter resulting in "deicing" runoff. In instances where water is to be softened (by ion-exchange) domestically, a three-line system is recommended so that only the water used for bathing and laundry would be softened—not the water for drinking.

Total review of the sources of sodium intake is urgently required along with publication of the sodium content of drinking water, beverages, foods, and drugs.

POTASSIUM

Potassium has four major biological functions. It contributes to the maintenance of electrolyte balance, the transmission of nerve impulses to muscle fibers and the control of normal muscle contractility, and the control of heart rhythm, and it acts as an insulin antagonist in intermediary carbohydrate metabolism.

Presence in Food and Water

According to Greathouse and Craun (1979), the mean concentration of potassium in household tap waters is 2.15 mg/liter (minimum, 0.721 mg/liter; maximum, 8.278 mg/liter). Concentrations of potassium in drinking water in Region V (defined by the U.S. Environmental Protection Agency) were 0.5 to 7.4 mg/liter in raw water and 0.5 to 7.7 mg/liter in finished water (U.S. Environmental Protection Agency, 1975).

Potassium is widely distributed in foods, both as a natural constituent and as an ingredient in food additives. In foods of plant origin, the commonest naturally occurring anions of potassium salts are nitrate (KNO_3), sulfate (K_2SO_4), phosphates (K_2HPO_4, KH_2PO_4, or K_3PO_4), and chloride (KCl). Amounts of these potassium salts vary with the plant as well as with methods of cultivation and fertilization (Grunes, 1978, personal communication).

Potassium-containing food additives include potassium alginate (a stabilizer, thickener, and emulsifier), potassium chloride [a gelling agent and a substitute for sodium chloride (NaCl) (Sopko and Freeman, 1977)], potassium iodate (KIO_3) and potassium bromate ($KBrO_3$) (dough conditioners that are added to bread mixes), potassium nitrate used as a

food preservative, monobasic and dibasic potassium phosphates (buffering agents and sequestrants), tribasic potassium phosphate (an emulsifier), potassium polymetaphosphate [$(KPO_3)_x$] (a fat emulsifier and a moisture retaining agent), potassium pyrophosphate ($K_4P_2O_7$) (an emulsifier and a texturizer), potassium sorbate ($CH_3CH = CHCH = CHCOOK$) (a preservative), potassium sulfate (a water corrective agent), and potassium bitartrate or cream of tartar ($KHC_4\ H_4\ O_6$) (an acidifying agent) (National Academy of Sciences, 1972).

TABLE V-9 FDA Total Diet Study ("Market-Basket") Estimates of Potassium Intake[a]

		Intake, mg/day	
Age	Classification	1977	1978
6 Months	Infants	1,551	1,590
2 Years	Toddlers	1,714	1,846
15-29 Years	Adults	4,549	4,735

[a] Data from Shank. 1978. Values are based on an intake of 3,900 cal/day.

Rich food sources of potassium are bran, dried brewer's yeast, cocoa, instant coffee, dried legumes, teas, spices, molasses, almonds, peanuts, raisins, peanut butter, avocados, pears, stewed prunes, parsley, bananas, potatoes, butter beans, dried, whole or nonfat milk, chocolate milk, oranges, orange juice, squash, and melon. Potassium is highly available in food.

The Food and Drug Administration's Total Diet Study ("market-basket" survey) estimates of potassium intakes in the United States for three age groups for 1977 and 1978 are shown in Table V-9.

Current dietary potassium intakes of adults are believed to range from 1,500 to 6,000 mg/day.

Dietary items with a very high potassium content may be consumed infrequently by young children and the elderly (Wilson *et al.,* 1966). Younger men and women obtain enough potassium from their diets to satisfy nutritional requirements (Wilde, 1962).

Requirements

The estimated adequate and safe intake of potassium for adults is between 1,875 and 5,600 mg/day. Intakes for infants and children are

given in Table V-31 at the end of this chapter (National Academy of Sciences, 1980).

Toxicity Versus Essential Levels

Deficiency

Older persons may have low potassium intakes. In a study of 46 men and 88 women aged 65 and over, who lived in their own homes in northern Glasgow, Judge *et al.* (1974) observed that the mean dietary potassium intake for men was 2,769 mg/day, and for women, 2,106 mg/day.

A gross reduction in dietary potassium intake can produce potassium depletion and a drop in serum potassium levels (Squires and Huth, 1959; Womersley and Darragh, 1955). Mohamed (1976) also observed low potassium intakes among elderly pensioners in southern Sweden. He analyzed actual food portions and beverages.

In an unpublished study of the diets of elderly housebound women and men in New York State during 1978, Roe (personal communication) discovered that the mean potassium intake was 2,071 mg/day (median, 1,893 mg/day; minimum, 703 mg/day; and maximum, 4,178 mg/day). Judge and Cowen (1971) showed that elderly people whose dietary intakes of potassium are less than 2,340 mg/day may have reduced handgrip strength. Overt potassium deficiency in adults is associated with intakes of 2.000 mg or less per day.

Major causes of potassium deficiency include prolonged vomiting or diarrhea, starvation, diabetic acidosis, surgery, use of diuretic drugs, use and abuse of cathartics, and intakes of corticosteroid hormones (Dargie *et al.,* 1974; Food and Drug Administration. 1975; Krause and Hunscher, 1972; Robinson, 1967).

Nardone *et al.* (1978) estimated that approximately 98% of total body potassium is contained in the intracellular compartment of the body. Less than 2% is located in the serum where it can be extracted for measurement. Low serum potassium levels usually reflect total body deficit. However, in alkalosis, insulin therapy and hypoosmolality may decrease serum levels of potassium (without a concomitant decrease in cellular potassium) so that they do not reflect actual body stores (Nardone *et al.,* 1978).

Potassium is excreted through the urine, gut, and skin. According to Nardone *et al.* (1978), the losses through the gastrointestinal tract and the skin are relatively minor under physiological conditions (Berliner, 1960; Suki, 1976).

Nardone *et al.* (1978) classified the origins of hypokalemia as follows:

- Gastrointestinal malabsorption
- Renal loss of potassium due to disease
- Loss of potassium induced by drugs [e.g., diuretics, including organomercurials, thiazides, furosemide (Dargie *et al.,* 1974), and ethacrynic acid; antibiotics, including carbenicillin and penicillin; laxatives; corticosteroids; and nephrotoxic drugs, e.g., outdated tetracycline] and by licorice and extracts of licorice
- Maldistribution of potassium caused by periodic paralysis (familial, acquired), drugs (e.g., insulin), and toxins (e.g., barium)

Drug-induced hypokalemia is extremely common. Hypokalemia resulting from low intake is less common. Several disorders, e.g., cancer, gross neurological disease, psychiatric illness, and chronic gastrointestinal disease, reduce total food intake and, thus, potassium intake. They can also lead to hypokalemia because of catabolism.

Hypokalemia can result from self-administration of excessive quantities of diuretics, laxatives, or licorice, or from self-induced vomiting. Patients who induce hypokalemia by these means usually have an underlying psychiatric illness. In the young, this illness may be anorexia nervosa (Fleming *et al.,* 1975; Katz *et al.,* 1972; Wallace *et al.,* 1968; Wrong and Richards, 1968).

Symptoms of potassium deficiency are weakness, anorexia, nausea, vomiting, listlessness, apprehension, and sometimes diffused pain, drowsiness, stupor, and irrationality. Hypokalemia can exist without any abnormal clinical findings. When symptoms are present, the most common is profound muscle weakness. Changes in electrocardiograms are also found (Zintel, 1968).

In hypertensive patients who are maintained on diuretics, potassium chloride can be used as a salt substitute to reduce sodium intake while providing a source of potassium.

Potassium depletion sensitizes patients to intoxication by cardiac glycosides such as digitalis. Potassium deficiency causes both structural damage and functional impairment of the kidney.

Toxicity

Keith *et al.* (1942) investigated effects of single large doses of potassium salts in seven normal persons. These subjects ingested from 12.5 to 17.5 g of potassium chloride or bicarbonate ($KHCO_3$). Their renal clearances were then determined. In two subjects, the potassium load disturbed normal renal excretion. The authors estimated that single doses of

potassium salts containing 80 to 100 mg of potassium per kilogram of body weight may be nephrotoxic. Extracellular potassium, if rapidly raised by intravenous injection from 125 to 2,500 mg/liter, is toxic and may be lethal (Comar and Bronner, 1962).

It is not possible to produce hyperkalemia or potassium toxicity by dietary means in people with normal circulatory and renal function (Burton, 1965). Hyperkalemia is caused mainly by diseases such as Addison's disease or by renal failure with gross oliguria. Potassium toxicity can be caused by ingestion of enteric-coated potassium chloride tablets. Symptoms of this toxicity include gastric irritation, ulceration of the small intestine, and perforation of late strictures (Mudge and Welt, 1975).

Malabsorption of vitamin B_{12} has been identified in patients receiving slow-release potassium chloride supplements. Schilling test values of vitamin B_{12} absorption were normalized when potassium chloride was withdrawn from the regimens of these patients (Palva *et al.,* 1972).

Symptoms of acute poisoning caused by eight 4-g potassium chloride tables were cyanosis, shallow respiration (Keith *et al.,* 1942), and life-threatening cardiac arrhythmia (Maxwell and Kleeman, 1972). According to Blum (1920), 25 g of potassium chloride per day can induce acute toxicity. Smaller doses can cause diarrhea. Intakes of potassium associated with acute toxicity are 7 to 10 g/day in adults and 2 g/day in young children.

Interactions

Metabolism of protein, amino acids, and glucose are affected by potassium status (Lehninger, 1970).

Focusing attention on the high sodium, low potassium environment in our society, Meneely and Ball (1958) and Meneely and Battarbee (1976) presented evidence that reduced sodium intake with a concurrent increase in potassium intake would benefit health, particularly for hypertensive or borderline hypertensive subjects. Potassium has a protective action against the hypertensive effect of high sodium intakes in humans and in laboratory animals, but the mechanism is unknown (National Academy of Sciences, 1970).

Contribution of Drinking Water to Potassium Nutrition

Because the levels of potassium in water are low in relation to those in foods, the contribution of water to the requirement or intake of potassium is negligible.

Conclusions

Table V-10 (see page 308) summarizes information on potassium in drinking water and the diet.

Potassium is abundant in the food supply, whereas water contributes little to total potassium intake.

Potassium deficiency is common in certain subgroups of the population, notably the elderly whose deficiency is attributed to low intake of potassium-rich foods as well as to the use of laxatives and diuretics. This deficiency is a cause of geriatric disability including severe muscle weakness. Frequently, it also produces digitalis toxicity, which is life-threatening.

Potassium excess leading to toxicity is not common and is not incurred through the diet. Acute potassium toxicity can be induced by oral potassium preparations including enteric-coated pills.

Research Recommendation

Studies should be directed toward defining more closely the RDA for potassium in different age groups, particularly the elderly.

CHLORIDE

Chloride is the most important anion in the maintenance of fluid and electrolyte balance and is necessary to the formation of the hydrochloric acid (HCI) in the gastric juices.

Presence in Food and Water

Rich sources of chloride are salt, breakfast cereals, breads, dried skim milk, teas. eggs, margarine, salted butter, bacon, ham, salted beef (corned beef), canned meats, canned fish, canned vegetables, salted snack foods, and olives. In the diet, chloride occurs mainly as sodium chloride (NaCl) (Harper *et al.,* 1977)).

Chloride is found in practically all natural waters. Surface waters contain only a few milligams per liter, whereas streams in arid or semiarid regions contain several hundred milligrams per liter, especially in drained areas where chlorides occur in natural deposits or are concentrated in soils through evaporation processes. Contamination with sewage increases the chloride content of river waters. Industrial wastes and drainage from oil wells or other deep wells and from salt springs

may add large quantities of chloride to streams. Most public water supplies contain less than 25 mg/liter. Groundwater usually contains larger quantities than surface water. Some public supply wells may contain as much as 100 mg/liter.

In the 1975 report on the Region V survey of the contents of selected drinking water supplies (U.S. Environmental Protection Agency, 1975b), the mean concentration of chloride in raw water was 18 mg/liter (SD ± 17) and the content of finished water was 21 mg/liter (SD ± 21). Concentrations as high as 179 mg/liter were recorded, although 95% of the samples analyzed fell below 40 mg/liter. In a chemical analysis of interstate carrier water supply systems (U.S. Environmental Protection Agency, 1975a), 11 of 684 samples (1.6%) failed to meet the recommended drinking water limit for chloride which was set in 1962 by the U.S. Public Health Service (1962) at 250 mg/liter.

The U.S. Environmental Protection Agency (1977) has similarly set the secondary maximum contaminant level for chloride content in drinking water at 250 mg/liter, based on findings that are described below.

The presence of particular concentrations of chloride ion (Cl) in drinking water can produce a taste that is sometimes objectionable to the consumer. Water may be rejected on the basis of its chloride content. Whipple (1907) reported that subjects showed a differential ability to detect the chloride content of water which varied with type of chloride salt added. The chloride content of the water sampled by his subjects ranged from 96 to 560 mg/liter.

Lockhart *et al.* (1955) reported that taste thresholds for the chloride anion in water varied from 210 to 310 mg/liter, according to the type of chloride salt added. They noted that a high chloride content of water may cause an unpleasant taste in coffee. Richter and Maclean (1939) found that the chloride taste threshold was lower than that found by other authors.

Current dietary intakes of chloride vary largely with intake of salt. Estimates range from 2.400 to 14,400 mg/day from sodium chloride (Dahl, 1960).

Distribution in Tissues

According to Forbes (1962), the chloride concentration in humans is approximately 2,000 mg/kg of fat-free body mass in the newborn and 1,920 mg/kg in the adult. Ziegler and Fomon (1974) believe it is reasonable to assume that the concentration of chloride in fat-free body weight gain after birth is approximately 1,920 mg/kg.

Requirements

The essentiality of chloride is generally recognized but no recommended dietary allowances (RDA's) have been determined.

Ziegler and Fomon (1974) suggested that the chloride requirements for growth (alone) was 28 mg/day from birth to 4 months of age, 21 mg/day from 4 months to 12 months, 12 mg/day from 12 months to 24 months, and 12 mg/day from 24 months to 36 months. Advisable total intakes of chloride for infants in these four age groups are 245 mg/day, 210 mg/day, 245 mg/day, and 350 mg/day, respectively. A daily chloride turnover in adults (intake/output) ranges from between 3,018 and 8,875 mg. Cotlove and Hogben (1962) found that the loss of chloride generally parallels that of sodium.

Toxicity Versus Essential Levels

Imbalance and Depletion

Electrolyte imbalances may disturb the absolute or relative amounts of chloride in the serum. Abnormalities of sodium metabolism are usually accompanied by abnormalities of chloride metabolism. When there are high losses of sodium, as in diarrhea, profuse sweating, or certain endocrine abnormalities, chloride deficit is also observed. However, when there is loss of gastric juice through vomiting, losses of chloride exceed sodium losses. This leads to a decrease in plasma chloride and a compensatory increase in bicarbonate (HCO_3^-). This results in hypochloremic alkalosis (Lennon, 1972). In Cushing's disease, or after the administration of an excess of corticotropin (ACTH), cortisone, or other corticosteroids, hypokalemia with an accompanying hypochloremic alkalosis may occur. Hypochloremia may also result when chloride is lost through profuse diarrhea, which impairs the reabsorption of chloride in the intestinal secretion (Harper *et al,* 1977).

Toxicity

The toxicity of salt containing the chloride ion depends mainly on the characteristics of the cation. The administration of hydrochloric acid (HCl), ammonium chloride (NH_4Cl), lysine hydrochloride $[NH_3(CH_2)_4CH(NH_2)COOH]^+[Cl]^-$, or arginine hydrochloride $[HN=C(NH_3)NH(CH_2)_3CH(NH_2)COOH]+[Cl]^-$adds to the quantity of readily dissociated acid (HCl) but is buffered by the bicarbonate ion (HCO_3), leading

to an increase in the plasma concentration of chloride and a decrease in plasma bicarbonate. This results in hyperkalemic metabolic acidosis.

Effects of Chloride Loading

Adaptation to sodium chloride load may occur in human subjects. In experiments on isolated frog skin, Watlington *et al.* (1977) showed that extracts of the urine of humans on a high sodium chloride intake produced a net active transport of mediated chloride ion efflux. On the other hand, such activity was not induced by the urine of either normal humans who had been deprived of sodium or humans with adrenal insufficiency who had been loaded. These findings suggest the presence of an adrenal corticosteroid, which may participate in adaptation to high salt intake.

Interactions

Normally, the only halogen in the extracellular fluid is chloride. Chloride may be partially replaced by bromide when bromide is taken as a medication over a prolonged period. Each mEq of bromide retained displaces 1 mEq of chloride, but total halide, i.e., total chloride and bromide concentration, remains unchanged. Many of the chemical and biological properties of chloride and bromide are similar, but renal tubular transport differs. The renal clearance of bromide is slightly less than that of chloride, indicating that the tubular epithelium retains bromide preferentially. A progressive rise in bromide and falling chloride concentrations result from long-term ingestion of rather small doses of bromide. Chronic bromism is treated by increasing urinary bromide excretion. Any treatment that increases chloride losses will also result in increased bromide losses. Therefore, administration of a chloride source, for example, such as sodium chloride, and such diuretics as furosemide ($C_{12}H_{11}ClN_2O_5S$) and ethacrynic acid ($C_{13}H_{12}Cl_2O_4$) has been used to treat bromism (Emmett and Narins, 1977).

Contribution of Water to Chloride Nutrition

Since no recommended dietary allowance (RDA) exists for chloride it is not possible to assess the contributions of drinking water to the nutritional requirement for chloride.

A typical chloride concentration in drinking water of 21 mg/liter would contribute 42 mg/day (assuming 2 liters/day consumption). This would be just under 2% of the lower estimates of total chloride intake.

The highest chloride concentration observed (179 mg/liter) would contribute 15% to the lowest total intake.

Conclusions

The chloride content of waters varies with the geochemistry of the area and contamination from sewage, industrial, or other wastes.

Concentrations above 250 mg/liter chloride cause a salty taste in water which is objectionable to many people.

Consumption of chloride in reasonable concentrations is not harmful to most people. However, if the chloride is present as sodium chloride, the sodium ion may be undesirable to persons requiring salt restriction.

Typical chloride concentrations in drinking water contribute relatively little to total chloride intakes.

Research Recommendations

Representative chloride intakes from water and food should be determined by region, by locality, and by sex/age groups.

Implications of high chloride ingestion require further investigation.

Means of minimizing the entry of excess chloride into drinking water supplies should be studied.

IODINE

In this section the term iodine, when used in a general sense, denotes all iodine-containing compounds, e.g., iodate (IO_3^-), iodide (I^-), etc.

Iodine is an essential micronutrient. It is an integral constituent of the thyroid hormones, thyroxine and triiodothyronine, which have important endocrine functions in metabolic regulation.

Presence in Food and Water

Sources of iodine include foods, water, internal and topical medications, and air (Underwood, 1971). In the United States, the major contributions to iodine intake come from iodized salt, bread, milk, marine fish, and seafood. Eggs, other animal protein foods, the food coloring erythrosine, water, human milk, kelp, vitamin-mineral supplements, and formula foods also contain iodine. These dietary sources of iodine are highly available as indicated by relationships between intake and urinary excretion of iodine (Kidd *et al.,* 1974).

The origins of iodine in foods are soils, water, commercial fertilizers, atmospheric iodine, iodine-containing antiseptics, food additives, and food or water pollutants. Major sources of iodine in milk are the iodophors—iodine-containing antiseptics that are used to cleanse cows' udders and to "sterilize" milking equipment or food preparation areas. The Wisconsin Alumni Research Foundation (WARF Institute, 1977) reported high iodine concentrations in milk shakes that were prepared in fast-food restaurants.

Iodine in seafoods is derived from ocean waters, which contain approximately 0.06 mg/liter, mainly as iodate, but also as iodine. Breads contain a variable amount of iodine, depending on the source and means of production. Breads made with dough conditioners consisting of calcium or potassium iodate [$Ca(IO_3)_2$, KIO_3] contain much higher iodine concentrations. Breads made by a continuous mixing process with iodate have a higher iodine content than those produced by conventional mixing with iodate (Hemken *et al.,* 1972; Kidd *et al.,* 1974; National Academy of Sciences, 1974a).

Erythrosine (the disodium salt of tetraiodofluorescein), a food-coloring agent, contributes iodine to the diet when it is used in the manufacture of certain breakfast cereals and other foods such as fruit jellies (Vought *et al.,* 1972).

Table salt is iodized to furnish 76 µg of iodine per gram of salt. As of 1968, 54.8% of the table salt sold in the United States was iodized. Use of iodized salt varies with region of the country. The amount of iodine added to the diet via table salt is extremely variable, not only because of differences in the use of iodized versus noniodized salt, but also because salt intake varies markedly. In a population containing both high and low salt users, it is difficult to use the average intake of salt at 10 g per day per person (National Academy of Sciences, 1974b) to calculate intakes of iodine from salt.

Iodine in marine fish and shellfish is presumably derived from sea water and, especially, from marine plants, which have the highest concentrations of iodine of any plant species (Chilean Iodine Education Bureau, 1950).

Drinking water contains a small and variable amount of iodine, which is determined by location, water treatment processes used, and the degree of pollution. Among water-processing methods, flocculation with alum and sedimentation appear to reduce iodine content. Chlorination, when used alone, results in only a small loss of iodine (<10% reduction in the iodine content of raw water).

Iodine enters drinking water from atmospheric iodine (via rain or

304 snow), soil, and, in the case of polluted drinking waters, from decaying plants, animal excretions, and commercial fertilizers. Water containing feces, urine, or plant debris contains more iodine than unpolluted water from the same area (Vought *et al.,* 1970).

Freshwater contains 0 to 2.4 µg/liter in areas where goiter is endemic and 8 to 9 µg/liter in goiter-free areas (Fisher and Carr, 1974). Surface water, more often consumed by domestic animals, contains 4 to 336 µg/liter (National Academy of Sciences, 1974b).

Concentrations of iodine at 4 to 8 µg/liter in raw (untreated) water and 3.4 to 3.8 µg/liter in treated water in Potomac, Maryland, and up to 18 µg/liter in polluted wells in Virginia have been reported.

Average dietary iodine intake has been estimated both from dietary studies and from analysis of thyroidal radioiodine uptakes by the thyroid. Oddie *et al.* (1970) studied radioiodine uptakes that were reported by 133 observers from approximately 30,000 euthyroid subjects throughout the United States. These estimates indicated that daily iodine intakes in various sections of the country varied from approximately 240 to 740 µg/day.

Requirements

The recommended dietary allowances (RDA's) for the intake of iodine by adults range from 0.08 to 0.140 mg/day, depending upon age and sex (National Academy of Sciences, 1974c). The full list of recommendations is shown in the overall summary section at the end of this chapter. An intake of less than 0.05 mg/day leads to endemic goiter (National Academy of Sciences, 1974a).

Toxicity Versus Essential Levels

Both iodine deficiency and excess can enlarge the thyroid, a condition termed goiter. Endemic goiter due to iodine deficiency, which was prevalent in the United States before salt was iodized, is now uncommon. A reduction in the incidence of endemic goiter may also be due in part to the use of breads containing iodate. Measurement of the urinary excretion of iodine suggests that moderate iodine deficiency still occurs in the United States. In the National Nutrition Survey conducted from 1968 to 1969, only a small percentage of persons sampled had visibly enlarged thyroid glands. For example, McGanity (1970) reported that 5.4% of the individuals examined in one study in Texas had palpable or visibly enlarged thyroid glands. Eleven individuals, or approximately 0.4% of this sample, had urinary iodine levels of less than 50 µg/g

creatinine, but none of their thyroids was enlarged. However, 9% of those whose iodine excretion was less than 100 µg did have enlarged thyroid glands (Matovinovic, 1970).

In the 1968-1969 Texas nutrition survey (McGanity, 1970), there was no evidence that the iodine content of drinking water was related to the incidence of enlarged thyroid. Furthermore, there was no relationship between the incidence of enlarged thyroid and the fluoride content or hardness of water.

In a study that was conducted in Virginia, Vought *et al.* (1967) reported that thyroid disease in children is not related to dietary iodine deficiency, but rather to contaminated water. They isolated cultures of microorganisms from contaminated waters and postulated that goitrogens known to be produced by these organisms might interfere with iodine uptake by the thyroids of the affected children.

Plant goitrogens have been implicated as a factor contributing to endemic goiter in many parts of the world, particularly in areas such as the Congo, where there is also dietary iodine deficiency (Delange and Ermans, 1971). While there is no evidence that plant goitrogens play a role in the production of enlarged thyroid or thyroid disease in the United States, too little is known about the possible effects of low doses of goitrogens on the availability of iodine to the neonate or to the developing fetus (Stanbury, 1970). During pregnancy iodine deficiency can impair the development of the fetal thyroid thereby producing cretinism. Endemic cretinism does not occur in the United States.

Goiters resulting from iodine overload have been well described in the United States and other countries. Although goiters can be produced by excessive dietary iodine intakes, the more common cause is ingestion of large quantities of iodine-containing medications. Wolff (1969) has divided iodine goiter into four groups: (1) adult iodine goiter, mostly in asthmatic subjects taking iodine-containing cough medicines; (2) iodine goiter of the neonate due to placental transfer of iodine from mothers who are being treated with iodine; (3) endemic iodine goiter, which is of dietary origin; and (4) hypothyroidism in patients with thyrotoxicosis (Graves' disease) who are being treated with potassium iodine (KI) or Lugol's solution (4.5-5.5 g of iodine and 9.5-10.5 g of potassium iodide per 100 liters of purified water). Most people who have developed iodine goiter have received very large amounts of iodine for prolonged periods. In the iodine goiter cases reviewed by Wolff, intakes of iodine ranged from 18 mg to more than 1 g/day over several months. When iodine goiter develops under these conditions, a secondary complication may be hypothyroidism with clinical signs of myxedema. Prenatal development of iodine goiter carries the risk of obstructed delivery or neonatal

tracheal obstruction. According to Wolff, there is a danger of iodine goiter from prolonged intakes of iodine above 1 to 2 mg/day.

In Northern Tasmania, two waves of increased prevalence of thyrotoxicosis have been attributed to iodine excess. In 1964, the incidence of thyrotoxicosis in Northern Tasmania increased. This was attributed to the use of iodophor disinfectants on dairy farms which, as previously stated, causes iodine residues to be present in milk. In 1966, another epidemic of iodine-induced thyrotoxicosis occurred in the same country. This time it was precipitated by the addition of iodate to bread to prevent endemic goiter (Stewart and Vidor, 1976).

Liewendahl and Gordin (1974) reported a case of iodine goiter in a woman who ingested seaweed for 2 years. Hyperthyroidism also occurred in this patient.

Stanbury (1970) cited an unpublished report of a study in Iceland, where the iodine intake is high (from 0.500 to 1.500 mg/day) because of the prevalence of fish in the diet. The investigator also reported that the incidence of papillary carcinoma of the thyroid was high in Iceland. In parts of Japan, where large intakes of iodine result from the local custom of eating seaweed, carcinoma of the thyroid is more prevalent than in any other country (Suzuki *et al.,* 1965). It has been suggested that the persons at risk of thyroid carcinoma from high iodine intake are those with preexisting thyroid adenoma or goiter.

Furszyfer *et al.* (1970) called attention to a rise in the prevalence of subacute (granulomatous) thyroiditis in Olmstead County, Minnesota, between 1960 and 1967. In a subsequent study (Furszyfer *et al.,* 1972), they reported that the prevalence of Hashimoto's disease (lymphomatoid thyroiditis) in Rochester, Minnesota, had increased from 6.5 per 100,000 females during 1935-1944 to 69.0 during 1965-1967. They suggested a relationship to excess iodine intake.

Iodine may produce acneiform skin eruptions. Sources of iodine cited as being responsible for production of iododermas are iodized salt, iodides in therapeutic vitamin-mineral preparations, and iodine in formula foods such as Metrecal.

Anaphylactic reactions as well as acneiform eruptions and furunculosis (boils) may follow intravenous administration of iodine preparations used as contrast substances for intravenous pyelograms and gall bladder or spinal X-rays (Baer and Witten, 1961).

Interactions

Lead has an adverse effect on the uptake of iodine by the thyroid gland. Persons with lead-poisoning from industrial exposure or from ingestion

of lead-contaminated, illicitly distilled whiskey have developed impairment of iodine uptake by the thyroid (Sandstead, 1977).

Contribution of Drinking Water to Iodine Nutrition

Contribution of Drinking Water to Iodine Requirements

Assuming 2 liters/day consumption of drinking water and total iodine requirements in the range of 0.080 to 0.0150 mg/day, low iodine waters (approximately 0.001 mg of iodine per liter) would provide 1% to 2% of the total requirement, medium iodine waters (0.004 mg/liter), 5% to 10%, and high (polluted) iodine waters (0.018 mg/liter), 24% to 44%.

Contribution of Drinking Water to Total Intake

Given the highest level of iodine in water at 0.018 mg/liter and a total intake of iodine equivalent to 0.240 mg/day, the contribution of water (2 liters/day) would be approximately 15%. Minimal contributions to total body burden would be made by low iodine waters if high iodine intakes from food were consumed (at a level of 0.740 mg/day). Under these conditions, iodine would contribute 0.3% to total intake. Where dietary intake of iodine is low and drinking water is obtained from polluted wells with high iodine content, the water could contribute to the prevention of iodine deficiency. However, in view of Vought's findings of bacterial goitrogens in polluted water (Vought *et al.,* 1970), this seems unlikely. Iodine toxicity is unlikely to be related to water intake unless water was highly contaminated with iodine.

Conclusions

Table V-10 summarizes information on iodine in drinking water and the diet. The average intake of iodine from all sources appears to be at least twice the RDA. Reduction of iodine intake to approximate the RDA is desirable. In some cases, reduction of the level in water would contribute to reduction of intake, but reduction of intake from other sources may be more practical.

Research Recommendations

Further data should be obtained on the iodine content of water supplies.

Microbiological examination of high iodine waters for fecal and urinary contamination should be performed.

Methods for the reduction in the iodine content of high iodine waters should be investigated.

TABLE V-10 Contribution of Drinking Water to Requirements for Iodine, Sodium, and Potassium (values based on a 70-kg adult man and water intake of 2 liters/day)[a]

	Sodium	Potassium	Iodine
Requirements			
Requirement per day, mg[b]	2,500	2,500	0.130
Ratio of toxic intake level to dietary requirement			
Acute[b]	4.7	2.2	
Chronic[c]	5.5	Unknown	7.7
Mean concentrations			
Mean concentration in water, mg/liter	27.7	2.15	0.004
Intake based on mean concentration in water, mg/2 liters	55.4	4.3	0.008
Intake from water based on mean, % of requirement	2.0	2.0	6.0
Highest concentrations observed			
Highest concentration in water, mg/liter	79.7	8.3	0.018
Intake based on highest observed concentration in water, mg/2 liters	159.4	16.6	0.036
Intake from water based on highest observed concentration, % of requirement	6.0	7.0	28

[a] References to the data in this table will be found in the text. Data available on chloride were not considered sufficient for compiling an assessment for that nutrient.

[b] These are intermediate values, selected for illustrative purposes, from recommendations of the National Academy of Sciences (1974c, 1980).

[c] Toxicity is expressed in terms of the ratio of intake to requirement which, over the long term, causes mild to severe signs of toxicity (Food and Drug Administration, 1975).

Changes in total iodine intake over time by the U.S. population should be studied by monitoring individual foods and total "market-basket" samples.

Extraneous sources of iodine due to air and water pollution, use of iodophors, use of erythrosine, and use in vitamin-mineral preparations should be reduced.

The relationships between acne and acneiform eruptions and iodine

intake from water as well as from food or other sources such as medication should be examined.

IRON

Throughout the world, including the United States, iron deficiency is one of the most commonly recognized signs of inadequate nutrition. This situation exists in spite of the fact that iron is among the most abundant elements in the earth's crust. There are several reasons for the anomaly. Man has developed an effective mechanism to prevent excess absorption of iron. This protective device is important because iron is poorly excreted and is highly toxic when tissue levels rise above the tolerance level. Iron compounds tend to be insoluble and the iron of such compounds is inefficiently absorbed. Thus, while the quantity of iron consumed is important, the chemical form of the iron is also a highly significant factor in meeting the dietary requirement. In view of these considerations it is understandably difficult to assess the dietary iron requirement of humans.

Presence in Food, Water, and Air

The concentration of iron in foods consumed by humans varies widely, ranging from less than 1 mg/kg in milk and related products to approximately 50 mg/kg in dry beans and cereals. See Table V-11 (page 318) for a tabulation of data on the amount of iron contained in food groups and the percentage of the mineral contributed by the food groups to the total food intake of persons in U.S. households (Consumer and Food Economics Institute, Science and Education Administration, U.S. Department of Agriculture, unpublished data). Approximately 35% of the dietary iron comes from meat, fish, and eggs, while 50% is supplied by cereals, root vegetables, and other foods of plant origin.

The median iron concentration in surface air layers at 38 U.S. nonurban sites was 0.255 μg/m^3 (National Academy of Sciences, 1979). Twenty cubic meters of such air (the average volume inhaled per day) would contain approximately 5 μg of iron. Even if totally absorbed, this quantity would make a negligible contribution to the daily intake of iron.

An estimate of the iron content of drinking water and its contribution to the iron requirement of the U.S. population are given in Table V-12 (in the section on zinc). While the concentrations of iron in raw water and waste waters are highly variable and, in some cases, quite high, this report is concerned only with finished water, most particularly with tap

water. Of 672 water samples collected from interstate carriers (suppliers) of water and analyzed for the U.S. Environmental Protection Agency (1975), 62.5% contained iron concentrations that could be estimated quantitatively. The average concentration in these 420 water samples was 0.240 mg/liter. The samples were collected from 10 regions in the continental United States. The mean of the maximum values was 2.180 mg/liter. In another EPA study (Craun *et al.,* 1977; Greathouse *et al.,* 1978), tap water samples from 3,834 residences in 35 regions of the United States were analyzed. The mean and maximum concentrations of iron in these samples were 0.245 and 2.180 mg/liter, respectively.

Requirements

The requirement for most nutrients, including iron, varies with the age and physiological state of the individual, but the difference between the male and female requirement for iron is greater than for most nutrients. This stems largely from the blood loss of females during the reproductive period and the increased demand during pregnancy.

Iron is inefficiently absorbed. Consequently, to meet the actual daily requirement for absorbed iron (approximately 1.0 mg for males and 1.5 mg for females), from 10 to 20 times that quantity must be ingested. The percentage of iron absorbed depends on the iron status of the individual, i.e., absorption is greater in persons with iron depletion. There are also differences in availabilities among the various iron compounds in the diet. To assure adequate intake for the majority of the population, the recommended dietary allowance (National Academy of Sciences, 1974) is 10 mg for adult males and 18 mg for females of reproductive age. See Table V-12 (in the section on zinc) for requirements and toxicity of iron compared to similar information for copper and zinc.

Toxicity Versus Essential Levels

When administered parenterally, iron is a highly toxic element. Humans are generally well protected from oral overdose, but children from 1 to 2 years of age are particularly vulnerable to iron toxicity from ingestion of iron supplements that have been commercially prepared for adults (Fairbanks *et al.,* 1971). In general, the long-term toxic levels of dietary iron for monogastric animals is 340 to 1,700 times greater than the requirement. Such continuous intake may give rise to signs of toxicity (Food and Drug Administration, 1975).

Interactions

The bioavailability of iron in foods varies widely. For example, iron in the form of heme is absorbed nearly 10 times as efficiently as iron in food of plant origin. Practically nothing is known about the absorption of iron from water. As a matter of fact, little is known about the chemical species of iron from drinking water at the tap. In a well-aerated river the dominant form is ferric iron (Fe^{3+}). Groundwater may contain appreciable ferrous iron (Fe^{2+}). Surface waters and groundwaters also contain organic complexes of iron (National Academy of Sciences, 1979). The fractions of these forms in water that are absorbed by humans are unknown, but it is clear that reducing agents, such as ascorbic acid, increase the absorption of iron in food (Monsen *et al.,* 1978). Ferrous iron appears to have better bioavailability than does ferric iron. The iron in certain chelates, such as ferric phytate, is poorly absorbed (Bowering *et al.,* 1976). Although it is generally assumed that trace elements in water are readily absorbed, there are few, if any, data relative to the bioavailability of iron in water.

Iron interacts physiologically with several nutritionally essential and nonessential elements. All of these elements, including copper, zinc, manganese, and lead, tend to increase the requirement for iron. Signs of copper toxicosis are eliminated by the addition of extra iron and zinc to the diet, and signs of zinc toxicosis are prevented by the addition of extra copper and iron (Magee and Matrone, 1960). The signs of lead toxicity are exacerbated by iron deficiency. Perhaps the most significant interaction of any mineral with iron is that of manganese. Excess manganese impairs hemoglobin regeneration by decreasing the absorption of iron (Underwood, 1977). (See the section on manganese for further discussion of the iron-manganese interaction.)

Contribution of Drinking Water to Iron Nutrition

Assuming 2 liters/day consumption of water containing an iron concentration equal to the mean value shown in Table V-12, water would contribute approximately 0.5 mg of iron, which is about 5% of the male requirement and less than 3% of the female requirement. For those persons consuming water containing the highest observed value, water would contribute from 17% to 44% of the daily requirement, depending on sex.

Conclusions

In the continental United States, most tap water probably supplies less than 5% of the dietary requirement for iron. This may be considered a

negligible contribution unless the iron in water has an appreciably higher bioavailability than iron in food. However, iron deficiency is common in the United States. Under severely limiting conditions, 0.5 mg of highly available iron from water would make a significant contribution to the daily dietary intake. If a local water supply contained unusually high concentrations of iron, it could contribute substantially to the total intake. The iron content of drinking water should not be reduced since there is little or no likelihood of toxicity from iron in natural foods and water. It should be noted that the present recommended limit for iron in water, 0.3 mg/liter, was based on taste and appearance rather than on any detrimental physiological effect from iron in water.

Research Recommendations

The chemical species of iron in drinking water and their bioavailability should be determined.

COPPER

While there is no evidence of copper deficiency in the U.S. population, except for isolated cases in patients maintained by total parenteral nutrition, copper is clearly an essential nutrient. There is some evidence that the intake is lower than required for optimal human nutrition. Klevay (1975) suggested that borderline deficiencies may occur among portions of the population. The concentration of copper in the earth's crust is estimated to be 50 mg/kg. It forms organic complexes readily and tends to concentrate in clay minerals, particularly in clays that are rich in organic matter. Copper in rocks is mobilized more readily under acidic rather than alkaline conditions (National Academy of Sciences, 1977). The species of copper in drinking water at the tap have not been determined, but copper presumably occurs in the oxidized, Cu(II) state complexed with various ligands. The reaction of soft water with the copper pipes that are used in some household plumbing systems contributes to the copper levels in water at the tap (Schroeder *et al.,* 1966).

Occurrence of Copper in Food and Water

The concentrations of copper in foods are highly variable. They are extremely low in dairy products and relatively high in cereals and roots.

Table V-11 (see page 318) shows the distribution of copper among food groups. These data suggest that the average copper intake is less than 2 mg/day. Klevay (1975) has presented evidence that many U.S. diets contain much less copper than required. For example, he quotes studies showing that the dietary copper intake of high-school girls and college women may be as low as 0.34 to 0.58 mg and that the diets of other individuals may supply less than 1 mg/day.

The estimated contribution of drinking water to an adult's copper requirement is shown in Table V-12 (in the section on zinc). Because the concentration of copper in drinking water is highly variable, means are of limited significance. Approximately 55% of the 604 water samples analyzed by the U.S. Environmental Protection Agency (1975) contained measurable levels of copper. The mean of these samples was 60 µg/liter. The mean of another study (Craun *et al.,* 1977; Greathouse *et al.,* 1978) was 150 µg/liter.

Requirements

Signs of copper deficiency have been observed in patients maintained totally by intravenous alimentation, but there is only one report of copper deficiency in children fed natural food by mouth (Meng, 1977). Since signs of dietary copper deficiency in the United States have not been observed among persons consuming commonly available foods, it has been assumed that the usual intake meets the requirement. The National Academy of Sciences Food and Nutrition Board did not previously set an RDA for copper, but has recently estimated an adequate and safe intake of 2 to 3 mg/day (see Table V-31; National Academy of Sciences, 1980). Copper intakes between 1.3 and 2 mg have been shown to maintain nutritional balance in preadolescent girls and adults of both sexes (National Academy of Sciences, 1974).

Toxicity Versus Essential Levels

Copper is toxic to monogastric animals when ingested in quantities that are 40 to 135 times greater than their respective requirements (Food and Drug Administration. 1975). Except for sheep, all animals absorb copper poorly because their gastrointestinal tracts provide an excellent barrier against oral toxicity. The greatest danger of toxicity arises when children consume acidic beverages that have been in contact with copper

containers or valves (Food and Drug Administration, 1975). The interim drinking water standard (U.S. Environmental Protection Agency, 1977) of 1 mg/liter is based on taste rather than toxicity and affords adequate protection to the general public. However, a few patients with Wilson's disease (hepatolenticular degeneration) are adversely affected by the estimated average intake of copper (Scheinberg and Sternlieb, 1965).

Interactions and Bioavailability

Copper probably occurs in drinking water in the form of the cupric ion (Cu^{2+}) complexed with organic ligands, but this has not been determined. It is reasonable to assume that it is as biologically available as the copper in food, if not more so. High levels of ascorbic acid adversely affect the absorption and metabolism of copper, but few other organic dietary constituents are known to affect its bioavailability (Carlton and Henderson, 1965; Hill and Starcher, 1965; Hunt *et al.,* 1970).

The interaction of two essential trace elements with copper increases the requirement of humans for copper. For example, high levels of zinc exacerbate the signs of copper deficiency in mammals. This effect can be reversed by feeding extra copper to the subject (O'Dell *et al.,* 1976). The antagonism of molybdenum to copper is augmented by sulfate (SO_4). This interaction is particularly significant in ruminant animals but may be of little importance in humans. Copper, sulfur, and molybdenum form an insoluble copper thiomolybdate complex (Dick *et al.,* 1975). Silver and cadmium, both nonessential elements, also interact with copper to exacerbate signs of deficiency (Underwood, 1977).

Contribution of Drinking Water to Copper Nutrition

If one assumes a typical concentration of copper in drinking water of 0.1 mg/liter, a human would obtain 0.2 mg of copper from 2 liters of water. This constitutes between 6.0% and 10% of the estimated adequate and safe intake. In view of the data assembled in Table V-11 (see page 318) indicating that the typical copper intake from food is less than 2 mg/day, and other observations (Klevay, 1975) suggesting even lower consumption of copper from food, the contribution of water to total copper intake becomes even more significant. Furthermore, some drinking water contains considerably higher levels of copper than 0.1 mg/liter and would contribute a correspondingly greater proportion of the total intake. Waters containing the average reported maximum copper

concentrations would supply approximately 40% of the requirement. Although overt signs of copper deficiency have not been reported in enterally nourished persons in the United States, the margin of safety may not be large and the contribution of drinking water to copper nutrition should not be overlooked.

Conclusions

Table V-12 (see page 320) provides a summary of information on the requirement for copper and its intake from various sources. Under present circumstances, copper deficiency in the typical U.S. diet is unlikely, but the total intake may be borderline in some sections of the population. Based on average food and water concentrations, most drinking water contributes a small proportion of the daily copper intake. Nevertheless, the extra copper contributed by water is a dietary safety factor which should be maintained if feasible. The potential for toxicity from the levels of copper in drinking water is extremely low.

Research Recommendations

The species of the copper in drinking water and their bioavailability should be determined.

ZINC

The importance of zinc to human nutrition has been recognized since 1962 when overt zinc deficiency was observed among rural inhabitants of the Middle East (Prasad *et al.,* 1963). There is also evidence of zinc deficiency among U.S. children who were not suspected of being nutritionally deprived (Hambidge and Walravens, 1976). These observations came as a surprise inasmuch as zinc is ubiquitous in food, water, and the general environment. This anomaly is explained in part by the low bioavailability of zinc in many foods, particularly plant seeds. The zinc ion (Zn^{2+}) forms strong chelates with many ligands, and it occurs commonly in nature in the form of complexes. The zinc in some of these complexes is readily absorbed, but in others, it is poorly absorbed (O'Dell, 1969). Unfortunately, the species and bioavailability of zinc in drinking water are unknown.

Presence in Food, Water, and Air

Zinc is widespread in commonly consumed foods, but tends to be higher in those of animal origin, particularly some seafoods (Table V-II). Furthermore, the zinc in foods of animal origin has a higher bioavailability than zinc in foods of plant origin. Unless foods are carefully selected, it is difficult to meet the 15 mg/day recommended dietary allowance with an intake of 2.0 kcal or less.

The zinc concentration in U.S. urban air is generally less than 1 μg/m^3. Assuming that 20 m^3 of air is inhaled per day (National Academy of Sciences, 1979), air contributes less than 20 μg of zinc to the daily intake of adults.

Of the 583 water samples analyzed by the U.S. Environmental Protection Agency (1975), 67% had detectable levels of zinc. The mean concentration in these 392 samples was 100 μg/liter, compared to 245 μg/liter observed in another study (Craun *et al.,* 1977; Greathouse *et al.,* 1978).

Requirements

The National Academy of Sciences Food and Nutrition Board (1974) has set the adult requirement for zinc at 15 mg/day. Although this may be higher than the actual requirement under many circumstances, it is designed to meet most needs under all conditions of bioavailability.

Toxicity Versus Essential Levels

The absorption of zinc is poorly understood, but appears to be well regulated. It is affected by such factors as the body's requirement for zinc as well as by the presence of chelating agents, e.g., phytates. Consequently, zinc has low toxicity when taken orally (Evans, 1976). As shown in Table V-12, there is a wide range of ratios (40-280) of toxic levels and requirements for zinc in the diets of monogastric animals. The greatest potential for zinc toxicity occurs when dietary copper is deficient or limited. Since copper deficiency is improbable in U.S. diets, zinc toxicity from food is highly unlikely.

The interim drinking water standard, 5 mg/liter (U.S. Environmental Protection Agency, 1977), is based on taste and appearance of the water rather than on toxicity. Considering the relatively low intake of zinc in food, this standard provides a wide margin of safety.

Interaction and Bioavailability

The bioavailability of zinc in foods varies widely. Phytate, a common constitutent of plant seeds, binds zinc strongly and decreases its absorption (O'Dell, 1969). Excess calcium in the presence of phytate accentuates the effect of the phytate. Dietary fiber also decreases the absorption of zinc along with that of other nutrients studied (Reinhold *et al.,* 1976).

Cadmium is closely related to zinc in its electronic configuration, and the two ions interact physiologically. Cadmium tends to increase the requirement for zinc or, stated in another way, zinc decreases the toxicity of cadmium (Underwood, 1977). Whereas calcium decreases the bioavailability of zinc in the presence of phytate, there is little evidence for a major direct calcium-zinc interaction. There is a copper-zinc interaction, which is described in the section on copper. Excess zinc exacerbates copper deficiency, but there is no evidence that excess copper aggravates zinc deficiency.

Contribution of Drinking Water to Zinc Nutrition

If one assumes a mean concentration of 200 µg/liter, drinking water would supply 0.4 mg, or approximately 3% of the daily requirement. Under most circumstances this is a negligible contribution to the total intake. However, in view of the potential for zinc deficiency in the U.S. population, even 0.4 mg should not be ignored. Some drinking water contains much higher concentrations of zinc than the mean value. The highest observed water concentrations might contribute up to 20% of the daily human requirement.

Conclusions

Zinc is an essential nutrient for humans. There is evidence of borderline deficiencies of the element in children in the United States as well as in other parts of the world (Hambidge and Walravens, 1976). Normally, drinking water contributes less than 5% of the dietary requirement. From that standpoint, it may be considered insignificant. However, in view of possible deficiency in U.S. diets, it is prudent to maintain all dietary sources of zinc, even those as small as 0.5 mg/day.

The possibility of detrimental health effects arising from zinc consumed in food and drinking water is extremely remote.

TABLE V-11 Iron, Copper, and Zinc in U.S. Diets [a]

Food Group	Mineral Contributed to Diet by Food Group								
	Content per kg of Food as Purchased [b]			Amount per Person per Day			Percentage of Total Food Intake [c]		
	Iron, mg	Copper, mg	Zinc, mg	Iron, mg	Copper, mg	Zinc, mg	Iron, %	Copper, %	Zinc, %
Milk, cheese, ice cream [d]	0.4	0.10	4.3	0.3	0.06	2.5	2	4	20
Meat, poultry, fish	20.1	0.49	18.6	5.9	0.14	5.5	31	10	43
Dried beans and peas, nuts	46.3	7.17	24.7	0.9	0.13	0.5	5	9	4
Eggs	20.5	0.89	12.7	1.0	0.04	0.6	5	3	5
Vegetables, dark-green and deep-yellow	9.5	0.88	2.8	0.3	0.03	0.1	2	2	1

	Mineral Contributed to Diet by Food Group								
	Content per kg of Food as Purchased[b]			Amount per Person per Day			Percentage of Total Food Intake[c]		
Citrus fruit, tomatoes	4.2	0.54	1.8	0.7	0.09	0.3	4	6	2
Potatoes	5.7	1.80	2.4	0.6	0.19	0.3	3	13	2
Other vegetables, fruit	5.7	0.70	2.0	2.0	0.25	0.7	10	17	6
Cereal, pasta	54.7	2.26	16.3	2.8	0.12	0.8	15	8	7
Flour, mixes	25.4	2.26	2.8	1.1	0.09	0.1	6	6	1
Bread	24.0	1.61	7.2	2.0	0.14	0.6	10	10	5
Other bakery products	11.0	1.26	6.0	0.8	0.09	0.4	4	6	3
Fats, oils	0.7	0.64	1.8	0	0.03	0.1	0	2	1
Sugar, sweets	7.9	0.88	0.6	0.6	0.06	0	3	4	0
TOTAL FOOD				19.0	1.46	12.5	100	100	100

[a] Unpublished data from the Consumer and Food Economics Institute, Science and Education Administration, U.S. Department of Agriculture, Hyattsville, MD 20782, January 1979. Food as purchased or brought into the kitchen from garden or farm except as noted. Mineral content of diet is estimated for the edible portion of food as purchased and does not allow for losses due to discarding of edible food during preparation and consumption in the home. Also, the mineral content of the diet and the contribution of food groups in any one person's diet may differ considerably from those shown, depending on kinds and amounts of foods consumed.

[b] Estimates for copper and zinc are based on a limited number of analyses.

[c] Excludes alcoholic beverages; nonalcoholic beverages, such as coffee, tea, cocoa, soft drinks, punches, and ades; seasonings; and leavening agents.

[d] Amounts are calculated for weights of dairy products containing the same amount of calcium as 1 kg whole fluid milk.

TABLE V-12 Contribution of Drinking Water to the Dietary Requirements for Iron, Copper, and Zinc

Ranges of Values Based on 70-kg Adult Human	Iron	Copper	Zinc
Requirement, mg/day[a]	♂ 10 : ♀ 18	2-3	15
Ratios of toxic intake levels to dietary requirement[b]	340-1,700	40-135	40-280
Likely concentrations in water, µg/liter[c]	240-245*	60-150*	100-245*
Daily intake based on likely concentrations in water, mg/2 liters	0.48-0.49	0.12-0.30	0.20-0.49
Likely water contributions to requirement, %	2.7-4.9	4-15	1.3-3.3
Highest concentrations observed in water, µg/liter[d]	1,560*-2,180	440*-450	1,320*-1,490
Daily intake based on highest observed concentrations in water, mg/2 liters	3.1-4.4	0.88-0.90	2.6-3.0
Water contribution to requirement based on highest observed concentration in water, %	17-44	30-45	17-20

[a] These are recommended dietary allowances (National Academy of Sciences, 1974) except the values for copper, which are estimated as adequate and safe intakes (National Academy of Sciences, 1980).

[b] Toxic levels for monogastric animals, which cause mild to severe signs of toxicity over the long term (Food and Drug Administration, 1975). Expressed as ratios of toxic levels to dietary requirements.

[c] The likely concentrations are based on analytical data from two sources: the U.S. Environmental Protection Agency (1975) report on interstate carrier water supplies and the study reported by Craun *et al.* (1977) and Greathouse *et al.* (1978). The asterisks indicate the values derived from the Craun and Greathouse study. In the U.S. Environmental Protection Agency study (1975), 672 samples from 10 regions were analyzed for iron. Analytical values were reported for 62.5% of the samples. The mean is based on these values. Of the 604 samples that were analyzed for copper, 55% contained sufficient copper to report and were averaged. Of the 583 samples analyzed for zinc, 67% were averaged.

[d] The maximum values reported by Craun *et al.* (1977) and Greathouse *et al.* (1978) are marked with an asterisk. The other values are the means of the maximum values found in the 10 regions sampled by the U.S. Environmental Protection Agency.

Research Recommendation

The chemical form and bioavailability of zinc in water should be determined in order to evaluate the contribution of water to the requirement for zinc.

SELENIUM

Presence in Food, Water, and Air

The amount of selenium in different foods is highly variable, depending upon where the plants were grown or where the animals were raised. There are great differences among the selenium contents of soils and, thus, the amount that is available for uptake by plants. This selenium is passed directly up the food chain through the plants to animals and humans. There are areas of the United States and other parts of the world where the selenium content of soil is either so low or so high that naturally occurring deficiencies or toxicities of selenium can occur in animals.

The estimated human daily intake of selenium from dietary sources tends to reflect the amount of selenium that is available in the soils (Table V-13). For example, in New Zealand, where the selenium contents of soils are low, the daily dietary intake of selenium is only 56 µg/day, whereas in Venezuela, where soils contain high selenium concentrations, the intake is 326 µg/day. Daily dietary intakes of selenium by humans range from 24 µg in certain diets in New Zealand (Stewart *et al.,* 1978) to 7.000 µg in highly seleniferous diets in South Dakota (Smith and Westfall, 1937).

Less than 0.5% of the samples taken from various public water supply systems in the United States exceeded the U.S. Environmental Protection Agency (1975) limit for selenium, 0.01 mg/liter (Lakin and Davidson, 1967; McCabe *et al.,* 1970; Taylor, 1963). Although there are exceptions, especially in areas where there are high levels of selenium in the soil, the National Academy of Sciences Committee on Medical and Biologic Effects of Environmental Pollutants (MBEEP) concluded that "waters rarely contain selenium at levels above a few micrograms per liter. Hence, they can rarely be considered a significant source of the element from either a nutritional or a toxicity standpoint" (National Academy of Sciences, 1976). This statement is confirmed by other analytical data from the United States as well as from Europe (Table V-14).

TABLE V-13 Estimated Daily Intake of Selenium by Humans from Dietary Sources

	Estimated Intake by Country, µg/day										
Food	New Zealand[a]	Japan[b]	Toronto, Canada[c] (1)	Maryland, USA[a]	Toronto, Canada[c] (2)	Canada[a]	"Market-Basket Survey," USA[d]	Winnipeg, Canada[a]	South Dakota, USA[e]	Halifax, Canada[c]	Caracas, Venezeula[f]
Vegetables, fruits, sugars	6	7	5	5	1	7	2	9	10	7	15
Cereals	4	24	62	45	112	74	103	80	57	105	88
Dairy products	8	2	7	14	5	23	5	28	48	22	70
Meat, fish	38	56	25	69	30	46	59	64	101	90	153
TOTAL	56	88	98	132	149	151	169	181	216	224	326

[a] Data from Watkinson, 1974. Intake estimates based on analyses of separate foods.

[b] Data from Sakurai and Tsuchiya, 1975. Intake estimates based on analyses of separate foods.

[c] Data from Thompson *et al.,* 1975. Intake estimates based on food composite data.

[d] Data from J.H. McLoughlin, personal communication. Estimated intake by adults consuming 3,900 cal/day based on food composite from fiscal year 1974 Total Diet Survey of the U.S. Food and Drug Administration ("Market-Basket Survey").

[e] Data from Olson *et al.,* 1978. Intake estimates based on analyses of separate foods.

[f] Data from Mondragon and Jaffe, 1976. Intake estimates based on analyses of separate foods, but assuming a food consumption pattern more typical of North America.

TABLE V-14 Concentration of Selenium in Drinking Water

Mean Selenium Concentration, and Range, μg/liter	Description of Sample	Comment	Reference
0.6 (range, 0.2-2.4)	Public water supplies from five European cities	Four of five samples contained < 1 μg/liter	Zoeteman and Brinkmann, 1977
0.36 (range, 0.08-0.9)	Tap water from three Swedish cities		Boström and Wester, 1967
3.5 (range, 1.0-6.5)	4,200 tap water samples from throughout the United States	Represents less than 10% of all samples; more than 90% were below the detection limit of the analytical method used (1 μg/liter)	Greathouse and Craun, 1978

Even if it is assumed that a person drinks 2 liters of water per day containing the EPA limit, the average daily intake of selenium via water would be only 20 μg. In most countries, this is still a small fraction of the selenium that is consumed in foods.

Zoller and Reamer (1976) reported that the atmospheric concentrations of selenium in most urban regions range from 0.1 to 10 ng/m^3. Hashimoto and Winchester (1967) and Pillay *et al.* (1971) measured concentrations of selenium in air at 0.3 to 1.6 ng/m^3 and 3.6 to 9.7 ng/m^3, respectively. The National Academy of Sciences MBEEP Committee concluded that the amount of airborne selenium is very small, probably well below 10 ng/m^3 (National Academy of Sciences, 1976). Assuming that a typical person ventilates 20 m^3 of air daily and that ambient air concentrations of selenium are not likely to exceed 10 ng/m^3, the maximum daily intake via this route would be only 0.2 μg, which is much less than that derived from foods. Therefore, it can be concluded that air contributes insignificantly to the average daily intake of selenium by the general population.

TABLE V-15 Selenium Content of Certain Human Tissues[a]

Tissue	Number of Samples	Mean Selenium Content and Range, mg/kg wet tissue
Kidney	6	1.09 (0.61-1.84)
Liver	6	0.54 (0.28-0.81)
Spleen	4	0.34 (0.28-0.47)
Pancreas	3	0.30 (0.27-0.34)
Testes	3	0.30 (0.15-0.38)
Heart	5	0.28 (0.25-0.37)
Muscle	5	0.24 (0.11-0.38)
Small intestine	3	0.21 (0.12-0.32)
Lung	6	0.16 (0.06-0.26)
Brain	2	0.13 (0.04-0.21)

[a] Adapted from Schroeder *et al.,* 1970.

Distribution in Tissues

Schroeder *et al.* (1970) analyzed from two to six samples each of various human tissues obtained at autopsy. They found that the kidney and liver contained the highest concentrations of selenium (Table V-15). Other tissues containing selenium, in order of decreasing concentrations, were: spleen, pancreas, testes, heart, muscle, and small intestine. The lungs and brain contained the lowest concentrations of selenium.

They calculated the total body content of selenium in subjects from the United States to be 14.6 mg (range, 13.0-20.3 mg) for 91.7% of the body. Stewart *et al.* (1978) estimated the total body selenium content in subjects from New Zealand to be either 6.1 mg (range, 4-10 mg) or 3.0 mg (range, 2-5 mg), depending on whether selenomethionine [$CH_3Se(CH_2)_2CH(NH_2)COOH$] or selenite ($SeO_3^{2-}$) was used in the estimation.

Requirements

The critical level of dietary selenium below which deficiency symptoms are observed is generally considered to be approximately 0.02 mg/kg of feed for ruminants and 0.03 to 0.05 mg/kg for poultry (National Academy of Sciences, 1971). A nutritionally generous level of dietary selenium is 0.1 mg/kg for livestock and 0.2 mg/kg for poultry. For a 70-kg human consuming 1 kg of diet per day (dry basis), this would

translate into a daily intake of 100 to 200 µg. An estimated adequate and safe intake of selenium for adult humans has been suggested to be between 50 and 200 µg with correspondingly lower intakes for children and infants (National Academy of Sciences, 1980; also see Table V-31 in the summary of this chapter). Any daily intake within the recommended ranges shown in the table is considered adequate and safe, but the recommendations do not imply that intakes at the upper limit of the range are more desirable or beneficial than those at the lower limit. Stewart *et al.* (1978) have recently indicated that the minimum dietary requirement of selenium for the maintenance of normal human health is probably not more than 20 µg/day.

Toxicity Versus Essential Levels

Several studies indicate that diets containing 5 mg/kg of selenium or more cause chronic toxicity in laboratory animals (see review by Moxon and Rhian, 1943). In seleniferous areas, 5 mg/kg of diet is generally accepted as the dividing line between toxic and nontoxic feeds (National Academy of Sciences, 1976). If 5 mg/kg is accepted as the dietary toxic level of selenium and 0.05 mg/kg is considered to be the dietary critical minimum for protecting against selenium deficiency, then the ratio between the toxic and beneficial doses is 100, a value not too different for many other minerals and nutrients.

On the other hand, some investigators have claimed to find toxic effects of selenium in rats fed diets containing less than 5 mg/kg under certain conditions (Table V-16). For example, Witting and Horwitt (1964) showed that 1 mg/kg of dietary selenium depressed growth in vitamin-E-deficient rats. Harr *et al.* (1967) reported that the addition of 0.5 mg/kg of selenium to a semipurified diet caused proliferation of the hepatic parenchyma. But these liver changes were observed to an even greater extent in rats fed a commercial diet not supplemented with selenium. Therefore, the relationship between the liver lesions and selenium intake is not clear.

Pletnikova (1970) administered selenium to rats in drinking water in doses that were roughly equivalent to a dietary selenium intake of 0.06 mg/kg. Such doses appeared to cause decreased blood glutathione levels, impaired liver function, depressed hepatic succinic dehydrogenase activity, and certain modifications in behavior. Since this level of selenium intake is similar to that needed to prevent selenium deficiency in animals, the toxicological significance of these observations is not clear unless a specific level of selenium in drinking water is more deleterious than an equivalent dose in the diet. However, there have been

few studies in which the toxicity of selenium given in these two ways has been compared directly.

TABLE V-16 Toxicity of Selenium in Rats

Experimental Conditions	Criteria of Toxicity	Dose of Dietary Selenium Causing Effect, mg/kg	Reference
Normal diet	Growth inhibition	4 to 5	National Academy of Sciences, 1976
Vitamin-Edeficient diet	Growth inhibition	1	Witting and Horwitt, 1964
Semipurified diet	Liver lesions	0.5	Harr *et al.,* 1967
Selenium given in drinking water	Blood GSH levels, liver function, enzyme activity, behavioral changes	0.06[a]	Pletnikova, 1970

[a] Calculated equivalent dose on dietary basis.

Obviously, if any of the effects discussed above prove to be actual toxic effects of selenium, the ratio of the toxic to beneficial dose of selenium would be decreased accordingly. However, the National Academy of Sciences Committee on Medical and Biologic Effects of Environmental Pollutants (MBEEP) concluded that the best indicator of chronic selenium poisoning is growth inhibition (National Academy of Sciences, 1976) and that 4 to 5 mg/kg dietary selenium is needed to demonstrate this effect in rats fed a normal diet. Clearly, more sensitive and specific criteria of selenium poisoning would be useful, and the development of such tests should be encouraged strongly.

Similarly, studies in people have been hampered by the lack of specific criteria for selenosis in humans. Smith *et al.* (1936) surveyed a rural population living in areas of the United States known to have a history of selenium poisoning in animals. They observed no symptoms that could be definitely related to selenium poisoning in humans and could link no serious illness to selenium poisoning. There were vague symptoms of ill health and symptoms suggestive of damage to the liver, kidneys, skin, and joints, but a causative role for selenium in these disorders could not be established. In a second survey, Smith and Westfall (1937) investigated the relationship between the incidence of these symptoms and the amount of selenium excreted in the urine.

Although they considered none of the symptoms to be specific for selenium poisoning, increased incidence of gastrointestinal disturbances, icteroid discoloration of the skin, and bad teeth seemed to be associated with elevated urinary selenium levels.

Lemley (1940) examined a South Dakota rancher whom he considered to be the first described case of chronic selenium dermatitis in a human caused by the ingestion of selenium from natural sources. However, the patient's urinary excretion of selenium was normal, i.e., <0.100 mg/liter (Glover, 1967). Moreover, administration of bromobenzene (C_6H_5Br), a compound known to increase urinary selenium output (Moxon *et al.,* 1940), caused only a mild elevation in urinary selenium levels.

Lemley and Merryman (1941) described a South Dakota family whose members excreted 0.200 to 0.600 mg/liter of selenium in the urine. One of these patients excreted 1.800 mg/liter 24 hr after a course of bromobenzene. Dermatitis was not cited as one of the symptoms in this family. Rather, these cases were afflicted with various psychological disturbances such as clouding of the sensorium, extreme lassitude accompanied by depression, and moderate emotional instability. All members of the family also suffered from slight, continual dizziness, and they complained that their powers of concentration were markedly impaired.

Lemley and Merryman justified their diagnosis of selenium poisoning in these subjects on the basis of the following evidence: knowledge that the subjects lived in a seleniferous area; presence of concentrations of selenium in the urine above 0.100 mg/liter: increased urinary elimination of selenium after administration of bromobenzene; and improvement of the subject's symptoms after elimination of selenium from the diet.

Jaffe (1976) found that the overall hemoglobin and hematocrit values of children living in a seleniferous area of Venezuela (Villa Bruzual) were lower than those of children living in Caracas, but there was no correlation between blood and urine selenium levels and hemoglobin or hematocrit values. Differences in hemoglobin were attributed to differences in nutritional or parasitological status and not to differences in selenium intake. Jaffe *et al.* (1972) showed that selenium poisoning decreased activities of prothrombin and serum alkaline phosphatase and transaminases in rats, but these activities were normal in all children studied. Dermatitis, hair loss, and abnormal nails were more frequent among the children in the seleniferous area than in those living in Caracas, but the cause of these clinical signs could not be determined definitively because of the lack of differences among the various biochemical tests performed.

The dose of selenium needed to cause chronic toxicity in humans is

poorly defined. Smith and Westfall (1937) calculated that most of their subjects, who lived in highly seleniferous areas of the United States, were probably absorbing approximately 10 to 100 µg/kg body weight/day and that some of their subjects may have absorbed as much as 200 µg/kg/day. For a 70-kg human, these rates of absorption would be equivalent to an intake of 0.70 to 14.0 mg/day, assuming that all of the selenium ingested was absorbed.

As discussed above, drinking water generally contributes little to the total daily selenium intake, but well water containing 9.0 mg/liter caused hair loss, weakened nails, and listlessness in a family of Indians living in Colorado (Anonymous, 1962). Assuming a daily water consumption of 2 liters, this would represent a selenium intake of 18.0 mg/day. On the other hand, consumption of water containing 0.050 to 0.125 mg/liter caused no increase in the incidence or prevalence of any of 85 health parameters measured in a population residing in a rural Colorado community (Tsongas and Ferguson, 1977).

Deliberate administration of selenium as selenite in oral doses of 50 µg/kg body weight/day for more than 1 year to patients with neuronal ceroid lipofuscinosis (NCL) caused no toxic manifestations (Westermarck, 1977). Rather, some of the NCL patients improved temporarily. A few of them exhibited a somewhat increased serum aspartate aminotransferase activity, but apparently this is not unusual in patients with NCL.

Schrauzer and White (1978) reported that consumption of 450 µg of selenium daily for 18 months in the form of a commercially available nutritional supplement along with 150 µ/g of selenium per day in the ordinary diet (total daily intake of 600 µg) produced no toxic effects in a well-fed individual although serum glutamic-oxaloacetic transaminase (SGOT) activities were somewhat elevated. Sakurai and Tsuchiya (1975) suggested a tentative maximum acceptable daily intake of 500 µg of selenium for the protection of human health. They obtained this value by multiplying a "low" mean normal daily selenium intake by humans (50 µg) by 10, a factor that appeared acceptable as a margin of safety within which the average human could tolerate selenium.

Interactions

In test animals, selenium has been shown to interact profoundly with several other elements. For example, selenium protects against the toxicity of several heavy metals including inorganic mercury (Parizek *et al.,* 1971), methyl mercury (Ganther and Sunde, 1974), cadmium (Parizek *et al.,* 1971), silver (Wagner *et al.,* 1975), and thallium (Rusiecki and

Brzezinski, 1966). Good correlations have been observed between the concentrations of mercury and selenium in the tissues of humans who have been exposed industrially to mercury (Kosta *et al.,* 1975). The molecular mechanisms of the protective effects of selenium are not well understood. It is known that mercury may increase the nutritional requirement of animals for selenium (Froseth *et al.,* 1974), while the toxicity of certain methylated selenium metabolites in rats is strongly potentiated by inorganic mercury (Parizek *et al.,* 1971). Thus, mercury exposure is yet another situation in which the ratio of toxic to beneficial dose of selenium may be decreased.

Levander and Baumann (1966) reported that arsenic has a remarkable protective action against the toxicity of selenium in rats, apparently because it increases biliary excretion. However, arsenic has a strong synergistic toxicity with trimethylselenonium ion, one of the urinary excretion products of selenium (Obermeyer *et al.,* 1971).

In rats, dietary sulfate (SO_4^{2-}) has some protective activity against the toxicity of selenium ingested as selenate (SeO_4^{2-}), presumably by increasing urinary excretion (Ganther and Baumann, 1962).

Selenium has a strong inverse nutritional relationship with vitamin E in that the amount of selenium required to prevent deficiency diseases in chicks is increased as the dietary level of vitamin E decreases (Thompson and Scott, 1969). As shown in Table V-16, selenium seems to exert a toxic effect at lower levels when animals are deficient in vitamin E. Therefore, vitamin E deficiency is another situation in which the ratio between the toxic and beneficial doses of selenium is decreased.

High levels of dietary protein afford some protection against selenium toxicity (Gortner, 1940). Linseed meal increases selenium residues in tissues but protects against selenosis (Halverson *et al.,* 1955; Levander *et al.,* 1970).

Bioavailability

Soluble inorganic salts of selenium are readily absorbed by animals, apparently without homeostatic control. Brown *et al.* (1972) reported that rats absorbed 95% or more of oral doses of selenite regardless of whether they were fed a selenium-deficient diet or a diet containing mildly toxic levels of selenium. Homeostatic control of selenium absorption in humans also appears to be lacking. Thomson (1974) observed that approximately 93% of milligram doses of sodium selenite (Na_2SeO_3) in solution was absorbed. However, only 60% of such doses was absorbed when the sodium selenite was given in the solid form

rather than in solution. This observation was puzzling in view of the high solubility of sodium selenite, and no explanation was offered.

The bioavailability of selenium in feeds was investigated by Cantor *et al.* (1975) who studied the ability of selenium in various poultry feedstuffs to prevent exudative diathesis, a selenium deficiency disease in chickens. In general, the selenium in plant products was more readily available than that in animal products. However, since the animal products consisted of highly processed fish and poultry meals, the availability of selenium in them may not resemble that in animal products consumed by humans.

Stewart *et al.* (1978) reported that intestinal absorption of selenium was approximately 79% in New Zealanders who consumed foods providing 24 µg/day.

Contribution of Drinking Water to Selenium Nutrition

The data in Table V-13 indicate that most diets in North America provide approximately 0.150 mg of selenium per day. At this level of dietary intake, selenium in drinking water would contribute 0.1%, 1.3%, or 11.8% of the total selenium intake, depending on the concentration of selenium in the drinking water (Table V-17). Thus, even when the selenium concentration in water is at the EPA limit of 0.01 mg/liter, water would contribute only about 12% of the total selenium intake under conditions found in Canada and the United States. The relative contribution of drinking water to total selenium intake would be even lower in countries where foods contain high levels of selenium because of the high selenium concentrations in the soil, e.g., in Venezuela and certain areas in the United States. Drinking water might prove to be a valuable source of selenium in countries such as New Zealand where the soil concentrations and dietary intakes of selenium are quite low.

Research Recommendations

More sensitive and specific indicators of selenium toxicity are needed.

The physiological significance of biochemical changes caused by the ingestion of relatively low levels of selenium in the drinking water should be evaluated.

TABLE V-17 Relative Contribution of Drinking Water to Total Selenium Intake at Different Concentrations of Selenium in Water and Different Dietary Intakes of Selenium[a]

Dietary Intake of Selenium, µg/day	Contribution of Water to Total Selenium Intake by Selenium Content of Water, %		
	0.1 µg/liter	1.0 µg/liter	10 µg/liter
Low selenium diet, e.g., New Zealand 24	0.8	7.7	45.5
Moderate selenium diet, e.g., North America 150	0.1	1.3	11.8
High selenium diet, e.g., Venezuela 326	0.06	0.61	5.8

[a] Assumes water consumption of 2 liters/day.

MANGANESE

Presence in Food, Water, and Air

Reviews of earlier data indicate that the average human dietary intake of manganese ranges from 2.0 to 8.8 mg/day (National Academy of Sciences, 1974; Underwood, 1977; World Health Organization, 1973). More recently, Wolf (1979) reported that the manganese intake of 22 subjects consuming self-selected diets (food *and* beverages) in the United States ranged between 1.1 and 6.4 mg/day (Figure V-1) and averaged 2.8 mg/day. Guthrie and Robinson (1977) reported that the mean dietary manganese intake of 23 New Zealand women was 2.7 mg/day (range, 0.8-7.1 mg/day).

The Total Diet Study ("market-basket" survey) conducted by the Food and Drug Administration (FDA) during fiscal year 1976-1977 indicated that typical American male teenagers and adults consuming a 3.900-cal diet ingested 3.8 and 3.7 mg of manganese per day, respectively (Food and Drug Administration, 1978). In this survey, grains and cereal products were the richest dietary sources of manganese, contributing 57% to total manganese intake. Fruits and vegetables provided another 22%. Dairy products, meat, fish, and poultry made up only 4% of the total dietary manganese. Beverages, including drinking water, contrib

uted 11% of the manganese intake, whereas oils, fats, shortening, sugar, and adjuncts contributed 6%.

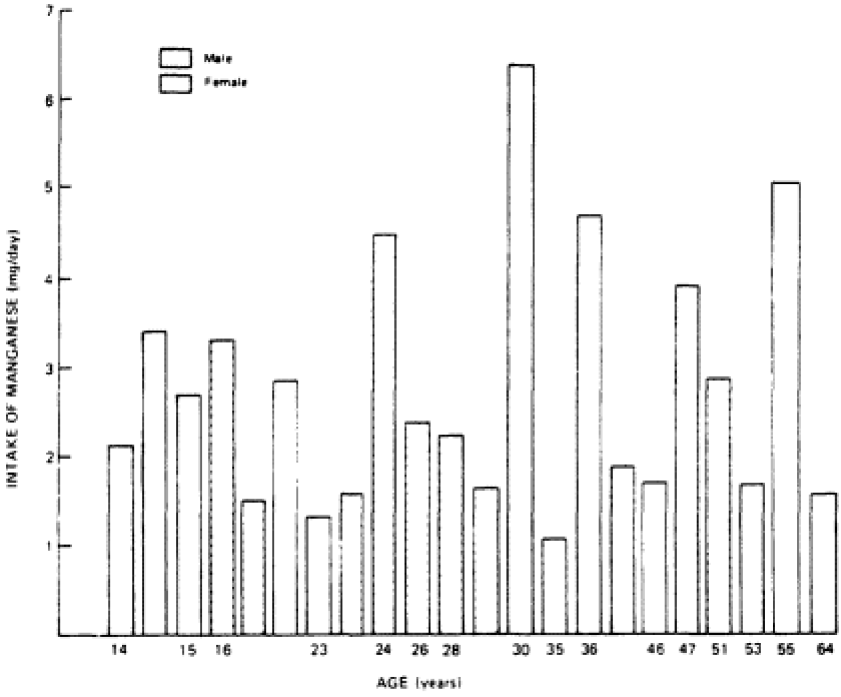

Figure V-l
Average daily intake of manganese per subject per day as determined by analysis of self-selected diets. Data from Wolf, 1979.

The mean concentrations of manganese observed in various surveys of drinking water samples collected in different parts of Europe and the United States are remarkably similar (Table V-18). Although occasional high values were reported, 92% to 95% of the samples analyzed was below the drinking water standard of 0.05 mg/liter (U.S. Public Health Service, 1962).

The concentration of manganese in ambient air is generally quite low. In 1968, the maximum average 24-hr manganese concentrations in three large cities in the United States were 0.09, 0.07, and 0.26 μg/m^3 for Washington, Los Angeles, and Chicago, respectively (U.S. Environmental Protection Agency, 1975). Assuming that the general population is exposed to atmospheric concentrations of manganese of 0.1 μg/m^3 and

TABLE V-18 Concentration of Manganese in Drinking Water

Mean Manganese Concentrations, mg/liter	Description of Sample	Comment	Reference
0.024 (range, <0.010-0.160)	Public water supplies from 19 European cities	18 samples contained <0.05 mg/liter; one sample (from Odense, Denmark) contained 0.160 mg/liter	Zoeteman and Brinkmann, 1977
0.022	969 public water systems located in nine geographic areas of the United States	8% of samples contained > 0.05 mg/liter; one sample contained 1.32 mg/liter	Craun and McCabe, 1975; McCabe *et al.,* 1970
0.032 ± 0.013 (SE)	161 water supplies located in 44 states of the United States		Hadjimarkos, 1967
0.02 (range, <0.010-0.32)	902 samples of drinking water from the Boston, Massachusetts area	5% of the samples contained >0.050 mg/liter	Karalekas *et al.,* 1976
0.026 (range, 0.0005-0.450)	380 drinking water samples obtained throughout the continental United States		Kopp, 1969
0.031 (range, 0.0026-0.152)	4,200 tap water samples obtained throughout the United States		Greathouse and Craun, 1979
0.017	Cold tap water samples from 50 households located in four municipalities in the Eastern United States		Strain *et al.,* 1975

that a person ventilates 20 m^3 of air daily, only 2 µg would be inhaled per day. This is less than 0.1% of the typical dietary intake of manganese.

Much higher concentrations of airborne manganese have been measured in cities with major manganese-emitting industries. For example, levels of 0.67, 1.10, and 14.0 µg/m^3 were reported in East Chicago, Pittsburgh, and Johnstown, respectively (U.S. Environmental Protection Agency, 1975). In the Johnstown case, atmospheric manganese could contribute 280 µg to the daily intake, approximately 10% of that provided by the average American diet.

Distribution in Tissues

The concentration of manganese in various human tissues is shown in Table V-19. Liver, pancreas, and kidney had the highest concentrations, and muscle, the lowest. Schroeder *et al.* (1966) estimated the total body pool of manganese in humans to be 20 mg.

Requirements

The National Academy of Sciences Committee on Animal Nutrition recommended a daily dietary intake of 50 mg of manganese per kilogram of diet to promote normal growth and gestation in rats (National Academy of Sciences, 1972). For poultry, the minimum dietary manganese requirement for normal growth, egg production, and hatchability is approximately 40 mg/kg of diet under normal dietary conditions; however. Underwood (1977) recommended a total intake of 50 mg/kg of diet to provide a margin of safety and to cope with variations in calcium and phosphorus intakes (see section on Interactions). A dietary manganese content of 50 mg/kg is sufficient to prevent perosis in chicks fed a diet based on ground corn and dried skim milk (Gallup and Norris, 1939).

Since no manganese deficiency has been recognized in humans, the usual manganese intake of 2 to 3 mg/day appears to be adequate for adults (World Health Organization. 1973). The intake of manganese estimated by the Food and Nutrition Board of the National Research Council as adequate and safe is 2.5 to 5.0 mg for adults. The Board estimated correspondingly lower values for children and infants (National Academy of Sciences, 1980: also see Table V-31 in the summary of this chapter). Figure V-1 indicates that a significant proportion of Americans are not consuming sufficient manganese in their food and beverages to meet the desirable level.

TABLE V-19 Manganese Content of Certain Human Tissues[a]

Tissue	Manganese Concentration, mg/kg wet tissue
Liver	1.69
Pancreas	1.21
Kidney	0.97
Brain	0.34
Lung	0.26
Prostate	0.23
Heart	0.25
Spleen	0.15
Adrenals	0.18
Ovaries	0.18
Testes	0.15
Aorta	0.19
Muscle	0.07

[a] From Tipton and Cook, 1963.

Toxicity Versus Essential Levels

Dietary manganese levels as high as 1,000 mg/kg have no adverse effect on chicks (Gallup and Norris, 1939), but 4,800 mg/kg causes severe mortality in this species (Heller and Penquite, 1937). Levels of 500 mg/kg cause growth retardation and appetite depression in growing pigs (Grummer *et al.,* 1950). and only 45 or 125 mg/kg is needed to impair hemoglobin repletion in anemic lambs and pigs, respectively (Hartman *et al.,* 1955; Matrone *et al.,* 1959).

Neurobehavioral changes are probably the most subtle and sensitive effects of manganese toxicty. The neurological effects in nonhuman primates are similar to those in humans suffering from manganism. However, the National Academy of Sciences Committee on Medical and Biologic Effects of Environmental Pollutants (MBEEP) (National Academy of Sciences, 1973) concluded that "experimentally induced behavioral changes after manganese intoxication have scarcely been reported and dose-response relations are unknown. . . . " Also, "experimental studies in animals, which appear to hold the key to full understanding of the pathophysiology of manganism, are extremely few and limited." Obviously, more work along this line of investigation is needed.

Underwood (1977) stated that "manganese toxicity in man arising from excessive intakes in foods and beverages has never been reported and is difficult to visualize ever arising, except where industrial contamination occurs." Kawamura *et al.* (1941) reported an epidemic of manganese intoxication in Japan resulting from contaminated well water. They reported neurological symptoms and the deaths of two patients whose organs contained large quantities of manganese (and zinc, since the well water was also contaminated by this metal). Neither the total doses of manganese consumed nor the period of consumption were known, but samples of the well water contained approximately 14 mg of manganese tetroxide (Mn_3O_4) per liter. This would represent a dose of approximately 20 mg of manganese per day. The World Health Organization (1973) found no evidence of manganese toxicity in individuals consuming 8 to 9 mg manganese/day in their food and has assumed that such levels are safe.

Underwood (1977) pointed out that "manganese is among the least toxic of the trace elements to mammals and birds," but the ratio of the highly toxic dose (4,800 mg/kg) to the minimum dietary requirement for the growth of chicks (40 mg/kg) is only 120—not so different from that for selenium.

Interactions

The ability of manganese to interfere with the repletion of hemoglobin in anemic animals has been discussed above. Hartman *et al.* (1955) suggested that the mechanism causing this effect may be a reduction by manganese of iron absorption. Others have shown that manganese inhibits the uptake of iron by perfused duodenal loops from iron-deficient rats (Thomson and Valberg, 1972). Increased manganese absorption is observed in iron-deficient animals (Diez-Ewald *et al.,* 1968; Pollack *et al.,* 1965) and humans (Mena *et al.,* 1969). Thus, while high levels of manganese may cause anemia by interfering with iron absorption, iron deficiency may increase an individual's susceptibility to manganese poisoning (Mena *et al.,* 1969).

Excess dietary calcium phosphate [$Ca_3(PO_4)_2$] increases the manganese requirement in chicks by reducing its bioavailability (Schaible and Bandemer, 1942).

Bioavailability

The gastrointestinal absorption of manganese is quite low, only 3% in healthy humans (Mena *et al.,* 1969) and from 3% to 4% in rats

(Greenberg *et al.,* 1943). As discussed above, manganese absorption is increased by iron deficiency but decreased by excess dietary iron or calcium phosphate. Although early work suggested that the bioavailability of several inorganic salts of manganese was more or less equal for chickens (Gallup and Norris, 1939), more recent research, using chick leg abnormality scores, has demonstrated differences (Watson *et al.,* 1971). Coupain *et al.* (1977) showed differences in the ability of various manganese chelates to promote normal reproduction rates and neonatal viability in rats.

Commenting on infant foods, Underwood (1977) said that "nothing is known of the form or availability of the manganese present in these foods, nor of how much is absorbed and retained." This is also true for the manganese in the foods and beverages of adult humans.

Contribution of Drinking Water to Manganese Nutrition

Assuming a daily water intake of 2 liters, typical drinking water sources should contribute anywhere from 0.040 to 0.064 mg to the total daily intake of manganese. The higher amount would be less than 3% of that derived from the usual dietary sources. Some isolated water samples contained manganese levels as high as 1.32 mg/liter which would contribute about as much as food to the total manganese intake.

Research Recommendations

More research should be conducted to establish dose-response relationships in experimentally induced behavioral changes and other toxic effects after manganese intoxication.

Further studies are needed to test the possible role of iron deficiency in potentiating manganese poisoning.

ARSENIC

Presence in Food, Water, and Air

For the past several years the U.S. Food and Drug Administration (FDA) has monitored arsenic and several heavy metals in its Total Diet Study ("market-basket" survey) program (Jelenik and Corneliussen, 1977). Each market-basket food composite represents the typical 2-week diet of a 15- to 20-year-old male in any one of four geographical regions

of the United States.: South, Northeast, North Central, and West. The results of this survey for arsenic during the fiscal years 1967 through 1974 are shown in Table V-20.

During 1971-1974, the average daily intake of arsenic was approximately 11.4 µg. About 82% of that amount was consumed in meat, fish, and poultry. No arsenic was detected in the beverage composite (including water) during these years. The difference between the values for 1967-1970 and those for 1971-1974 does not represent an actual drop in the dietary arsenic intake. Rather, it reflects an analytical artifact due to the development of a more sensitive and reliable method of arsenic analysis (Horwitz, personal communication). Estimates of the human dietary arsenic intake for earlier years were much higher (0.4-4.2 mg/day: Schroeder and Balassa, 1966; World Health Organization, 1973) than the values reported by the FDA for 1971-1974. This suggested either that similar analytical problems were involved or that there has been a drastic reduction in arsenic intake from dietary sources during recent years.

The lack of arsenic in the typical drinking water sampled by the FDA market-basket surveys has also been verified by surveys directed specifically at drinking water. McCabe *et al.* (1970) reported that only 0.4% of samples taken from various public water supplies in the United States exceeded the recommended limit for arsenic of 0.01 mg/liter established by the U.S. Public Health Service (1962). Most other surveys have shown the average concentration of arsenic in drinking water to be only a few micrograms per liter (Table V-21). One exception involved some deep wells in Indiana that produced water containing concentrations of arsenic up to 2.0 mg/liter; but by careful blending of this water with a source with a lower arsenic content, it was possible for the supply management to bring the arsenic content of the total water supply within the mandatory (as opposed to recommended) limit of 0.05 mg/liter (U.S. Public Health Service, 1962). Elevated levels of arsenic have also been found in the drinking water of Lane County, Oregon (Whanger *et al.,* 1977), Antofagasta, Chile, (Borgono *et al.,* 1977), the southwest coast of Taiwan (Tseng, 1977) and in certain European bottled mineral waters (Zoeteman and Brinkmann, 1977). Although 5 of the 13 types of bottled water analyzed contained less than 1 µg/liter, the arsenic content of the other eight averaged 34 µg/liter (range. 2-190 µg/liter).

The concentration of arsenic in air is generally quite low. In nonurban areas, the maximal average concentration was 20 ng/m^3, but most values were less than 10 ng/m^3 (National Academy of Sciences, 1977a). In New York City the average concentration of airborne arsenic was approximately 30 ng/m^3. Assuming that 20 m^3 of air is ventilated by humans

daily, atmospheric arsenic in urban areas would contribute about 0.6 µg to the daily intake or about 5.3% of that derived from dietary sources. Of course, in certain regions, the concentration of arsenic in air may be much higher because of industrial or agricultural activities. For example, values as high as 2.5 mg/m^3 have been reported in the vicinity of a smelter treating arsenical ores, and up to 141.0 mg/m^3 was detected downwind from a west Texas cotton gin.

Distribution in Tissues

The concentrations of arsenic in certain human tissues are listed in Table V-22. The liver had the highest concentration, and the heart, the lowest. The total normal human body content of arsenic, which apparently tends to increase with age, has been given as 3 to 4 mg (National Academy of Sciences. 1977a).

Requirements

An increasing amount of evidence suggests that trace amounts of arsenic may play a beneficial nutritional role in animals. For example, Nielsen *et al.* (1975) reported that rats fed diets containing only 0.030 µg/g of arsenic had a roughness of their hair coat, low growth rate, anemia, splenomegaly, and increased erythrocyte osmotic fragility when compared to control rats that were supplemented with 4.5 µg/g arsenic. More recent work by Nielsen and Shuler (1978a.b) has indicated that arsenic contributes to the maintenance of water and mineral balance in the chick and that the dietary requirement of the chick for arsenic is approximately 0.045 µg/g. Also, Anke and coworkers (1976, 1978) described the consequences of apparent arsenic deficiency in goats and minipigs fed semisynthetic rations containing less than 0.050 µg/g. The low-arsenic diet impaired reproduction and, in animals fed the diet for two generations, decreased weight gains. Schwarz (1977) reported that 0.5 to 2 µg/g of arsenic as sodium arsenite ($NaAsO_2$) stimulated the growth of rats fed diets containing less than 0.050 µg/g.

Toxicity Versus Essential Levels

Experiments with laboratory animals have shown that several factors can influence the toxicity of arsenic (for a review, see National Academy of Sciences, 1977a). One of the most important of these factors is the chemical form of the arsenic itself. In general, soluble trivalent arsenic compounds are more toxic than pentavalent ones, and inorganic

TABLE V-20 Average Human Daily Intake of Arsenic from Dietary Sources[a]

	Average Intake by Fiscal Year of Sample Collection, µg/day							
Food Class	1967	1968	1969	1970	1971	1972	1973	1974
Vegetables, fruits, sugars, oils, fats	9.1	23.6	7.6	2.3	1.5	ND[b]	ND	3.0
Grain and cereal products	3.0	22.1	8.4	ND	ND	ND	0.8	ND
Dairy products	9.9	6.1	3.8	4.6	ND	ND	1.5	ND
Meat, fish, and poultry	22.0	34.2	25.8	36.5	11.4	9.1	4.7	12.2
Beverages, including water	7.6	18.2	11.4	ND	ND	ND	ND	ND
TOTAL	51.6	104.2	57.0	43.4	12.9	9.1	7.6[c]	16.0[c]

[a] Recalculated from Jelenik and Comeliussen, 1977.

[b] ND, not detected.

[c] Total arsenic value is somewhat higher than sum of food class composites because traces of arsenic in certain vegetables and fruits were not given numerical values.

TABLE V-21 Concentration of Arsenic in Drinking Water

Mean Arsenic Concentration and Range, µg/liter	Description of Sample	Comment	Reference
0.9 (range, 0.5-1.6)	Public water supplies from six European cities		Zoeteman and Brinkmann, 1977
1.1 (range, 0.08-3)	Tap water from three Swedish cities		Boström and Wester, 1967
Range, < 1-50[a]	Finished drinking water in midwestern United States	Single value of 50 µg/liter in blended water due to water from deep wells containing up to 2 mg/liter	U.S. Environmental Protection Agency, 1975
2.4 (range, 0.78-7.9)	4,200 tap water samples obtained throughout the United States	Represents two-thirds of all samples; one-third was less than detection limit of the analytical method used (0.78 µg/liter)	Greathouse and Craun, 1979

[a] Mean not calculated.

arsenicals also are more toxic than organic arsenicals. Elemental arsenic, being insoluble, is essentially nontoxic.

TABLE V-22 Arsenic Content of Certain Human Tissues[a]

Tissue	Arsenic Concentration, mg/kg wet tissue
Liver	0.09-0.30
Lung	0.08-0.17
Kidney	0.07-0.14
Spleen	0.05-0.08
Thyroid	0.06-0.13
Pancreas	0.07
Intestine	0.07
Stomach	0.04
Heart	0.0001-0.016

[a] Adapted from National Academy of Sciences, 1977a.

The physical state of the arsenic compound is also important since arsenic trioxide (As_2O_3) given orally in the solid form is much less toxic than the compound given in solution. Moreover, there are species-specific differences in susceptibility to arsenic poisoning. For example, Kerr *et al.* (1963) reported that 3-nitro-4-hydroxyphenylarsonic acid was much more toxic to turkeys and dogs than to chickens and rats.

A considerable body of literature provides qualitative descriptions of the signs and symptoms of arsenic poisoning in humans (reviewed in National Academy of Sciences, 1977a, b), but few data concern quantitative dose-effect relationships. Vallee *et al.* (1960) estimated that the acute fatal dose of arsenic trioxide for humans is between 70 and 180 mg. This would be equivalent to a total arsenic dose of 53.2 to 136.8 mg, or, for a 70-kg human, 0.76 to 1.95 mg As/kg body weight. This is considerably less than the oral LD_{50} reported by Harrison *et al.* (1958) for arsenic as arsenic trioxide (As_2O_3) in the rat whether given in the solid form (145 mg As/kg body weight) or in solution (15.1 mg As/kg body weight). Thus, on a mg/kg body weight basis, humans appear to be much more susceptible than rats to the toxic effects of arsenic trioxide. The National Academy of Sciences Committee on Medical and Biologic Effects of Environmental Pollutants (MBEEP) pointed out that the rat may not be a suitable model for studying arsenic toxicity because of its peculiar metabolism of arsenic (National Academy of Sciences, 1977a). However, Harrison *et al.* (1958) reported that the oral LD_{50} for arsenic in Swiss mice was 39.4 mg/kg when administered as arsenic trioxide solution.

Therefore, on a mg/kg body weight basis, rodents appear to be more resistant than humans to the toxic effects of arsenic. These results illustrate the caution that is needed when extrapolating arsenic toxicity data from rodents to humans.

Silver and Wainman (1952) described chronic human arsenic poisoning in their report of a patient who used Fowler's solution (which contains 10 g of arsenic trioxide per liter of solution) to treat asthma. This individual ingested approximately 6.69 mg of arsenic as arsenic trioxide daily for 9 months and then 3.35 mg daily for an additional 19 months. Increased freckling and darkening of nipples, indicative of arsenic poisoning, was first seen after 13 months along with intermittent bouts of nausea, cramps, and diarrhea. After approximately 1.5 years, the patient noted redness and puffiness about her eyes and patches of thickened skin (hyperkeratosis) on her palms and soles. Symmetrical, tender hepatomegaly was also observed. After 2.5 years, the patient became aware of neurological symptoms such as paresthesia and slight weakness of both hands. Once the arsenic was withdrawn, the pigmentation lightened, but the hyperkeratotic condition remained. Also, the asthma remained difficult to control. This history is useful because it allows a reasonably good estimate of the amount and duration of exposure necessary to produce toxic effects of arsenic in humans, whereas incidents of accidental arsenic poisoning generally do not permit this. However, many reports suggest that humans are highly variable in their degree and kind of response to arsenic exposure (National Academy of Sciences, 1977a).

Numerous epidemiological studies have suggested an association between chronic arsenic overexposure and certain diseases such as cardiovascular disease or cancer (reviewed in National Academy of Sciences, 1977a,b). For example, people in Taiwan who consumed well water that contained 0.6 mg/liter or more of arsenic had a 3 to 4 times higher rate of blackfoot disease (a peripheral vascular disorder resulting in gangrene of the extremities) than did people who consumed well water that contained 0.29 mg/liter or less of arsenic (Tseng, 1977).

An increased incidence of bronchial and pulmonary diseases as well as cardiovascular pathology was observed in residents of Antofagasta, Chile, where the drinking water contained 0.8 mg/liter of arsenic (Borgono and Greiber, 1972). These people also had increased abnormal skin pigmentation (Borgono *et al.,* 1977) and increased cutaneous lesions such as leukoderma, melanoderma, hyperkeratosis, and squamous-cell carcinoma (Zaldivar. 1974). Moreover, Tseng (1977) observed an increased prevalence rate for skin cancer in the Taiwanese population that drank the arsenic-contaminated well water. Epidemiological studies

have also indicated an association between occupational exposure to airborne arsenic and excess mortality due to lung cancer. Blejer and Wagner (1976) have suggested that a no-effect level, i.e., no increased risk of respiratory cancer mortality, might lie in the range of a few micrograms of arsenic per cubic meter of air.

The role of arsenic in carcinogenesis remains controversial. It has not been accepted universally as a carcinogen, largely because laboratory studies have not succeeded in producing tumors in animals (Fraumeni, 1975).

Although arsenic is generally considered to be highly toxic, it is actually much less toxic than selenium, a trace element with established nutritional value. For example, 31 mg/kg of dietary arsenic as sodium arsenite has no effect on the growth of rats (Byron *et al.,* 1967), whereas 5 mg/kg of dietary selenium as sodium selenite (Na_2 SeO_3) is sufficient to cause growth retardation. If the nutritional requirement for arsenic as arsenite is 0.05 mg/kg of diet, then the ratio of the toxic to nutritional dose is approximately 1,250 since 62.5 mg/kg of arsenic as arsenite inhibited growth in rats (Byron *et al.,* 1967).

If 0.05 mg/kg of dietary 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 (Table V-20). On the other hand, these levels of intake are not very far removed from those that have been associated with various human diseases (see above). Consequently, the possible roles of arsenic in human health and disease urgently need to be clarified.

Interactions

The strong interaction between arsenic and selenium was discussed in the section on selenium. Administration of both arsenic and cadmium depressed weight gains in rats more than either metal did alone, but there did not seem to be any strong interactive effects of arsenic and lead (Mahaffey and Fowler, 1977).

Bioavailability

Water-soluble inorganic salts of arsenic are readily absorbed, but elemental arsenic and the insoluble arsenic sulfides are not. The unknown organic form of arsenic in shrimp and other shellfish is apparently absorbed well but excreted rapidly in the urine (National Academy of Sciences, 1977a).

Contribution of Drinking Water to Arsenic Nutrition

Since the nutritional requirement of arsenic for humans has not been proven, calculations on the contributions of drinking water to the arsenic requirement must necessarily be speculative. However, the extrapolation of animal data to humans (see above) gives a possible (calculated) need for 0.025 to 0.050 mg of arsenic daily. Two liters of water at an arsenic concentration of 0.0024 mg/liter (which may be slightly higher than a typical value; see Table V-21) would supply 10% to 19% of this speculated need, or 30% of the total intake of arsenic.

Conclusions

It is not possible to estimate the nutritional value of arsenic intake from water since the contributions calculated above are based on speculative values.

Research Recommendations

If the preliminary estimates of the need for arsenic in animals can be extrapolated to humans, the typical American diet may be marginally low in arsenic. Therefore, the possible beneficial nutritional effects of arsenic in animals and humans should be investigated further.

Additional research should be conducted in order to elucidate the relationship between levels of arsenic in the drinking water and the incidence of various human diseases, including cancer. A suitable animal model for the study of arsenic-induced cancer should be developed.

NICKEL

Presence in Food, Water, and Air

There is a wide variation in the nickel content of various foodstuffs. Thus, it is difficult to ascertain a definitive average daily dietary intake of nickel by humans. Various reported dietary intakes of nickel by humans are listed in Table V-23. The daily intake averages range from 165 to 500 µg.

In 1962, the nickel content of selected samples of public water supplies from the 100 largest cities in the United States ranged from 4 to 56 µg/liter (Durfor and Becker, 1964). The samples of water were obtained at the source, in storage, and in various stages of treatment. Most of the

samples contained less than 10 µg of nickel per liter. Only three samples contained more than 20 µg of nickel per liter. In 1969-1970, the average concentration of 969 U.S. water supplies (from 2,503 samples that were taken at the tap) was 4.8 µg/liter (National Academy of Sciences, 1975). No nickel was detected in 21.7% of the samples, and only 25 of the 2,503 samples (1%) contained more than 20 µg/liter. In another study, 3,676 tap water samples were collected in 35 different areas. Nickel was found in 83% of the samples in concentrations ranging from 0.4 to 5.1 µg/liter (Greathouse and Craun, 1979).

TABLE V-23 Oral Intake of Nickel by Humans

Average Intake, mg/day	Range, mg/day	Reference
NC[a]	0.300-0.600	Schroeder *et al.,* 1961
0.300	—	Taktakishvili, 1963
NC	0.170-0.330	Tipton *et al.,* 1969
0.500	—	Louria *et al.,* 1972
NC	0.280-0.310	Nodiya, 1972
0.451	0.288-0.696	Muhy *et al.,* 1973
0.165	0.107-0.221	Myron *et al.,* 1978

[a] NC, not calculated.

The concentration of nickel in ambient air is generally quite low in nonurban areas. The Division of Atmospheric Surveillance of the National Air Sampling Network (NASN) reported that nonurban air averaged 0.006 µg/m^3 during 1965-1969 (National Academy of Sciences, 1975). McMullen *et al.* (1970) found that the ambient air of 30 nonurban stations scattered across the United States ranged from 0.002 to 0.008 µg/m^3. Thus, if a person ventilates 20 m^3 of air per day, only 0.04 to 0.16 µg of nickel would be inhaled daily in nonurban areas. The higher value is less than 0. 1% of the dietary intake of nickel.

Urban air contains more nickel than does nonurban air. In urban areas, nickel is most concentrated near heavily traveled highways where it is probably derived from asphalt and automobile tires (Nielsen *et al.,* 1977). The NASN Division of Atmospheric Surveillance reported that urban ambient air contained 0.017 µg/m^3 in spring and summer and 0.025 µg/m^3 in winter (National Academy of Sciences, 1975). The average was 0.021 µg/m^3. Cities with the highest air nickel level were Boston, East Chicago, Indiana, and Philadelphia, where the levels were near 0.100 µg/m^3. McMullen *et al.* (1970) found that the ambient air

from 217 urban stations contained 0.017 μg/m^3. Assuming that urban air contains an average of 0.021 μg/m^3 of nickel, an individual would inhale 0.42 μ*g* of nickel daily, or less than 0.3% of the typical dietary intake. Even in cities with the highest concentrations of nickel in ambient air, the inhalation of nickel would be less than 2% of the typical dietary intake.

TABLE V-24 Selected Examples of the Nickel Content of Human Tissues and Fluids

	Nickel Content, μg/kg		
Tissue	Fresh	Dry	Reference
Bone	190-640	—	Nomoto, 1974
Hair	—	190-240	Nechay and Sunderman, 1973
Hair	—	1,010-4,050	Katz *et al.,* 1975
Heart	6.0	23	Sundermanet *et al.*, 1971
Liver	9.0	32	Sunderman *et al.*, 1971
Lung	16.0	86	Sunderman *et al.,* 1971
Fluids	Nickel Content, μg/liter		Reference
Blood	23		Szadkowski *et al.,* 1970
	4.8		Nomoto and Sunderman, 1970
	22		Delves *et al.,* 1971
	6.0		Zachariasen *et al.,* 1975
Saliva, parotid	1.9		Catalanatto *et al.,* 1977

Distribution in Tissues

Schroeder and Nason (1971) estimated that the human body contains 10 mg of nickel, or approximately 0.1 μg/g. They also suggested that 18% of the body nickel is found in the skin. Tissue analyses indicate that nickel is widely distributed in low concentrations (Table V-24) and that bone and hair contain the highest concentrations.

Requirements

Because nickel is essential for animals (Nielsen, in press a), it is highly probable that it is also essential for humans. However, no nickel

deficiency has been recognized in humans. Nielsen *et al.* (1975) and Schnegg and Kirchgessner (1978) suggested that the critical level of dietary nickel, below which deficiency symptoms are observed, is about 50 µg/kg of diet for chicks and rats. If animal data can be extrapolated to humans, then a 70-kg human consuming 1 kg of diet per day (dry basis) would have a daily requirement of 50 µg of nickel. The reported daily dietary intakes of 165 to 500 µg of nickel would adequately satisfy the presumed human nickel requirement.

Toxicity Versus Essential Levels

The toxicity of nickel or nickel salts through oral intake is low, ranking with such elements as zinc, chromium, and manganese. Nickel salts exert their action mainly by gastrointestinal irritation and not by inherent toxicity (Schroeder *et al.,* 1961). The cause of this relative nontoxicity appears to be a mechanism in mammals that limits intestinal absorption of nickel.

Nickel also has little tendency to accumulate in tissues during lifetime exposure. Large oral doses of nickel salts are necessary to overcome the homeostatic control of nickel. Generally, 250 µg or more of nickel per gram of diet is required to produce signs of nickel toxicity in rats, mice, chicks, rabbits, and monkeys (Nielsen, 1977). Thus, the ratio of the minimum toxic dose and the minimum dietary requirement for chicks and rats is approximately 5,000. If animal data can be extrapolated to humans, this translates into a daily dose of 250 mg of soluble nickel to produce toxic symptoms in humans.

One isolated report indicates that lower levels of dietary nickel are toxic. Schroeder and Mitchener (1971) reported that 5 µg of nickel per milliliter of drinking water may be moderately toxic to rats during reproduction. After three generations of this exposure, the signs of toxicity included increased perinatal deaths and number of runts. The investigators reported that the size of the litters decreased with each generation and that few males were born in the third generation. These findings have not been confirmed.

Nickel dermatitis is a relatively common form of nickel toxicity. Several surveys indicated that the incidence of sensitivity to nickel is between 4% and 13% (National Academy of Sciences, 1975). Reviews (National Academy of Sciences, 1975; Nielsen, 1977) attributed nickel dermatitis to percutaneous absorption of nickel. However, Christensen and Moller (1975) suggested that the ingestion of small amounts of nickel may be of greater importance than external contacts in maintaining hand eczema. They observed that an oral dose of 5.6 mg of nickel as

nickel sulfate ($NiSO_4$) produced a positive reaction in nickel-sensitive individuals within 1 to 20 hr after ingestion. That dose is only 11 to 34 times as high as the reported daily human dietary intake of nickel but 112 times as high as the human daily requirement of nickel that may be postulated from animal studies (50 µg/day; see above).

Interactions

Nickel interacts with at least 13 essential minerals in animals, plants, and microorganisms (Nielsen, in press b). Perhaps the most important interaction occurs with iron. Nielsen *et al.* (1978) reported that the interaction between dietary nickel and iron in rats affected hematocrit, hemoglobin level, plasma alkaline phosphatase activity, plasma phospholipid level, the ratio of liver weight to body weight, liver lipid extract yellow pigment, liver copper concentration, and, perhaps, liver manganese and nickel concentration. They also found that signs of nickel deprivation developed more rapidly and severely in rats when their diet contained borderline levels of iron. The data obtained from the borderline iron-deficient rats indicate that nickel deficiency impairs iron absorption. Schnegg and Kirchgessner (1976) suggested that the depressed levels of hemoglobin, erythrocytes, and hematocrit during nickel deficiency were caused by impaired iron absorption.

Bioavailability

Most ingested nickel remains unabsorbed by the gastrointestinal tract and is excreted in the feces. The limited data in Table V-25 indicate that only 2% to 3% of ingested nickel is absorbed. Nielsen *et al.* (1978) suggested that elevated levels of dietary iron may enhance absorption of nickel.

Contribution of Drinking Water to Nickel Nutrition

Assuming an estimated daily intake of 2 liters of water, these data indicate that typical drinking water sources would contribute anywhere from negligible amounts to 40 µg of nickel daily. The average contribution would probably be near 10 µg of nickel daily, which would be 2% to 6% of that derived from usual dietary sources. However, isolated water samples assay as high as 75 µg of nickel per liter, and some individuals may be consuming diets that contain low amounts of nickel. Consequently, in some atypical instances, drinking water would contribute as much as or more than food to the total nickel intake.

TABLE V-25 Percent of Ingested Nickel Found in Feces and Urine

	Ingested Nickel Found, %		
Species	Feces	Urine	Reference
Cow	96-98	—	O'Dell *et al.,* 1971
Dog	90	10	Tedeschi and Sunderman, 1957
Human	—	3	Schroeder and Nason, 1971
Human	90	10	Nodiya, 1972
Rabbit	81	11	Babedzhanov, 1973
Rat	94-98	2-6	Elakhovskaya, 1972

Conclusions

Nickel deficiency in the typical U.S. diet is unlikely under present circumstances. Based on average food, water, and air concentrations, most drinking water contributes a very small proportion of the daily nickel intake. Only under unusual circumstances would nickel in drinking water contribute toward satisfying the presumed nutritional requirement of nickel or cause detrimental body burdens (i.e., help maintain hand eczema in nickel dermatitis).

VANADIUM

Presence in Food, Water, and Air

Recent studies have shown that the vanadium content of most foods is very low (Byrne and Kosta, 1978; Myron *et al.,* 1977; Soremark, 1967; Welch and Cary, 1975), i.e., less than 1 ng/g. These studies differ from those of Schroeder *et al.* (1963) which are now generally assumed to be erroneous. Consequently, the 2 mg that Schroeder *et al.* (1963) estimated to be the daily average intake of vanadium is probably incorrect.

Only Myron *et al.* (1978) and Byrne and Kosta (1978) provide reasonable suggestions concerning the typical daily dietary intake by humans. Myron *et al.* (1978) ascertained the vanadium content of nine

institutional diets and found that they would supply 12.4 to 30.1 µg of vanadium daily (average, 20 µg). Byrne and Kosta (1978) stated: "It can be estimated that the daily dietary intake is of the order of a few tens of micrograms, though it may vary over wide limits."

Vanadium occurs in water in several chemical forms of which the dioxyvanadium cation (VO_2^+) and the vanadate anion (VO_4^-) are readily soluble (Hopkins *et al.,* 1977). However, the mobility of those ions in water is probably low because of easy adsorption on clay and precipitation with organic matter.

In 1962 the vanadium content of selected samples from the public water supplies of the 100 largest cities in the United States ranged from undetectable levels to 70 µg/liter (median, <4.3 µg/liter) (Durfor and Becker, 1964). The samples of water were obtained at the source, in storage, and in various stages of treatment. Water supplies from the Southwest generally contained higher vanadium concentrations than did supplies from the eastern states. Vanadium was detected in 26% of 3,676 tap water samples from 34 areas in the United States. The detected concentrations ranged from 1.3 to 33 µg/liter (mean, 4.85 µg/liter) (Greathouse and Craun, 1979).

Petroleum and other naturally occurring hydrocarbons such as asphaltite contain appreciable quantities of vanadium (Hopkins *et al.,* 1977). Consequently, the combustion of petroleum products may contribute detectable amounts of vanadium to the atmosphere.

The amount of vanadium in ambient air is quite variable (ranging from undetectable to >2.0 µg/m^3), depending upon location and season of the year (National Academy of Sciences, 1974). Concentrations of vanadium in urban atmospheres are generally higher than those in the air of nonurban areas. However, in 1965-1969 the National Air Sampling Network measured concentrations of vanadium in the rural areas of nine eastern seaboard states from Maine to South Carolina that were significantly higher than those in other rural areas of the country (National Academy of Sciences, 1974). The concentrations in rural areas in the nine eastern seaboard states were similar to those in urban air from the midwestern and western portions of the United States (2 to 64 ng/m^3).

Assuming that 20 m^3 is inhaled daily, ambient air would contribute 0.2% to 6% to the total daily intake of vanadium in some regions and cities in the United States. In some eastern cities, ambient air would contribute as much or more vanadium as the typical diet. However, in regions other than the eastern United States, ambient air would contribute probably less than 0.1% to the total daily intake of vanadium.

TABLE V-26 Vanadium Content of Human Tissue and Fluids[a]

Tissue	Vanadium Content, µg/kg
Bile	0.65-1.85
Bone	0.8-8.3
Blood	<0.5
Brain	<0.7-0.75
Fat	0.3-0.8
Heart	1.1
Hair	12-87 (average = 40)
Kidney	2.6-3.3
Liver	4.5-7.5
Lung	19-140 (median = 30)
Muscle	0.45-0.62
Teeth	<2.0-5.1
Thyroid	3.0-3.2

[a] From Byrne and Kosta, 1978.

Distribution in Tissues

Byrne and Kosta (1978) estimated that the total body content of vanadium in healthy, adult humans is approximately 100 µg. Tissue analyses indicated that vanadium is widely distributed in very low concentrations in humans (Table V-26). Hair and lung contain the most vanadium, probably as the result of exposure to atmospheric vanadium. The next highest concentrations occur in the bone, kidney, liver, teeth, and thyroid. The lowest concentrations are found in blood, brain, fat, and muscle tissue. The report of Byrne and Kosta (1978) showed that the estimate of Schroeder *et al.* (1963) (i.e., 17 to 43 mg of vanadium in the human body, of which 16 mg was in fat and 1.4 mg was in serum) was most likely erroneous.

Requirements

Several reports suggest that vanadium is an essential element for chicks and rats (Nielsen, in press). However, the evidence for this is tenuous because of difficulties in obtaining a consistent set of signs that are indicative of vanadium deprivation. Apparently, the difficulty is related to the sensitivity of vanadium metabolism to changes in the composition of the diet (Nielsen, in press). It may be necessary to find a specific physiological role for vanadium in order to establish its essentiality.

The nutritional significance of vanadium is unclear because of the incomplete knowledge concerning the conditions that produce apparent vanadium deficiency and the dietary components that affect vanadium metabolism. As a result, it is difficult to suggest a vanadium requirement for any animal species, including humans. Estimates of the vanadium requirement of rats and chicks have ranged from 50 to 500 µg/kg of diet (Underwood, 1977). Those estimates are most likely too high. Under certain conditions, at least four independent laboratories have found that feeding less than 25 µg of vanadium per kilogram of diet adversely affected rats or chicks (Nielsen, in press). If animal data can be extrapolated to humans, then a 70-kg human consuming 1 kg of diet per day (dry basis) may have a daily requirement of approximately 25 µg of vanadium under certain dietary conditions. If that were true, the estimated daily dietary intake of vanadium (20 µg) would be borderline inadequate or adequate.

Toxicity Versus Essential Levels

Diet composition also significantly alters the response of animals to elevated levels of dietary vanadium. For example, vanadium toxicity in chicks was alleviated by feeding corn, dehydrated grass, cottonseed meal, ascorbic acid. ethylenediaminetetraacetic acid (EDTA), and chromate (CrO_4^{2-}) (Berg, 1966; Berg and Lawrence, 1971; Hathcock *et al.,* 1964; Hill, 1976). Nonetheless, under certain dietary conditions, 10 mg of vanadium per kilogram of diet was found to be slightly toxic to chickens (Berg *et al.*. 1963). That level of dietary vanadium is approximately 45 to 50 times the level at which apparent vanadium deficiency signs occurred in chicks. If animal data can be extrapolated to humans, a daily dose of 10 mg of vanadium may be slightly toxic in humans under certain conditions. That extrapolation is supported by Dimond *et al.* (1963) who gave oral doses of 4.5 to 18 mg vanadium per day to volunteers for 6 to 16 weeks. Cramps and diarrhea were produced only by the larger dose. Schroeder *et al.* (1963) fed up to 9 mg of vanadium per day for 6 to 16 months to older individuals who were confined to a mental institution. They observed no ill effects due to the vanadium supplementation.

Interactions

The apparent interaction of vanadium with various dietary components is discussed above in the sections on nutritional requirements and toxicity. The mechanisms by which various dietary components affect

vanadium metabolism are not known. Furthermore, knowledge as to which specific dietary components affect vanadium metabolism is incomplete.

Bioavailability

Little is known about the availability of ingested vanadium. Apparently, most ingested vanadium remains unabsorbed by the gastrointestinal tract and is excreted in the feces. Curran *et al.* (1959) found that only 0.1% to 1.0% of a 100-mg dose of diammonium oxytartarovanadate was absorbed from the human gut and that 60% of the absorbed vanadium was excreted by the kidneys within 24 hr. The remainder of the absorbed vanadium was retained by liver and bone.

Contribution of Drinking Water to Vanadium Nutrition

Assuming a daily intake of 2 liters of water, data indicate that typical drinking water sources would contribute between negligible amounts and 140 µg of vanadium daily. The average contribution is probably near 8 µ*g* of vanadium daily which would be 40% of the amount of vanadium derived from usual dietary sources. In some instances, the contribution of drinking water to the daily intake of vanadium may be much greater than the contribution of the diet. For example, if the drinking water contained 33 µg of vanadium per liter, it would contribute 3 times as much vanadium as a typical diet.

Conclusions

Because the essentiality of vanadium has not been proven, no requirements have been established. However, it may have nutritional significance. For example, less than 25 µg of vanadium per kilogram of diet adversely affected rats and chicks under certain conditions. The typical diet in the United States probably supplies only 20 µg of vanadium daily, which may not provide an optimal intake of vanadium through the diet. Therefore, any contribution drinking water may give to the daily intake of vanadium may be beneficial.

SILICON

Presence in Food, Water, and Air

Since the essentiality of silicon as a trace element has only been established recently (Carlisle, 1969, 1970), little is known about its concentration in food. Older reports may be inaccurate because of contamination problems occurring during analysis. However, since food consumed by humans may also be contaminated, results obtained under clean laboratory conditions may also not be representative of human consumption. Generally, plants contain more silicon than do animal products (Hopps *et al.,* 1977). In plants, the proportions of silicon present as monosilicic acid (H_2SiO_3) and solid silica (SiO_2) vary with the species, stage of growth, and soil conditions (Bezeau *et al.,* 1966). Substantial losses may occur during food processing, particularly in the refining of sugar (Hamilton and Minski, 1972/1973). Hamilton and Minski (1972/1973) determined that the mean total silicon intake from the diets of human adults in Great Britain was 1.2 ± 0.1 g/day.

The maximum, median, and minimum concentrations of silicon as silica in finished water from water supplies of the 100 largest cities of the United States were 72, 7.1, and 0 mg/liter (Durfor and Becker, 1964). No mean concentrations were given. Natural waters may contain from a few to several thousand milligrams of silicon per liter (Carlisle *et al.,* 1977).

The information on concentrations of silicon in air is limited to special situations, e.g., the silicon in airborne asbestos particles.

Distribution in Tissues

Silicon enters the alimentary tract from the food as monosilicic acid, as solid silica, and in organic bound form with pectin mucopolysaccharides. Little is known about its absorption (Jones and Handreck, 1965; Underwood, 1977).

Increased urinary silicon output with increasing intake up to fairly well-defined limits has been demonstrated in humans (Holt, 1950), rats (Keeler and Lovelace, 1959), and guinea pigs (Sauer *et al.,* 1959). The rate of excretion does not seem to be dependent on the renal capabilities to excrete silicon but. rather, on the extent of silicon absorption. Body retention of silicon is small and occurs primarily in the bone.

Underwood (1977) reported that tissue levels of silicon vary greatly, that they are higher in humans than in the rat or the monkey, and that they decrease with age. The highest concentrations are found in human dental enamel (mean, 243 mg/kg). In the head and epiphysis of the

femur of monkeys, silicon concentrations average 456 mg/kg. The silicon content of the normal human aorta decreases with age, and less silicon is present in arterial walls as arteriosclerosis develops. These are very interesting findings, but, because of the inherent difficulties encountered in analytical procedures, additional careful studies should be undertaken to substantiate them.

Requirements

The requirements for silicon in humans are unknown. Limited experiments with rats and chicks have shown that silicon is necessary for normal growth and satisfactory skeletal development (Carlisle, 1974). In rats, 50 mg/g of dry diet provided as water-soluble sodium metasilicate (Na_2SiO_3) fulfilled these requirements (Schwarz and Milne, 1972).

From studies of silicon-deficient and silicon-supplemented chickens, Carlisle (1974) concluded that silicon is involved in mucopolysaccharide and hexosamine synthesis. Thus, silicon affects the formation of cartilage and connective tissue. It also seems to hasten the rate of bone mineralization. Carlisle *et al.* (1977) reported that it reduced the water content of the bones of silicon-deficient chickens.

Toxicity Versus Essential Levels

When inhaled into the lungs, particles of silica and asbestos may stimulate a severe fibrogenic reaction in the lungs and elsewhere in the body. Silica particles are phagocytized by alveolar macrophages where they interact with lysosomes (Nash *et al.,* 1966). Furthermore, asbestos is now generally recognized as a carcinogen in humans. The potentials of silicate to induce pneumoconiosis and carcinoma are beyond the scope of this brief report. Further information on this subject can be found in *Environmental Factors in Respiratory Disease* (Lee, 1972) and in *Occupational Lung Disorders* (Parkes, 1974).

Ruminants consuming plants with a high silicon content may develop silicious renal calculi. Renal calculi in humans have been shown to contain silicates (Carlisle *et al.,* 1977).

Grasses and sedges contain higher concentrations of silicon than do forbs and shrubs growing in the same locality (Bezeau *et al.,* 1966). Thus, the proportional consumption of different plant material may determine whether ruminants would develop urolithiasis in specific areas.

The essentiality of silicon in chickens was established through carefully designed studies (Carlisle, 1972). Silicon deficiency was apparent when the diet contained 1 mg of silicon per kilogram of diet, while

the presence of 100 mg of silicon in 1 kg of diet resulted in increased growth rate and increased water content of the long bones. Similarly, 50 mg of silicon per kilogram of diet prevented silicon deficiency in rats. It has not been established whether lower levels would have been equally effective.

Requirements for Nutrition

No information is presently available on essential dietary levels in humans.

Interactions

The interaction of silicon with molybdenum has been discussed by Carlisle (1979) but there is no well-substantiated information on the interaction of silicon with other trace elements. Ingestion of large amounts of silicon in the form of silica may interfere with the absorption of other nutrients.

Conclusions and Recommendations

Animal studies indicate that silicon is an essential trace element, but this has not been established in humans. There is no information on required daily intakes of humans.

Additional studies should be conducted to determine the availability of silicon in different forms and whether calcium and phosphates interact with silicon.

Since silicon can be found in appreciable amounts in mucopolysaccharide-rich tissues such as cartilage, it should be determined how silicon affects the development and maintenance of cartilage and calcification of bone. It should also be established whether silicon plays any role in the healing of fractures. Further careful studies should be conducted to determine levels of silicon in food and human tissues.

MOLYBDENUM

Presence in Food, Water, and Air

Molybdenum occurs in nature with valences of 4^+, 6^+, and, possibly, 3^+ and 5^+. It is contained in minerals such as molybdenite (MoS_2).

wulfenite (PbMo4), ferrimolybdate ($FeMoO_3 \cdot$ ` H_2O), and jordisite (amorphous MoS_2).

The widely varying environmental concentrations of molybdenum are caused by regional geological factors. Concentrations in water and soil may vary by a factor of more than 10 causing both deficient and excessive intake of molybdenum for plants and ruminants, depending on their location. In areas where molybdenum ore is processed, concentrations in soil and water may increase considerably. In soil, the available molybdenum is of greater importance for plant nutrition than is the total amount of molybdenum. The availability is dependent on pH (i.e., it is greater in alkaline soils) and other factors in the soil (Swaine. 1955).

The amount of molybdenum in vegetable crops is greatly influenced by the molybdenum content of soil. Engel *et al.* (1967) determined that the molybdenum in the dry matter of the diet ranged from 94 to 189 µg/kg. Jacobson and Webster (1977) stated that ordinary hospital diets provided from 44 to 1,000 µg of molybdenum per day. Schroeder *et al.* (1970) determined molybdenum concentrations in a variety of foods on a wet-weight basis. The concentrations ranged from none to 21.40 mg/kg in beef kidney. Molybdenum was associated with most foods that contained purine. Table V-27 shows some representative food contents of molybdenum. The richest sources were meats, grains, and legumes; the poorest were vegetables other than legumes, fruits, sugar, oils, and fats. However, Friberg *et al.* (1975) concluded that diets based on leafy vegetables and legumes would result in a higher intake of molybdenum than those based mainly on meat products.

In balance studies with healthy children, Alexander *et al.* (1974) determined that a total molybdenum intake of 3.02 µg/kg body weight resulted in the absorption of 2.32 µg of molybdenum per kilogram of body weight. Of this, 42% was retained and 1.76 µg/kg was excreted.

Schroeder *et al.* (1970) estimated that the average diet in the United States contained 335 µg of molybdenum (range, 210-460 µg). In the USSR, estimates of intake by children were 156 to 161 µg/day (Vorob'eva and Osmolovskaya, 1970) and by adults, 329 to 376 µg/day (Gabovich and Kulsyaya, 1964). Hamilton and Minski (1972/1973) studied total diets from different regions of the United Kingdom. They reported an average daily intake of 128 µg of molybdenum (SD ± 34).

Kopp and Kroner (1967) measured trace metals in rivers and lakes of the United States from 1962 to 1967. They detected molybdenum in 32.7% of 1,577 samples (range, 2-1,500 µg/liter; mean, 68 µg/liter). Durfor and Becker (1964) reported that the median concentration of molybdenum in finished water in public water supplies was 1 to 4 µg/liter and that the maximum concentration was 68 µg/liter. Great-,

house and Craun (1979) detected molybdenum in 30% of 3,676 tap water samples. The maximum concentration was 52.7 μg/liter, the mean concentration was 8.05 μg/liter, and the minimum concentration was 1.1 μg/liter.

TABLE V-27 Molybdenum Concentration in Food[a]

Food Item	Molybdenum Concentration, μg/g wet tissue
Seafood	0-0.56
Ground beef	0
Beef liver	1.97
Beef kidney	21.40
Pork loin	3.68
Lamb chops	5.00
Milk products	0-0.49
Grains and cereals	0.46-5.87
Vegetables	
Legumes	0-4.8
Roots	0-0.59
Vines	0-0.34
Leafy	0-0.11
Fruits	0.3-0.79

[a] Data from Schroeder *et al.,* 1970.

Air quality data from the National Air Sampling Network indicated that molybdenum concentrations in ambient air in the United States during 1966 ranged from 10 to 30 ng/m^3 in urban areas and from 0.1 to 3.2 ng/m^3 in nonurban areas (U.S. Public Health Service, 1968). Fly ash from power stations may contain as much as 10 to 40 mg/kg (Smith, 1958).

Distribution in Tissues

The hexavalent compounds molybdenum trioxide (MoO_3) and calcium molybdate ($CaMoO_4$) are absorbed quite well from the gastrointestinal tract but, apparently, molybdenum sulfide and molybdenite are not (Gray and Daniel. 1954). The absorption of molybdenum given orally as molybdenum trioxide to guinea pigs was 85% (Fairhall *et al.,* 1945). Similarly, Van Campen and Mitchell (1965) showed ready absorption of ammonium molybdate [$(NH_4)_2{}^{99}MoO_4$] from the gastrointestinal tract of rats. In humans, between 25% and 80% of ingested molybdenum is

absorbed (Alexander *et al.,* 1974; Boström and Wester, 1968; Robinson *et al.,* 1973; Tipton *et al.,* 1969).

TABLE V-28 Molybdenum in Human Tissues[a]

Organ	Molybdenum Concentration, µg/g wet tissue
Adipose tissue	0.0055
Blood	0.041
Liver	1.1
Lungs	0.016
Heart	0.017
Kidneys	0.37

[a] Data from Schroeder *et al.,* 1970.

Hexavalent molybdenum is rapidly excreted by animals over a 2-week period (Amon *et al.,* 1967). In humans, 30% to 40% of an intravenous dose was excreted within 10 days (Rosoff and Spencer, 1964). The highest concentrations of molybdenum in tissue were found in kidney, liver, and bone (Anke *et al.,* 1971; Durbin *et al.,* 1957).

Normal blood values in humans vary a great deal but are usually in the ng/ml whole-blood range. Molybdenum levels seem to increase in the kidneys and liver in the second and third decade of life. On the average, the liver reaches a concentration of 0.5 to 1 µg/g, and the kidneys, 0.25 µg/g, on a wet-weight basis (Friberg *et al.,* 1975). Molybdenum concentrations in tissues from 150 victims of accidental deaths in the U.S. are listed in Table V-28. When tissues were analyzed on a wet-weight basis, molybdenum was usually not found in other organs. However, on an ash basis µg/g concentrations of molybdenum were found in a number of other organs.

Venugopal and Luckey (1978) reported that 9.3 mg was the average content of molybdenum in the human adult body.

Requirements

Minimum dietary requirements for molybdenum in animals and humans are presently unknown. Requirements for molybdenum in rats and chickens are very low (Higgins *et al.,* 1956). Attempts at inducing molybdenum deficiency have been successful only when tungstate (WO_4^-), an inhibitory metal, was given concomitantly (Friberg *et al.,* 1975). Recently, Anke *et al.* (1978) were able to induce molybdenum deficiency in goats by feeding them a diet containing less than 60 µg of

molybdenum per kilogram of diet. Similar molybdenum deficiencies in poultry have been described by Payne (1977).

The daily intake of molybdenum from dry diet in adults has been estimated as 0.1 mg/day, or approximately 0.13 mg/kg of diet. Several balance studies in humans (Engel *et al.,* 1967; Tipton *et al.,* 1966) suggested that a slightly positive balance is maintained if the diet provides approximately 2 µg of molybdenum per kilogram of body weight per day. An estimated adequate and safe intake of molybdenum for humans of 0.15 to 0.5 mg/day has been established (National Academy of Sciences, 1980); also see Table V-31 in the summary of this chapter). The essentiality of molybdenum is based on the fact that it is an integral part of many enzymes.

The flavoprotein enzyme xanthine oxidase is a molybdenum-containing metalloenzyme. Aldehyde oxidase and sulfite oxidase are also dependent for their activity on the presence of molybdenum (Cohen *et al.,* 1971; DeRenzo *et al.,* 1953; Higgins *et al.,* 1956; Richert and Westerfeld, 1953).

The beneficial effect of molybdenum on the incidence and severity of dental caries has been refuted by Hadjimarkos (1968). He pointed out that the lower prevalence of caries in children from the high molybdenum area in the earlier studies could have resulted from the presence of fluoride since teeth from the children of this group were reported to contain 34% more fluoride than the teeth of the children from the low molybdenum area (Hadjimarkos. 1973).

Toxicity Versus Essential Levels

In rats and rabbits, dietary levels ranging from 500 to 5,000 mg/kg diet produce weight loss and, in most instances, anemia (Arrington and Davis. 1953; Gray and Daniel. 1954; McCarter *et al.,* 1962). Bone deformities were also noted (Arrington and Davis, 1953; Lalich *et al.,* 1965; McCarter *et al.,* 1962; Valli *et al.,* 1969).

Among livestock, cattle seem to be more sensitive than sheep, horses, and pigs to the toxic effects of molybdenum. Severe molybdenosis in cattle occurs under natural grazing conditions in many parts of the world (Britton and Goss, 1946; Cunningham, 1957; Dye and O'Hara, 1959; Ferguson *et al.,* 1938).

Both occupational and high-level dietary exposure to molybdenum have been linked to elevated uric acid levels in blood and an increased incidence of gout.

Akopajan (1964a) examined 73 workers in a copper-molybdenum

plant and 10 control subjects. He observed the highest levels of uric acid in the blood of miners who had been exposed to the highest concentrations and who complained of arthralgia. However, his results are difficult to evaluate since he did not report the blood levels of uric acid or the concentrations to which the subjects were exposed.

Kovalsky *et al.* (1961) and Yarovaya (1964) reported a high incidence of gout in an area of Armenia where the concentrations of molybdenum and copper in the soil were 77 mg/kg and 39 mg/kg, respectively. In 1961, using molybdenum and copper levels in different food products as a basis, the investigators calculated the total daily intakes for an adult man in this area to be 10 to 15 mg molybdenum and 5 to 10 mg copper compared to the intake of a man in a control area which was 1 to 2 mg molybdenum and 10 to 15 mg copper.

A survey of 262 18-year-old and older subjects from two villages in the molybdenum-rich area revealed a prevalence of symptoms similar to gout in 31% of the subjects from one village and 18% from the other. The authors claimed that similar symptoms normally occurred in 1% to 4% of the population of the USSR. The symptoms were characterized as arthralgia in the knees, hands, and feet. Deformities of the joints were also reported.

In some areas of India, where sorghum apparently contains high concentrations of molybdenum, a bone-crippling disease has been observed among the population. A definite cause-effect relationship has not been established, but it is possible that molybdenum increases the toxicity of fluoride (Food and Drug Administration, 1975).

Depending on the amount of copper, fluorides, and other nutrients in the diet and the solubility of the molybdenum salt, a concentration of 10 to 15 mg of molybdenum in the diet may result in chronic toxicity in humans (Underwood, 1977). This concentration is at least 20 times higher than the upper limit of the estimated adequate and safe intake.

Interactions

Copper compounds have had a beneficial effect on molybdenosis (Cook *et al.,* 1966; Cunningham, 1957; Wynne and McClymont, 1955). In New Zealand, Cunningham (1950) found that the onset of scouring (molybdenosis) was delayed until copper stores in the tissue were depleted. Based on experimental evidence, Miltmore and Mason (1971) claimed that for the prevention of molybdenosis, the critical ratio of copper to molybdenum is 2.0; however, Alloway (1973) believes that it is closer to 4.0. Since

the levels in New Zealand are generally lower than those in England, ratios may vary with different molybdenum concentrations.

Experimental studies further elucidate this interaction between molybdenum and copper, which is modified by other dietary factors such as the amount of sulfate, manganese, and protein in the diet. Gray and Daniel (1954) showed that small amounts of molybdenum could produce toxicity in rats on copper-deficient diets. This effect was intensified by the simultaneous addition of sulfate (SO_4). In rats with adequate dietary intake of copper, large amounts of molybdenum were necessary to produce the same effect while molybdenosis could be completely prevented by the addition of sulfate to the diet. Studies primarily in sheep seem to indicate that sulfates in the diet may have either adverse or beneficial effects depending upon the copper status. Dick (1956) found that daily doses of 0.3 to 100 mg molybdenum in sheep on a low dietary intake of sulfate had no effect on blood copper levels, but when sulfate intakes were high, blood copper levels rose rapidly in response to 60- or 90-mg doses of molybdenum. Sulfates also increase the urinary excretion of molybdenum (Dick, 1953) in sheep but at high concentrations decreased the molybdenum excretion in milk (Hogan and Hutchinson, 1965).

Sulfides also seem to affect the metabolism of molybdenum and copper. A number of investigators have attempted to explain the complex interaction between copper, molybdenum, and sulfide. Apparently, exposure to molybdenum decreases the activity of sulfide oxidase in the liver (Mills *et al.,* 1958). Excessive dietary intake of cystine by rats that were given high concentrations of molybdenum increased molybdenum toxicity (Halverson *et al.*. 1960). An accumulation of sulfide in the tissues as a consequence of depressed sulfide oxidase activity occurs in molybdenotic rats (Van Reen, 1959; Williams and Van Reen, 1956). In the digestive tract, copper interacts with molybdenum and copper availability may be depressed through the precipitation of insoluble cupric sulfide (Cu_2S_3) (Mills, 1961). Furthermore, cupric molybdate [$Cu_2(MoO_4)_3$], a biologically unavailable compound, may form in the digestive tract, and, if sulfate at relatively neutral pH is reduced to sulfide, thiomolybdate may form. This molybdate may complex with copper (Huisingh *et al.,* 1973; Suttle, 1975). Much of the research on the interaction of molybdenum with copper in the digestive tract has been done in sheep. It is presently not known whether similar interactions would occur in humans.

Interactions between molybdenum, iron, and fluorides are not well understood.

Contribution of Drinking Water to Molybdenum Nutrition

Assuming that the estimated dietary intake of molybdenum from dry diet is 100 µg/day in areas where the concentration in water is high, the amount contributed by drinking water may be substantial. For a daily consumption of 2 liters of water this contribution could range from approximately 2 to 136 µg, according to Durfor and Becker (1964). However, high concentrations in public water supplies seem to be infrequent. Durfor and Becker reported the median concentration of molybdenum in finished water to be 1 to 4 µg/liter. This amount could result in a daily intake of 2 to 8 µg molybdenum, which would constitute only 1.3% to 5.3% of the lower limit of the estimated adequate and safe intake (National Academy of Sciences, 1980).

Conclusions and Recommendations

Evidence seems to indicate that molybdenum is an essential trace element. Since it is only required in very low concentrations, it has been difficult to produce deficiency diseases in animals. Further studies should be conducted in this area under well-controlled conditions.

Our understanding of chronic molybdenum toxicity or deficiency in humans is presently extremely limited. This topic should be studied. Further studies should also be conducted to determine the interaction of molybdenum with other elements and nutrients in humans.

Factors affecting absorption and availability of molybdenum in humans should also be determined. The reduction of molybdenum in water in areas with high molybdenum concentrations should not be attempted until it has been determined whether molybdenum is absorbed in the form in which it occurs in water. The effect of such a reduction on other nutrients such as copper and fluoride should be determined.

CHROMIUM

Presence in Food, Water, and Air

Although some of the difficulties in analyzing biological material for chromium have been overcome during the past few years, the methods

are still not satisfactory (Mertz, 1976; Mertz *et al.,* 1978). Because of these analytical difficulties, the accuracy of many of the chromium values in the literature is suspect.

The nutritional value of chromium in foods is complicated by the fact that it is not all present in the most biologically active form, i.e., as glucose tolerance factor (GTF) (Mertz, 1976). The bioavailability of inorganic chromium in foods is very low. Trivalent chromium is the nutritionally useful form; hexavalent chromium is not. Thus, total chromium content may not be a valid indicator of the contribution a food will make toward meeting the chromium requirement. Although a reproducible method for assaying biologically active chromium has been developed (Brantner and Anderson, 1978), a direct and accurate quantitative analysis of GTF in tissues and foods has not. Most likely, when a routine quantitative analysis for GTF is developed, the nutritional value of chromium will be expressed in that manner, just as the nutritional value of cobalt in foods is expressed as vitamin B_{12} activity. Because there is no better measure, total chromium content is still used.

Perhaps the most accurate determination of the mean daily chromium intake in the United States was made by Kumpulainen *et al.* (in press). They found that 14 "typical self-selected American diets"—typical with regard to fat and calories—supplied 37 to 130 µg of chromium daily (average, 62 µg). The mean daily intake was similar to that found earlier for subjects consuming institutional diets and for young people on a free choice diet (Levine *et al.,* 1968: Schroeder *et al.,* 1962).

The limited data on the chromium content of drinking water in the United States are summarized in Table V-29.

The chromium concentration in air varies with location. Towill *et al.* (1978) cited data from the U.S. Environmental Protection Agency which showed that the chromium concentrations in most nonurban areas were below detection levels. In urban areas, the yearly average concentrations varied from below detection levels to as high as 0.120 µg/m^3. Yearly averages were greater than 0.01 µg/m^3 in only 59 of 186 urban areas. Those values were similar to the values compiled by the National Air Sampling Network (cited by Towill *et al.,* 1978), which showed the national average for chromium in air to be 0.015 µg/m^3 and the maximum to be 0.350 µg/m^3. Thus. if a person ventilates 20 m^3 of air/day, ambient air in most areas of the United States would contribute less than 0.5% to the daily intake of chromium. Even in cities with the highest concentration of chromium in ambient air, the inhalation of chromium would probably be less than 4% of the typical dietary intake.

TABLE V-29 Studies on Chromium in Drinking Water in United States[a]

	Chromium Concentration, µg/liter		
Number of Samples	Minimum	Maximum	Mean
100[b]	ND[c]	35	0.43 (median)
2,595	NR[d]	79	NR
1,577	1	112	9.7
380	1	29	7.5

[a] Data from Durfor and Becker, 1964; Kopp, 1969; and U.S. Department of Health, Education, and Welfare, 1970.
[b] Samples from public water supplies of 100 largest cities in the United States.
[c] ND, not detected.
[d] NR, not reported.

Distribution in Tissues

Because the analytical methods are inadequate, most of the reported values for the chromium content of tissue are probably inaccurate. It is generally accepted that the concentrations of chromium are very low. This is supported by Doisy *et al.* (1976), who reported 1 to 5 ng of chromium/ml in plasma, and by Guthrie *et al.* (1978), who found less than 1 ng of chromium per milliliter of urine.

Requirements

Data on older subjects, diabetics, pregnant women, and malnourished children suggest that chromium deficiency does occur in humans (Doisy *et al.,* 1976; Gurson, 1977). Jeejeebhoy *et al.* (1977) described a case of chromium deficiency in a woman who was maintained on total parenteral nutrition for several years. Their findings suggested that the signs of chromium deprivation in humans include glucose intolerance, inability to utilize glucose for energy, neuropathy with normal insulin levels, high free fatty acid levels, a low respiratory quotient, and abnormal metabolism of nitrogen. Although it is apparent that chromium is an essential nutrient, the estimation of the dietary chromium requirement is difficult because of the great difference in bioavailability of this element in different foods. Nonetheless, an estimated adequate and safe intake for chromium of 50 to 200 µg/day has been established

(National Academy of Sciences, 1980). The lower value of 50 µg was based on the average chromium intake in the United States from mixed diets and the lack of evidence of widespread, serious chromium deficiency related to this intake. Thus, the 62-µg average daily dietary intake of chromium, which was estimated by Kumpulainen *et al.* (in press), would be just adequate to meet the minimal value.

Toxicity Versus Essential Levels

The toxicity of chromium depends upon its valence. Trivalent chromium, which is the nutritionally active form, has such a low order of toxicity that a wide margin of safety exists between amounts ordinarily ingested and those that induce deleterious effects. The National Academy of Sciences Committee on Chromium stated: "Compounds of chromium in the trivalent state have no established toxicity. When taken by mouth, they do not give rise to local or systemic effects and are poorly absorbed. No specific effects are known to result from inhalation. In contact with the skin, they combine with proteins in the superficial layers, but do not cause ulceration" (National Academy of Sciences, 1974).

Hexavalent chromium is much more toxic than trivalent chromium, but has no nutritional value. Hexavalent chromium may be absorbed by ingestion, through the skin, and by inhalation. Generally, hexavalent chromium compounds cause irritation and corrosion. Signs of toxicity by these compounds include hemorrhage of the gastrointestinal tract after ingestion, ulceration of the nasal septum and cancer of the respiratory tract from inhalation, and cutaneous injury (ulceration and both eczematous and noneczematous contact dermatitis) upon dermal exposure (National Academy of Sciences, 1974).

Trivalent chromium also provokes allergic skin responses in chromium-sensitive subjects. However, a concentration of trivalent test material greater than that of hexavalent chromium is needed because the trivalent forms are less soluble and less absorbable. Hexavalent chromium is reduced within the skin to the trivalent state. Methionine, cystine, and cysteine can bring about this reduction (Samitz and Katz, 1963). The National Academy of Sciences Committee on Medical and Biologic Effects of Environmental Pollutants (MBEEP) suggested that the trivalent form resulting from the reduction may form haptene-protein complexes thereby initiating sensitization (National Academy of Sciences, 1974).

Hexavalent concentrations of 50 µg chromium per gram of diet have been associated with growth depression and liver and kidney damage in

laboratory animals (Mackenzie *et al.,* 1958). Symptoms of excessive dietary intake of chromium in humans are unknown. Thus, if the most toxic form of chromium (hexavalent) is used and if the minimum toxic dose is assumed to be near 50 µg/g of diet, the ratio of the minimum toxic dose and the minimum RDA for humans is approximately 1,000. This ratio would be much higher if the minimum toxic dose of trivalent chromium were known.

Interactions

Perhaps the best known interaction between chromium and another trace element is that with vanadium. Wright (1968) found that high dietary chromium (500-2,000 µg/g) was effective in overcoming growth depression and mortality in rats that had been fed 20 mg of vanadate per kilogram of diet. On the other hand, Hunt and Nielsen (1979) reported that 500 µg of chromium (as the acetate) per gram of diet caused relatively small amounts of vanadium (5 µg/g of diet) to be toxic for the chick.

Bioavailability

The mechanisms through which chromium is absorbed from the gastrointestinal tract are poorly understood. It is known that the bioavailability of chromium is dependent upon its chemical form. Biologically active chromium (i.e., as GTF) is quite available. For example, the absorption in the rat of chromium extracted from Brewer's yeast (a good source of GTF) ranged from 10% to 25% of the given dose (Mertz, 1976). On the other hand, the absorption of trivalent inorganic chromium is presumed to be 1% or less (Doisy *et al.,* 1976). However, Polansky and Anderson (1978) reported that rats absorbed from 5% to 10% of radioactive trivalent chromium (as the chloride) within 5 min after it was administered by stomach intubation. The chromium retained by the rats decreased from 5% to 10% at 5 min to less than 1% at 1 hr. Apparently, most of the quickly absorbed chromium was lost via the gastrointestinal tract. Their data indicated that the absorption of trivalent chromium is approximately 10-fold higher than previously presumed, that it occurs within minutes of administration, and that it is retained for less than 1 hr. Contrary to current thinking, their data suggest that urine may not be the most important excretory pathway for all absorbed chromium.

Contribution of Water to Chromium Nutrition

Table V-29 shows that typical drinking water sources would contribute anywhere from negligible amounts to 224 µg of chromium daily if 2 liters of water were consumed. The average contribution would probably be near 17 µg of chromium daily, which would be approximately 27% of that derived from the usual food sources or 22% of the total intake from food and liquids. In some instances, the contribution of drinking water to the daily intake of chromium may be greater than the contribution of the diet. For example, if the drinking water contained 50 µg of chromium per liter, the Environmental Protection Agency (1975) maximum contaminant level for drinking water, it would contribute almost twice as much chromium as does food in a typical diet.

Conclusions and Recommendations

Chromium is an essential nutrient for humans. Based on average concentrations in food, water, and air, drinking water could contribute a substantial proportion of the daily chromium intake. Unfortunately, drinking water probably contains the inorganic form of chromium. This form has poor bioavailability and retention. Nonetheless, any amount that drinking water contributes to the daily intake of chromium may be beneficial because chromium intake via the diet may not always meet the estimated adequate and safe intake for humans. Moreover, evidence suggests that chromium deficiency may be a problem in the United States.

The subcommittee recommends that regulations governing the presence of chromium in drinking water distinguish between the nutritionally useful trivalent and the more toxic hexavalent forms.

SUMMARY

Table V-30 shows the recommended dietary allowances (RDA's) for the various nutrients discussed in this report. Table V-31 shows estimated adequate and safe intakes for nutrients for which a RDA has not been established.

Table V-32 shows the contribution that drinking water can make to the requirement for or the intake of these nutrients. These calculations are generally based on concentrations of elements in water from large water supply systems. A sizeable portion of the population (16.7%) is served by systems supplying less than 25 persons. The minerals in water

TABLE V-30 Recommended Dietary Allowances (RDA's)[a]

			RDA, mg/day					
Group	Age, yr	Weight, kg	Calcium	Magnesium	Phosphorus	Iodine	Iron	Zinc
Infants	0.0-0.5	6	360	60	240	0.035	10	3
	0.5-1.0	9	540	70	400	0.045	15	5
Children	1-3	13	800	150	800	0.060	15	10
	4-6	20	800	200	800	0.080	10	10
	7-10	30	800	250	800	0.110	10	10
Males	11-14	44	1,200	350	1,200	0.130	18	15
	15-18	61	1,200	400	1,200	0.150	18	15
	19-22	67	800	350	800	0.140	10	15
	23-50	70	800	350	800	0.130	10	15
	51+	70	800	350	800	0.110	10	15

			RDA, mg/day					
Group	Age, yr	Weight, kg	Calcium	Magnesium	Phosphorus	Iodine	Iron	Zinc
Females, not pregnant	11-18	44-54	1,200	300	1,200	0.115	18	15
	19-50	58	800	300	800	0.100	18	15
	51 +	58	800	300	800	0.080	10	15
Females, pregnant			1,200	450	1,200	0.125	18+[b]	20
Females, lactating			1,200	450	1,200	0.150	18	25

[a] From National Academy of Sciences, 1974. The allowances are intended to provide for individual variations among most normal persons as they live in the United States under usual environmental stresses. Diets should be based on a variety of common foods to provide other nutrients for which human requirements have been less well defined.

[b] This increased requirement cannot be met by ordinary diets; therefore, the use of supplemental iron is recommended.

TABLE V-31 Estimated Adequate and Safe Intakes[a]

		Estimated Adequate and Safe Intakes, mg/day							
Group	Age, yr	Fluoride	Sodium	Potassium	Copper	Selenium	Manganese	Molybdenum	Chromium
Infants	0-0.5	0.1-0.5	115-345	350-925	0.5-0.7	0.1-0.04	0.5-0.7	0.03-0.06	0.01-0.04
	0.5-1	0.2-1.0	250-750	425-1,275	0.7-1.0	0.02-0.06	0.7-1.0	0.04-0.08	0.02-0.06
Children	1-3	0.5-1.0	325-975	550-1,650	1.0-1.5	0.02-0.08	1.0-1.5	0.05-0. 1	0.02-0.08
	4-6	1.0-2.5	450-1,350	775-2,350	1.5-2.0	0.03-0.12	1.5-2.0	0.06-0.15	0.03-0.12
	7-10	1.5-2.5	600-1,800	1,000-3,000	2.0-2.5	0.05-0.2	2.0-3.0	0.1-0.3	0.05-0.2
	11+	1.5-2.5	900-2,700	1,525-4,575	2.0-3.0	0.05-0.2	2.5-5.0	0.15-0.5	0.05-0.2
Adults		1.5-4.0	1,100-3,300	1,875-5,600	2.0-3.0	0.05-0.2	2.5-5.0	0.15-0.5	0.05-0.2

[a] From National Academy of Sciences, 1980. Any daily intake within the ranges recommended in this table is considered adequate and safe. The recommendations do not imply that intakes at the upper limit of the range are more desirable or beneficial than those at the lower limit.

from such systems may exceed the concentrations used in these calculations, but extensive information on such systems is not yet available.

CONCLUSIONS

At their typical levels in drinking water, the nutrients reviewed in this chapter usually make a small, but by no means negligible, contribution to the mineral nutrition of humans.

When the intake of a particular nutrient by the general population or a particular group is marginal, the contribution by water may be important in preventing deficiency and ill health. This may be the case for magnesium, fluoride, iron, copper, zinc, vanadium, and chromium.

For the overwhelming majority of the nutrients studied, the risk of toxicity to normal individuals from typical levels in drinking water is negligible. When the level of a nutrient in drinking water is typical and reduction of total intake is prudent (e.g., for iodine or for sodium), reduction from sources other than water would seem to be the option by which the largest initial reduction could be made.

The accurate assessment of the contribution of water to nutrition is hampered by the lack of information on the speciation and bioavailability of elements, particularly in drinking water. Additionally, water treatment practices, e.g., addition of phosphates for corrosion control, may alter nutrient composition of water, as might the use of chelators in food preparation.

RESEARCH RECOMMENDATIONS

The speciation of essential elements in drinking water and food and their respective bioavailabilities should be investigated.

Information on the mineral composition of water to be collected in the U.S. Environmental Protection Agency's National Statistical Assessment of Rural Water Conditions should be evaluated to determine if mineral levels in such waters and the contributions of the water to nutrition differ greatly from those reviewed above.

A review of water treatment and distribution practices is desirable to define the ways in which such practices contribute to or alter the nutrient composition of water.

Suggested changes in water treatment practice, e.g., the introduction of granular activated carbon treatment, should be evaluated on a pilot-plant scale to determine how they would affect the nutrient composition of drinking water.

TABLE V-32 Contribution of U.S. Drinking Water to Mineral Nutrition of Humans

Line No.	Nutrient	Body Content, mg[a]	Level in water, mg/liter		Requirements, mg/day[d]		
			Typical[b]	Maximum[c]	RDA	Adequate and Safe Intake	Calculated[e]
1	Calcium	1,000,000	26	145	800		
2	Magnesium	19,000	6.25	120	300-400		
3	Phosphorus	780,000	Undetectable	3.0	800		
4	Fluoride	2,600	0.4	7.0	See text[g]		
5	Sodium	80,000	28	220		1,100-3,300	
6	Potassium	145,000	2.15	8.3		1,875-5,600	
7	Chloride	95,000	21	179	See National Academy of Sciences, 1980, p. 176		
8	Iodine	20	0.004	0.018	0.14 ♂ 0.10 ♀		
9	Iron	4,000-5,000	0.240	2.20	10 ♂ 18 ♀		
10	Copper	80	0.100	0.450		2-3	
11	Zinc	1,400-2,300	<0.200	1.50	15		
12	Selenium	14.6	0.001	0.0066		0.050-0.200	
13	Manganese	20	0.025	1.32		2.5-5.0	
14	Arsenic	3.5	<0.0024[i]	(MCL=0.050)[j]			0.025-0.050
15	Nickel	10.0	0.005	0.075			0.050
16	Vanadium	0.10	0.004	0.070			0.025
17	Molybdenum	9.3	<0.002[i]	0.068		0.15-0.50	
18	Silicon	NRD[k]	7.1	72	No recommendation exists		
19	Chromium	NRD	0.0085	0.112(>MCL of 0.050)		0.05-0.20	

[a] Values are based on a 70-kg adult. Sources are referenced in the text or data are from Masironi (1978) or the International Commission on Radiological Protection (1975).

[b] This level is considered by the committee to be a realistic assessment of a "typical" level. It is not derived by any particular statistical method because water composition data are often reported in such a manner that it is difficult to apply such methods to them.

[c] This is the highest level observed in the studies cited in this report but should not be taken as the maximum level that might occur.

[d] The values given are for young adults. As can be seen from Tables V-30 and V-31, recommendations vary with age and reproductive status. RDA's (recommended daily allowances) were taken from National Academy of Sciences (1974). Adequate and safe intake values were taken from National Academy of Sciences (1980).

[e] This value was derived by extrapolating from animal studies. It was done merely for illustrative purposes and should not be taken as a recommendation.

Line No.	Typical Intake (F, Food; T, Total), mg/day	Contribution from Water, %				Comments
		To Requirement		To Intake[f]		
		Typical	Maximum[c]	Typical	Maximum[c]	
1	500-1,000 (F)	6.5	36	5-10	29-58	
2	240-480 (F)	3-4	60-80	2.4-4.8	33-50	
3	1,500 (F)	Negligible	Negligible	Negligible	Negligible	
4		See text[g]		See text[g]		
5	1,600-9,600 (T)	1.7-5.0	13.3-40	0.6-3.4	4-27	Intake generally too high
6	1,500-6,000 (T)	Negligible	Negligible	Negligible	Negligible	Low intake by the elderly
7	2,400-14,000 (T)	Requirement not clear		0.3-2	2.5-15	
8	0.240-0.740 (F)	8 ♀ : 6 ♂	19-44	1-3	5-15	Intake generally high
9	19.0 (F)	<3 ♀ : 5 ♂	24 ♀ : 44 ♂	2.5	23	Bioavailability important
10	1.5 (F)	6-10	30-45	12	38	
11	12.0 (F)	2.7	20	3.1	20	
12	0.150 (T)[h]	Negligible	7-26[h]	Negligible	8[h]	MCL = 0.01 mg/ liter
13	1-6.4 (F)	1-2	50-100	<1-5	29-72	
14	0.0114 (T)	<10-19	See footnote[j]	<30	See footnote[j]	
15	0.165-0.500 (F)	20	100	2-6	23-50	
16	0.02 (F)	32	100	29	Near 90	
17	0.100 (F)	<0.8-2.7	27-91	<3.8	57	
18	NRD	Requirement not clear		NRD	NRD	
19	0.062 (F)	8.5-34	50-100 at MCL	22	62 at MCL	Valence form important

[f] The percent contribution from water is calculated as a proportion of the total intake from both food and water.

[g] Major benefit from correct fluoride intake accrues to children. This is discussed more fully in the text.

[h] Intake varies regionally and in different countries. See section of text on selenium.

[i] The typical level is probably much lower than this value since many of the samples tested did not contain detectable levels of the element and were not included in this average.

[j] When higher concentrations were observed, e.g., 2.0 mg/liter in water from some deep wells (see section on arsenic), the water was blended with water containing less arsenic in such a way that the maximum contaminant level (MCL) of 0.05 mg/liter was not violated. Arsenic intake from 2 liters of water/day containing the MCL would be far greater than that from food.

[k] NRD, no reliable data.

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Appendix

1977 Amendment to Safe Drinking Water Act

Appendix A in the 1977 National Academy of Sciences publication *Drinking Water and Health* (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 (PL93-523).

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 two (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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