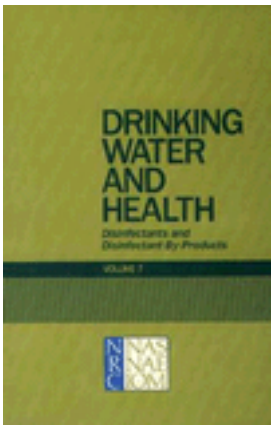

Drinking Water and Health, Volume 7 Disinfectants and Disinfectant By-Products

Safe Drinking Water Committee, National Research Council

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Drinking Water and Health

Disinfectants and Disinfectant By-Products
Volume 7

Subcommittee on Disinfectants and
Disinfectant By-Products
Safe Drinking Water Committee
Board on Environmental Studies
and Toxicology
Commission on 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

The Safe Drinking Water Act of 1974 (PL 93-523) mandated that the U.S. Environmental Protection Agency (EPA) establish federal standards to protect humans from harmful contaminants in drinking water. This law authorized EPA to seek the expertise of a National Research Council committee to identify the health effects associated with specific contaminants, identify areas of insufficient knowledge, and to make recommendations for future research. Since 1977, committees of the National Research Council have issued six volumes of *Drinking Water and Health*, each of which includes a review of toxicological data and estimates of the risks associated with specific contaminants found in drinking water.

The most recently constituted Safe Drinking Water Committee directed the Subcommittee on Disinfectants and Disinfectant By-Products to conduct the study reported in this seventh volume of the series. At the request of EPA, the subcommittee examined current practices of water disinfection and assessed the human health effects and animal toxicological data for several currently used disinfectants and disinfectant by-products. This volume updates material published in Volume 2 on the chemistry and efficacy of disinfectants and in Volume 3 on their toxicity and the toxicity of the by-products formed. In addition, the volume contains evaluations of several epidemiological studies relating to drinking water disinfection and provides new risk assessments for several by-products. The findings of this study are briefly summarized in the Executive Summary.

To help in the preparation of this volume, subcommittee members attended the Second International Symposium on Health Effects of Drinking Water Disinfectants and Disinfection By-Products, convened by EPA

in Cincinnati, Ohio, on August 27-29, 1985. In addition to an intensive literature search, the subcommittee used EPA data summaries for each substance as a further indication of the range of available toxicological data. Many of the data resulted from 2-year chronic feeding studies in rodents, reflecting past interest in carcinogenesis testing. However, the subcommittee carefully examined toxicological data on teratogenesis, mutagenesis, reproductive effects, metabolism, and neurological effects as well.

The data summaries, symposium papers, and published toxicological literature served as the basis for a subcommittee workshop on disinfectants and disinfectant by-products held in October 1985. Whenever possible, the subcommittee evaluated published, peer-reviewed literature pertaining to the substances under study. For several compounds, important new information was made available by researchers of current projects. When unpublished information was provided, the subcommittee conducted its own peer review of the unpublished studies and in some cases subjected the data to additional independent review.

The principal goal of disinfecting water supplies is the elimination of pathogens that are responsible for waterborne diseases. Chlorination is a very successful method for achieving this goal in the United States. Nonetheless, the formation of trihalomethanes (THMs) and other chlorination by-products has prompted the introduction of other disinfection techniques. Chlorination and other major methods of disinfection are examined individually. Their chemical characteristics and biocidal efficacy are assessed and compared. Economic considerations were not part of this study.

Richard Thomas was project director for this volume, and Leslye Wakefield served as research associate. Project editor was Jacqueline Boraks, and Barbara Ream was bibliographer. Tracy Brandt and Mireille Mesias typed the manuscript. The subcommittee extends special thanks to its consultants Keith Jacobson, James Reisa, and Henry Wills, without whose technical support this volume could not have been completed. We are also grateful for the contribution of workshop papers and advice from Mirat Gürol, John Hoff, Vincent Olivieri, and Sally Zierler. Kulbir Bakshi, Ruth Hodges, Alison Kamat, Victor Miller, and Edna Paulson, staff of the Board on Environmental Studies and Toxicology, assisted in research. Devra Davis and Alvin Lazen provided helpful advice and guidance.

J. DONALD JOHNSON, CHAIRMAN

SUBCOMMITTEE ON DISINFECTANTS AND DISINFECTANT BY-PRODUCTS

SAFE DRINKING WATER COMMITTEE

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Drinking Water and Health

1

Executive Summary

New knowledge about disinfection and disinfectant by-products has led to changes in procedures for disinfecting drinking water in the 6 years since the Safe Drinking Water Committee last reviewed the potential health effects of these practices (Volumes 2 and 3 of *Drinking Water and Health*). This report, prepared by the Safe Drinking Water Committee's Subcommittee on Disinfectants and Disinfectant By-Products, examines these innovations and assesses their implications for human health.

The predominant method of drinking water disinfection practiced in the United States today is chlorination. Studies of the toxicity of the by-products of disinfectants have focused on the trihalomethanes (THMs), which are formed during chlorination and for which considerable data on carcinogenicity have been developed. The level of total THMs in finished drinking water, currently regulated at 100 micrograms (μg) per liter, should be reduced. Noting that chloroform is the principal THM produced by chlorination, the subcommittee found this level to be unsupportable on the basis of the risk values for chloroform developed in this review.

Other, nonvolatile by-products of chlorination may be important in contributing mutagenic properties to drinking water, especially when the natural water being treated contains high levels of organic matter. Short-term animal skin tests, although not conclusive, provide indications that organic concentrates from chlorinated water are tumorigenic under some experimental conditions. Studies by routes other than dermal application have not shown such an effect. The subcommittee has developed risk assessments and recommended SNARLs for some of these by-products based on the available data.

Unfortunately, many by-products of chlorination and other disinfection practices have not been identified. Consequently, the risks of ingesting these by-products cannot be quantified at present, but are potentially high enough to warrant continued efforts to analyze them. Further studies of reaction mechanisms, controlling factors, and by-product identification are needed. Improved methods for characterizing the nonvolatile products are also needed to support such studies. Methods should be sought to follow the risk associated with multiple chlorination by-products even in the absence of individually quantifiable compound risks. The fact that THM levels may also indicate the presence of unidentified by-products of chlorination is further reason to reduce the total THMs in finished drinking water whenever possible.

The use of alternative methods of drinking water disinfection is increasing, largely due to health and regulatory concerns about trihalomethanes. Thus, the nature and toxicity of the by-products of some other widely used water treatments (chloramination, ozonation, and chlorine dioxide) are also evaluated in the report to the extent allowed by available data. Research is also needed to improve understanding of their relative efficacy in eliminating the currently most resistant viruses and protozoan cysts and the major factors affecting such efficacy under treatment plant operating conditions. To prevent overestimation of the degree of disinfection achieved by alternative practices (especially chloramination, which is becoming widely used), methods must be developed for fully quantifying both organic nitrogen precursors of toxic by-products and an organic chloramine fraction in the presence of inorganic monochloramine. Recognizing the paucity of information on these alternative practices, the subcommittee urges that the direct and indirect implications of their potential widespread use be investigated more thoroughly.

A major health concern is the chronic ingestion of low levels of disinfection by-products. In some epidemiological studies of the effects of chlorination, investigators have found increased rates of bladder cancer associated with trends in the levels of certain contaminants in water supplies. Interpretation of these studies is hampered by a lack of control for confounding variables (e.g., age, sex, individual health, smoking history, other exposures). Nevertheless, the subcommittee recommended that epidemiologists continue to improve protocols and conduct such studies, particularly of drinking water and bladder cancer, wherever exposure data can be obtained directly from individuals rather than by estimation from exposure models.

Humans may also be exposed to disinfectants and their by-products from sources other than drinking water and routes other than ingestion. For example, cooking, showers, bathing, swimming, and other activities could provide additional toxic exposures through inhalation or skin absorption.

Given the absence of data on these noningestion routes of exposure and the lack of methods for estimating the magnitude of such exposures, the subcommittee declined to include them in its risk estimates. Recognizing their potential for producing toxic effects, however, it recommends that methods be developed for estimating both noningestion exposures to contaminants in drinking water and exposures to the same contaminants in other media, such as food, air, and dust, so that total exposure can be considered by regulators in setting acceptable levels of contaminants in water.

The subcommittee calculated quantitative risk assessment for disinfectants or their by-products when there were sufficient data. These assessments include four distinct components: hazard identification, exposure assessment, dose-response assessment, and characterization of human risk at projected levels and patterns of exposure. The first six volumes of this series provided such assessments for approximately 220 drinking water contaminants. Some of those were disinfectants that are reevaluated in this volume along with other compounds not reviewed previously.

2

Disinfection Methods and Efficacy

CURRENT PRACTICES

More than 1.5 billion people in developing nations are still without safe drinking water. Waterborne diseases such as typhoid, cholera, dysentery, amebiasis, salmonellosis, shigellosis, and hepatitis A are still estimated to be responsible for the deaths of more than 30,000 people daily (IRC, 1984). In that context, the United Nations General Assembly has declared 1981-1990 as the International Drinking Water Supply and Sanitation Decade (WHO, 1984).

In the last century, major outbreaks of waterborne diseases also occurred in the United States and other affluent nations. Cholera and dysentery were rampant in the 1800s, and typhoid fewer was responsible for about 25,000 deaths in the United States as late as 1900 (Akin et al., 1982).

Current drinking water disinfection practices in the United States provide the means to control most pathogenic bacteria, viruses, helminths, and protozoa responsible for the major waterborne diseases. Some outbreaks still occur in this country (Figure 2-1) owing to continuing problems involving consumption of untreated water, errors of insufficient or interrupted disinfection, failures to maintain adequate levels of residual disinfectant in potable water distribution systems, and/or breaches in the systems (Akin et al., 1982). Moreover, as discussed later in this chapter, the etiology of waterborne disease has changed dramatically since the early 1900s; most outbreaks in recent years have been caused by viruses and protozoan cysts that are generally more resistant to disinfection than are pathogenic bacteria, the primary targets of concern in past decades.

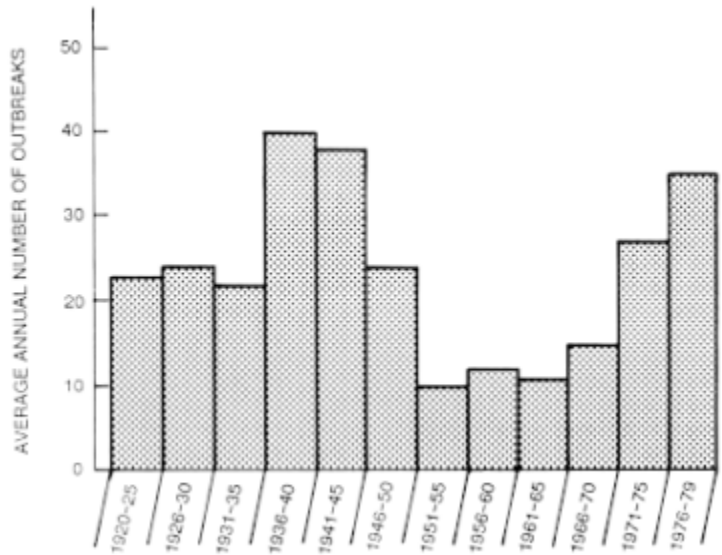

Figure 2-1 Average annual number of waterborne disease outbreaks occurring in the United States from 1920 through 1979. From Akin et al. (1982), with permission.

Regardless of the method employed, disinfection is only one of the requirements of a potable water supply system. Disinfection requirements and efficacy are often highly interrelated with other water supply and treatment operations. A complete system of potable water supply operations may be considered in three general phases: collection, treatment, and distribution.

These operations and the principal disinfection practices are briefly discussed below. The historical development of potable water treatment and more detailed aspects of disinfection have been reviewed in previous volumes of *Drinking Water and Health* (NRC, 1977, 1980a, b).

Collection

Surface and groundwater sources of potable water vary locally in terms of their physical, chemical, particulate, biological, and aesthetic characteristics. Each characteristic may be an important factor in water supply operations, including disinfection. Water quantity, temperature, pH, suspended particulates, solid aggregates, dissolved inorganic constituents (e.g., hardness, ferrous ions, nitrites, and ammonia), nonparticulate organic

constituents (e.g., fulvic and humic acids), microbiota (e.g., bacteria, viruses, protozoa, helminths, and algae), and taste, odor, or color problems, both natural and anthropogenic, may cause treatment practices appropriate for one set of conditions to be inappropriate for others.

Treatment

Besides disinfection, drinking water treatment practices at a given facility may include coagulation, flocculation, settling, and filtration to remove suspended particles; stripping and chemical oxidation to reduce objectionable taste, odor, or color; and precipitation, softening, pH control, or other operations designed to produce safe and aesthetically acceptable finished water from a raw water source, reliably and cost effectively. More than 1.2 million tons of about 60 bulk chemicals were used for potable water treatment in the United States in 1981; chemicals used for disinfection and oxidation amounted to about 42% of that total (Rehwoldt, 1982).

The biocidal efficacy of a chemical or physical disinfectant can depend on the method of application as well as the methods and staging of other treatment practices. Thorough mixing is important to ensure uniform dispersal and exposure of pathogens to the disinfectant. Pretreatment is often important to minimize solid particles and aggregates that would shield pathogens from the disinfectant. If the disinfectant is chemical, pretreatment such as sedimentation of suspended matter, coagulation with alum, or filtration may also be needed to reduce potential reactants that would, in effect, consume a disinfectant, thereby reducing the biocidal efficacy of a given dosage.

Distribution

A drinking water distribution system is more than a means of transporting finished water to the tap. It also acts as a storage system and a potential source of inorganic, organic, and biological contamination that must be considered in the design and operation of a potable water supply system. The distribution system imposes a requirement that adequate postdisinfection residuals continue biocidal activity.

CHLORINATION

Chlorination has been the predominant method of drinking water disinfection in the United States for more than 70 years. When concerns about the formation of trihalomethanes and other halogenated hydrocarbon by-products began to stimulate the reexamination of chlorination practices

in the early 1970s, chlorine was being used to disinfect about 95% of the potable water supplied in the United States (Morris, 1971).

Chlorine, a strong oxidizing and disinfecting agent, is an effective microbiocide against most waterborne pathogens. It is inexpensive and relatively convenient to produce, store, transport, and use. Nonetheless, because it is a gas at room temperature it can present safety problems, especially during transportation and storage. Its high solubility in water makes it easy to apply in controlled amounts either as chlorine gas, which readily dissolves in water at room temperature, or as a salt of hypochlorite, which is formed by the reaction of chlorine and water as follows:

$$Cl_2 + H_2O \rightleftharpoons HOCl + H^+ + Cl^-$$

$$HOCl + H_2O \rightleftharpoons H^+ + OCl^-$$

During chlorination, the relative concentrations of the hypochlorous acid (HOCl) and hypochlorite ions (OCl^-), together termed "free chlorine," are determined mainly by measurement of pH. HOCl, a more effective biocide than OCl^-, dissociates into OCl^- between a pH of 7.0 and 8.0, the range in which most potable water undergoes treatment (Figure 2-2).

Inorganic and organic molecules, suspended particles, and microbiota in raw water produce what is termed "chlorine demand," because they react with and consume free chlorine, requiring a higher dose of additional chlorine for equivalent biocidal activity. Addition of chlorine beyond the chlorine-demand "breakpoint" produces a free-chlorine residual, which, together with time of exposure, forms the practical basis for determining required amounts of disinfectant.

Sedimentation, coagulation, filtration, aeration, or any other practices that remove chlorine-demanding substances before chlorination reduce the amount of chlorine required to produce equivalent disinfection. Such practices may also remove humic acids and other organic precursors before chlorination, thereby reducing the formation of trihalomethanes and other by-products of concern (NRC, 1977).

Postdisinfection biocidal activity persists in a chlorinated drinking water distribution system. This residual activity, an important advantage of chlorination, is primarily due to the reaction of hypochlorous acid with ammonia and amines in raw water to form chloramines, which are less effective as biocides but persist longer than chlorine.

The mechanism of chlorine's highly effective biocidal action against indicator bacteria appears to involve alteration of cell membrane permeability and disruption of enzymatic reactions within the cell (NRC, 1980a). The relative efficacies of HOCl, OCl^-, and chloramines against bacteria, viruses, and protozoan cysts, compared with those of several

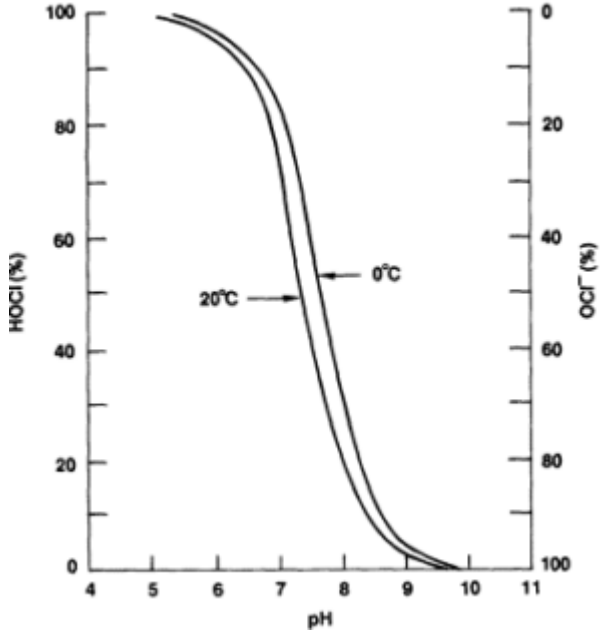

Figure 2-2 Effect of pH on quantities of hypochlorous acid (HOCl) and hypochlorite ion (OCl^-) that are present in water (NRC, 1980a).

alternative disinfectants (see next section), are summarized in Tables 2-1 and 2-2.

ALTERNATIVE METHODS

The suitability of any method for drinking water disinfection can be evaluated on the basis of its efficacy against waterborne pathogens, the accuracy with which it can be monitored and controlled, its ability to provide the necessary residual biocidal activity in the distribution system, the aesthetic quality of the treated water, the applicability of the technology to large-scale operations, and the formation of toxic by-products (NRC, 1977, 1980a, b). Cost may also be a factor, although the costs of several alternative disinfection methods compared by Clark (1981) did not vary by more than threefold to fourfold. Also relevant are the comparative hazards of production, use, transport, disposal, and cleanup.

Restricting itself to toxicological and technological criteria, the Safe Drinking Water Committee has previously (NRC, 1980a) judged three of

the many possible alternatives to chlorine to be suitable for primary or secondary drinking water disinfection: ozone, chlorine dioxide, and chloramines (Table 2-3).

Ozone

Ozone (O_3) is a strong oxidizing gas that reacts rapidly with most organic (and many inorganic) molecules. Its short half-life in water, approximately 10 to 30 minutes in practical treatment applications, requires ozone to be generated on-site for use as a disinfectant. Ozone does not produce a disinfecting residual, so a second disinfectant must usually be added to the treated water to furnish the necessary protection in the distribution system.

Ozone is used as the primary disinfectant in many drinking water treatment plants, mostly in Europe and Canada. Small-scale applications have been limited in the past owing to maintenance and repair requirements for a reliable power source; but the large-scale technology is well established, and both the reliability and efficiency of ozone technology are improving rapidly. A typical ozone disinfection system consists of modular solid-state generators, air predrying equipment (necessary to produce ozone efficiently), and contactors designed to produce good mixing with the water being treated.

Ozone is an efficient biocide that appears to attack the double bonds of fatty acids in bacterial cell walls and the protein capsid of viruses (NRC, 1980a). Its overall efficacy against waterborne pathogens is summarized in Table 2-1.

Chlorine Dioxide

Chlorine dioxide (ClO_2) is used mainly as an industrial bleaching agent for wood pulp, textiles, flour, fats, oils, and waxes, but it has been widely used at drinking water treatment plants for taste, odor, and algal control; iron and manganese removal; and (mainly in Europe) disinfection. Since ClO_2 is unstable; sensitive to temperature, pressure, and light; and explosive in air at concentrations of about 4% or more, it is usually generated and used on-site to avoid problems of bulk storage and distribution.

ClO_2 is highly effective as a biocide against bacteria and viruses under the temperature, pH, and turbidity conditions of drinking water treatment (Table 2-1).

Chloramination

Although chloramines are less effective than free chlorine as biocides (Table 2-1), they are more persistent and do not react to form trihalomethanes.

TABLE 2-1 Summary of Major Possible Disinfection Methods for Drinking Watera

		Efficacy in Demand-Free Systems[b]			
Disinfection Agent[c]	Technological Status	Bacteria	Viruses	Protozoan Cysts	Persistence of Residual in Distribution System
Chlorine[d]	Widespread use in U.S. drinking water				Good
As hypochlorous acid (HOCl)		++++	+++++	++	
As hypochlorite ion (OCl-)		+++	++	NDR[e]	
Ozone[d]	Widespread use in drinking water outside United States, particularly in France, Switzerland, and the province of Quebec	++++	++++	++++	No residual possible
Chlorine dioxide[d]	Widespread use for disinfection (both primary and for distribution system residual) in Europe, limited use in United States to counteract taste and odor problems and to disinfect drinking water	++++	++++	NDR[e]	Fair to good (but possible health effects)

		Efficacy in Demand-Free Systems[b]			Persistence of Residual in Distribution System
Disinfection Agent[c]	Technological Status	Bacteria	Viruses	Protozoan Cysts	
Iodine	No reports of large-scale use in drinking water				Good (but possible health effects)
As diatomic iodine (I2)		+ + + +	+ + +	+ + +	
As hypoiodous acid (HOI)		+ + + +	+ + + +	+	
Bromine	Limited use for disinfection of drinking water	+ + + +[f]	+ + + +[f]	+ + +[f]	Fair
Chloramines	Limited present use on a large scale in U.S. drinking water	+ +	+	+	Excellent

[a] Data from NRC (1980a), pp. 114-115.

[b] Ratings: + + + +, excellent biocidal activity; + + +, biocidal activity; + +, moderate biocidal activity; +, low biocidal activity; ±, of little or questionable value.

[c] The sequence in which these agents are listed does not constitute a ranking.

[d] By-product production and disinfection demand are reduced by removal of organics from raw water before disinfection.

[e] Either no data reported or only available data were not free from confounding factors, thus rendering them not comparable to other data.

[f] Poor in the presence of organic material.

TABLE 2-2 Comparative Efficacy of Disinfectants in the Inactivation of 99% of Microorganisms in Demand-Free Systemsa

	Escherichia coli			Poliovirus 1			*Entamoeba histolytica* Cysts		
		Temperature,			Temperature,			Temperature,	
Disinfection Agent	pH	°C	$C \cdot t$[b]	pH	°C	$C \cdot t$[b]	pH	°C	$C \cdot t$[b]
Hypochlorous acid	6.0	5	0.04	6.0	0	1.0	7	30	20
				6.0	5	2.0			
				7.0	0	1.0			
Hypochlorite ion	10.0	5	0.92	10.5	5	10.5		[c]	
Ozone	6.0	11	0.031	7.0	20	0.005	7.5-8.0	19	1.5[d]
	7.0	12	0.002	7.0	25	0.42			
Chlorine dioxide	6.5	20	0.18	7.0	15	1.32			
	6.5	15	0.38	7.0	25	1.90		[c]	
	7.0	25	0.28						
Iodine	6.5	20-25	0.38	7.0	26	30	7.0	30	80
	7.5	20-25	0.40						
Bromine		[c]		7.0	20	0.06	7.0	30	18
Chloramines									
Monochloramine	9.0	15	64	9.0	15	900		[c]	
	9.0	25	40	9.0	25	320			
Dichloramine	4.5	15	5.5	4.5	15	5,000		[c]	

[a] From NRC (1980a), p. 117.

[b] Concentration of disinfectant (mg/liter) times contact time (min).

[c] Either no data were reported or only available data were not free from confounding factors, thus rendering them not comparable to other data.

[d] This value was derived primarily from experiments that were conducted with tap water; however, some parallel studies with distilled water showed essentially no differences in inactivation rates.

TABLE 2-3 Status of Possible Methods of Drinking Water Disinfection[a]

Disinfection Agent	Suitability as Inactivating Agent	Limitations	Suitability for Drinking Water Disinfection[b]
Chlorine	Yes	Efficacy decreases with increasing pH; affected by ammonia or organic nitrogen	Yes
Ozone	Yes	On-site generation required; no residual; other disinfectant needed for residual	Yes
Chlorine dioxide	Yes	On-site generation required; interim MCL 1.0 mg/liter	Yes
Iodine	Yes	Biocidal activity sensitive to pH	No
Bromine	Yes	Lack of technological experience; activity may be pH sensitive	No
Chloramines	No	Mediocre bactericide; poor virucide	No[c]
Ferrate	Yes	Moderate bactericide; good virucide; residual unstable; lack of technological experience	No
High pH conditions	No	Poor biocide	No
Hydrogen peroxide	No	Poor biocide	No
Ionizing radiation	Yes	Lack of technological experience	No
Potassium permanganate	No	Poor biocide	No
Silver	No	Poor biocide; MCL 0.05 mg/liter	No
UV light	Yes	Adequate biocide; no residual; use limited by equipment maintenance considerations	No

[a]Data from NRC (1980a), p. 118.
[b]This evaluation relates solely to the suitability for controlling infectious disease transmission. See Conclusions.
[c]Chloramines may have use as a secondary disinfectant in the distribution system in view of their persistence.

Concerns about halogenated by-products of chlorination, and the maximum contaminant level (MCL) of 0.10 mg of total trihalomethanes per liter set by the Environmental Protection Agency under the Safe Drinking Water Act (EPA 1979, 1980), have caused treatment facilities in several states to increase or switch to chloramination (Hack, 1985).

Kansas now requires the use of ammonia to convert all the free-chlorine residual to chloramines following 30 minutes of chlorination. The Metropolitan Water District of Southern California has changed from chlorination to chloramination for distribution system disinfection. In contrast, several states continue to prohibit chloramination, as Kansas formerly did.

WATERBORNE PATHOGENS

Outbreaks of waterborne disease associated with drinking water from 1978 to 1984 are shown in Table 2-4. During this time, 261 outbreaks were observed, with almost 72,000 cases. The average annual number of outbreaks corresponded to 37, with more than 10,000 cases.

The etiology of disease found in drinking water has changed dramatically since the early 1900s. While the early diseases associated with drinking water were those with a bacterial etiology, the more recent outbreaks appear to be dominated by gastrointestinal illness associated with viruses and protozoa. The agents associated with the waterborne outbreaks for 1984 are shown in Table 2-5. The data in the table are dominated by acute gastrointestinal illness, which was responsible for nine outbreaks, with 426 cases. Despite the fact that no agent was identifiable in these episodes of waterborne illness, a significant percentage of these outbreaks is believed to be caused by Norwalk or Norwalk-like virus (Kaplan et al., 1982; Kappus et al., 1982; Taylor et al., 1981; Wilson et al., 1982). In 1983, three outbreaks with 164 cases were due to hepatitis A virus (CDC, 1984), while in 1984 only one outbreak with seven cases was reported

TABLE 2-4 Disease Associated with Drinking Water, 1978-1984[a]

	Number of Outbreaks According to Water Source				
Year	Community	Non-community	Private	Total	Number of Cases
1978	10	18	4	32	11,435
1979	23	14	4	41	9,720
1980	23	22	5	50	20,008
1981	14	16	2	32	4,430
1982	22	12	6	40	3,456
1983	29	6	5	40	20,905
1984	13	4	9	26	1,755
TOTAL	134	92	35	261	71,709
Average				37	10,244

[a] Data from CDC (1985).

TABLE 2-5 The Etiology of Disease Associated with Drinking Water in 1984[a]

Etiology	Outbreaks	Cases
Acute gastrointestinal illness	9	426
Giardia	6	879
Campylobacter	4	41
Chemical	3	30
Hepatitis A	1	7
Cryptosporidium	1	117
Norwalk virus	1	251
Entamoeba	1	4
TOTAL	26	1,755

[a] Data from CDC (1985).

for that virus (CDC, 1985). The majority of cases reported for 1984 were due to the flagellated protozoa, *Giardia lamblia*, which forms a cyst.

Giardia

During the past 5 years, a considerable body of information has been generated in laboratory-scale investigations on the inactivation of *Giardia* with drinking water disinfectants. Unless otherwise stated, the studies were conducted under carefully controlled conditions with stable disinfectant concentrations. Although some of the studies were conducted at higher temperatures, much of the disinfection research has been conducted at low water temperatures (≤5°C) because inactivation rates are slower at low temperatures and thus represent more difficult conditions for disinfectant efficiency. Low water temperatures would be typical for several months each year in many locations where giardiasis outbreaks have occurred. When possible, results are expressed as the disinfectant concentration and exposure time necessary for inactivation of 99% of the cysts or other microbial populations that are compared. The results are also expressed as $C \cdot t$, which is the disinfectant concentration C in milligrams/liter times the time t in minutes required to inactivate a given percentage of the population (e.g., 99%) under defined pH and temperature conditions.

Data from several investigations showing the comparative resistance of *Giardia* cysts and several other types of microorganisms to free chlorine in the form of hypochlorous acid are shown in Table 2-6. The $C \cdot t$ data indicate that the resistance of *Giardia* cysts is approximately 2 orders of magnitude higher than that of the enteroviruses, represented by poliovirus 1, and more than 3 orders of magnitude higher than the enteric bacteria,

TABLE 2-6 C · t Products for 99% Inactivation of Various Microorganisms by Free Chlorine at 5°C, pH 6.0

Microorganism	Chlorine Concentration (mg/liter)	Time (min)	$C \cdot t$	Reference
E. coli	0.1	0.4	0.4	Scarpino et al. (1972)
Poliovirus 1	1.0	1.7	1.7	Scarpino et al. (1972)
E. histolytica[a] cysts	5.0	18	90	Snow (1956)
G. lamblia[b] cysts	1.0	50	50	Jarroll et al. (1981)
	2.0	40	80	Jarroll et al. (1981)
	4.0	20	80	Jarroll et al. (1981)
	8.0	9	72	Jarroll et al. (1981)
G. lamblia[c] cysts	2.5	30	75	Rice et al. (1982)
G. lamblia[b] cysts	2.5	100	250[a]	Rice et al. (1982)
G. muris cysts	2.5	100	250[a]	Rice et al. (1982)

[a] Extrapolated data.
[b] Cysts from asymptomatic carriers.
[c] Cysts from symptomatic carriers.

represented by *Escherichia coli*. $C \cdot t$ results for *G. lamblia*, based on cysts from the same source using different chlorine concentrations and exposure times, are similar. The results also indicate that *G. lamblia* from different sources may vary in resistance and that *G. muris* cysts are similar in resistance to *G. lamblia* cysts.

Additional data showing the effects of temperature and pH on cyst inactivation by free chlorine are presented in Table 2-7 (*G. lamblia*) and Table 2-8 (*G. muris*). For cysts of both species, the general decrease in inactivation rates at lower temperatures is evident. The decrease in free-chlorine efficiency with increasing pH is due to the shift from the more effective hypochlorous acid form to the less effective hypochlorite form.

Table 2-9 presents data on inactivation of *G. muris* by chloramine. The results point up the lower disinfection efficiency of chloramine. The differences between free-and combined-chlorine efficiency appear to be greater at higher temperatures.

The literature on mechanisms of inactivation of other microorganisms by monochloramines is limited. Nusbaum (1952) proposed that the mechanism of bactericidal action of monochloramine is similar to that of hypochlorous acid; that is, the chloramine molecules enter the cytoplasm and interfere with enzymatic reactions. Ingold (1969) suggested that monochloramine

TABLE 2-7 C · t Products for 99% Inactivation of Giardia lamblia Cysts by Free Chlorine

		Range					
Temperature (°C)	pH	Disinfectant Concentration (mg/liter)	Time (min)	$C \cdot t$	Mean $C \cdot t$	Number of Experiments	Reference
5	6	2.5	20-100	75-250	162	2	Rice et al. (1982)
5	6	1.0-8.0	6-47	47-84	65	4	Jarroll et al. (1981)
5	7	2.0-8.0	7-42	56-152	97	3	Jarroll et al. (1981)
5	8	2.0-8.0	72-164	72-164	110	3	Jarroll et al. (1981)
15	6	2.5-3.0	7	18-21	20	2	Jarroll et al. (1981)
15	7	2.5-3.0	6-18	18-45	32	2	Jarroll et al. (1981)
15	8	2.5-3.0	7-21	21-52	37	2	Jarroll et al. (1981)
25	6	1.5	<6	<9	<9	1	Jarroll et al. (1981)
25	7	1.6	<7	<10	<10	1	Jarroll et al. (1981)
25	8	1.5	<8	<12	<12	1	Jarroll et al. (1981)

TABLE 2-8 C · t Products for 99% Inactivation of Giardia muris Cysts by Free Chlorine

Temperature (°C)	pH	Range Disinfectant Concentration (mg/liter)	Time (min)	$C \cdot t$	Mean $C \cdot t$	Number of Experiments	Reference
1	7	1.3-2.2	597-1,038	1,280-1,400	1,330	3	Rubin (1986)[a]
3	6.5	0.24-1.1	32-297	39-106	68	4	Jarroll et al. (1984)
3	7.5	0.24-1.0	150-770	111-184	140	5	Jarroll et al. (1984)
5	7	0.41-2.73	236-467	173-637	370	3	Rubin (1986)[a]
5	6	2.5	100	250	250	1	Rice et al. (1982)
25	5	4.4-13	3.9-16.3	51-72	66	3	Leahy (1986)[b]
25	7	2.9-7.1	3.6-16.0	26-46	29	3	Leahy (1986)[b]
25	9	11.6-72.6	3.0-15.6	181-223	206	4	Rubin (1986)[a]

[a] A. J. Rubin, Professor of Civil Engineering, The Ohio State University, Columbus, Ohio, personal communication, 1986.

[b] J. G. Leahy, Research Assistant, Department of Civil Engineering, The Ohio State University, Columbus, Ohio, personal communication, 1986.

TABLE 2-9 C · t Products for 99% Inactivation of Giardia muris Cysts by Chloramine

		Range							
Temperature (° C)	pH	Concentration (mg/liter)	Time (min)	$C \cdot t$	Mean $C \cdot t$	Number of Experiments	Ratio[a] Cl_2:N	Preformed Chloramine	Reference
3	6.5	1.6-2.6	188-276	430-496	463	2	1.7:1	No	Glicker (1986)[b]
3	7	1.5-2.4	236-276	425-566	496	2	1.7:1	No	Glicker (1986)[b]
3	7.5	1.5-2.3	225-296	443-580	514	3	1.7:1	No	Glicker (1986)[b]
10	7	1.3-2.7	122-227	295-357	327	3	1.7:1	No	Glicker (1986)[b]
10	8.5	1.3-2.3	164-263	331-371	351	2	1.7:1	No	Glicker (1986)[b]
18	6.5	1.1-2.1	58-225	119-248	197	3	1.7:1	No	Glicker (1986)[b]
18	7	1.0-1.9	75-241	144-246	184	3	1.7:1	No	Glicker (1986)[b]
18	7.5	1.1-2.0	68-256	135-282	217	3	1.7:1	No	Glicker (1986)[b]
1	8	3.8	502	1,883	1,880	1	1:4	Yes	Rubin (1986)[c]
5	7	6.4-17.7	107-220	1,400-1,890	1,720	3	1:4	Yes	Rubin (1986)[c]
5	8	3.8	380	1,429	1,430	1	1:4	Yes	Rubin (1986)[c]
15	7	5.0-16.6	55.3-182	900-1,040	970	4	1:4	Yes	Rubin (1986)[c]
15	8	3.2-8.4	58.3-133	420-733	530	4	1:4	Yes	Rubin (1986)[c]

[a] Weight ratios.
[b] J. Glicker, Supervising Engineer, Portland Bureau of Water Works, Portland, Oregon, personal communication, 1986.
[c] A. J. Rubin, Professor of Civil Engineering, The Ohio State University, Columbus, Ohio, personal communication, 1986.

was known to oxidize sulfhydryl groups immediately and irreversibly. On the other hand, Jacangelo and Olivieri (1985) have shown that monochloramine reacts rapidly with several amino acids including cystine. In the presence of excess monochloramine, reactions with other amino acids may also occur. A less rapid reaction, but still more rapid than with other amino acids, occurs between monochloramine and asparagine, aspartic acid, histidine, lysine, and tyrosine (Jacangelo and Olivieri, 1985). The inactivation of enzymes that occurs during monochloramine oxidation is believed to be the lethal event in the killing of bacteria.

Nucleic acids, particularly deoxyribonucleic acid (DNA), react comparatively rapidly with monochloramine. The purine and pyrimidine bases react with monochloramine about 0.6 times as rapidly as the nucleosides (Jacangelo and Olivieri, 1985). Scission of the nucleic acid polymer, rather than substitution reactions on the purine or pyrimidine bases, is believed to be responsible for the inactivation of DNA or ribonucleic acid (RNA). In a study carried out by Shih and Lederberg (1976), when monochloramine was applied to *Bacillus subtilis* cells *in vivo* or to the extracted bacterial DNA, it caused double- and single-strand breaks.

Comparative inactivation of *G. muris* and other types of microorganisms by ozone is shown in Table 2-10. The overall resistance pattern is similar to that for chlorine, with cyst resistance being approximately 1 order of magnitude higher than that for poliovirus 1 and 2 to 3 orders of magnitude higher than that for *E. coli*. The much lower $C \cdot t$ products also point up the much higher efficiency of ozone compared with chlorine.

Inactivation of *G. muris* cysts by chlorine dioxide has been studied by one group of researchers (A. J. Rubin, Professor of Civil Engineering, The Ohio State University, Columbus, Ohio, personal communication, 1986). The results are shown in Table 2-11. The data indicate that chlorine dioxide is considerably more effective than free chlorine but not so effective as ozone for inactivating *Giardia* cysts.

The use of ultraviolet (UV) radiation for low-maintenance, cost-effective disinfection in small water supply systems is under active consideration. The results of laboratory studies on the effectiveness of UV radiation against *G. lamblia* cysts (Rice and Hoff, 1981) indicate that at the maximum dose used (63,000 μW-sec/cm^2), less than 80% of the cysts were inactivated, whereas a dose of 3,000 μW-sec/cm^2 inactivated 99.9% of an exposed *E. coli* population. This is very significant when one considers that the maximum designed dose range of many commercially available UV treatment units is 25,000 to 35,000 μW-sec/cm^2. Other studies in progress confirm the high resistance of Giardia cysts to UV radiation (Carlson et al., 1985).

The results of recent studies indicate that, contrary to our general impressions 10 years ago, *Giardia* cysts can be inactivated by drinking water

TABLE 2-10 C · t Values for 99% Inactivation of E. coli, Poliovirus 1, and Giardia Cysts by Ozone

			Range					
Microorganism	Temperature (°C)	pH	Disinfectant Concentration (mg/liter)	Time (min)	$C \cdot t$	Mean $C \cdot t$	Number of Experiments	Reference
E. coli	1	7.2	0.07	0.083	0.006	0.006	1	Katzenelson et al. (1984)
	1	7.2	0.065	0.33	0.022		1	Katzenelson et al. (1984)
Poliovirus 1	5	7.2	0.15	1.47	0.22		1	Roy et al. (1982)
G. muris	5	7	0.2-0.7	2.5-9.6	1.8-2.0	1.9	4	Wickramanayake et al. (1985)
G. lamblia	5	7	0.1-0.5	1.0-5.0	0.5-0.6	0.6	3	Wickramanayake et al. (1985)
G. muris	15	7	0.1-0.3	1.7-7.7	0.3-0.4	0.4	3	Wickramanayake et al. (1984)
G. muris	25	7	0.03-0.2	1.3-8.2	0.2-0.3	0.3	6	Wickramanayake et al. (1985)
G. lamblia	25	7	0.03-0.2	1.0-5.5	0.1-0.2	0.18	3	Wickramanayake et al. (1985)

TABLE 2-11 C · t Products for 99% Inactivation of G. muris Cysts by Chlorine Dioxide

		Range					
Temperature (°C)	pH	Disinfectant Concentration (mg/liter)	Time (min)	$C \cdot t$	Mean $C \cdot t$	Number of Experiments	Reference
5	7	0.11-5.55	1.3-168	7.2-17.6	11.0	5	Rubin (1986)[a]
25	7	0.22-1.13	3.3-28.8	3.7-6.2	5.0	5	Rubin (1986)[a]
25	9	0.16-0.82	2.1-19.2	1.7-3.7	2.8	4	Rubin (1986)[a]

[a] A. J. Rubin, Professor of Civil Engineering, The Ohio State University, Columbus, Ohio, personal communication, 1986.

disinfectants. The order of efficacy of disinfectants conventionally used for drinking water treatment is ozone > chlorine dioxide > free chlorine > chloramine. *Giardia* cysts are among the most resistant pathogens known, however, and the disinfection step must be conducted rigorously under well-controlled conditions. This is especially important during periods when water temperatures are low. Employing additional treatment processes to remove substantial numbers of cysts before disinfection is also important in order to decrease reliance on disinfection.

Viruses

The majority of tests described in the literature pertaining to inactivation of human viruses with drinking water disinfectants have been conducted with human enteroviruses, poliovirus, Coxsackievirus, and echovirus. While the data collected about these viruses are useful, data on the viruses that are responsible for the diseases observed in drinking water are even more important. Culture methods for hepatitis A virus have recently become available, and several have been reported (Grabow et al., 1983; Peterson et al., 1983). Recent information is summarized in Table 2-12 in the light of other information for the human enteroviruses. The information is presented as the product of the disinfection concentration times the contact time necessary for 99% inactivation. The data on the human enteroviruses were taken from an earlier summary (NRC, 1980a). The $C \cdot t$ product for poliovirus was about I to 2 and 10.5 for hypochlorous acid and hypochlorite ion, respectively. The $C \cdot t$ product estimated from the data reported by Peterson et al. (1983) for 99% inactivation of hepatitis A virus was about 60 at pH 7 (a mixture of hypochlorous acid and hypochlorite ion). The C · t for 99% inactivation is at best a crude estimate and was approximated from data presented for the sero

TABLE 2-12 The Inactivation of Selected Viruses with Chlorine Disinfectants

Test Microorganism	Disinfectant	pH	Temperature (°C)	$C \cdot t$	Reference
E. coli	HOCl	6.0	5	0.04	NRC (1980a)
	OCl^-	10.0	5	0.92	NRC (1980a)
	NH_2Cl	9.0	5	175.00	NRC (1980a)
Poliovirus 1	HOCl	6.0	5	1–2	NRC (1980a)
	OCl^-	10.0	5	10.5	NRC (1980a)
	NH_2Cl	9.0	15	900.0	NRC (1980a)
Rotovirus	HOCl	[a]	[a]	0.25	NRC (1980a)
	OCl^-	[a]	[a]	1.4	NRC (1980a)
	NH_2Cl	[a]	[a]	[a]	
Hepatitis A	HOCl	7.0	5	60[b]	Peterson et al. (1983)
	HOCl	6.0	[a]	<0.32[c]	Grabow et al. (1983)
	OCl^-	10.0	[a]	<1.04[c]	Grabow et al. (1983)
	NH_2Cl			[a]	
Norwalk agent	$HOCl/OCl^-$	7.4	25	[a]	Keswick et al. (1985)

[a]Not reported.
[b]$C \cdot t$ estimated from animal infectivity data.
[c]$C \cdot t$ estimated from disinfection curves. Chlorine residual data suggested that the test mixtures contained significant demand. Concentration used for calculation was the dose reported.

conversion of marmoset monkeys (*Saguinus* spp.). Grabow et al. (1983) reported that the infectious hepatitis agent was much more sensitive to chlorine. Hepatitis A virus was titrated with a multiple-tube dilution procedure coupled with a radioimmune assay that allowed the determination of the probable number of viruses during disinfection with chlorine. The $C \cdot t$ product for 99% inactivation of hepatitis A virus from their graphic presentation was <0.32 for hypochlorous acid at pH 6.0 and <1.04 for hypochlorite ion at pH 10.0. The study reported comparative data for other microorganisms, and the hepatitis A virus did not appear to be particularly resistant to chlorine. The conditions normally specified for the disinfection of drinking water with free chlorine would successfully inactivate hepatitis A virus. The $C \cdot t$ products reported for 99% inactivation of poliovirus by combined chlorine are considerably higher, and $C \cdot t$ products as high as 900 have been reported. No data are available at present on the inactivation of hepatitis A virus by the combined forms of chlorine. As with other viruses, the suspected resistance of this agent may be due to the dramatic differences in the rates of inactivation with free and combined chlorine.

While culture methods are not available for Norwalk agent, limited information has become available from human volunteer studies. Keswick et al. (1985) reported that Norwalk agent appeared to be more resistant

to chlorine than two strains of rotovirus, one strain of poliovirus, and F2 bacterial virus. Close inspection of the reported data shows that for chlorine doses of 3.75 to 6.25 mg/liter, the majority of the chlorine was in the combined form. Free chlorine was observed after 30 minutes in the rotovirus and poliovirus trials, and these viruses were not recovered. However, after 30 minutes no free chlorine was found in the Norwalk trial, and only trace quantities of free chlorine were found in the F2 trial. In each case the viruses were not completely inactivated. The data suggesting the resistance of Norwalk agent to free chlorine are difficult to interpret without a clear definition of the nature of the chlorine species present in the reaction system. The reported resistance may be due to the marked difference in the viricidal activity of free and combined chlorine that has been reported for other viruses (NRC, 1980a).

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3

Chemistry and Toxicity of Disinfection

Concerns about possible adverse health effects of drinking water disinfection have centered on chemical by-products produced by reactions of chlorine with various organic precursors during water treatment. The presence of certain organic compounds in raw water prior to treatment can be attributed to chemical manufacturing, processing, distribution, uses, or urban and agricultural land runoff. However, most of the carbon in typical surface waters is found in natural humic materials, which are potential precursors of toxic disinfection by-products (Rook, 1976; Thurman, 1985).

Many recent studies discussed in this chapter have addressed disinfection by-products produced from these aquatic humic materials, which consist of complex natural mixtures of humic and fulvic acids plus neutral and basic components produced mainly by decaying vegetation.

CHLORINATION

Reactions and By-Products of Chlorination

Although chlorination has the desired effect of inactivating pathogenic microorganisms through the disinfecting reactions of chlorine, as well as the additional desired effect of oxidizing many organic molecules to form CO_2 (Helz et al., 1980; Jolley et al., 1985), this method of disinfection also produces chlorinated by-products and other incompletely oxidized compounds of potential concern. Noteworthy contributions to the chemistry of drinking water chlorination over the past few years have included

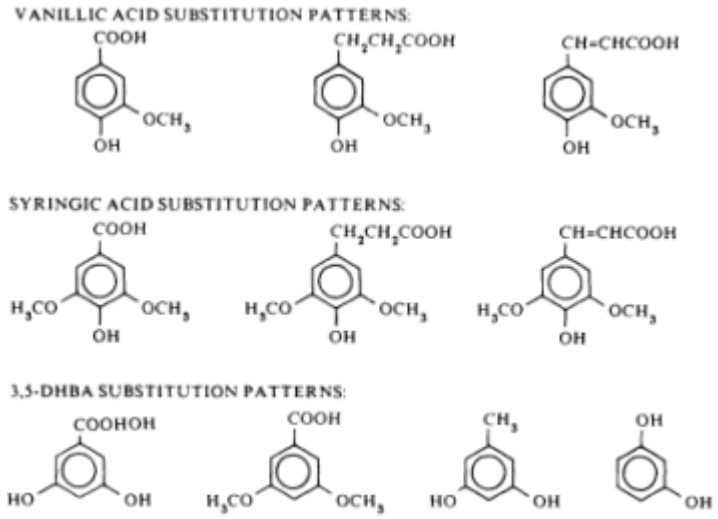


Figure 3-1 Aquatic humic model compounds. From Norwood et al. (1980) with permission.

a number of studies of the reaction mechanisms and types of by-products formed from chlorination of aquatic humic materials.

Model Compound Studies

Mechanisms of chlorination by-product formation have been investigated through the use of isolated humic and fulvic acids, as well as simple compounds that are viewed as models of the complex molecules found in natural humic materials. Many humic molecules (and study models) contain electron-rich phenolic structures and/or aliphatic side chains that are vulnerable to attack by chlorine (Liao et al., 1982). It has generally been found during the studies discussed below that the specific by-products depend on the molecular structures of the humic and fulvic acids undergoing chlorination, the chlorine-to-carbon ratio, pH, and several other factors. The by-products fall into two general categories: volatile hydrophobic and nonvolatile hydrophilic compounds.

Christman et al. (1978) and Norwood et al. (1980) used resorcinol, orcinol, 3,5-dihydroxybenzoic acid, 3-methoxy-4-hydroxycinnamic acid, and 3,5-dimethoxybenzoic acid as models of humic molecules, based on copper oxide degradation products of humic materials (Figure 3-1). Their model compounds all consumed significant amounts of chlorine and produced measurable

levels of chloroform. Resorcinol, as suggested earlier by Rook (1977), consumed a large quantity of chlorine (7 moles per mole of resorcinol) and rapidly produced 1 mole of chloroform. Similar results were produced with their other model compounds, suggesting that chloroform is a primary reaction product of chlorination of aquatic humic materials that contain substructures similar to these model compounds. Other by-products produced by their model compounds are shown in Table 3-1. High chlorine-to-carbon ratios favored the production of nonvolatile hydrophilic by-products.

Boyce and Hornig (1983) studied chloroform production from chlorination of 1,3-dihydroxyaromatic compounds and simple methyl ketones, which they confirmed to be efficient at producing chloroform. With isotope labeling, they unambiguously demonstrated that the C_2 position of resorcinol is responsible for chloroform generation, as previously hypothesized by Rook (1977) and Norwood et al. (1980). Boyce and Hornig (1983) further demonstrated that the specific types of chlorinated products depend on both pH and the relative concentrations of chlorine and substrate in solution. The by-products that they obtained from resorcinol at various chlorine concentrations and pH values are shown in Table 3-2 and confirm the previous observations of Norwood et al. (1980) regarding by-products formed at neutral pH.

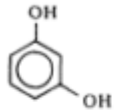
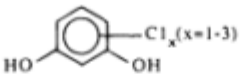
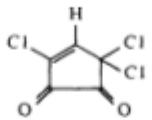
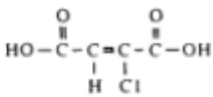
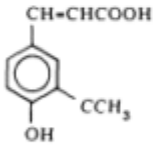
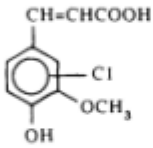
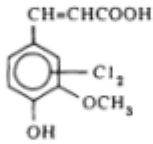
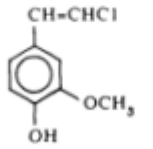
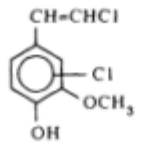
Based on these results and previous hypotheses of Moye (1967) and Rook (1980), Boyce and Hornig proposed a comprehensive mechanism for the conversion of 1,3-dihydroxyaromatic structures to chloroform by aqueous chlorination. A portion of this proposed mechanism, modified and reproduced in Figure 3-2, involves successive electrophilic attack of chlorine to produce substituted resorcinols (I) with the eventual loss of aromatic character to produce the intermediate pentachlororesorcinol (II). This is followed by hydrolytic ring cleavage and a number of other substitution and hydrolysis reactions to produce chloroform and short-chain chlorinated acids, in this case chloromatic acid (VI).

De Leer and Erkelens (1985) attempted to support the mechanism proposed by Boyce and Hornig (1983) by synthesizing the proposed intermediate pentachlororesorcinol according to the method of Zincke (1890) and subjecting it to aqueous chlorination at neutral pH. Although the chlorination of resorcinol and pentachlororesorcinol produced several identical products, large discrepancies were seen in apparent reaction rate, chloroform production, and products, indicating that pentachlororesorcinol is not a major intermediate. De Leer and Erkelens (1985) further concluded that the principal reaction and most important side reaction are

$$C_6H_6O_2 + 7Cl_2 + 4H_2O \rightarrow CHCl_3 + CO_2 + \textit{cis}\text{-}HOOCCCl = CHCOOH + 10HCl, \text{ and}$$
$$C_6H_6O_2 \rightarrow \textit{cis}\text{-}HOOCCCl = CHCH_2COOH + CO_2 \text{ or } CHCl_3.$$

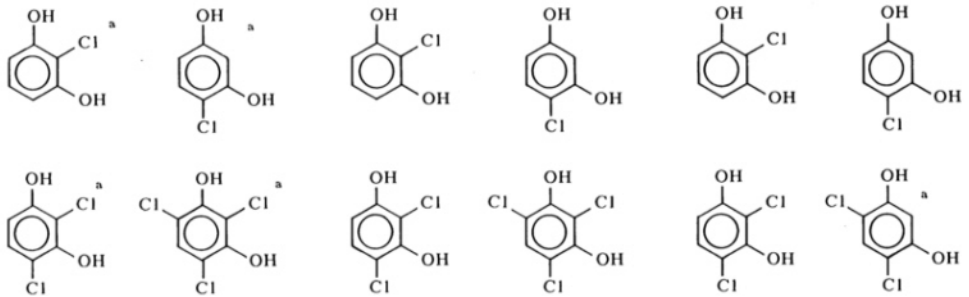
but that many side reactions producing other chloroform precursors and highly oxidized products occur.

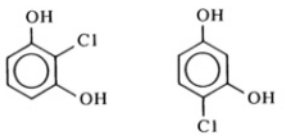
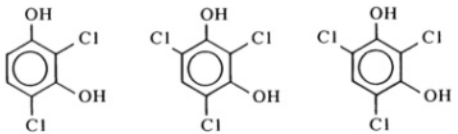
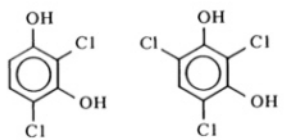
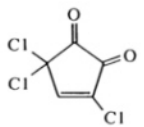
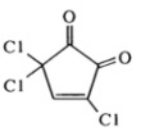
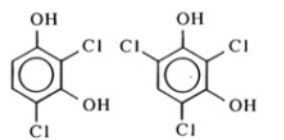
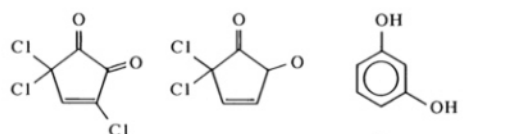
TABLE 3-1 Reaction Products from Model Compounds and Hypochlorous Acid (HOCl)[a]

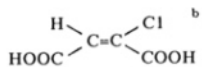
Reactant	Products Identified	
	At 0.5 Cl_2/C	At 2.0 Cl_2/C
1,3 DIHYDROXY BENZENE 	  $CHCl_3$	$CHCl_3$  CCl_3COOH
3-METHOXY-4-HYDROXY-CINNAMIC ACID 	    $CHCl_3$	$CHCl_3$ $CHCl_2COOH$ CCl_3COOH

[a] From Norwood et al. (1980) with permission.

TABLE 3-2 Reaction Products Identified from the Chlorination of 5×10^{-4} *M* Resorcinol Using 5×10^{-4} *M* to 5×10^{-3} *M* Chlorine Dioxide at 10°C[a]

Mole Ratio (Cl_2)/ Resorcinol	pH		
	4	7	10
1			
	$C_1{}_2CHC(O)CCl{=}CHCH_2Cl$	$CHCl_3$ [a]	$CHCl_3$

Mole Ratio (Cl_2)/ Resorcinol	pH		
	4	7	10
3			
			
	$Cl_2CHC(O)CCl{=}CHCH_2Cl$	$CHCl_3$	$CHCl_3$
5			
	$Cl_2CHC(O)CCl{=}CHCH_2Cl$	$CHCl_3$	
	$Cl_2CHC(O)CCl{=}CHCHCl_2$		
	$CHCl_3$		$CHCl_3$

10	$Cl_3CC(O)CCl{=}CHCH_2Cl$	$CHCl_3$	$CHCl_3$
	$Cl_3CC(O)CCl{=}CHCHCl_2$	chlorinated acids	Cl_3CCOOH
	$Cl_3CCH(OH)_2$ [a]		
	$Cl_2CC(O)CHCl_2$ [a]	Cl_3CCOOH	
	$CHCl_3$ [a]	 [b]	
	$Cl_2CHCOOH$		
	Cl_3CCOOH [a]		
	$Cl_3CC(O)CCl{=}CHCHClCOOH$		

[a]From Boyce and Hornig (1983) with permission.
[b]Structure of compound confirmed through matching of gas chromatography retention time and mass spectrum with analysis of standard sample. Other structural assignments are considered to be tentative.
[c]Detected by gas chromatography/mass spectrometry (GC/MS) in ether extracts obtained at reactant concentrations of 1×10^{-3} *M* resorcinol and 1×10^{-2} *M* chlorine.

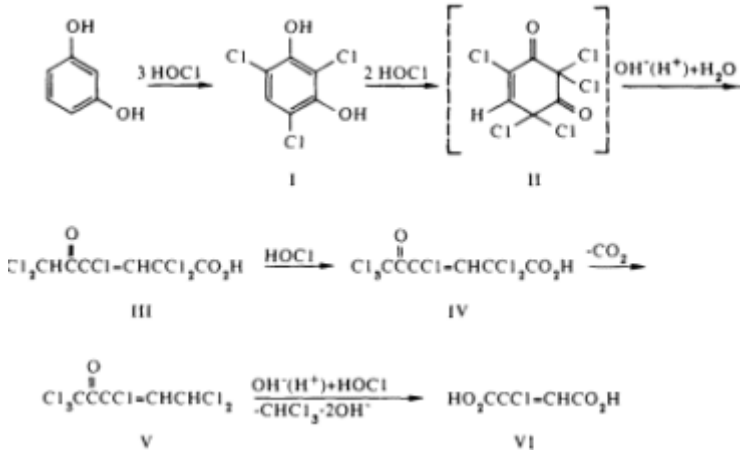


Figure 3-2 Abbreviation of mechanism proposed by Boyce and Hornig (1983) for the aqueous chlorination of resorcinol (adapted from Norwood, 1985).

Thus, it appears from the above studies of model compounds that nonselective aqueous chlorination of activated aromatic ring systems produces not only chloroform (a volatile hydrophobic by-product) but many nonvolatile hydrophilic chlorinated aromatic by-products as well.

Isolated Acids

Working with isolated aquatic humic and fulvic acids, Christman and co-workers (Christman et al. 1980, 1983; Johnson et al., 1982; Norwood et al., 1983) identified more than 100 different chlorination products by gas chromatographic/mass spectroscopic methods at a 4:1 chlorine-to-carbon mole ratio. Some of these products are shown in Tables 3-1 and 3-2. Chlorination of several humic and fulvic acid samples from the same source produced significant differences in product mixtures. A notable difference was that most products of fulvic acid chlorination contained chlorine, whereas most humic acid samples produced at high pH did not. In both cases, however, the dominant chlorinated products were chloroform and chlorinated aliphatic acids, especially dichloroacetic acid (DCA), trichloroacetic acid (TCA), chloroform, dichlorosuccinic acid, and dichloromalonic acid.

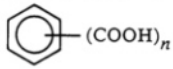
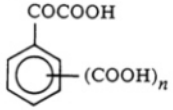
A variety of short-chain, nonvolatile aliphatic halogenated products (listed by Norwood, 1985) result from the exposure of aquatic humic and fulvic acids to chlorine:

Name	Molecular Formula
Trichloromethane (chloroform)	$CHCl_3$
Bromodichloromethane	$CHBrCl_2$
Trichloroethanal (chloral)	CCl_3CHO
Chloroethanoic acid (chloroacetic acid)	H_2CClCO_2H
Dichloroethanoic acid (dichloroacetic acid, DCA)	$HCCl_2CO_2H$
Trichloroethanoic acid (trichloroacetic acid, TCA)	CCl_3CO_2H
2, 2-Dichloropropanoic acid	$CH_3CCl_2CO_2H$
3, 3-Dichloropropenoic acid	$CCl_2 = CHCO_2H$
2, 3, 3-Trichloropropenoic acid	$CCl_2 = CClCO_2H$
Dichloropropanedioic acid (dichloromalonic acid, DCM)	$HO_2CCCl_2CO_2H$
Butanedioic acid (succinic acid)	$HO_2C(CH_2)_2CO_2H$
Chlorobutanedioic acid (chlorosuccinic acid)	$HO_2CCH_2CHClCO_2H$
2, 2-Dichlorobutanedioic acid (α, α-dichlorosuccinic acid, DCS)	$HO_2CCCl_2CH_2CO_2H$
cis-Chlorobutenedioic acid (chloromaleic acid)	$HO_2CCH = CClCO_2H$
cis-Dichlorobutenedioic acid (dichloromaleic acid)	$HO_2CCCl = CClCO_2H$
trans-Dichlorobutenedioic acid (dichlorofumaric acid)	$HO_2CCCl = CClCO_2H$

The apparent dominance of C_2-chlorinated acids is in agreement with the findings of Quimby et al. (1980), who reported the tentative identification of TCA and halogenated phenols after soil extract chlorination, and Rook (1980), who found that DCA and TCA were the principal constituents in methylene chloride extracts of Rotterdam drinking water after breakpoint chlorination. However, no halogenated aromatic products were detected after chlorination of actual aquatic humic and fulvic acids under high pH conditions.

A large number of monobasic and dibasic unchlorinated aliphatic acids, from oxalic up to the C_{27} monobasic fatty acid, were identified from the humic acid fraction (Table 3-3). Only a few of the dibasic acids were associated with the fulvic acid fraction, and almost none of the monobasic

TABLE 3-3 Non-Chlorine-Containing Products of Aquatic Humic and Fulvic Acids[a]

Compound Class	Number Identified[b]	Major Compounds
Benzenecarboxylic acid	16	 n^c = 1-5
(Carboxyphenyl)-glyoxylic acids	8	 n^d = 2-4
Monobasic acids	17	$2H_3C—(CH_2)_n—COOH$ n^e = 7-25
Dibasic acids	9	$HOOC—(CH_2)_n—COOH$ n = 0-8

[a]From Norwood (1985) with permission.
[b]Includes only the more confident identifications.
[c]All possible isomers detected.
[d]Several isomers detected in each case; identifications considered very tentative.
[e]Not all n values detected; some may have been below the detection limit.

acids were detected. The dibasic aliphatic acids are generally of low molecular weight, containing 2 to 10 carbons. Most of these were detected in relatively low yield. Aromatic acids were also detected, including monobenzoic to hexabenzoic acid in all isomers, as well as small quantities of methyl-substituted aromatic acids (tentatively identified) and isomers of (carboxyphenyl) glyoxylic acids (tentatively identified). These non-chlorine-containing products of each acid are similar to the polybasic aromatic and aliphatic acids reported from potassium permanganate ($KMnO_4$) oxidation (Christman et al., 1981; Liao et al., 1982).

Recently de Leer et al. (1985) subjected humic acid extracted from a peat soil to aqueous chlorination under degradation-scale conditions (0.38 g humic acid per liter of solution, pH 7.2, 24-hour reaction time, ambient temperature, chlorine-to-carbon ratios of 0.39:1 and 3.35:1). The lower chlorine-to-carbon mole ratio was chosen to represent typical drinking water disinfection practice, while the higher ratio was chosen to maximize product yields. Utilizing gas chromatography/mass spectrometry (GC/MS) methods, structures were assigned to more than 100 products. The product distribution was different for the two reaction mixtures.

The products detected in ether and ethyl acetate extracts of the acidified high chlorine-to-carbon ratio aqueous reaction mixture were a series of

unchlorinated aliphatic monobasic and dibasic acids, aromatic carboxylic acids, and chlorinated aliphatic monobasic and dibasic acids, both saturated and unsaturated, that correspond well to those reported in the experiments on isolated aquatic humic and fulvic acids (Tables 3-1 and 3-2). The predominant chlorinated compounds were DCA, TCA, and 2,2-dichlorobutanedioic acid (α,α-dichlorosuccinic acid), also in agreement with the earlier findings.

Aqueous chlorination of humic acid derived from soil at a high chlorine-to-carbon ratio (3.35:1) produced two new classes of compounds (Figure 3-3) (de Leer et al., 1985). These were the cyano-substituted alphatic monobasic acids, 3-cyanopropanoic acid and 4-cyanobutanoic acid, and the chlorinated aromatic carboxylic acids, 4-chlorobenzoic acid, 2-chlorobenzoic acid, 2-chlorophenylacetic acid, 4-chlorophenylacetic acid, 2,6-dichlorophenylacetic acid, and 2,4-dichlorophenylacetic acid. This constituted the first definitive report of the production of chlorinated aromatic compounds from the aqueous chlorination of humic material.

De Leer and coworkers (1985) found that a greater number of compounds with higher boiling points were formed at the lower chlorine-to-carbon ratio than at the higher ratio, although the classes of compounds formed were similar. Also produced at the lower ratio was a group of compounds termed "chloroform precursors" because they contained a trichloromethyl group adjacent to a group susceptible to further oxidation. These structures, described above, may be divided into two groups: one with the trichloromethyl group next to a hydroxyl group and the other with the trichloromethyl group next to a carbonyl group conjugated with a carbon-to-carbon double bond (Figure 3-3).

Holmbom et al. (1981, 1984) discovered a series of acids, the furanones, in chlorinated kraft pulp waste. Recently, Hemming and colleagues (1986) showed that low concentrations (μg/liter) of these compounds were formed when aqueous humic and drinking water samples were chlorinated at 1:1 chlorine-to-carbon weight ratios at pH 7. After chlorination, these nonvolatile compounds were concentrated and separated by high-pressure liquid chromatography (HPLC). Almost all of the mutagenic activity injected by chlorination was found to be in a relatively narrow HPLC fraction. After methylation by CI and EI mass spectrometry, the major contributor was tentatively identified as 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5 *H*)-furanone. This same compound was also found by Meier et al. (1986).

A number of studies have been conducted with commercial materials of unknown origin sold as humic acid (Bull et al., 1982; Coleman et al., 1984; Meier et al., 1983; Seeger et al., 1985). These materials appear to be European lignitic coal extract rather than soil or aquatic humic acid (Malcolm and MacCarthy, 1986). Chlorination products included chloroacetonitriles, chloroketones, and chlorobenzenes (Coleman et al., 1984).

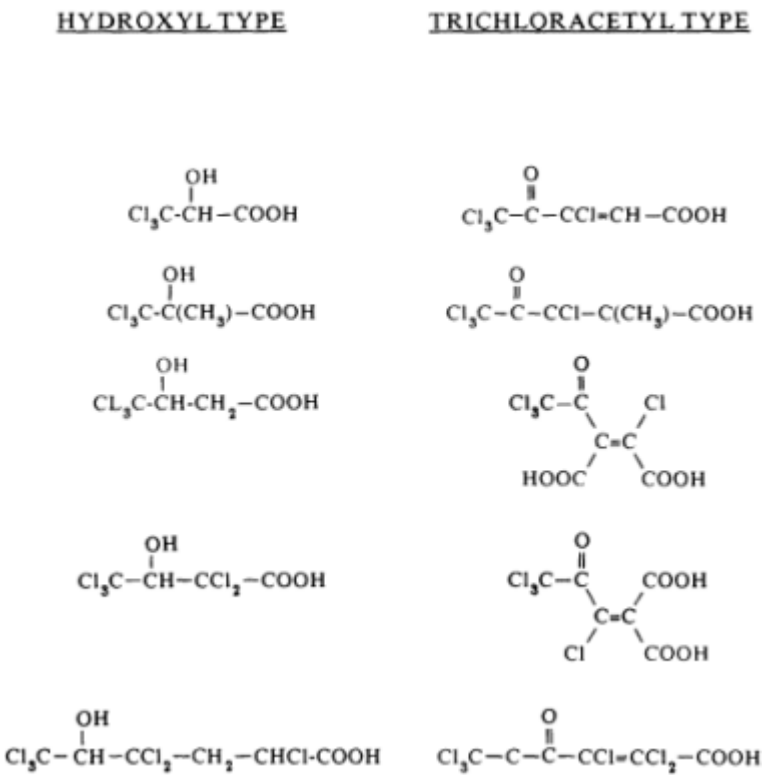


Figure 3-3 Chloroform precursors detected from the aqueous chlorination of a soil-derived humic acid at chlorine-to-carbon ratios of 0.39 and 3.35 by de Leer and colleagues (1985).

Small quantities of dichloroacetonitrile (0.2 µg/ml), 3,3-dichloro-2-butanone (0.4 µg/liter), and 1,1-dichloro-2-propanone (0.6 µg/liter) and relatively large quantities of pentachloro-2-propanone (1.1 µg/liter) and 1,1,1-trichloro-2-propanone (11 µg/liter) were also identified from a 1-g/liter Ohio River humic fraction chlorinated at pH 7 with a 1:1 chlorine-to-carbon mole ratio for 90 hours. The major products were similar to the 14 µg/ml of DCA, 35 µg/ml of TCA, and 66 µg/ml of chloroform previously found. Thus, large quantities of DCA and TCA were recovered even though extraction into organic solvents from water was carried out at pH 3.1, where recoveries of the salts of these very strong acids (pK_a <1) are poor. Even larger quantities of the chloroacetic acids (77-122 µg/ml) and significant quantities of the chloroacetonitriles (4.3-4.4 µg/ml) were found in the commercial humic material. The identifiable products for both the Ohio River sample and the commercial humic material, however, were only 23% to 28% of the measured total organic halogen (TOX) produced even though long reaction times (90 hours) and very high concentrations of starting material (1 g total organic carbon [TOC]/liter) and chlorine (35.5 g/liter) were used. Such low yields of identifiable products, even under conditions expected to produce highly degraded humic material and short-chain cleavage products, are typical of the identifiable yields of products found by others. Even under conditions where chloroform would be expected to predominate, it represents only a small quantity of the TOX produced, and DCA and TCA are produced in nearly identical amounts.

The yield of identifiable products from chlorination of fulvic and humic acids isolated from natural surface water is a small fraction of the starting organic material. In the work of Coleman et al. (1984), the 28% of the TOX identified represents less than 10% of the starting organic material identified in the Ohio River humic fraction and a much lower percentage of the TOC in the river. Some of the highest recoveries reported (Christman et al., 1983) are 14% of starting aquatic fulvic material and 53% of TOX. Both of these studies were conducted under conditions of high initial carbon concentration (0.5 to 1 g/liter) and high chlorine-to-carbon (4:1 to 1:2) mole ratios designed to maximize identifiable product yield. Christman et al. (1983) showed that the yields of chloroform and the C,—C_4 chlorinated aliphatic acids make up 17% and 36% of the TOX, respectively. The high yield of TCA in these samples is confirmed by the use of isotope-dilution MS (Norwood et al., 1986) with ^{13}C-labeled TCA added to the aqueous chlorination mixture before any separation or analysis is performed (Christman et al., 1983). The confirmed dominance of TCA is in agreement with Quimby et al. (1980), who used a GC microwave-plasma emission method (Miller et al., 1982).

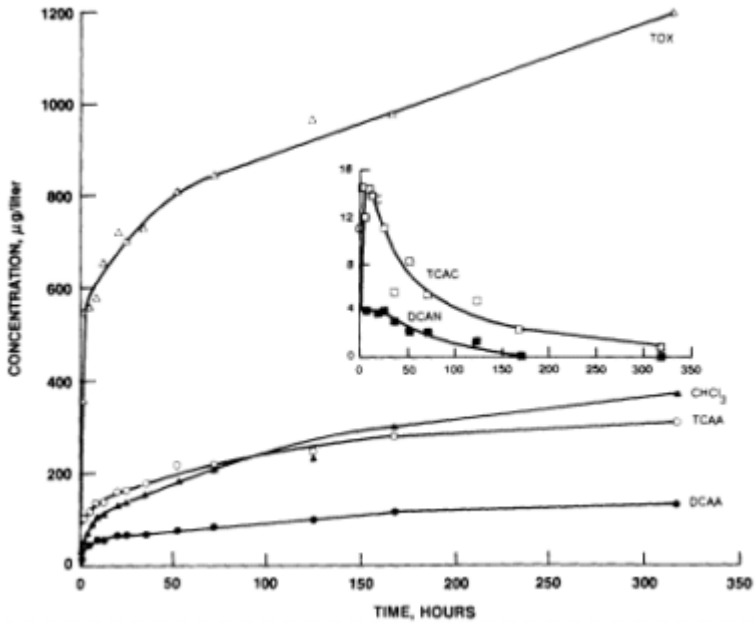


Figure 3-4 Formation of organic halides from Black Lake fulvic acid as a function of chlorine contact time. Conditions: 4.1 mg TOC/liter, pH 7.0, 20 mg applied hypochlorous acid/liter. From Reckhow and Singer (1985) with permission.

Miller and Uden (1983) have shown the relative concentration of chloroform, DCA, TCA, and chloral hydrate in chlorinated reaction mixtures of soil fulvic acid to be a function of pH, chlorine-to-carbon mole ratio, and time. The quantity of chloroform produced generally increased with increasing pH, chlorine-to-carbon ratio, and time. Based on the mechanism of de Leer et al. (1985), this is to be expected because of the greater number of intermediates formed from the chlorination of humic materials.

Using chlorine-to-carbon mole ratios and TOC values more typical of natural water, Reckhow and Singer (1985) showed that when aquatic fulvic acid (4.1 mg of TOC/liter at pH 7) was treated with 20 mg of HOCl/liter of water, TOX, chloroform, TCA (Figure 3-4), and DCA all increased with time. TCA and dichloroacetonitrile reached their maximum concentrations within the first few hours, then decreased rapidly with time. Their results with pH were similar to those of Fleischacker and Randtke (1983), an increase in chloroform but a decrease in TOX with increasing pH (Figure 3-5). It is clear from these studies

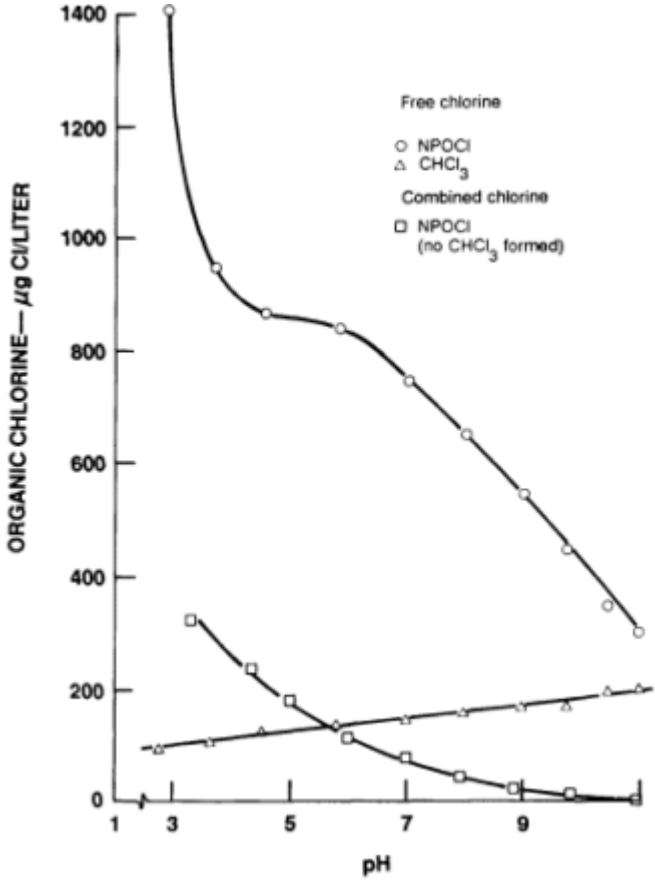


Figure 3-5 Effect of pH on the formation of organic chlorine by free and combined chlorine. Conditions: 3.0 mg TOC from peat fulvic acid/liter, 20 mg chlorine dosage/liter, 100 hours contact time, combined chlorine formed by addition of free chlorine to samples containing excess ammonium chloride. From Fleischacker and Randte (1983) with permission of the copyright holder, American Water Works Association.

that the importance of trihalomethanes is overstated in quantitative studies done at high concentration for long time periods and at a high chlorine-to-carbon mole ratio. Trihalomethanes are also easily quantified by GC procedures (EPA, 1979, 1980). No simple and accurate method exists, however, for the identification and quantification of even the major individual nonvolatile chlorinated compounds formed in

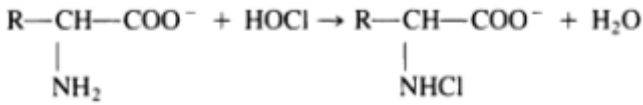
chlorinated surface water. The principal method has been solvent extraction, followed by derivatization and GC/MS (Norwood et al., 1983) and, more recently, by isotope dilution, GC/MS (Norwood et al., 1986), and GC microwave-plasma emission (Miller and Uden, 1983). Unfortunately, even using these sophisticated methods we are unable to identify the majority of products formed in water chlorination.

Isolated Bases

Numerous organic nitrogen compounds are present in natural surface waters (C. Le Cloirec et al., 1983a, b, c; P. Le Cloirec et al., 1983; Mallevialle et al., 1984; Ram and Morris, 1980; Thurman, 1985). These include a number of man-made nitrogen-containing pesticides and industrial compounds in trace quantities. However, the most abundant nitrogen-containing compounds are the naturally occurring amino acids, nucleic acids, amino sugars, natural porphyrin-based pigments (such as chlorophyll), and proteins. The higher molecular weight members of this group of compounds have been difficult to isolate and characterize, and little is known about the products of their reactions with aqueous chlorine other than the low-molecular-weight by-products such as chloroform.

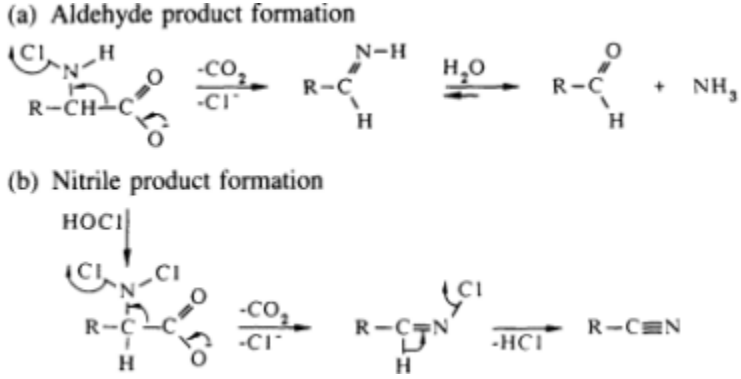
Within the past several years, research using model compounds has elucidated the reactions of aqueous chlorine with some of the more nucleophilic examples of these compounds, which explain the origins of some of the chlorine demand of natural waters and by-products of water disinfection.

Amino acids react rapidly with one equivalent of aqueous chlorine to form *N*-chloramino acids (Morris, 1967):



After isolating colloidal particles from river water Helz et al. (1983) showed that amino acids associated with the particles in either a free or proteinaceous form are depleted by chlorination. The amino acids containing reactive side groups were the most reactive. A number of the *N*-chloroamino acids have comparatively short lifetimes and decompose losing carbon dioxide to produce aldehydes (see Scheme Ia) (Dakin, 1916; Friedman and Morgulis, 1936; Golschmidt et al., 1927; Langheld, 1909a, b; C. Le Cloirec et al., 1983a, b, c; P. Le Cloirec et al., 1983; Stanbro and Smith, 1979).

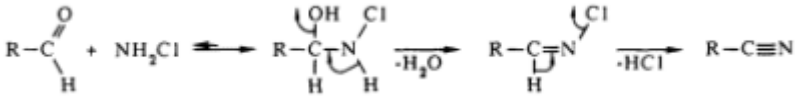
Scheme I



The intermediacy of an imine (Schiff base) that hydrolyzes to an aldehyde is suggested. Stanbro and Smith (1979) have studied the effect of pH on the decomposition of *N*-chloroalanine and found that the decomposition is independent of pH in the range between pH 3 and pH 9. At lower pH values the rate of decomposition accelerates. At higher chlorine-to-amino acid mole ratios, the amino acid becomes dichlorinated (Scheme Ib), and the dichloroamino group, which is even less stable than the monochlorinated derivative, decomposes to a nitrile group. The amount of nitrile increases relative to the aldehyde as the pH of the solution increases and likely involves chlorination of the imine intermediate followed by dehydrohalogenation.

Recently, C. Le Cloirec and Martin (1985) demonstrated that inorganic monochloramine can react with acetaldehyde to produce acetonitrile (Scheme II).

Scheme II



In 1976 McKinney et al. reported the presence of dichloroacetonitrile in tap water in Raleigh, North Carolina. Trehy and Bieber (1981) identified both dichloroacetonitrile and bromochloroacetonitrile in chlorinated lake and well waters in south Florida. Using model solutions of a number of naturally occurring nitrogen-containing compounds, Trehy and Bieber (1981) and Bieber and Trehy (1983) have shown that the amino acids, aspartic acid, tyrosine, and tryptophan, as well as the catabolites of tryptophan,

kynurenine, and kynurenic acid, react with hypochlorite at pH 7-8 to produce significant quantities of dichloroacetonitrile. A model protein was also shown to produce considerable quantities of dihaloacetonitriles.

Trehy and Bieber (1981) have proposed that dichloroacetaldehyde and its reaction product with chlorine, trichloroacetaldehyde (chloral), would be formed by chlorination of aspartic acid, but suggest that because these aldehydes are extremely water soluble, their presence has not yet been reported.

Proteins (Scully et al., 1985) and amino acids (Bieber and Trehy, 1983; Trehy and Bieber, 1981) also react with hypochlorite to produce trihalomethanes. Although the yields of chloroform from the individual amino acids are generally low, the overall yield of chloroform from proteins is comparable to that of humic acid for solutions containing equivalent amounts of organic carbon.

Chloropicrin has been identified in chlorinated surface waters (Duguet et al., 1985; Mallevialle et al., 1983; Sayato et al., 1982). Sayato et al. (1982) showed that it can be formed by the reaction of chlorine with humic acid, amino acids, and nitro-or nitrosophenols (Sayato et al., 1982). However, the yields are not appreciable at pH values normally present during water treatment. At extremely basic pH, the yield of chloropicrin is enhanced. Duguet et al. (1985) showed that the presence of nitrite greatly enhanced the formation of chloropicrin both in chlorinated model solutions and in chlorinated natural waters. They suggest that chemists might overlook the presence of chloropicrin in water samples, if they dechlorinate those samples before analysis with thiosulfate, sulfite, or ascorbic acid. They concluded that "for customary TOC levels, low nitrite concentrations are sufficient to explain the levels of chloropicrin actually found in full-scale water treatment plants." Becke et al. (1984) demonstrated that preozonation of a natural lake water enhanced the formation of chloropicrin over nonozonation water. They confirmed the significance of nitrite in the generation chloropicrin and further noted that N_2O_5, which is present in ozone generated from air by silent electric discharge, also reacts with natural organic components of water to produce this by-product.

Uracil has been identified in river water and, since the identification of the mutagen, 5-chlorouracil, in chlorinated wastewater effluent (Jolley, 1975), the products of the reaction of nucleic bases (purines and pyrimidines) with chlorine have been of concern. Gould and Hay (1982), Gould et al. (1984a, b), and Dennis et al. (1978, 1979) have studied the reaction of several biologically important purines and pyrimidines with aqueous chlorine. Uracil reacts with hypochlorous acid to produce 5-chlorouracil as well as several other products including an *N*-chlorinated product (Gould

et al., 1984b). Cytosine reacts to produce an unusually stable chloramine product (Gould et al., 1984a; Patton et al., 1972).

Chlorination Toxicity

Short-term toxic effects associated with chemicals found in drinking water are observed only at concentrations substantially above the levels occurring in typical water supplies. The principal health concern, if one exists, pertains to chronic ingestion of low levels of disinfection by-products.

Toxicological evaluation of complex mixtures is difficult, especially for mixtures derived from environmental sources such as water. No single sample represents the total body of water. No two samples are identical, and variations in the same sample occur over time. Comparisons among samples often fail to improve understanding of the potential toxicity of the source.

Numerous studies of the mutagenic and carcinogenic properties of treated (disinfected) and untreated drinking water have been reported (Cumming et al., 1983; Loper et al., 1983; Van Hoof, 1983). One finding common to most studies performed throughout the world is that chlorination introduces mutagens that are not present (or are present in lower amounts) in raw, untreated water (Cheh et al., 1980a, b; de Greef et al., 1980; Douglas et al., 1986; Loper et al., 1985; Marouka and Yamanaka, 1980; Nestmann et al., 1979). Since chlorine itself has not been found to be mutagenic, attention has focused on the reaction products formed by the chlorination of compounds already existing in untreated surface water.

Besides implicating chlorination of humic and fulvic acids as the source of much, if not most, of the mutagenic activity observed in drinking water samples, Meier et al. (1983) conclusively showed that most (about 80%) of the mutagenic activity of the chlorinated humic acid was due to nonvolatile compounds, as previously shown for extracts of drinking water (Kool et al., 1982). Until recently (see previous section), most organics identified in drinking water (Coleman et al., 1984), and the mutagenic components of drinking water that had been characterized previously (Nestmann et al., 1980; Simmon et al., 1977) were volatile compounds. Meier et al. (1983) showed that the volatile component of mutagenic activity (20%) could be eliminated either by lyophilization or by purging the samples during their preparation for testing. Further work by Meier et al. (1985) involved calculating the theoretical contribution of mutagens whose activities had been reported in the literature (e.g., Douglas et al., 1983). In addition, artificial mixtures of these compounds were tested, but the collective activities accounted for only 6.5% and 8% of the mutagenicity

of the total sample. Clearly, chemical identification of the nonvolatile compounds responsible for most of the mutagenicity of drinking water remains a prime area for further investigation.

Another approach that has been used successfully in the identification of mutagens in an archived sample of drinking water residue (Tabor, 1983) and in chlorinated pulp and paper mill effluent (Douglas et al., 1985; Holmbom et al., 1984) is mutagenicity-directed fractionation, i.e., sequential subfractionation of extracts using mutagenicity as a guide.

Toxicity testing of water has been limited almost exclusively to short-term assays for genetic toxicity and short-term animal skin tests for tumorigenicity. In one 90-day study, Condie et al. (1985) found enlarged livers and hemoglobin in the urine in male Sprague-Dawley rats fed chlorinated humic acid (1.0 g/liter) daily in their drinking water. Apparently, the bleeding was caused by crystalline deposits in the renal pelvis.

Genetic toxicity studies are employed in the toxicological evaluation of mutagenicity (Health and Welfare Canada, 1986) as well as for predicting carcinogenic potential. One hundred percent association between mutagenicity and carcinogenicity is not expected because of important toxicological considerations, such as differences between *in vitro* and *in vivo* conditions and the complex, multistage process of carcinogenesis (Nestmann, 1986). Their reliability as indicators of carcinogenic potential for rodents and humans ranges from 60% to 75% for a broad range of chemical classes. Some classes of chemical carcinogens (e.g., aromatic amines and polycyclic hydrocarbons) are identified with greater accuracy than others (e.g., halogenated organics and metals), so genetic toxicity test results should be interpreted with caution. Samples devoid of activity should not be assumed to be noncarcinogenic, and some relatively strong responses in a test like the Ames assay can be produced by noncarcinogenic agents (e.g., some nitroarenes).

Rodent skin studies, while possibly more relevant as indicators of tumorigenicity, also fail to respond to all classes of chemical carcinogens and are confounded by secondary mechanisms involving irritation. In addition, the results from these assays cannot be directly extrapolated to ingestion exposures.

Toxicity of Concentrated Drinking Water

Little if any genetic toxicity has been found in unconcentrated drinking water (Forster et al., 1983; Harrington et al., 1983), so a number of studies have addressed concentrated drinking water samples and their subfractions. Numerous methods have been used to concentrate drinking water prior to its evaluation in mutagenicity and carcinogenicity bioassays. The methods employed most frequently utilize macroreticular resin chromatography

(commercial XAD-2) and subsequent testing with the *Salmonella* /mammalian-microsome mutagenicity test (EPA, 1985; Nestmann et al., 1979).

The Ames *Salmonella* assay has been the primary source of toxicity information on drinking water samples (Cumming et al., 1983; Kool et al., 1985b; Meier and Bull, 1985). It requires only minimal amounts of material and is compatible with the broad range of solvents used to reconstitute concentrated solids or elute resin columns.

Concentrated residues from both chlorinated and untreated drinking water samples have been evaluated in the Ames test; most show some mutagenic activity, a subject that has been reviewed in depth (Loper, 1980a; Nestmann, 1983). The mutagens appear mixed between frameshift and base-pair substitution types and are, by and large, direct-acting mutagens. Some mutagenic species are quite stable. The levels found in tap water have been considered difficult to eliminate by such purifying methods as distillation, reverse osmosis, or carbon filtration (Cheh et al., 1983), although activated carbon systems have recently been used successfully (Loper et al., 1985). The mutagenicity of drinking water also appears to fluctuate in direct proportion with the organic content of the water. Water disinfection, particularly chlorination, has been shown to affect the mutagenicity of concentrated samples (Douglas et al., 1986; Loper, 1980a).

Using a model system in which aqueous solutions of organics were chlorinated, Bull et al. (1982) showed that by-products formed by chlorination of either humic or fulvic acids were mutagenic in salmonellae. This report was followed by a more detailed study of the reaction conditions required to produce the mutagens and to maintain mutagenic activity (Meier et al., 1983). Certain parallels were noted between mutagenic activity of drinking water samples and the model reaction involving chlorinated humic acids. For example, unchlorinated samples were nonmutagenic in salmonellae; and the mutagenic activity observed in chlorinated samples was higher without an extract of mammalian enzymes (S9) for metabolic activation (Meier et al., 1983).

The toxicological significance to humans of bacterial mutagens found in drinking water concentrates is not clear, and the Ames assay may best be used as a biological monitor for drinking water sources over time or to assess the consequences of various treatment procedures. While there may be epidemiological evidence supporting an association between chronic toxicity and drinking water contaminants (organics, for example), one cannot assume that biological activity in the Ames test is a reflection of the cause of the increased risk.

Lang et al. (1980) reported that organic residues from drinking water samples were able to transform BALB/C3T3 cells in culture and that the transformed cells were capable of producing tumors when transplanted to

TABLE 3-4 Dependence (pH) of Mutagenic Activity in the Ames Test and SCE Induction in CHO Cells on Treatment of Humic Acid with Chlorine (HOCl/OCl)[a]

	Mutagenic Activity[b]		
Humic Acid Sample	TA98	TA100	SCE Induction[c]
Chlorinated (pH 7.0-2.5)	339 ± 29 (100)	1,696 ± 148 (100)	2.96 (100)
Chlorinated[d] (pH 7.0-6.5)	62 ± 10 (100)	367 ± 34 (22)	2.15 (73)
Chlorinated (pH 11.5-6.5)	NS[e]	490 ± 33 (29)	1.10 (37)
Nonchlorinated[f]	NS[e]	NS[e]	0.62 (21)

[a] From Meier and Bull (1985) with permission.
[b] Net revertants per milliliter of sample, calculated from the linear portion of the dose-response curve from assays without S9 added. Parentheses indicate the percentage of activity in sample A.
[c] SCEs per cell per percent sample incorporated into medium, calculated from the linear portion of the dose-response curve from assays without S9 added. Parentheses indicate the percentage of activity in sample A.
[d] 0.25M sodium phosphate buffer was added during chlorination to stabilize the pH.
[e] NS, Not significant (i.e., less than twofold above background at any dose tested).
[f] The nonchlorinated humic acid was prepared at pH 7; the pH was then lowered to 2.5 with HCl.

athymic mice (Kurzepa et al., 1984). This assay responds to many of the same classes of chemicals that are active in the Ames test. These results, however, add some significance to the biological activity of the organic residues in that *in vitro* transformation is performed with animal cells and the tumorigenic properties of the transformed cells can be verified in *vivo*.

Table 3-4 compares bacterial mutation with sister chromatid exchange (SCE) activity in unchlorinated and chlorinated humic acid. These data provide further evidence of the genetic toxicity of drinking water organics but also show that humic acid alone may have some biological activity. *In vivo*, however, chlorinated humic acid samples were not active in assays designed to detect alterations in chromosome structure or spermhead morphology (Meier and Bull, 1985).

Rodent skin tumorigenesis studies have been used to evaluate drinking water concentrates (Kool et al., 1985b). Responses in these tests were variable but did seem to be associated with the concentrations of total organics applied. Mouse skin initiation/promotion studies also suggested that some drinking water concentrations contain tumor initiators but do not act as promoters or complete carcinogens. Subcutaneous injection and skin painting studies are considered to be reasonably reliable models for

some chemical classes; but like the Ames test and *in vitro* cell transformation assays, this group of tests may be responding to a class of chemicals that are not particularly relevant to carcinogenic risk in humans, whose primary route of exposure is by ingestion (OSTP, 1985, p. 10414).

Another explanation is that some chemicals interfere with the expression of mutagenic properties of other agents. Numerous examples of chemical interference resulting in inhibition or elimination of mutagenicity have been reported for the Ames test.

A much more relevant *in vivo* approach would be lifetime exposures to drinking water. Two studies of this type have been reviewed (Kool et al., 1985b). One addressed chronic carcinogenicity in rats, employing a synthetic residue containing 11 chlorinated hydrocarbons most commonly detected in drinking water samples. The results of this study, in which the high-dose animals received 22 mg/day for 27 months, were negative. In the second study (Kool et al., 1985a), rats were exposed to drinking water concentrates obtained from commercial XAD-4/8 resins. The duration of the study was 26 months, and no increases in tumor incidence were observed. The samples used in this study were mutagenic in the Ames assay.

Toxicity of Fractionated Drinking Water Concentrates

Some drinking water concentrates produced by resin columns exhibited little or no bacterial mutagenicity until the complex residues were fractionated by HPLC. After separation, several subfractions showed activity (Cumming et al., 1983). These observations may indicate toxicity associated with unfractionated concentrates, or they may indicate chemical interference. The expression of weakly mutagenic components requiring large doses may be prevented by premature target cell cytotoxicity from other nonmutagenic components. Once the components are separated from each other by HPLC, the weak mutagens can easily be detected.

On the other hand, other research efforts have identified methods either to prevent the formation of mutagens during disinfection processes or to reduce their levels subsequent to formation. For example, using ozone instead of chlorine as a disinfectant, with fulvic acids as a model mixture, ozonated fulvic acids were found to produce only weak (Kowbel et al., 1982) or no mutagenic activity (Kowbel et al., 1984) compared with the results of Bull et al. (1982) with chlorinated fulvic acids. Depending on the dose of ozone and the pH of the reaction mixtures, preozonation of soil or water fulvic acids could partially or even totally prevent the formation of mutagens during subsequent chlorination treatment (Kowbel et al., 1984, 1986). In addition, Meier et al. (1983) showed that mutagenic activity of chlorinated humic acid can be prevented or reduced either by

chlorination at alkaline pH or by raising the pH of samples following chlorination. This change in activity is probably due to the lability of the direct-acting, chlorine-substituted mutagens at alkaline pH, as also observed in drinking water samples by Loper (1980b) and in an experimental system by Nazar and Rapson (1982).

One observation derived from analysis of HPLC fractions was that disinfection processes alter the total mutagenicity of pooled HPLC fractions. This suggests that even though water concentrate samples may be mutagenic both before and after disinfection, the mutagenic components of the residue change. Some mutagenic species seem to disappear, while new ones are formed.

It is not clear whether comparisons of pooled HPLC fractions are relevant to an assessment of biological activity. If nonmutagenic compounds are capable of reducing or suppressing mutagenicity of other agents in concentrates, the combined mutagenicity obtained from pooling fractions may give misleading indications of activity. Conversely, concentrated residues are not comparable with normal water in chemical/chemical interactions. Mutagens in dilute samples may act more like the HPLC subfractions.

EPIDEMIOLOGICAL STUDIES

The importance of drinking water for human life creates powerful incentives for epidemiological studies of the effects of contaminants in this essential, ubiquitous medium. The use of such studies in risk assessment is reviewed in Volume 6 of *Drinking Water and Health* (NRC, 1986, pp. 226-249).

Epidemiological studies of drinking water typically rely on a dichotomous characterization of the water treatment as chlorinated or nonchlorinated combined with a dichotomous classification of the water supply source as surface water or groundwater. Some studies infer trends in levels of contaminants such as trihalomethanes (THMs) and other carcinogens in drinking water by modeling past exposures from currently monitored levels or from histories of water-treatment practices. However, most epidemiological studies of drinking water are seriously hampered by the universal exposures that occur and by the need to control for potentially confounding variables, such as patterns of diet, smoking, and geographic migration in large populations.

Volume 3 of *Drinking Water and Health* (NRC, 1980) reviewed 13 epidemiological studies, beginning with the initial mortality associations of Harris and colleagues (Page et al., 1976). The majority of these studies were correlational in nature, using mortality as the outcome measure; only three used specific chemical assays (for THMs) as exposure variables. In

Volume 3 the committee noted methodological problems associated with these early studies and the generally low and inconsistent risks to specific cancer sites. They concluded that with the large array of possible confounding factors it would be difficult to ascribe an effect to any factor with certainty. Nonetheless, it was believed that continued epidemiological studies of drinking water in relation to cancers of the bladder and possibly the colon and rectum were warranted, particularly studies in which exposure to the water variables of interest and other potential confounding variables could be obtained directly from individuals rather than being inferred on an ecological basis.

The present committee reviewed studies subsequent to those of the 1980 report and briefly examined those discussed previously. These studies are grouped according to broad categories of epidemiological design and show progressively greater ability to obtain data from individuals. Additional reviews of epidemiological evidence have been prepared by Cantor (1982), Crump (1983), Crump and Guess (1982), and Williamson (1981).

Correlational Studies

Erie County, New York

Carlo and Mettlin (1980) studied 4,255 cases of esophageal, stomach, colon, rectal, bladder, and pancreatic cancers reported through the New York State Tumor Registry for Erie County, New York (Buffalo and environs), between 1973 and 1976. Age-adjusted incidence rates were calculated by census tract and related to water source, level of THMs from a single survey in July 1978, and a variety of socioeconomic parameters of the tracts. Statistically positive associations were found between surface water and esophageal and pancreatic cancer and between pancreatic cancer in white males and THM levels. The authors themselves placed little credence on these findings, noting that the pancreas-THM relationship was found only in one sex-race subgroup and that only 10% of the census tracts were served by groundwater. Finally, the range of THM measurements was narrow (the largest variation was 71 ppb), and no trend data were obtained. Given only a single measurement per source, the opportunity to form meaningful associations was limited.

Massachusetts

Tuthill and Moore (1980) related cancer mortality rates for the 1969 to 1976 period in Massachusetts communities supplied by surface water to chlorination exposure data as measured by average past chlorine dose, recent total THM levels, and recent chlorine dose. Stomach and rectal

cancers significantly correlated with recent THM levels and chlorine dose but not with estimates of past chlorine dose. In addition, when stepwise regression models with migration patterns and ethnic data were used, the significance of the associations between cancer rates and recent total THM and chlorine dose disappeared. The authors believed that failure to control first for social variables and then for changing patterns of chlorination over time may have led in previous studies to spurious associations of chlorination of drinking water with cancer.

Iowa

Bean et al. (1982a, b) examined age-adjusted cancer incidence rates in Iowa communities supplied by a single major source of drinking water for the period 1969-1978 and related these to the source and characteristics of the water supply after stratification for population density. In each population group, rates of lung and rectal cancers were higher in communities supplied by surface water than in communities supplied by groundwater; the risk ratio for colon cancer (1.38) was higher only in the 1975-1978 period. When communities supplied only by groundwater were included, risk ratios of male (1.32) and female (1.29) lung and female rectal (1.39) cancers were found to be higher in communities with chlorinated water, while for male rectal cancer, rates were higher in communities with nonchlorinated water.

Isacson et al. (1985) examined cancer incidence in communities of 1,000 to 10,000 inhabitants supplied by groundwater with nonchlorination-induced contamination as indicated by levels of 1,2-dichloroethane or nickel in the finished supplies. Significantly elevated rates of colon and rectal cancers were found in residents of communities with detectable levels of 1,2-dichloroethane and of bladder and lung cancer in residents of communities with detectable levels of nickel. The associations were independent of chlorination status. Results did not necessarily indicate a specific relationship between nickel or 1,2-dichloroethane, but rather that these variables served as indicators of likely contamination from external sources. These results suggest that water-quality variables other than THMs may be associated with cancer.

Mortality Case-Control Studies

Illinois

Brenniman et al. (1980) conducted a case-control study of gastrointestinal and genitourinary cancers in Illinois residents, excluding Cook County (Chicago). The cases were cancer deaths from 1973 to 1976; controls

were noncancer deaths over the same time period. Noting that the composition of surface waters differed from groundwaters in many respects other than chlorination and THM production, the authors limited their analysis to communities served by groundwater supplies. Although elevated relative risks of chlorination were found for colon and rectal cancer, particularly in females, the authors believed that the results showed no clear associations, since little consistency appeared in the analysis by subgroups, especially in degree of urbanization. In a detailed review, Crump and Guess (1982) observed that the numbers in the Illinois study limited the power to detect significant but relatively small associations because only a dichotomous chlorination variable was used, no control for population migration was employed, and the restriction of analysis to groundwater limited the range of THM values that could be used in analysis.

Wisconsin

Young et al. (1981) and Kanarek and Young (1982) examined associations among gastrointestinal, genitourinary, brain, lung, and breast cancers in white females in Wisconsin from 1972 to 1977 by a death-certificate-based case-control design. Detailed information on past source and treatment characteristics of the community water supplies was obtained by interviewing plant operators to elicit those factors presumed to influence the organic content of the raw water. Based on these factors, estimates of by-products were constructed. Other variables included in the analysis were occupation, urbanicity, and marital status. Only colon cancer was significantly associated with the estimated chlorine dose for the past 20 years. No relative risk gradient was found according to high, medium, or low chlorine dose, but an approximate doubling of the risk (1.5 to 3.0) occurred when the analysis was restricted to chlorinated sources affected by rural runoff. This was presumed to be related to the increased THM formation that occurred when added substrate was present. Rural runoff was not evaluated as an independent risk factor, nor was population mobility assessed.

Louisiana

Gottlieb et al. (1981, 1982) compared cancer and noncancer deaths from 1960 to 1975 in Louisiana parishes selected for similarities in industrialization and approximately equal exposure of the population to surface water and groundwater. The length of time of water source exposure was estimated by relating place of birth to place of death. The study also compared cardiovascular death of controls to death of controls

from all other causes. No evidence was found of bias resulting from the use of cardiovascular deaths among controls. Three types of cancer (rectum, breast, and lung) showed significant association with drinking surface water. The risk considered to be most suggestive of a causal relationship was found for rectal cancer. Elevated odds ratios were seen in both sexes, and a dose-response gradient was noted, with odds ratios of 2.50, 1.57, and 1.00 for lifetime surface, some surface, and lifetime groundwater use, respectively. Odds ratios for males were highest, increasing to over 3.0 when the lifetime water use variable was used. The association of lung cancer and surface water was statistically significant only among nonwhite males and only for water source at death. Breast cancer also showed a gradient effect, but significance was found only for white females. For all cancers, the effect of chlorination, as expected, paralleled the relationship to surface water, but for breast cancer the odds ratio increased when chlorination was considered independently. It was suggested that confounding by population density may have occurred. Kidney and liver cancers also showed elevated odds ratios, but to a lesser degree and without statistical significance.

In one of the early studies of cancer mortality in Louisiana, Page et al. (1976) used multivariate regression analysis to show an association of cancer mortality rates with drinking water obtained from the Mississippi River. (Some parishes in Louisiana, mostly in the southern part of the state, receive all or most of their drinking water from the river, while other parishes do not.) They found an apparent association between the use of water from the Mississippi River and mortality rates from all cancers, from cancers of the urinary organs, and from cancers of the gastrointestinal tract. The possible role of water disinfection was not postulated, but the investigators pointed to the high incidence of bladder cancer in New Orleans and the finding of carcinogens in water from that river.

New York

Lawrence et al. (1984) used the New York State retirement system to identify public school teachers and recorded deaths among them between 1962 and 1978. A total of 395 colon and rectal cancers in white females in the central geographic corridor of the state were identified and matched by age and year of death (2 years) with noncancer deaths from the same pool. Water source and treatment were recorded for each study subject for a 20-year period at home or work prior to death. Cumulative chloroform exposure was modeled from previous THM surveys, with significant predictor variables being prechlorine and postchlorine dose, chlorine residual, and type of water source. Calculation of odds ratios showed no associations

among colon or rectal cancers and surface water or cumulative distribution of chloroform exposure after control by logistic analysis for average source type, population density, marital status, age, or year of death.

Massachusetts

Zierler et al. (1986) examined the patterns of mortality of residents of Massachusetts who died from 1969 to 1983 and lived in communities using drinking water that was disinfected by either chlorine or chloramine. There were 51,645 deaths due to selected cancers and 214,988 controls who died from cardiovascular, cerebrovascular, or pulmonary disease or from lymphatic cancer. Data were analyzed by calculating standardized mortality ratios for cancer and other diseases in residents of communities with chloraminated drinking water. Expected rates were derived from cause-specific deaths in Massachusetts and also by examining mortality ratios of selected cancer sites in comparison with mortality ratios of controls in communities with chlorinated versus chloraminated drinking water; these were termed the mortality odds ratio. Bladder cancer mortality was elevated (the mortality odds ratio was 1.7 with a 95% confidence interval of 1.3-2.2) in residents of communities with chlorinated water relative to mortality in residents of communities with chloraminated drinking water, a factor possibly related to the higher levels of THMs produced by chlorination. Also of interest was a small increase in deaths due to pneumonia and influenza among residents of communities using chloramine as their drinking water disinfectant.

Case-Control Studies Using Personal Interview

North Carolina

Cragle et al. (1985) performed an incidence-based case-control study of colon cancer and water chlorination in North Carolina in which detailed personal interviews were used to collect information on pertinent risk variables, including exposure to chlorinated water through a 25-year residence history. Cases were hospitalized males and females with primary colon cancer; controls were patients with the closest admission date who matched on age, race, sex, vital status, and hospital and who had no previous history of cancer of any type, mental disorder, or major chronic intestinal disorder. The sources of water exposure were initially classified as unchlorinated groundwater, chlorinated groundwater, or chlorinated surface water, with no consideration of levels of pollutants. When it was found that chlorinated groundwater represented only a small fraction (7%), this group was lumped with chlorinated surface water. Thus, the results

represent essentially a dichotomous comparison of chlorinated surface water versus nonchlorinated groundwater sources. Nonwater variables positively associated with colon cancer were genetic risk and a factor that is alcohol consumption times a high-fat diet, while smoking and number of pregnancies were negatively associated.

For reasons that are not clear, an interaction between age and chlorination was found even after adjustment for length of exposure. Above the age of 60, there was a statistically significant relationship between chlorination and colon cancer, using a logistic regression model with control of confounders. Although this effect was not seen in younger individuals, for all age groups the odds ratio was higher for persons who drank chlorinated water at their home for 16 years or more than it was in those who drank chlorinated water for 15 years or less. For persons 80 years of age or older, the odds ratio reached 3.36 in those exposed for more than 15 years. In this study no attempt was made to model the levels of THMs in the drinking water in past years, nor were current levels reported.

Wisconsin

Young et al. (in press) conducted a case-control study of colon cancer and drinking water trihalomethanes in white males and females between the ages of 35 and 90 in Wisconsin. There were 400 living colon cancer cases selected from the Wisconsin Cancer Reporting System; 600 controls came from two sources: a random selection from a statewide listing of motor vehicle operators and cancer cases other than gastrointestinal or genitourinary from the Wisconsin reporting system. Lifetime residential and drinking water source histories, diet, medical history, social class, and other life-style factors were obtained by questionnaire. Highly detailed historical data on community water source and treatment were collected, as well as data from individuals on the amount of water consumed per day. These data, together with current levels of THMs from a recent survey, allowed the construction of a model for estimating period-specific THM concentrations for the length of the study period. Logistic regression was used to estimate risks associated with THMs at 10-year periods up to 50 years before cancer diagnosis. Small risks of marginal significance were found for exposure to chlorinated water at the time of diagnosis, but no significant risk ratios were found for any other time period, for any specific relation to THMs, or for any specific age groupings. This held true when each control group was analyzed separately.

These results were of particular interest in view of the earlier results, discussed above, of a case-control mortality study conducted by the same investigators in the same general population of Wisconsin, in which a

significant positive association was found between colon cancer and sources affected by rural runoff. The reasons for the difference in outcome are not definitively known, but the second Wisconsin study comes as close to meeting ideal methodological criteria for cancer-THM associations as any study yet presented. This suggests that design differences—specifically, the inclusion of residential mobility in the latter study—could have been responsible. As noted by the authors themselves, however, another possible explanation could be the generally low levels of THMs found in Wisconsin surface waters, which would limit the ability to detect significant differences.

National Bladder Cancer Study

In 1979 the National Cancer Institute launched a nationwide collaborative study of the relationship between bladder cancer and the use of artificial sweeteners. Cantor et al. (1985) were able additionally to analyze the effects of the chlorination of drinking water on bladder cancer. The drinking water regions were metropolitan Atlanta, Detroit, New Orleans, San Francisco, and Seattle and the states of Connecticut, Iowa, New Jersey, New Mexico, and Utah. All had population-based cancer incidence registries from which live cases were selected. Controls were randomly picked from the general population and frequency matched to cases by sex, 5-year age group, and geographic area. Detailed information on geographic mobility and water source was collected, as well as information on other pertinent variables. In a separate data collection, water utilities serving more than 1,000 persons were surveyed, and information on source, chlorination, and protection of watershed was noted.

Risk of bladder cancer among white respondents was examined in logistic regression models that included age, smoking of cigarettes, sex, study area, and usual employment as a farmer. These initial analyses were restricted to the 1,244 cases and 2,550 controls who were never employed in a high-risk occupation for bladder cancer and whose residential water was supplied from either a nonchlorinated ground source or a chlorinated surface source for at least 50% of their lifetime. When risk of bladder cancer was evaluated by the usual use of chlorinated surface source, as compared with the usual use of nonchlorinated groundwater, there was no overall association. However, among nonsmokers, a group generally at low risk for bladder cancer, those whose usual source was of chlorinated surface origin had an odds ratio of 1.4, relative to usual users of nonchlorinated groundwater, and there was a relationship between risk and duration of chlorinated surface water use. Among nonsmokers, the relative risk increased from 1.3 among users of chlorinated surface water for less

than 20 years to 2.3 among those who used chlorinated surface water for 60 or more years.

When relative risks by duration of chlorinated surface water use were examined by reporting area, risk appeared to be significantly higher in the relatively rural areas (Iowa, Utah, and New Mexico) than in the metropolitan areas. No reasons for this difference could be positively identified, but it was noted that high levels of chlorination by-products are present in many community water supplies serving towns in farming areas. An elevated risk could also potentially be related to the use of agricultural chemicals or, conversely, to unknown, and therefore uncontrolled, independent causal variables in the urban areas that masked a chlorination effect.

Further analyses of the NCI data set (Kenneth Cantor, National Cancer Institute, Bethesda, Maryland, personal communication, 1986) have revealed positive associations of bladder cancer risk with level of tap water ingestion and duration of exposure, predominantly among study subjects with long-term residence in communities served by chlorinated surface waters.

Groups at Increased Risk

While there has been a considerable amount of research on the chemistry of disinfection by-products, the data base is often limited with respect to the toxicological effects of such products. Even less attention has been directed to the effects of such chemical by-products on individuals and groups within the human population who are at potentially increased risk. Nevertheless, with increasing knowledge of the nature of the chemical properties (i.e., oxidation potential) and emerging toxicological profiles revealing the end points affected, it is possible to make predictions. Population subgroups who have previously shown enhanced risk from exposure to agents that damage DNA or affect red-blood-cell membranes, endocrine functions, or cholesterol formation and metabolism would appear to be likewise at enhanced risk to these products of drinking water disinfectants.

Several oxidant-stressor by-products of disinfection with chlorine dioxide, such as chlorite, have been evaluated for their potential effects on individuals with a compromised ability to deal with oxidant stress to their red blood cells (i.e., those with a glucose-6-phosphate dehydrogenase [G6-PD] deficiency). In the one published study addressing this issue (Lubbers et al., 1983, 1984), the researchers administered 5 mg of chlorite in 500 ml of drinking water per day for 12 weeks to three healthy adult males with an A-variant form of the G-6-PD deficiency. The researchers found an increase in methemoglobin in the treated subjects. Although actual

values were not reported, the authors found them to be in the normal range and therefore dismissed as unimportant this indication of increased oxidant stress. The A-variant form of the G-6-PD deficiency is the most prevalent found in the United States, comprising 13-16% of black American males. The less frequently occurring Mediterranean variant affects no more than 8% of males of Mediterranean origin, but more severely limits enzyme activity to only 1-8% of normal males as compared with the A-variant's 20-33% of normal activity. Those with the Mediterranean variant are known to be more sensitive than those with the A-variant. Not only is the dosage initiating the hemolytic process lower, but also the adverse effect is more intense (Calabrese, 1984). Lubbers et al. (1983) did not investigate the responses of variants other than the A-variant to chlorite; neither did they address the issue that the process of hemolysis in G-6-PD-deficient persons exposed to oxidant stressor agents may be markedly enhanced or potentiated by the copresence of an infection (Baehner et al., 1971), by a diet low in antioxidants (Calabrese, 1984), and possibly by chemical interactions (Calabrese et al., in press). Due to the small number of participants in this study and the very low dose administered, it is premature to offer any generalizations on the responses of individuals with a G-6-PD deficiency to the oxidant-stressor activity of agents such as chlorite based on the Lubbers et al. (1983) study.

A major challenge in addressing the potential health effects in G-6-PD-deficient persons is the lack of a general animal model adequate for both qualitative and quantitative predictions of human responses. A recent comprehensive evaluation has indicated that no rodent model is suitable for this purpose (Horton and Calabrese, in press). One possible model, the Dorset sheep, displays a G-6-PD deficiency in terms of absolute enzyme activity like that of the human with a Mediterranean variant deficiency. Similarly, it displays the heightened sensitivity to a number of oxidant-stressor compounds shown by humans with G-6-PD deficiency. Although the Dorset sheep might avoid false-positive predictions of response, it is inadequate as a model because red cells in sheep are less dependent upon glucose for energy metabolism than are those in other mammals, and their response to other known oxidant-stressor agents is different from G-6-PD-deficient red blood cells in humans. Many large communities in the United States have been treating their drinking water either with chloramines (AWWA, 1985) or chlorine dioxide (Aieta and Berg, 1986). This opens the possibility for initiating epidemiological investigations on the effects of these agents on currently exposed populations.

Newborns, especially those with enzymatic deficiencies, are the group most likely to be at increased risk from the effects of such oxidant-stressor agents on red blood cells. Neonates have low levels of several antioxidant

enzymes including catalase (Jones and McCance, 1949) and methemoglobin reductase (Ross, 1963). They also have difficulty in detoxifying bilirubin as a result of a developmental deficiency of glucuronyl transferase (Vest, 1965). In the single, very limited epidemiological study considering the potential enhanced susceptibility of the very young to oxidant stressor agents, Tuthill et al. (1982) reported findings consistent with the theory that the red cells of infants are at increased risk from the by-products of chlorine dioxide disinfection.

Hemodialysis patients are also at potentially increased risk from exposure to contaminants in water. Researchers (Eaton et al., 1973; Kjellstrand et al., 1974) have demonstrated that when tap water containing chloramines is used for dialysis baths, methemoglobin and Heinz bodies are formed and red cell reductive metabolism is inhibited in these patients (see monochloramine section of Chapter 4).

In summary, there have been only limited attempts to assess the effects of by-products of alternative disinfection processes on potential high-risk groups via the use of animal models or epidemiological studies. This gap in the available data base precludes confident prediction of the effects of such products on the U.S. population.

ALTERNATIVE METHODS

Chloramination

Monochloramine is becoming more widely used as a disinfectant (Dice, 1985; Kreft et al., 1985), primarily because it limits the concentration of trihalomethanes produced (Fleischacker and Randtke, 1983; Johnson and Jensen, 1986). Monochloramine produces chlorine substitution into humic and fulvic material to produce an organic halogen that cannot be purged (Fleischacker and Randtke, 1983; Jensen et al., 1985; Johnson and Jensen 1986) and that can be measured using the TOX method (EPA, 1980, Method 450.1). The quantity of TOX produced by monochloramine is only 5% to 50% of the TOX produced by a similar dose of free chlorine, but the concentration of monochloramine used in water treatment is generally greater because it is less effective as a disinfectant. Few individual, ether-extractable, and GC/MS-identifiable products are produced in the chloramination of humic materials compared with the large number of such compounds produced by chlorine. DCA and TCA, which are identifiable by such methods, are produced in extremely small quantities (Johnson and Jensen, 1986).

Monochloramine may be a by-product of drinking water chlorination, or it may be added to maintain residual disinfection activity in a potable water distribution system. Operationally, chloramination has been practiced

in three different ways. Each method produces a finished water of different chemical and bacteriological quality (Arber et al., 1985).

First, marginal chlorination is practiced when chlorine is added to a water source that contains ammonia in order to generate monochloramine as the primary disinfectant. The amount of chlorine added by weight is usually less than five times the amount of ammonia present by weight. Because the interaction between free chlorine and the trace organic precursors of THM in the water is minimized, THM levels produced in the finished water are low. However, because monochloramine is a much poorer disinfectant than chlorine (Feng, 1966; Johnson, 1978; Marks and Strandskov, 1950; Wolfe et al., 1984, 1985), disinfection levels may not be sufficient to prevent bacterial growth in the system (Arber et al., 1985). In addition, for the reasons discussed below, it is more likely that part of the chloramine formed is an organic chloramine.

More potent disinfection can be obtained if sufficient chlorine is added beyond the amount needed to remove ammonia from source water, producing a free-chlorine residual. The contact time sufficient to obtain optimum primary disinfection can then be kept to a minimum before commercially available ammonia is added to the water. Although this method does produce chlorinated by-products, it is generally preferred when it is important to produce maximum disinfection.

A third method of chloramination involves the generation of a concentrated solution of monochloramine off-line (preformed) and the addition of this solution to water as both the primary and residual disinfectant. For water containing significant concentrations of organic amino-nitrogen, the bactericidal quality would be better, at least initially (see discussion below), if preformed monochloramine is used than if marginal chlorination is practiced. However, the poorer disinfection capability of monochloramine may still pose a problem (Feng, 1966; Johnson, 1978; Marks and Strandskov, 1950; Wolfe et al., 1984, 1985).

Chloramine Analysis

The analysis of chloramines in natural water samples has been of two types. The most widely used methods are oxidant or chlorine residual measurements. Chloramines are strong oxidants that, like chlorine, can oxidize iodide to iodine. The measurement of iodine, or iodometry, is a classical method of analysis, although total oxidant methods such as iodometry are notoriously susceptible to interferences. The oxidation of iodide to iodine is relatively easy; the standard oxidation potential of the couple is -0.54 V. Thus, most oxidizing agents such as manganese (IV) (Strupler and Rouault, 1979), hydroperoxides, and at least some *N*-chloroorganic compounds (Gray and Workman, 1983) are capable of making

iodine under the conditions used to measure monochloramine (NH_2Cl). The compounds measured as chloramines, therefore, include a wide variety of oxidants that may contain no chlorine.

Thus, all the common chlorine residual measurements are relatively nonspecific or nonselective for the compound that it is desirable to measure. The most selective methods include the free-chlorine procedures, such as FACTS and amperometric titration without the addition of iodide. The least selective methods are the total chlorine residual techniques. The latter methods include nearly all of the oxidants because they use either high concentrations of iodide (e.g., the *N*,*N*-diethyl-*p*-phenylenediamine ferrous ammonium sulfate [DPD-FAS] total chlorine method) or low pH (e.g., amperometric titration for dichloramine at pH 4) (APHA, 1985). The second and more selective type of method measures the chloramine compounds after a separation process. These methods include the amperometric membrane electrode (Stanley and Nossel, 1983) and chromatographic methods (Kearney and Sansone, 1985; Scully et al., 1984). Although less precise, these methods are less subject to interferences than the iodometric methods.

Organic Nitrogen Compounds

In all methods of chloramination, the generation of the disinfectant relies on the fact that ammonia reacts rapidly with hypochlorite to produce monochloramine (Morris, 1967):

$$NH_3 + HOCl \rightarrow NH_2Cl + H_2O.$$

Most organic amines and amino acids, however, react even more rapidly with hypochlorite to form organic *N*-chloramines (Morris, 1967; Weil and Morris, 1949). In water containing both ammonia and organic amino-nitrogen compounds, the relative amounts of organic (versus inorganic) chloramines formed when the water is chlorinated depend on the concentration ratios of ammonia to organic amino-nitrogen, the temperature, the pH, and the relative reaction rates (Isaac and Morris, 1980). However, Isaac and Morris (1980) have explained that chlorination of water containing 20 mg ammonia nitrogen per liter and 2 mg organic amino-nitrogen per liter will form 54% inorganic chloramine and 46% organic chloramine within 0.3 seconds at pH 7 and 20°C if the relative specific rates of reaction are 1:8.5 (NH_3 to organic amino-nitrogen).

In Volume 2 of *Drinking Water and Health* (NRC, 1980), some of the problems with the analysis of free and combined residual chlorine were discussed briefly. However, since that time, considerable uncertainties surrounding the interpretation of conventional measurements of free and combined residual chlorine have been pointed out (Cooper et al., 1982;

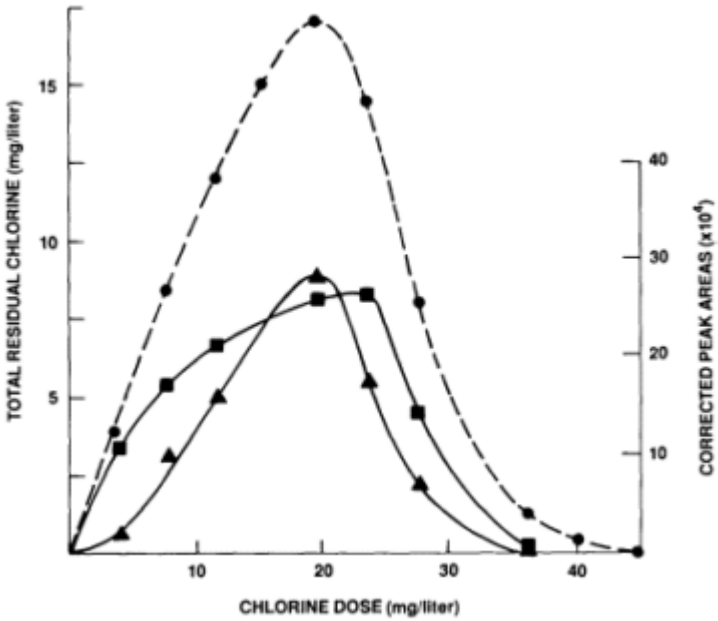


Figure 3-6 *N*-Chloroglycine (■) and NH_2Cl (▲) recovered after derivatization of dilute aqueous solutions of glycine and ammonium sulfate in 0.01 *M* phosphate buffer (pH 7.2) that had been chlorinated to different levels. After chlorination, each solution was incubated in the dark for 1 hour at room temperature before a portion was analyzed for total residual chlorine (•) and then derivatized.

Gould, 1986; Johnson, 1978; Jolley and Carpenter, 1983; Ram and Malley, 1984; Scully, 1986; Wajon and Morris, 1980; Wolfe and Olson, 1985; Wolfe et al., 1984, 1985). Both organic and inorganic *N*-chloramines respond in an identical manner to conventional methods of analysis (APHA, 1985) because both oxidize iodide to iodine in the determination of "combined residual" chlorine.

As a result, the breakpoint curve of water containing both ammonia and organic amines is a composite of the individual breakpoint curves of ammonia and every organic amino-nitrogen compound in the water that can react with hypochlorite. Figure 3-6 illustrates this using a recently reported method for the derivatization and analysis of organic and inorganic *N*-chloramines in dilute aqueous solution (Scully et al., 1984). Equimolar solutions of glycine and ammonia (4 mg of total nitrogen/liter) were chlorinated to different levels along the breakpoint curve. Figure 3-6 plots the relative amounts of chloramine derivatives recovered along

with the total residual chlorine measured by the DPD-ferrous ammonium sulfate (DPD-FAS) method (APHA, 1985). The plot demonstrates how *N*-chloroglycine is formed to a greater extent than NH_2Cl at low chlorine dosages.

From a water treatment standpoint, organic *N*-chloramines are undesirable because they are not effective disinfectants (Feng, 1966; Johnson, 1978; Marks and Strandskov, 1950; Wolfe et al., 1984, 1985). Consequently, a water treatment facility that practices marginal chlorination of water containing high concentrations of organic amino-nitrogen compounds runs the risk of overestimating the ability of its systems to maintain adequate disinfection.

The implications of this have been demonstrated by Wolfe et al. (1984, 1985). Using water from the San Joaquin Reservoir, they examined the effect of added glycine on the reduction of total count bacteria after chlorination or chloramination. Total count bacteria were reduced by 2 log units within 60 minutes when preammoniated water was chlorinated to a chlorine-to-nitrogen ratio of 3:1 by weight. However, when increasing amounts of glycine (0.1, 0.25, and 0.55 mg/liter) were added to the ammoniated samples before they were chlorinated to the same residual as in the initial experiment, inactivation of the bacteria was significantly inhibited to an extent proportional to the concentration of the glycine added (see Figure 3-7). Nevertheless, both amperometric titration and DPD-FAS determination of the "combined residual" chlorine suggested that all solutions had equivalent bactericidal capabilities. These results could only be explained by the competition between glycine and ammonia for reaction with chlorine and formation of the less-bactericidal *N*-chloroglycine. By contrast, preformed inorganic monochloramine was an effective disinfectant when added to water whether or not it contained glycine.

Organic *N*-chloramines can also form slowly by the reaction of inorganic chloramine with organic amines (Isaac and Morris, 1983, 1985; Snyder and Margerum, 1982):

$$NH_2Cl + RNH_2 \rightarrow NH_3 + RNHCl.$$

However, because the chlorine transfer reaction is slow, its significance may be limited to water distribution systems that use inorganic chloramine as the disinfectant when detention time in the system is considerable.

Several studies (Cooper et al., 1982; Wajon and Morris, 1980; White et al., 1983) have shown that a "false" free residual can be obtained by conventional methods of analysis in the presence of a number of organic chloramine compounds. White, for instance, failed to obtain adequate disinfection of wastewater that contained low concentrations of ammonia and significant concentrations of organic nitrogen. The effluent showed an apparent free residual chlorine level that should have been sufficient.

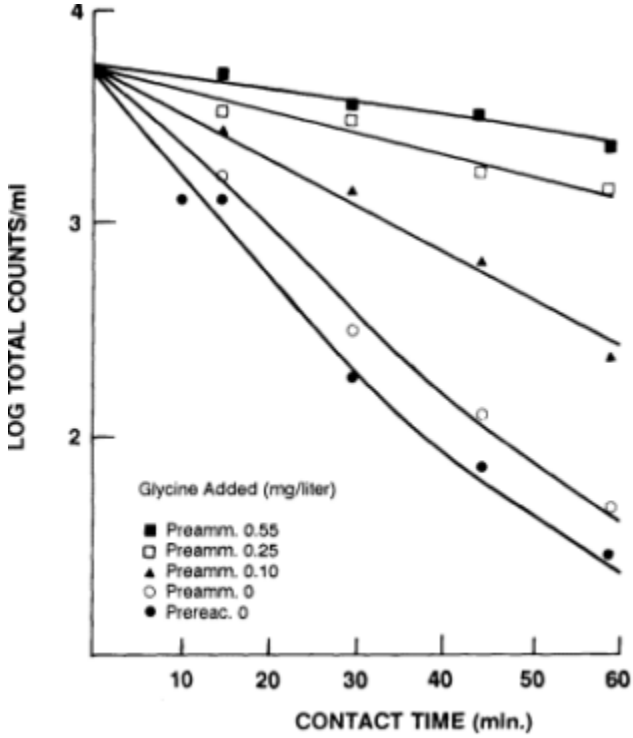


Figure 3-7 Inactivation of total count bacteria in a San Joaquin Reservoir sample using preammoniation and prereacted application techniques. Nitrogen (as glycine) was added to the samples at levels of 0.1, 0.25, and 0.55 mg/ liter prior to preammoniation treatment. Each datum point represents the mean of two observations. The pH was 8.2, chloramine concentration was 1.60, and the ratio of chlorine to nitrogen was 3:1. From Wolfe et al. (1985) with permission.

Ram and Malley (1984), on the other hand, examined the disinfecting ability of a number of model organic chloramino-nitrogen compounds that produce a free-chlorine residual. All appeared to exhibit bactericidal effectiveness toward *E. coli* when the bacterial cultures were inoculated so that an apparent free residual of 0.2 mg/liter of chlorine was maintained after 15 minutes.

Although interference of disinfection by organic nitrogen compounds can be demonstrated in laboratory experiments and these used to implicate interferences in treatment plants, there is still a poor understanding of the specific compounds responsible for interferences and their true significance

in actual treatment practice. The studies discussed here suggest that conventional methods of chemical analysis of residual chlorine tend to overestimate the effectiveness of disinfection. Until these processes are better understood, an awareness of such potential interferences is needed in the water treatment industry.

Chlorine Dioxide and Ozonation

Chlorine dioxide is a reddish-yellow gas that is stable only in the dark. A strong oxidant, it is used in drinking water principally for taste and odor control and as a residual disinfectant in the distribution system. Although it does not form chloramines or THMs, it yields chloride and chlorate in strongly acidic solutions (Bray, 1906) and chlorite and chlorate in alkaline solutions (Gordon and Feldman, 1964). Chlorite is also a by-product when chlorine dioxide reacts with any volatile organic material. Other by-products are unknown. Chapter 4 includes a discussion of the toxicity of chlorine dioxide, chlorite, and chlorate.

Ozone is a colorless gas, a dark blue liquid, and blue-black when in crystalline form. The gas is unstable at ambient temperature; the liquid and solid phases are particularly unstable. Its solubility in water is 49 ml/100 µl at 0°C; its melting point is -197.7 ± 2°C, and its boiling point is -111.9°C. In the gaseous state its density is 2.144 g/liter at 0°C and as a liquid it is 1.614 g/liter at -195.4° C.

Ozone is used as a disinfectant for air and water and as a mold and bacteria inhibitor in cold storage, in synthesis of organic chemicals, in water treatment for taste and odor control, and in bleaching agents. It is also used in the ozonolysis of unsaturated fatty acids to pelargonic acid, to azelatic acid, and to other acids; in the oxidation of furnace carbon black for ink black manufacturing; and as a catalyst in the production of peroxyacetic acid.

Ozone and its by-products were described in Volume 2 of *Drinking Water and Health* (NRC, 1980). This brief section discusses the current state of knowledge on the chemistry of ozone as it pertains to water treatment.

Use Patterns of Ozone and Chlorine Dioxide

Concern over by-products of chlorine has caused municipal water authorities to consider alternatives for disinfection and oxidation of drinking water. As a result, use of chlorine dioxide and ozone is on the increase in the United States, and many more utilities are considering these alternatives to chlorine.

New research has shown that ozone has the property of improving coagulation (flocculation is a more effective disinfectant for resistant pathogens) and can control taste and odor compounds and manganese at least as well as chlorine. The city of Los Angeles (Department of Water and Power) is currently building a 600-million-gallon per day (26 m^3/sec) direct-filtration plant with 1 mg of ozone/liter of water for pretreatment, making it the site of largest ozone use in water treatment in the world. This plant has also given the industry a new standard for the cost of ozone in a large-scale plant, one that is at least 20% lower than projected costs only 5 years ago. In summary, all indicators point to the increased use of ozone in water treatment.

How extensive this adoption of ozone and chlorine dioxide technology will be is not yet clear. However, it is clear that we know very little about the potential impact of these disinfectants (oxidants) if they are used in place of chlorine. This section does not focus on problems of engineering, thought to be particularly challenging for ozone, or the problems of disinfection efficacy. Rather, we emphasize the lack of information on by-product formation.

OXIDATION PROCESSES

It is not well appreciated that in water treatment chlorine acts primarily as an oxidant. That is, most of the chlorine added ends up as chloride ion (Cl^-), indicating that a redox process has taken place. This is the desirable result in many cases, i.e., to aid coagulation/flocculation and Mn+ + control. In addition, we can expect that the principal by-products of organic substrates will be oxidized, not substituted. Indeed, aqueous chlorine is capable of substituting halogen (for hydrogen, usually) only in a very few types of organic compounds. Research has focused on halogenated organics partly because they are often toxic as a class, but also because they are conveniently measured (by GC, electron capture and GC/MS). Oxidation products, either from chlorine, ozone, or chlorine dioxide, are not so easily detected. This is due to the fact that they are devoid of any convenient "marker" atoms (such as Cl in halo-organics), and also because they are similar to the organic compounds formed by natural oxidation processes. In other words, a surface water source such as a lake will be experiencing oxidative processes for months, perhaps longer. These oxidative processes (both prebiological and chemical) are quite similar in their chemistry to oxidation processes used in water treatment. Thus, it is no surprise that these oxidation processes produce by-products that are analytically difficult to distinguish from background organics. Nonetheless, with sophisticated analytical procedures by-products can be observed.

One of the difficulties in drawing conclusions about the risks associated with alternative oxidation processes is that these processes are chemically complex. Hoigné and coworkers (Hoigné and Bader, 1978a,b; Staehelin and Hoigné, 1985) have elucidated ozone decomposition, which becomes an example in point. What these studies show is that ozone reactions can occur by direct reaction of O_3 (a selective reagent) and by reaction of OH (hydroxyl radical) formed by O_3 decomposition. Moreover, the relative amounts of these two routes will be determined by variations in the matrix (e.g., pH, alkalinity, total TOC, and perhaps by the extent of the reaction). Superoxide ion is often a by-product of oxidation processes. Superoxide, hydrogen peroxide, formic acid, and other oxidation by-products can initiate the decomposition of ozone and change its route from direct reaction to radical character.

In toxicological studies on water with and without oxidative treatment, changes in reaction conditions may cause changes in reaction mechanisms, and therefore in reaction by-products. This perhaps explains some of the apparently contradictory findings of studies, that in some cases have shown carcinogenicity and mutagenicity of ozonation water greater than unozonated water, and in some cases vice versa (Bull et al., 1982; Kowbel et al., 1986; Zoeteman et al., 1982).

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4

Chemistry and Toxicity of Selected Disinfectants and By-Products

Volume 3 of the *Drinking Water and Health* series examined the toxicity of several major disinfectants and many of the by-products formed during drinking water disinfection. This chapter updates that material by assessing current research data. Recommendations for future research are also provided.

Risk quantification is an essential tool for rationalizing regulatory actions. Where sufficient data are available, quantitative risk assessments are calculated for the substances reviewed in this chapter. Quantitative risk assessment includes four distinct components: hazard identification, exposure assessment, dose-response assessment, and characterization of human risk at projected levels and patterns of exposure.

Following a thorough review of the toxicological data, compounds were classified according to whether they were or were not known (or suspected) carcinogens. For carcinogens, the multistage model was chosen for extrapolating from the high doses used in animal studies to the lower doses common in the environment of humans. This model appears to have a greater biological basis than most other models and in most cases is more conservative, usually producing higher estimates of risk at low doses. It incorporates the reasonable assumption of background additivity and is thus linear at low doses.

For carcinogens, the risk to humans is expressed as the probability that persons weighing 70 kg would develop cancer sometime in their lives as a consequence of ingesting I liter of water containing 1 μg of the substance daily over a lifetime of 70 years. Although risks to a 10-kg child were not calculated, the disproportionately high intake of drinking water by children compared with that of adults would place them at greater risk.

For substances not identified as known or suspected carcinogens and for which there were adequate toxicity data from prolonged ingestion studies in humans or animals, the subcommittee calculated a suggested no-adverse-effect level (SNARL), using methods developed in earlier volumes of *Drinking Water and Health* and estimating dose-response relationships when data were sufficient. This conventional approach was taken by default in the absence of suitable low-dose extrapolation models and because a "safe" level has not been demonstrated for these noncarcinogenic effects.

The SNARL was derived by estimating a no-observed-effect level (NOEL) for any given compound and then dividing it by an uncertainty or safety factor. Because of the pitfalls encountered in estimating NOELs, evidence supporting such a level in any given study was carefully weighed. Safety factors should be properly interpreted to indicate levels of confidence in the underlying studies. For some substances, the data base was adequate to permit an estimate of the magnitude of interspecies or intraspecies variability and to suggest a safety factor based on that estimation. Where such an estimate was not possible, safety factors devised in the first volume of *Drinking Water and Health* (1977, pp. 803-804) were used: 10 when satisfactory data from chronic epidemiological or clinical studies were used; 100 for well-conducted long-term animal studies; and 1,000 for short-term studies or studies with some potential inadequacies.

Ingestion may not be the sole route of exposure to substances in drinking water that are examined in this review. Cooking, showers, bathing, swimming, and other activities could theoretically provide important toxic contributions. Given the absence of data on these noningestion routes, this report does not include specific estimates of their contribution to total exposure. Further, drinking water is not the only medium or source of exposure to many of the substances evaluated here. To allow for exposures through other routes, the subcommittee generally assumed that drinking water provides 20% of the total exposure to a given substance.

CHLORINE

CAS No. 7782-50-5

$$Cl_2$$

Chlorine was previously reviewed in Volume 2 of *Drinking Water and Health* (NRC, 1980, pp. 18, 39, 144-166). At room temperature, chlorine is a greenish-yellow gas. It has a melting point of -102°C and a boiling point of -35° C. Chlorine is widely used as a water supply disinfectant as well as an oxidizing or chlorinating agent in producing chlorinated organic compounds. When chlorine is added to water, the following reactions occur:

$$Cl_2 + H_2O \rightarrow HOCl + H^+ + Cl^-$$
$$HOCl \rightleftarrows H^+ + OCl^-$$

Very little chlorine is available in molecular form (Cl_2) at pH values greater than pH 3.0. The hypochlorous acid (HOCl) that is formed may further ionize to produce hypochlorite ion (OCl^-) and hydrogen ion (H+). The dissociation of hypochlorous acid to hypochlorite and hydrogen ions is dependent on the pH of the solution.

Before analytical methods were capable of distinguishing free chlorine (HOCl + OCl^-) from combined chlorine (chloramines), it was recognized that chlorine residuals depended on chlorine dose in a complex manner. Low concentrations of added chlorine produce an equivalent amount of chlorine residual (oxidant), but subsequent addition of chlorine causes a reduction in the residual. After the loss of most of the residual chlorine, a point is reached beyond which additional chlorine produces a chlorine residual that is clearly more effective as a bactericide but less stable with time of contact. This point is known as the "breakpoint." Thus, chlorination of drinking water is practiced in two distinct ways, depending on the level of nitrogen present and the level of chlorine added. Marginal chlorination, or the use of the first residual produced, is really chloramination. Breakpoint chlorination, or addition of chlorine beyond the dip in the curve of residual produced versus chlorine added, is free residual chlorination (White, 1972).

Health Aspects

Based on relatively early literature, the American Conference of Governmental Industrial Hygienists (ACGIH, 1986) recommends a threshold limit value (TLV) expressed as an 8-hour time-weighted average for workroom air of 1 ppm (approximately 3 mg/m^3) for occupational exposures to protect against chronic lung changes, accelerated aging, and corrosion of teeth.

Observations in Humans

Occupational and domestic poisonings to chlorine gas have been reported (Philipp et al., 1985). No other recent studies were found.

Observations in Other Species

Potential mutagenicity to germ cells was studied by Meier et al. (1985). Oral administration of chlorine (pH 8.5) to $B6C3F_1$ mice at 4 and 8 mg/ kg of body weight (bw) per day for 5 weeks induced significant increases in sperm-head abnormalities. In another study by Chang and Barrow

(1984), sensory respiratory tolerance was shown to develop in F-344 male rats after repeated exposure up to 10 days to chlorine gas at 2.5 and 10 ppm. As indicated earlier in this report, the principal health concerns, other than exposure directly to chlorine gas, arise from the use of chlorine as a disinfectant in drinking water supplies, when various chlorinated by-products such as the trihalomethanes are formed. However, Vogt and coworkers (1979) showed *in vivo* production of chloroform after ingestion of sodium hypochlorite; several halogenated by-products produced *in vivo* were found in blood plasma and stomachs of rats 1 hour after NaOCl injection; and various halogenated organics are known to be produced by chlorination of amino acids (Trehy and Bieber, 1981), nucleic acids (Olivieri et al., 1980), uracil (Dennis et al., 1978), and nucleotides (Hoyano et al., 1973).

Revis and co-workers (1986) studied the prevention, by calcium ion in drinking water, of atherosclerotic plaques and the effects on serum cholesterol concentrations induced in pigeons by lead and cadmium. Chlorine, chlorine dioxide, chlorite, and monochloramine were added individually to the drinking water of separate groups of pigeons at 2 and 15 mg/liter for 3 months. The chlorine was tested at two pH levels (6.5 and 8.5) to provide conditions under which essentially 99% HOCl and 99% OCl^- were being administered. While methods were not specifically stated, apparently the investigators examined major blood vessels for lipid-containing material, counted the number of plaques, and measured their area on the vessel wall. Plaque formation was reduced but low-density-lipoprotein (LDL) cholesterol levels increased. The sample size in this study was too small with proportionately too few controls to produce statistically significant results. Marked effects were observed on serum thyroid T3 and T4 levels at very low doses of the disinfectants. The marked increases in serum T3 and T4 need to be further evaluated, as well as the relevance of the pigeon as an animal model. This study is also described in the section on chlorine dioxide.

CHLORINE DIOXIDE

CAS No. 10049-04-4

$$ClO_2$$

Chlorine dioxide is a reddish-yellow gas that freezes at -59.5°C, boils at 10°C, and is explosive in air at concentrations of about 4% or more. It decomposes in water and dissolves in alkalies, forming a mixture of chlorite and chlorate. In addition to its utility in water treatment, it is used as a bleach for wood pulp, fats, and oils; a maturing agent for flour; a biocide; and an

odor controller. The toxicity of chlorine dioxide was reviewed in *Drinking Water and Health*, Volume 4 (NRC, 1982, pp. 174-178); the following material updates and reevaluates information on this disinfectant.

Metabolism

Abdel-Rahman and coworkers (1980a, 1982) studied the absorption of 3 ml of a solution of 100 mg $^{36}ClO_2$/liter of water given per os to male Wistar rats. The rate constant for absorption of the labeled Cl was 3.8/ hour. Within 72 hours, 31% of the label was excreted in the urine and 10% in the feces. The form in which Cl was excreted in feces was not identified; however, in buccal scrapings from monkeys, chlorine dioxide was reduced to a nonoxidizing substance rapidly (Bercz et al., 1982), suggesting that chlorine dioxide is rapidly altered after ingestion. Most of the labeled Cl found in the urine by Abdel-Rahman and associates was as chloride ion, with some chlorite and chlorate. Less than 5% of the administered dose of labeled Cl was found 72 hours after gavage in plasma, kidney, lung, stomach, duodenum, ileum, liver, spleen, and bone marrow.

Moore and Calabrese (1980) administered 100 mg chlorine dioxide per liter of drinking water to A/J and C57L/J mice (18 mg/kg bw per day) for 30 days and studied possible effects on blood components, including glucose-6-phosphate dehydrogenase (G-6-PD), red blood cells, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, reticulocyte count, and osmotic fragility. They found no significant effects on any of these parameters, though the G-6-PD activity in C57L/J mice was said to be reduced slightly.

Bercz et al. (1986) summarized the conclusions from some of their studies of endocrine effects in Wistar and Sprague-Dawley rats and African Green monkeys, especially of chlorine dioxide on thyroid function. In both species ingestion of chlorine dioxide affects the mucosal surfaces of the alimentary tract and the chemical composition of nutrients and hormones within it, apparently by oxidation and covalent binding of bioavailable iodide, which is ubiquitous in the digestive tract. Absorption of the iodinated molecules may be the mechanism for inhibition of activity by the thyroid and for an accelerated decrease in the concentration of thyroxin in the blood.

Health Effects

Observations in Humans

Lubbers et al. (1981, 1982, 1983) exposed human volunteers to chlorine dioxide in a triphasic study; statistical analysis of the data was reported

by Lubbers and Bianchine (1984) and Lubbers et al. (1984). In phase I, the acute effects of increasing doses were investigated (Lubbers et al., 1981). Ten normal volunteers ingested two 500-ml portions of water containing chlorine dioxide 4 hours apart every fourth day for a total of 6 dosing days. Each portion was consumed within 15 minutes. The concentration of chlorine dioxide was increased in steps on each of the 5 subsequent dosing days, from 0.1 mg/liter on the first day to 24 mg/liter on the final day. The maximum dose for a 70-kg person was 0.34 mg/kg bw. Hematic, blood chemical, urinary, and other values of the volunteers who ingested chlorine dioxide did not differ significantly from those of 10 volunteers who ingested plain water. Nevertheless, the investigators did not rule out the possibility that effects might become significant upon increased exposure.

In phase II, 10 volunteers drank 500 ml of a solution containing chlorine dioxide concentrations of 5 mg/liter of water daily for 12 weeks. Weekly physical examinations and laboratory studies of blood and urine showed a statistically significant group-time interaction ($p < 0.05$) for group mean urea nitrogen values in the volunteers who ingested chlorine dioxide, but the investigators questioned the clinical significance of these changes.

Phase III was concerned only with the effects of consumption of water containing sodium chlorite on G-6-PD-deficient subjects, which will be described in the section on that compound.

Michael et al. (1981) conducted a prospective study of 197 inhabitants of a rural village using water disinfected with chlorine dioxide. They compared the hematological profiles of 87 males and 110 females (23 being less than 15 years of age) from this village with those of a group of 112 people (48 males and 64 females, 12 of whom were under 15 years of age) using unchlorinated water. The chlorinated water contained chlorine dioxide concentrations of 0.25-1.11 mg/liter and free-chlorine concentrations of 0.45-0.91 mg/liter during the 12-week period of the study. The concentrations of chlorite and chlorate in the water were 3.2-7.0 mg/ liter and 0.87-1.8 mg/liter, respectively. The water treatment plant operated for only 8 hours a day, which was responsible, at least in part, for the variable concentrations measured. Neither the exposed group nor the comparison group of 118 persons showed any significant changes in hematocrit, hemoglobin, erythrocyte count, white-cell count, reticulocyte count, mean corpuscular volume, methemoglobin level, serum creatinine, or serum total bilirubin from the preexposure levels to those measured after 115 days of exposure. Only blood urea nitrogen (BUN) was changed, the mean values of the test population at the end of the experiment being lower than at the beginning, with a slight but opposite trend in the comparison group.

The researchers believed that they had not ruled out the possibility of transient effects, such as a transient hemolytic anemia. They pointed out the lack of racial and ethnic diversity in their study population. Only one person exhibited a G-6-PD deficiency; his erythrocyte count, hemoglobin, and hematocrit declined by the end of the 3 months of exposure, but without a noteworthy change in methemoglobin or in any other effect studied. These variables had returned at least partly to normal by 90 days after the end of the exposure.

Analysis of variance showed a small but significant association of methemoglobin with sex, of total bilirubin with age, of BUN with exposure (as mentioned earlier), of hemoglobin with age, and an exposure-sex-age association with erythrocyte count. The investigators thought that BUN changes might reflect a mild dehydration during the summer weather prevailing at the end of the study and pointed to a similar pattern in the BUN/creatinine ratio consistent with this interpretation. They recommended that further research on chlorine dioxide disinfection focus on high-risk persons who might be especially susceptible to oxidants.

Tuthill et al. (1982) examined a population that had used water disinfected with chlorine dioxide in the 1940s, comparing its morbidity and mortality with those of a neighboring community. The chlorine dioxide-exposed group had a significantly greater proportion of premature births, but this difference between the two communities disappeared when the effect of the age of the mother on premature parturition was taken into account. The only other significant difference after compensating for variations in feeding methods was a greater postnatal loss of weight by infants born into the exposed population.

Three older studies deserve mention here: those by Gloemme and Lundgren (1957), Elkins (1959, pp. 89-90), and Ferris et al. (1967). Gloemme and Lundgren studied 12 men who had experienced acute symptoms usually related to the respiratory tract, and who worked in a Swedish sulfite pulping plant during the mid-1950s. They were exposed usually to less than 0.1 ppm chlorine dioxide and of Cl, with occasional exposures to low concentrations of sulfur dioxide or to comparatively large concentrations of chlorine dioxide. Slight chronic bronchitis was identified in seven of these men. In one case, a detected bronchitis disappeared, proving reversibility of the lesion. There were complaints among the 12 men also of irritation of eyes, respiratory tract, and G.I. tract. Elkins found that 5 ppm of chlorine dioxide in air was definitely an irritant to the respiratory and the G.I. tracts. A concentration of 19 ppm of chlorine dioxide in the air within a bleach tank was reported by Elkins to have caused the death of a man assigned to work within the tank. Ferris et al. compared two populations of workers: one of 124 individuals working in a plant making Kraft paper and the other of 147 people working in a sulfite pulp mill and

with an average duration of possible exposure to chlorine dioxide, chlorine, and sulfur dioxide of 6 times that of the workers in the paper mill. Ferris and coworkers found no significant differences in ventilatory function or respiratory symptoms between the two populations.

Observations in Other Species

Acute Effects

No studies of acute effects were found.

Subchronic Effects

Bercz et al. (1982) studied possible hematological effects in African Green monkeys given water that contained 100 mg chlorine dioxide/liter to yield a daily dose of 9.5 mg/kg bw during a 6-week period. This dose was without effects on measured components, but the monkeys were said not to have tolerated higher doses. Monkeys given chlorine dioxide at this dose level, but not those given 3.5 mg/kg bw per day, had decreased serum levels of thyroxine. The effect on secretions of thyroxine appears not to have been due to formation of chlorite or chlorate, as daily doses of 43 or 44 mg/kg bw of these compounds did not alter the concentration of thyroxine in the serum.

Abdel-Rahman et al. (1980b) and Couri and Abdel-Rahman (1980) gave male Swiss Webster mice and Sprague-Dawley rats 1, 10, 100, or 1,000 mg chlorine dioxide per liter of drinking water. If the rats drank 0. 1 ml/kg bw per day and the mice 0.18 ml/kg bw per day, the first three concentrations would yield daily doses of 0.1, 1, and 10 mg/kg bw for rats and 0.18, 1.8, and 18 mg/kg bw for mice. (Because the highest concentration was expected to induce a decrease in water consumption, a dose corresponding to that concentration was not estimated.) At 2, 4, 6, and 12 months of exposure, the following were assayed: glutathione reductase, glutathione peroxidase, catalase, glutathione, and methemoglobin. There were various statistically significant changes, as judged by use of multiple t-tests, but there was no consistency with respect to dose or to period of exposure, except for an increase in both species in catalase activity at 1,000 mg/liter and at 10 and 100 mg/liter in mice. At no time nor at any dose was there an increased methemoglobinemia in either species.

In another experiment from the same laboratory (Abdel-Rahman et al., 1984), osmotic fragility was studied in rats given drinking water containing 10 to 1,000 mg chlorine dioxide/liter (about 1 to 100 mg/kg bw per day). There was a dose-related increase in resistance of erythrocytes to hemolysis in hypotonic media (i.e., decreased fragility) in animals given water containing 10 to 100 mg/liter. When the concentration was increased from 100 to 1,000 mg/liter, the increase in resistance was not proportional to

the earlier ones, perhaps because of the markedly decreased water consumption mentioned above.

Mutagenicity

Meier et al. (1985) observed no increase in sperm-head or chromosomal aberrations or in micronuclei formation in CD-1 mice administered aqueous chlorine dioxide by gavage (3.2, 8, or 16 mg/kg bw per day) for 5 days. The sperm heads were examined at 1, 3, and 5 weeks after the last dose, to detect effects at all stages of spermatogenesis. No evidence that ClO_2 induces mutational change was found.

Carcinogenicity

No investigation of possible carcinogenicity of ClO2 was found.

Reproductive Toxicity

Abdel-Rahman et al. (1984) found a dose-related decrease in testicular uptake of ^{3}H-thymidine in male rats given 10 or 100 mg of chlorine dioxide/liter or 10 mg/kg bw per day) in their drinking water (65% and 38% of control levels, respectively). This suggests a reduction in cell division in the testes but does not indicate what effect, if any, there was on spermatogenesis or on hormone production.

Teratogenicity

Orme et al. (1985) gave female rats drinking water containing chlorine dioxide concentrations of 2, 20, or 100 mg/liter starting 2 weeks prior to mating and continuing through lactation, 21 days after parturition. At the highest concentration, 100 mg/liter (14 mg/kg bw per day), there was a significant depression of the concentration of thyroxine in serum in the pups, but not in the dams, at the time of weaning. No significant thyroid effects were seen in pups from the group ingesting 20 mg of chlorine dioxide/liter. There was a decrease ($p = 0.08$) in exploratory and locomotor activity in pups born to dams given 100 mg/liter but not in pups from dams given 20 mg/liter (3 mg/kg bw per day). In a second experiment, pups born to dams drinking plain water were administered chlorine dioxide at concentrations of 14 mg/kg bw by stomach tube per day between days 5 and 20 after birth. There was a larger depression in serum thyroxine and a greater and more consistent delay in development of exploratory and locomotor activity ($p < 0.05$) than in the first experiment.

Taylor and Pfohl (1985) observed a significant reduction in cell number, as judged by total DNA content, in the cerebella of rat pups born to dams given water containing chlorine dioxide concentrations of 100 mg/liter through gestation and lactation. Pups given 14 mg/kg bw per day by stomach tube had reduced numbers of cells in both cerebellum and forebrain at 11 days postpartum and exercised less than normal on a voluntary running wheel at 50-60 days postpartum (though administration of chlorine dioxide had ended at 20 days of age).

Suh et al. (1983) examined the effects of chlorine dioxide and its metabolites on fetal development in the rat. Female rats were administered chlorine dioxide at 0, 1, 10, or 100 mg/liter of drinking water for 2.5 months prior to and throughout gestation. The small number of dams bred (six to nine per test group), an unusually high percentage of abnormal control fetuses (31%), and uncertainty as to the unit of statistical comparison (fetus or litter) preclude statistical significance of observed skeletal variations and decreased number of implants per dam. A statistically significant increase in fetal body weight at the 100-mg/liter dose level may be a consequence of the reduced litter size observed at this dose.

Suh et al. (1983) gave groups of six to eight female Sprague-Dawley rats drinking water containing chlorine dioxide concentrations of 0, 1, 10, or 100 mg/liter (0, 0.1, 1, or 10 mg/kg bw per day) for 2.5 months, before they were bred with untreated males. Exposure was continued throughout gestation. At the highest dose, there were reductions in mean number of implants and mean number of live fetuses per dam. The small number of litters and the unusually high percentage of abnormal fetuses among the control litters (approximately 31%) preclude statistical significance of skeletal variations observed and invite questions as to the validity of the study.

Other Effects

Revis et al. (1986) investigated the effects of drinking water containing chlorine dioxide at 2 or 15 ppm on thyroid function and on plasma cholesterol in rabbits and pigeons. In pigeons supplied drinking water containing 15 ppm chlorine dioxide for 3 months, concentrations of T_4 in the plasma were reported to be significantly reduced whether they were on a normal or a high-cholesterol diet, as compared with those of controls. In most of the groups, T_4 levels were reported to be significantly lower after imbibing water containing a 2-ppm concentration of chlorine dioxide. Increases in plasma cholesterol were seen frequently in groups with the lower T_4 levels, especially in those given the high-cholesterol diet and the water at 15 ppm chlorine dioxide. Revis et al. suggest that these effects are mediated by-products formed by the reaction of chlorine dioxide, hypochlorite, and monochloramine with organic matter in the upper gastrointestinal tract. The significance of this study for humans is unknown because little information exists on pigeon thyroid function. Further, the statistical and hormone measurement methods used in this study appear inappropriate to the experimental design.

Conclusions and Recommendations

Chlorine dioxide produces hematological effects in both humans and laboratory animals. The mechanism of these effects is not known; however, it is believed to be related to the oxidant properties of chlorine

dioxide and its aqueous reaction products, chlorite and chlorate. In addition, thyroid and developmental neurological effects have been observed in laboratory animals. The thyroid effects of chlorite are thought to be caused by its oxidation of dietary iodide in the gastrointestinal tract. The oxidized iodide then binds to either food or tissue and is unavailable for absorption. The mechanisms of the neurological anomalies are unknown. Orme et al. (1985) found that levels of 14 mg/kg bw per day produced drops in T_4 levels and abnormal neurological development in rat pups born to dams exposed during gestation and lactation. The investigators were able to show a no-observed-effect level (NOEL) of 3 mg/kg bw per day. In addition, Bercz et al. (1982) were able to show a NOEL of 3.5 mg/kg bw per day for thyroid effects in monkeys, supporting the more recent results by Orme et al. (1985).

The committee selected the NOEL of 3.0 mg/kg bw per day and an uncertainty factor of 100 to estimate a chronic suggested no-adverse-effect level (SNARL) assuming that a 70-kg human consumes 2 liters of water daily, which contributes 20% of total intake:

$$\frac{3 \text{ mg/kg bw/day} \times 70 \text{ kg} \times 0.2}{100 \times 2 \text{ liters}} = 0.21 \text{ mg/liter, or } 210 \text{ µg/liter.}$$

A SNARL may also be estimated for a 10-kg child consuming 1 liter of water daily, which contributes 20% of total intake:

$$\frac{3 \text{ mg/kg bw/day} \times 10 \text{ kg} \times 0.2}{100 \times 1 \text{ liter}} = 0.06 \text{ mg/liter, or } 60 \text{ µg/liter.}$$

CHLORAMINES

Monochloramine

CAS No. 10599-90-3

$$NH_2Cl$$

Pure monochloramine is a colorless, unstable, and pungent liquid with a freezing point of -66°C. It decomposes above -50°C and forms nitrogen, chlorine, and nitrogen trichloride (Colton and Jones, 1955; Kovacic et al., 1970). Monochloramine is used as an intermediate in the Raschig process for the industrial production of hydrazine. However, aqueous solutions of monochloramine formed by the chlorination of natural waters containing ammonia hold the primary environmental significance of the compound.

Some confusion exists over the use of the term chloramine because it implies simply that a compound, organic or inorganic, contains both a chlorine atom and an amino-nitrogen functional group. This would then include the highly carcinogenic nitrogen mustards, which are not formed when water containing ammonia or organic amines is chlorinated. Furthermore, monochloramine should be recognized as different from the commercial products known as chloramine B, chloramine T, and dichloramine T, which are organic compounds made by chlorinating benzenesulfonamide or *para*-toluenesulfonamide.

The reactions of chloramine with organic materials have been extensively reviewed (Kovacic et al., 1970). In general, monochloramine is a less potent oxidant than chlorine, with a standard oxidation potential of -1.16 V compared with -1.49 V for chlorine (Rosenblatt, 1975). Chloramine is able to transfer either the chlorine or the nitrogen onto reactive organic substrates. For example, chloramine reacts with phenols under certain conditions in dilute aqueous solution to form chlorophenols (Burttschell et al., 1959; Carlson and Lin, 1985), or it can form phenolamine coupling reaction products (first observed by Berthelot in 1859). In addition, C. Le Cloirec and Martin (1985) have shown that chloramine can react with aldehydes to form nitriles. However, it has not been shown that these products are formed in chloraminated natural waters. Even though monochloramine can substitute a chlorine atom into organic compounds present in water, it does so to a much lesser extent than chlorine. It is for this reason that it has been recommended as an alternative disinfectant to limit the formation of trihalomethanes in disinfected water (EPA, 1983).

Metabolism

Few studies have been reported on the fate of monochloramine following ingestion. Stomach fluid contains high concentrations of proteins and amino acids, and monochloramine reacts with some amino acids (Jacangelo and Olivieri, 1985). Sulfur-containing amino acids and proteins are readily oxidized by monochloramine, and their presence may cause reduction of the monochloramine to innocuous products. Under conditions that the reducing mechanisms in stomach fluid may overcome (Scully et al., 1986), the transfer of a chlorine atom from monochloramine to organic amines or amino acids to form organic chloramines may be important (Isaac and Morris, 1980, 1983, 1985; Snyder and Margerum, 1982). In the absence of reducing mechanisms, the concentrations of organic amino-nitrogen compounds in stomach fluid (Scully et al., 1985, 1986) are sufficient to allow half of the monochloramine in a 2-mg/liter (as Cl_2) solution to transfer its chlorine atoms to amino acids in less than 1.5

minutes at pH 6 (concentrations from Scully et al., 1985, 1986; rate data from Isaac and Morris, 1985). In this case the toxicological effects of monochloramine may be related to the organic chloramines formed by this chlorine transfer reaction. Absorption of an orally administered organic chloramine (*N*-chloropiperidine) into the blood of Sprague-Dawley rats has been shown (Scully et al., 1985, 1986), but the full toxicological implications of these results are still to be determined.

Under conditions designed to simulate the gastrointestinal tract, Bercz and Bawa (1986) found that monochloramine caused covalent binding of radioiodide to nutrient biochemicals. Monochloramine was believed to oxidize iodide to iodine, which subsequently reacted with nutrient chemicals to form iodinated organic compounds. Tyrosine, 4-aminobenzoic acid, arachidonic acid, and folic acid were among the compounds that became iodinated under the conditions of the experiment. Some of the reactions were carried out under conditions of pH that were somewhat basic. The observed percent binding was generally higher for reactions carried out at higher pH levels. Although complex mixtures of nutrients, such as gastric juice and saliva, appeared to bind iodine in dilute aqueous solution, it is important that these results be correctly extrapolated to physiological pH before their significance is fully understood.

Grisham et al. (1984) published evidence that through the oxidation of chloride by the myeloperoxidase/hydrogen peroxide/chloride system, human neutrophilic leukocytes may produce organic chloramines. The proposed function of the organic chloramines is to provide a reserve of oxidizing equivalents for killing bacteria. The organic chloramines are then believed to react with ammonium ions to form the more bactericidal monochloramine. However, because no transfer of chlorine from organic chloramines to ammonia has ever been demonstrated, the validity of this mechanism has not yet been proven.

Abdel-Rahman et al. (1983) administered doses of 3 ml of freshly synthesized aqueous chloramine[^{36}Cl] (370 mg/liter) orally to four male Sprague-Dawley rats that had been fasted overnight. Suh and Abdel-Rahman (1983) reported a study of the control compound chloride-36. The rate of absorption of the radiolabel into blood was considerably faster in the case of chloramine[^{36}Cl] ($T_{1/2}$ = 2.5 hours) than it was in the case of chloride-36 ($T_{1/2}$ = 19.2 hours). The plasma maximum was reached in 8 hours for both compounds, followed in each case by a plateau in the plasma concentration. Following this plateau, the rate of elimination of the label from plasma was similar in both studies ($T_{1/2}$ = 38.8 hours for chloramine[^{36}Cl] and $T_{1/2}$ = 51.9 hours for $^{36}Cl^-$).

In their study of chloramine[^{36}Cl], Abdel-Rahman et al. (1983) collected the urine, feces, and expired air over 4- and 5-day periods from four male Sprague-Dawley rats. During the first 24 hours after administration of chloramine[^{36}Cl], only 0.40% and 0.08% of the total dose administered

were eliminated in the urine and feces, respectively. The proportion eliminated through the urine and feces at the end of the 120-hour study period was 25.15% and 1.98%, respectively. By comparison, Suh and Abdel-Rahman (1983) found that over twice as much of the ^{36}Cl-label was eliminated over the 120-hour study period when 36Cl-labeled chloride was administered. They found that 57.2% of the administered chloride-36 was eliminated in urine and 3.0% was eliminated in feces. Unlike chloramine[^{36}Cl], a considerable amount of the chloride-36 was eliminated in the first 24 hours: 16.1% in urine and 0.92% in feces. After 48 hours, radioactivity was eliminated with a half-time similar to that found after 24 hours in the chloramine[^{36}Cl] study (T1/2 = 24 hours). Only 27.1% of the administered amount of the label was excreted in the chloramine study over 5 days, whereas 60.2% of the radiolabel was excreted in the chloride-36 study. Abdel-Rahman et al. (1983) reported that the principal (88%) excreted metabolite of chloramine[^{36}Cl] is chloride-36. However, the identity of the 73% of the label retained by the body is unknown.

The subcellular distribution of ^{36}Cl activity in rat liver preparations was similar at 24 hours following oral administration of either chloramine[36Cl] or chloride-36 (Abdel-Rahman et al., 1983; Suh and Abdel-Rahman, 1983).

Abdel-Rahman et al. (1983) analyzed plasma for metabolites at 120 hours after administration of chloramine[^{36}Cl] to rats. Neither 36Cl-labeled chlorite nor chlorate was detected in rat plasma. Most of the total ^{36}Cl was identified as chloride-36, which, according to the authors, indicated that the chlorine moiety was eliminated primarily in this form.

The control reagent for a pharmacokinetic study of chloramine[^{36}Cl] is ^{36}Cl^{-}. It is not likely that an oxidant as strong as NH2Cl will survive intact absorption, distribution, and excretion from an animal. If the chloramine is rapidly detoxified in the stomach to ^{36}Cl-labeled chloride and ammonia, the observed pharmacokinetics will be identical to the control kinetics. There are aspects of the pharmacokinetics of both chloramine[^{36}Cl] and chloride-36 that are similar, but there are also notable differences. The fate of much of the ^{36}Cl label is unknown. If the chloramine acts as a chlorinating agent, other, more stable chlorinated organic compounds may form. The observed kinetics of the radioactivity seen after administration of chloramine[^{36}Cl] appear to be quite complex, possibly owing to a combination of chloride-36, ^{36}Cl-chlorinated compounds, and their metabolites.

Health Effects

Observations in Humans

Acute Effects

One case has been reported of a woman who was overcome by inhalation of gaseous monochloramine in a poorly ventilated

bathroom (Laakso et al., 1982). Monochloramine and dichloramine were formed when household ammonia was mixed with chlorine bleach (5% sodium hypochlorite). The vapors caused burning of the eyes and throat, dyspnea, coughing, and vomiting. The resultant pneumonitis did not lead to permanent pulmonary damage. In another reported instance, treatment of a dental abscess with a 2% solution of monochloramine resulted in the development of a type I allergic reaction (Beck, 1983).

Subchronic Effects

Lubbers et al. (1981) administered drinking water containing chlorine, chloramine, chlorine dioxide, sodium chlorite, or sodium chlorate to adult male human subjects. Sixty volunteer subjects were randomly assigned to six treatment groups of 10 subjects each; the members of one group (the control group) received untreated water, while the members of the other groups each received one of the disinfectants or disinfectant reaction products. In phase I, the acute effects of increasing doses were investigated; in phase II, the effects of ingestion of the disinfectants or reaction products at a concentration of 5 mg/liter of water for 12 consecutive weeks were investigated. A third phase of the study did not include monochloramine.

In the first phase, each subject ingested 1 liter of the untreated water in two half-portions; the second 500-ml portion was given 4 hours after the first. Each portion was consumed within 15 minutes. Following this day of disinfectant administration, there were 2 days free of such administration, during which blood and urine were collected and physical examinations were conducted. Five such consecutive 3-day segments constituted this phase of the monochloramine study, and doses were increased from 0.01 to 24.0 mg/liter. Assays of a number of serum chemical components and of blood cells were performed, as well as analyses of urine and special tests, such as G-6-PD, thyroid hormones, and electrocardiograms (ECGs). Physical examinations included observations of systolic and diastolic blood pressures, respiratory and pulse rates, and oral temperature. Using analysis of variance techniques, group main effects (G), time main effects (R), and group-time interaction (RG) were estimated. In all instances, group mean values were and remained within normal ranges. Any trends identified by the analysis of variance were concluded not to be of clinical importance.

In phase II, the group of 10 subjects administered monochloramine was divided into three subsets; each subset entered the study sequentially to facilitate management of the experiment. Each subject consumed 500 ml of water containing monochloramine in concentrations of 5 mg/liter daily for 12 weeks. Physical examinations, collection of blood and urine, and taste evaluations were performed weekly during the treatment period and for 8 weeks afterward. Compared with the common control group, significant

RG values ($p < 0.05$) were found in group mean corpuscular hemoglobin in the case of chlorine. No linear trends were detected by linear regression analysis for monochloramine. The findings with regard to other disinfectants and their by-products are discussed in their respective sections.

Several studies of the effect of monochloramine on blood components and the risk to hemodialysis patients have been reported (Eaton et al., 1973; Kjellstrand et al., 1974). When tap water containing chloramines was used for dialysis baths, two major effects were observed: oxidation of hemoglobin to methemoglobin and denaturation of hemoglobin. The amount of oxidative damage was proportional to the amount of monochloramine formed. Furthermore, exposure of red blood cells to monochloramine also inhibited the hexose monophosphate shunt that protects red cells from oxidative damage. Kjellstrand et al. suggested that chloramine-induced hemolysis might be reduced by the addition of ascorbic acid to the treatment water.

Observations in Other Species

Acute Effects

Abdel-Rahman et al. (1984) investigated the toxicity of monochloramine in male Sprague-Dawley rats. Acute exposure to a single dose (3 ml) at 10, 20, or 40 mg/liter induced a significant increase in blood glutathione levels within 30 minutes of administration of an aqueous solution by gavage.

Subchronic Effects

Bercz et al. (1982) studied the subchronic toxicity of monochloramine administered to African Green monkeys in drinking water following 30- to 60-day subchronic, exponentially rising step doses. At 100 mg/liter, monochloramine had no detectable effect in 18 hematological tests on the 12 monkeys, including red-cell glutathione (GSH) levels. No evidence of thyroid suppression was detected in serum.

In a draft of a report of the Gulf South Research Institute for the National Toxicology Program, peer-reviewed by the committee (GSRI, 1981), Fisher 344 rats and the $B6C3F_1$ mice were given monochloramine in drinking water at concentrations of 0, 25, 50, 100, 200, and 400 mg/liter for 90 days. The investigators reported decreased body weight gain and liver damage including increased mitotic figures, cellular hypertrophy, and unusual chromatin patterns in the liver cells of mice exposed to chloramines in concentrations of 100, 200, and 400 mg/liter. They also observed decreased body weight gain and decreased relative liver weight in male and female rats and increased protein excretion in male rats given 200 and 400 mg/liter. The investigators stated that 100 mg/liter "appeared to

be the threshold level for lower toxicity in mice." So a no-observed-effect level would be 50 mg/liter, approximately 8.3 mg/kg bw per day.

Chronic Effects

Moore and Calabrese (1980) reviewed the health effects studies previously conducted on monochloramine. Few drinking water studies had been reported. Maziarka et al. (1976) had found no observable effects in rats exposed for 12 months to chloramine at concentrations of 9.0 mg/liter. Moore et al. (1980) exposed male A/J mice for 30 days to monochloramine in bicarbonate-buffered solution (pH 8.9) at concentrations of 2.5 to 200 mg/liter. They found no significant changes in nine measured blood parameters. Following chronic treatment at 1, 10, or 100 mg/liter, they found no significant changes in measured blood parameters.

Chronic treatment at 1, 10, or 100 mg/liter doses in drinking water induced a significant decrease in glutathione levels after 4 months of treatment at the 1-mg/liter and 100-mg/liter dose levels (Abdel-Rahman et al., 1984). Results varied over the 12-month study period, but at 6 and 12 months after initiation of the study, statistically lower glutathione levels were observed at all dosages. After 3 months of treatment, significant decreases in red-blood-cell count and hematocrit were observed at the higher dosage levels. However, there was an apparent lack of dose-response or time-dependent response in both glutathione levels and hematological parameters.

Bull (1980) reported the results of a 45-day study in which monochloramine in drinking water was administered to laboratory rats. Body weight gain and hematological parameters in exposed animals did not differ significantly from those observed in control animals. The only significant finding was a decrease in the amount of methemoglobin present in the blood—the opposite result of what was expected.

Mutagenicity

In a study by Shih and Lederberg (1976), chloramine reacted with *Bacillus subtilis* deoxyribonucleic acid (DNA) *in vivo* and *in vitro* ; it was shown to be weakly mutagenic to a strain of *B. subtilis*, causing reversion of *trp*C to *trp*$^+$. Shih and Lederberg studied the biological and physical effects of chloramine on *B. subtilis* after treating the bacterial cells (*in vivo*) and the bacterial DNA (*in vitro*). Both kinds of treatment resulted in single-strand breaks and a few double-strand scissions (at higher chloramine doses) with loss of DNA-transforming activity. Chloramine used as a bactericide may target DNA since some DNA-repair mutants seem to be more sensitive to chloramine.

Fetner (1962) found that distilled water containing monochloramine produced chromosome breakage when it was used for soaking *Vicia faba* seeds. A 1-hour exposure to 10^{-4} *M* monochloramine produced 24%

abnormal anaphases. Monochloramine produced chromosome breakage at concentrations that exhibited little evidence of tissue damage.

On the other hand, Cheh et al. (1980b) found that the organic concentrate from drinking water treated with chloramine produced only half as many revertants in the Ames *Salmonella* mutagenicity assay as the same water treated with chlorine. The addition of sulfite to reduce chemically the oxidants in chloramine-treated drinking water sharply decreased the mutagenic response (Cheh et al., 1980a, b; Wilcox and Denny, 1985).

Carcinogenicity

In two reports (Bull, 1980; Bull et al., 1982), settled, coagulated, and sand-filtered Ohio River water was treated with monochloramine (3 mg/liter). The residual disinfectant was dissipated within 48 hours. The water was then concentrated by reverse osmosis, and the concentrate was subjected to a mouse skin initiation-promotion assay in Sencar mice. Lesions macroscopically observed at autopsy included papillomas, squamous carcinomas, and lung adenomas. In these studies, monochloramine was not the primary carcinogen but was believed to be reacting with trace organics in the water and producing carcinogenic by-products. Bull observed variations in the quality of the source water (which produced tumors in one case prior to disinfection) and cautioned against extrapolation of these results until further examples have been examined.

Herren-Freund and Pereira (1986) used a bioassay that involved an increased incidence of γ-glutamyl transpeptidase (GGT) foci as an indicator of carcinogenicity. This bioassay consisted of administration of a candidate initiator to rats 18 to 24 hours after removal of two-thirds of their livers. Seven days after this initiation, the rats were given a candidate promoter in drinking water for at least 10 weeks. The assay detected such initiators as 2-acetylaminofluorene, aflatoxin B_1, diethylnitrosamine, dimethylhydrazine, and urethane. In this assay, chloramine was not found to act as an initiator when a dose of 14.75 mg/kg bw was administered by gavage 1 day after partial hepatectomy and promotion with 500 ppm phenobarbital in the drinking water was begun 7 days after the dose of chloramine and continued for 10 weeks. The rats were killed 1 week after the end of promotion. Diethylnitrosamine was used as a positive control for initiation at 0.3 mole/kg.

Teratogenicity

Abdel-Rahman et al. (1982) investigated the effects of monochloramine administered in drinking water to mature virgin female Sprague-Dawley rats. Six animals per group were administered monochloramine daily in concentrations of 0, 1, 10, or 100 mg/liter of drinking water, both 2.5 months prior to and throughout gestation. Sacrifice of the rats on the twentieth day of gestation was performed for soft-tissue and skeletal examination. Monochloramine did not produce any significant

changes in rat fetuses at any dose level; in fact, there was a slight increase in fetal weight in all monochloramine groups compared with controls.

Meier et al. (1985) evaluated the ability of 40-, 100-, and 200-mg/liter solutions of monochloramine and other oxidants to induce chromosomal aberrations and micronuclei in the bone marrow of CD-1 mice and spermhead abnormalities in $B6C3F_1$ mice. Monochloramine showed no evidence of any significant effects in any of the tests.

Carlton et al. (1986) administered chloramine by intragastric catheter at doses of 0, 2.5, 5.0, or 10 mg/kg bw per day to male and female Long-Evans rats that were 4 to 6 weeks old at the beginning of the experiment. Males were treated for 56 days and females for 14 days prior to mating; the administration was continued during the 10-day mating period, and thereafter females were given chloramine daily through gestation and lactation. Males were necropsied at the end of the mating period. Their sperm were examined for normalcy, and microscopic changes in the anatomy of the reproductive tract were sought. Dams and some offspring were necropsied at weaning, 21 days after birth. Other offspring were administered chloramine after weaning until they were 28 or 45 days old; these pups were evaluated for vaginal patency and thyroid hormone levels.

No differences between control and exposed rats were found in fertility, viability, litter size, day of eye opening, or day of vaginal patency. There were no alterations in sperm count, direct progressive sperm movement, percent mobility, or sperm morphological characteristics in adult males. Weights of male and female reproductive organs were not significantly different among test and control groups, and there were no significant morbid anatomic changes evident on tissue examination. There were no signs of toxicity, changes in blood counts, or body weight suppression in adult rats of either sex at any dose level. The mean weight of the pups was unchanged from that for control litters.

Other Effects

Revis et al. (1986) studied the effects of monochloramine on thyroid function and on plasma cholesterol in rabbits and pigeons. In pigeons supplied drinking water containing monochloramine at 2 ppm for 3 months, concentrations of plasma thyroxine (T_4) and plasma cholesterol were increased, although a clear dose-response effect for plasma cholesterol was not observed. The sample size in this study was too small with proportionately too few controls to produce statistically significant results. The marked increase in serum T_4 should be further evaluated, as well as the relevance of the pigeon as an animal model.

Conclusions and Recommendations

Monochloramine can produce hepatocellular changes in laboratory animals. A subchronic bioassay (GSRI, 1981) showed decreased body weight

gain and liver toxicity in exposed mice. A no-observed-effect level (NOEL) for liver toxicity from the GSRI study was 8.3 mg/kg bw per day. Using this as a basis, and assuming that a 70-kg human consumes 2 liters of water daily, which contributes 20% of total intake, the committee estimates a suggested no-adverse-effect level (SNARL) as:

$$\frac{8.3 \text{ mg/kg bw/day} \times 70 \text{ kg} \times 0.2}{100 \times 2 \text{ liters}} = 0.581 \text{ mg/liter, or } 581 \text{ µg/liter.}$$

A SNARL may also be estimated for a 10-kg child consuming 1 liter of water daily, which contributes 20% of total intake:

$$\frac{8.3 \text{ mg/kg bw/day} \times 10 \text{ kg} \times 0.2}{100 \times 1 \text{ liter}} = 0.166 \text{ mg/liter, or } 166 \text{ µg/liter.}$$

CHLORITE

Sodium chlorite

CAS No. 7758-19-2

$NaClO_2$

CHLORATE

Sodium chlorate, Atlacide

CAS No. 7775-09-9

$NaClO_3$

Sodium chlorite can be crystalline, flake, or powder in form. It is slightly hygroscopic but does not cake. In crystalline form its density is 2.468 g/cm^3. At 17°C its solubility is 39 g/100 ml of water and at 60°C, 55 g/100 ml. The melting point of sodium chlorite is between 180°C and 200°C.

Chlorite is used for the on-site production of chlorine dioxide, in water purification, in paper pulp, and as a bleaching agent for textiles. It is also used in shellacs, varnishes, waxes, and straw products.

Sodium chlorate is colorless, odorless, and crystalline in form with a cooling saline taste. It is soluble in cold water (79 g/100 ml water at 0°C) and in 0.5 ml of boiling water (230 g/100 ml water at 100°C); its melting point is 248°C, and its boiling point is 122°C with a density of 2.490.

Sodium chlorate is used as an oxidizing agent and as a bleach (especially to make chlorine dioxide). It is also used in paper pulps, matches, explosives,

flares and pyrotechnics, ore processing, herbicides and defoliants, medicine, and as a substitute for potassium chlorate.

Both sodium chlorite and sodium chlorate were reviewed in Volumes 3 and 4 of *Drinking Water and Health* (NRC, 1980, pp. 193-202; 1982, pp. 174-176). The following information updates and reevaluates what is known about these compounds.

Metabolism

As a part of an investigation of the kinetics of ClO_2, Abdel-Rahman et al. (1980) investigated the effects of chlorite and chlorate on blood components. Male rats weighing 150 to 170 g and white leghorn roosters weighing 250 to 300 g were given drinking water that contained chlorite or chlorate in concentrations of 10 or 100 mg/liter for 20 hours/day, 7 days/week for 4 months. In all groups of rats and chickens intoxicated by chlorite or chlorate there were decreases in blood glutathione, in osmotic fragility of erythrocytes, and in the morphology of erythrocytes from rats and chickens. No methemoglobin was detected.

Abdel-Rahman and coworkers (1984a) administered 36Cl-labeled sodium chlorite and sodium chlorate to rats in a study of their absorption, distribution, and excretion. Groups of four male rats drank 3 ml of solutions of chlorite (10 mg/liter) or chlorate (5 mg/liter); some groups were kept for periodic drawing of blood before being killed at 72 hours for analysis of various organs and tissues, while others were kept in metabolism chambers for collection of excreted air, urine, and feces. Peak plasma levels of ^{36}Cl were reached 2 hours after administration of chlorite; the half-life for elimination of the labeled Cl was 35 hours. Peak plasma levels of labeled Cl from chlorate were reached in 30 minutes, with a half-life for rapid elimination of about 6 hours, followed by a slower phase with a half-life of 36.7 hours. At 72 hours after administration of the chlorite, ^{36}Cl was highest in whole blood, followed by packed cells, plasma, stomach, testes, skin, lungs, kidney, duodenum, carcass, spleen, ileum, brain, bone marrow, and liver. But with chlorate, ^{36}Cl was highest in plasma, followed by whole blood, stomach, testes, lungs, kidney, skin, duodenum, spleen, brain, packed cells, ileum, carcass, liver, and bone marrow.

In the 72-hour period following administration of chlorite or chlorate, during which the rats were kept in metabolism chambers for collection of urine, feces, and expired air, 39% of the ^{36}Cl from administered chlorite was recovered, about 35% in the urine and about 5% in the feces. No labeled Cl was detected in expired air. In the case of chlorate, about 43% of the labeled Cl was collected in the 72-hour period, about 39% in the

first 24 hours. As in the case of chlorite, no labeled Cl was found in expired air.

Labeled Cl excreted after chlorite administration was in the form of chlorite or chloride; labeled Cl excreted after chlorate administration was in the form of chlorate, chlorite, or chloride.

The investigators pointed to the prolonged retention of chlorite or chlorate metabolites in the testes as evidence of possible action at this site.

Abdel-Rahman and coworkers (1980, 1982) also studied the tissue distribution of ^{36}Cl-labeled chlorite and chlorate. The amounts found in various fluids and tissues, expressed as percentage of the initial dose after 72 hours, were as follows: for chlorite, 0.55% was found in the plasma, 0.63% in packed cells, 0.64% in whole blood, and a total of about 3% in kidneys, lungs, stomach, duodenum, ileum, liver, spleen, bone marrow, testes, skin, and carcass, with the highest concentrations (about 0.4%) being found in skin, testes, stomach, and lungs; for chlorate, 0.68% was found in plasma, 0.23% in packed cells, 0.57% in whole blood, with a total of 3.6% in the same tissues listed for chlorite, with about 0.4% in each of several tissues, namely, kidney, lungs, stomach, testes, and skin. Chlorite was administered as a 10-mg/liter solution and chlorate as a 5-mg/liter solution.

According to Abdel-Rahman (1985), the amounts of labeled chlorine remaining in various tissues after 72 hours can be largely accounted for as chloride. Together with the other data, this suggests that neither chlorite nor chlorate bioaccumulates.

Unlike chlorite the excretion of ^{36}Cl from chlorate was biphasic. The first phase of decay from the plasma of rats had a half-life of 6 hours, and a second phase had a half-life of 36.7 hours (Abdel-Rahman et al., 1982, 1984a). Most of the intact chlorate excreted in the urine appeared within the first 8 hours after administration; thereafter, no intact chlorate was detected.

Bercz et al. (1982) observed that one effect of ClO_2 in African Green monkeys was a decrease in serum thyroxine. On the chance that this might reflect an effect of chlorite or chlorate resulting from ClO_2 transformation, they administered chlorite or chlorate to these monkeys at concentrations equivalent to doses of up to 60 mg/kg bw per day without developing a depression of serum thyroxine.

Health Aspects

Observations in Humans

Because of its use as a weed killer, there are many case reports of chlorate intoxication. Most of these were available at the time of the

publication of Volume 3 of *Drinking Water and Health* (NRC, 1980). Several recent ones add to that information.

Stavrou et al. (1978) described the effects in a 13-year-old boy who ingested an unknown amount of $NaClO_3$ by licking the crystals adhering to his moistened finger placed in a bag of crystals. He became very ill and was seen by the family physician the next day; on the third day he was admitted to the hospital. He was cyanotic, passing little urine, and febrile. His liver was enlarged, he was jaundiced, and he felt tenderness in the epigastrium and loins. His blood was brown and Heinz bodies were seen. Methemoglobin was found in the plasma, and protein was detected in the urine. He had renal failure, managed by peritoneal dialysis, for 21 days.

Bloxham et al. (1979) described a 29-year-old man who had ingested about 20 g $NaClO_3$ (230 mg chlorate/kg bw). He became cyanotic, and his hemoglobin dropped to 11 g/100 ml within 24 hours; methemoglobin and methemoalbumin were detected in his plasma. He was anuric for 14 days, then gradually improved, and he was released from the hospital after 6 weeks.

Helliwell and Nunn (1979) reported on 14 cases of $NaClO_3$ poisoning. The patients' ages ranged from 3 to 55 years. Doses estimated to be in excess of 100 g or 79 g as chlorate ion were uniformly fatal. One 46-year-old woman given supportive therapy died 20 hours after a dose estimated to be 15 g (218 mg chlorate/kg bw). This was the lowest dose found to be fatal in these cases. Another female of unreported age died 5 days after ingesting 30 g (436 mg chlorate/kg bw), despite treatment with methylene blue, peritoneal dialysis, and exchange transfusion. However, an 18-year-old male survived a dose estimated at 100 g (1.45 g chlorate/kg bw) after treatment with methylene blue, exchange transfusion, and hemodialysis. Cyanosis was seen in 50% of the patients, abdominal pain in 36%, diarrhea in 21%, dyspnea in 21%, anuria within 48 hours in 50%, coma in 12%, and methemoglobinemia in 93%. Sixty-four percent died.

Steffen and Seitz (1981) saw a 26-year-old woman 5 hours after she ingested 150 to 200 g chlorate (2.145 g chlorate/kg bw). Methemoglobin was an early sign of intoxication; although the administration of methylene blue appeared to be helpful in treating the methemoglobinemia, it did not prevent massive hemolysis and disseminated intravascular coagulation. Renal function was said to be completely absent for 10 days, but dialysis was continued another month, and renal output then began to exceed 1,000 ml/day.

Lubbers and associates (1981, 1982, 1983) conducted controlled clinical investigations of volunteer human subjects exposed to sodium chlorite and sodium chlorate (as well as to some other materials). Statistical analyses

of the data were reported by Lubbers and Bianchine (1984) and Lubbers et al. (1984). The subjects were given repeated physical examinations and extensive laboratory tests.

Described in more detail in the section on chloramine, Lubbers et al. (1981) gave groups of 10 male volunteer subjects sodium chlorite in drinking water in two separate phases. In the first phase, the 10 subjects drank 1 liter of water every fourth day for 6 days, over a 16-day period. The first day, the concentration of sodium chlorite in water was 0.01 mg/ liter, and this was increased each exposure day to a final concentration of 2.4 mg/liter (average 0.34 mg/kg bw per day). Subjects were given the test substance once every 3 days. Group mean values determined for 2 days following each exposure of all investigated effects, including many serum chemistry components and blood-cell counts as well as results from ECG analysis and physical examinations, were within normal ranges, and no trends or interactions judged to be of clinical significance were found.

In a second phase, 10 volunteers drank 500 ml of water containing 5 mg sodium chlorite/liter daily for 12 weeks (average 0.034 mg/kg bw per day). Significant ($p < 0.05$) group-time interaction was found in the case of group mean corpuscular hemoglobin; however, linear trend regression analysis did not show a significant linear trend, and the physiological significance of this interaction was doubted.

In a third phase of the study on sodium chlorite, three subjects found to be deficient in G-6-PD were given 500 ml of drinking water containing 5 mg chlorite/liter of water every day for 12 weeks, as in the second phase. The rationale for this phase was that those deficient in G-6-PD might be expected to be especially susceptible to oxidative stress. The small number of subjects made some of the otherwise desirable statistical procedures of questionable use, and linear regression analysis was chosen. There were several laboratory analyses with a significant probability ($p < 0.05$) of a change with respect to time over the 12-week treatment period; these were in albumin/globulin ratio, T_4 radioimmunoassay (RIA), free thyroxine, mean corpuscular hemoglobin concentration, and methemoglobin values. The authors cautioned that these were only trends, that caution should be used in interpreting their significance because of the small number of subjects and the possibility of "laboratory drift," and that attribution of physiological consequence would be premature.

Lubbers and coworkers (1981) also administered chlorate in drinking water to groups of male volunteer subjects in the same experiments, described more fully in the section on chloramine. In phase I, 10 subjects drank 1 liter of chlorate-containing water for 6 days, with 2 days intervening between each exposure day, so that the six exposures occurred over a span of 16 days. The concentration of sodium chlorate rose gradually during the experiment; it was 0.01 mg/liter the first day and rose to 2.4

mg/liter by the sixth day (average 0.34 mg/kg bw per day). There were changes concluded to be statistically significant but not clinically significant in serum bilirubin (total) and iron.

In a second experiment, 10 subjects drank 500 ml of water containing sodium chlorate at 5 mg/liter every day for 12 weeks (average 0.034 mg/ kg bw per day). There was a significant ($p < 0.05$) group-time interaction in the case of group mean corpuscular hemoglobin and of group mean blood urea nitrogen, but linear regression analysis did not show a significant linear trend in the means of these effects, and they were judged not to be clinically significant.

Observations in Other Species

Acute Effects

There were no studies available.

Subchronic Effects

Bercz et al. (1982) administered drinking water containing $NaClO_2$ or $NaClO_3$ as well as ClO_2 or NH_2Cl to African Green monkeys for 30-60 days in a study of possible thyroid effects. The drinking water contained chlorite or chlorate in concentrations of 25, 50, 100, 200, or 400 mg/liter in a rising-dose experiment. Equivalent doses were 4, 7.5, 15, 30, or 58.4 mg/kg bw per day. Neither the chlorite nor the chlorate induced any thyroid depression at any dose. The chlorite but not the chlorate induced a dose-dependent oxidative stress on hematopoiesis, resulting in a decreased hemoglobin and erythrocyte count and an increased methemoglobin. Serum glutamic pyruvate transaminase (SGPT) was increased in a statistically significant and dose-dependent manner; however, the investigators believed that the effect was not clinically important and pointed out that this elevation was not corroborated by elevations in other enzymes or in serum bilirubin. The blood changes in the chlorite-treated monkeys started to reverse before the end of the period of administration.

Heffernan et al. (1979a) found that the hemoglobin in blood from both rats and human beings was oxidized to methemoglobin by the chlorite, the hemoglobin in rat blood being somewhat more sensitive to concentrations of 10^{-3} to 10-2 M $NaClO_2$ than that of human blood. Above a concentration of NaClO2 of about 4 x 10^{-2}, the situation seemed to reverse, the hemoglobin of human blood being more sensitive to oxidation by chlorite than that of rat blood. Hemoglobin of rat blood was more sensitive to oxidation by NO_2 than by chlorite. The concentration of glutathione in erythrocytes was reduced almost to zero (3.8% of original) by added $NaClO_2$ to yield a concentration of 50 mM; added $NaNO_2$ to yield the same concentration of added material reduced the concentration of glutathione in the erythrocytes to only 79.5% of the original value.

Anaerobic conditions did not alter appreciably the reduction in glutathione concentration by chlorite. The finding that reduction of glutathione concentration in erythrocytes preceded the production of measurable amounts of methemoglobin suggests that chlorite-induced oxidative injury of red cells involves targets other than hemoglobin. EM-visible distortion of the erthrocyte membrane appeared at a concentration of $NaClO_2$ of 7.4 x 10^{-4} M and increased in number on single membranes and in populations of red blood cells as the concentration of $NaClO_2$ in the suspension of erythrocytes was increased. At a concentration of $NaClO_2$ of 1.5 x 10-2 M, the envelope of the red cell became completely permeable to both hemoglobin and methemoglobin. The activity of catalase in red blood cells, used as a measure of production of H_2O_2, decreased practically to zero as the concentration of $NaClO_2$ in the erythrocyte suspension was increased from 10-4 to 10-3 M, indicating that the active center of the enzyme became almost completely occupied by endogenously produced H_2O_2. These various findings in vitro indicate that the chlorite ion belongs to the class of chemicals that induces production of H_2O_2 and that is likely to produce hemolytic anemia in vivo.

In fact, Heffernan et al. (1979b) found that rats given drinking water containing $NaClO_2$ at 100 mg/liter for 30 days had a mean concentration of glutathione in their red blood cells that was only 66. 1% of normal, that the concentration of Hgb in their blood was reduced to about 87% of normal, that their mean red-blood-cell (RBC) count was 96.9% of normal, and that their mean packed-cell volume was 93.5% of normal. Higher concentrations of $NaClO_2$ in the drinking water, up to 500 mg/liter, produced greater changes in these measures. Administration of these amounts of drinking water for longer periods of time, up to 90 days, generally yielded smaller terminal deviations from normal than the shorter period, indicating the marshaling of compensatory mechanisms. The single exception to this generalization was the glutathione concentration within the red blood cell, which was insignificantly lower after 90 days of exposure to $NaClO_2$ in drinking water than after 30 days. When single doses of $NaClO_2$ were injected intraperitoneally into rats in amounts of 1, 10, 20, 30, and 50 mg/kg bw, there was a dose-related increase in the percentage of methemoglobin in total heme pigment and a less clearly dose-related decrease in total heme pigment. Oral doses of 20 and 64 mg $NaClO_2$/kg bw given to cats resulted in fairly sharp (1.5 hours) increase in the percentage of methemoglobin in total heme pigment to a mean of 22.8% in three cats given the smaller dose and of 46.7% in one cat given the larger dose. By 6 hours after the doses, these increases had decreased considerably from their maxima. Only the cat given the smaller dose that had had the least maximum increase in its percentage of methemoglobin (10%) had returned completely to normal within that time.

In a companion report, Heffernan et al. (1979a) described some of these effects further. They incubated sodium chlorite with blood from male rats and studied some of the reactions that occurred. They found chlorite to be slightly less potent than nitrite as an oxidant of hemoglobin and less specific in its oxidation of cellular constituents. As in the earlier paper, they found that chlorite depleted red-cell glutathione, accompanied by an increased generation of hydrogen peroxide. They described changes in the structure of erythrocyte membranes. They commented that chlorite acts primarily as a hemolytic agent and secondarily as an oxidant of hemoglobin.

In an investigation of subchronic toxicity, Heffernan et al. (1979b) gave rats and cats $NaClO_2$ in their drinking water. There was a dose-related decrease in RBC, hemoglobin, and packed-cell value at 30 and at 60 days in rats given water containing chlorite at 100 mg/liter, equivalent to 10 mg/kg bw per day, and higher. After 90 days, some adaptation had occurred. RBC glutathione concentrations were significantly decreased at chlorite concentrations as low as 50 mg/liter (5 mg/kg bw per day). Unlike the other blood effects studied, this effect on glutathione did not recover on longer exposure. Erythrocytes from rats earlier intoxicated with chlorite were less able than is normal to control generation of hydrogen peroxide when incubated *in vitro* with chlorite. The investigators attributed this to chlorite-induced depletion of glutathione in erythrocytes.

In cats, there was a 20-30% decrease in packed-cell volume and in hemoglobin concentrations. They were given drinking water containing $NaClO_2$ at 500 mg/liter (7 mg/kg bw per day). Doubling the chlorite concentration greatly increased this effect. The use of ^{51}Cr-labeling of erythrocytes in cats given water containing 0, 100, 250, or 500 mg $NaClO_2$/liter (0.6, 3.6, or 7 mg/kg bw per day) showed a dose-related increase in turnover of RBCs at levels of 100 mg/liter and above. (Water consumption at the highest dose was markedly decreased.) It was concluded that chlorite can induce hemolytic anemia.

An increase in the ratio of kidney weight to body weight was observed in male CD rats given drinking water containing 500 mg $NaClO_2$/liter (50 mg/kg bw per day) for 60 days (Heffernan et al., 1979b). This was not seen at lower water concentrations (100 and 150 mg/liter).

Moore and Calabrese (1980) found an increase in mean corpuscular volume, in osmotic fragility and G-6-PD activity, and in the number of acanthocytes in A/J and C57L/J mice given drinking water containing 100 ppm (but not at I or 10 ppm) $NaClO_2$, equivalent to 13-18 mg chlorite/kg bw per day for 30 days. Moore and Calabrese concluded that the main effect of chlorite on erythrocytes is disruption of the cell membrane. Similarly, Heffernan et al. (1979a) had earlier described effects of chlorite on erythrocyte membranes of rats.

Couri and Abdel-Rahman (1980) administered water containing chlorite at 100 mg/liter (10 mg/kg bw per day) to rats for 6-12 months and found marked decreases in RBC glutathione. There were smaller decreases at 10 mg/liter (1 mg/kg bw per day) at 6 but not at 12 months. This was more extensively studied by Abdel-Rahman et al. (1984b), but questions about the statistical treatment of the data need to be considered in light of the inconsistency of results. They reported a progressive decrease in RBC osmotic fragility as chlorite intoxication was extended beyond a few months. Hemoglobin, hematocrit, and RBC were decreased from control values after 9 months of exposure to chlorite at concentrations as low as 10 mg/liter.

Chronic Effects

Haag (1949) gave month-old rats sodium chlorite in their drinking water at concentrations of 1, 2, 4, 8, 100, and 1,000 ppm for 2 years. There were three groups of controls; two received distilled water and the third received water containing enough sodium chloride to be ionically equivalent to 1,000 ppm of sodium chlorite. There were seven female and seven male rats in each group except that no female rats were included in one of the drinking water controls. The alkalinity of the solution of 1,000 ppm sodium chlorite was adjusted by the addition of hydrochloric acid (HCl).

At the end of the first year, all rats receiving 1, 2, or 4 ppm were switched to the 8-ppm solution, and they continued to receive sodium chlorite at that level throughout the second year.

Rats of both sexes receiving sodium chlorite at 1,000 ppm consistently drank less water than did controls, while male rats receiving sodium chloride had an elevated fluid consumption. Consistent with the reduced ingestion of water, rats getting 1,000 ppm chlorite grew at a slower rate, whereas the male rats getting sodium chloride grew at a greater rate than did negative controls. There was no trend of increased mortality in any group.

Microscopic studies of morbid anatomy were performed on representative animals and on all rats with macroscopically evident tumors. The only findings judged to be related to the treatment were in the kidneys of male rats that imbibed 100 or 1,000 ppm sodium chlorite and in male rats receiving sodium chloride in their drinking water. These changes consisted of a marked distention of the glomerular capsules by fluid as well as the filling of tubules with a pale, pink-staining material. The changes were most severe in the rats that imbibed sodium chloride.

Haag (1949) wondered whether the affected rats might have developed a beginning nephritic condition at some point in the experiment that might have become exacerbated by the prolonged absorption of these salts. While he did not describe the source of his animals, it is doubtful that he had

specific-pathogen-free rats or other rats of excellent health in an experiment conducted about 40 years ago.

Mutagenicity

Eckhardt et al. (1982) reported in an abstract that $NaClO_3$ was mutagenic to *Salmonella typhimurium* strain TA1535 in the presence of an S9 supernatant from an unspecified source. They also reported that $NaClO_3$ was mutagenic in a *Drosophila* system but was inactive in the micronucleus assay; they did not give details.

Meier et al. (1985) investigated the mutagenicity of $NaClO_2$ and $NaClO_3$ (as well as ClO_2 and other disinfectants) in the mouse micronucleus assay, in the mouse sperm-head assay, and in the mouse bone-marrow chromosomal aberration assay. Swiss (CD-1) mice were used for the micronucleus and bone-marrow studies, and $B6C3F_1$ mice were used for spermhead studies. Doses, administered orally for 5 days, were 1 ml of a solution of 1 g/liter in water, or about 50 mg/kg bw if the mouse weighed 20 g. One group of animals was killed 6 hours after the last dose and examined for types and numbers of chromosomal aberrations. Another group, also killed at 6 hours, was examined for micronuclei in polychromatic erythrocytes. Sperm-head abnormalities were looked for in animals killed at 1, 3, or 5 weeks after the last dose, to ensure that all major stages of spermatogenesis would be included. Evidence of mutagenicity was not found in any of these tests with either salt.

Carcinogenicity

Kurokowa et al. (1984) tested chlorite as a possible tumor promoter or as a complete carcinogen. In the test of complete carcinogenicity, 0.2 ml of a 20-mg/ml solution in acetone was applied to the shaved backs of female Sencar mice twice weekly for 51 weeks; this is 200 mg/kg bw if the mouse weighed 20 g. No tumors were detected in the group of 20 animals. In the test of tumor promotion, dimethylbenzanthracene (DMBA) was applied once prior to the 51-week application of $NaClO_2$ as in the test of complete carcinogenicity. Six of the 20 mice receiving the prior dose of DMBA developed skin tumors, whereas those that received DMBA followed by acetone alone did not develop tumors. Five of the mice had squamous-cell carcinomas, but this did not meet the investigators' criterion of statistical significance ($p = 0.01$) on Fisher's exact test or the chi-squared test. The investigators' conclusion on sodium chlorite was that a "potential promoting effect was suspected. ..."

Developmental Effects

Suh et al. (1983) gave groups of six to nine pregnant rats drinking water containing chlorite or chlorate in concentrations of 0, 1, or 10 mg/liter for 21/2 months prior to and throughout gestation in a teratogenicity study of these disinfectants. Some alterations in number of fetuses resorbed or with skeletal or visceral anomalies were observed,

but the small number of litters and a high rate of abnormalities among control fetuses precluded statistical significance or clear interpretation of the results.

Suh et al. (1983) also gave groups of eight to nine pregnant rats drinking water containing chlorate at the same concentrations (1 or 10 mg/liter). The incidence of these same skeletal anomalies was 52% in the group receiving 1 mg chlorate/liter and 55% in the group receiving 10 mg/liter, compared with 31% in controls. Again, statistical significance was not demonstrated. An animal at 10 mg/liter was found to have hydronephrosis.

Reproductive Effects

Moore and Calabrese (1982) gave female A/J mice water containing 100 ppm $NaClO_2$ as soon after mating as vaginal plugs, indicative of conception, were seen. There were no significant differences from controls, as judged by a *t*-test, in litter size, number alive at weaning, gestation time, number of stillbirths, number of pups dying between birth and weaning, average litter birth weight, and the weight and age of dams. However, there were significant decreases ($p < 0.03$) in the case of offspring of chlorite-treated dams, in body weights at weaning, and in the growth rate between birth and weaning.

Couri et al. (1982) found fetal resorptions, sometimes involving all of a litter, in dams given high levels of $NaClO_2$ (20,000 mg/liter, or 212 mg/kg bw per day). However, resorptions were not induced at 5,000 mg/ liter (122 mg/kg bw per day). The investigators suggested that these resorptions might be a consequence of anemia-induced hypoxemia.

Suh et al. (1984) gave rats water containing chlorite or chlorate in concentrations of 100 mg/liter for 3 weeks. They found incorporation of ^{3}H-thymidine (2-hour labeling period) into nuclei of testes was inhibited 50% in rats given chlorite at 100 mg/liter but was not decreased in rats given chlorate at that concentration. After exposure for 3 months, there was an inhibition of thymidine uptake (8-hour labeling period) at drinking water concentrations of 10 mg chlorate/liter (1 mg/kg bw per day) in another study (Abdel-Rahman et al., 1984b).

Carlton and Smith (1985) described effects of chlorite on several aspects of reproduction. Long-Evans rats ingested water containing sodium chlorite at 0, 1, 10, or 100 ppm for 66-76 days. Males were treated for 56 days and females for 14 days before being allowed to mate, and the treatment was continued during the 10-day breeding period; other females were continued on the chlorite regimen throughout gestation and lactation. Following breeding, males were killed and evaluated for sperm changes and for morbid anatomic changes in the reproductive tract. Dams and some pups were necropsied at weaning time, 21 days after birth.

No differences between control and test animals were seen with respect to litter size and viability or days of vaginal patency and of eye opening.

Body weights of adults were not affected. Additional male rats imbibed sodium chlorite in drinking water at 0, 100, or 500 ppm for 72-76 days; and in these rats given 500 ppm, a 28% decrease in water consumption occurred.

Methemoglobin levels were not increased nor were other chorite-induced changes observed in blood elements. Reproductive tract anatomy was not altered. Organ weights and ratios of organ to body weights were not changed.

A slight trend toward decreasing motility of sperm was seen in rats given 10 ppm and higher, but this was not statistically significant. There was a decrease in sperm drive range in rats given 10 ppm and higher, and this decrease was statistically significant ($p < 0.01$) in groups imbibing 100 or 500 ppm. Increases in abnormal sperm forms ($p < 0.001$) occurred in rats given 100 or 500 ppm.

Conclusions and Recommendations

Both chlorate and chlorite produce damage in erthyrocytes and produce methemoglobin. It is thought that these effects are related to their oxidative properties. Hematological effects have been observed both in humans and in laboratory animals. As in the case of chlorine dioxide, decreased thyroid function has been associated with exposure to chlorate and chlorite. Nonetheless, this has not been confirmed by other studies. The decreased thyroid function may be related to oxidation of dietary iodide in the gastrointestinal tract, which then binds to food or tissue and is unavailable for absorption. Further research is necessary before this association can be accepted.

A previous Safe Drinking Water Committee (NRC, 1980) has recommended a suggested no-adverse-effect level (SNARL) for chlorite of 0.21 mg/liter based on work by Heffernan et al. (1979b), where the no-observed-effect level (NOEL) for hematological effects in cats was 0.6 mg/ kg bw per day. There are no new studies in humans or animals that would show lower NOEL values, with the exception of the clinical studies by Lubbers and coworkers (1981), where a NOEL of 0.034 mg/kg bw per day was indicated for both chlorate and chlorite. An observed-effect level was not determined in these studies, therefore the relevance of a dose of 0.034 mg/kg bw per day to a threshold for hematological effects in humans cannot be determined. Nonetheless, the committee prefers to use human data when they are available. Thus a SNARL may be calculated for chlorate and chlorite using a NOEL of 0.034 mg/kg bw per day, and assuming that a 70-kg human consumes 2 liters of water daily, which contributes 20% of the total intake:

$$\frac{0.034 \text{ mg/kg/day} \times 70 \text{ kg} \times 0.2}{10 \times 2 \text{ liters}} = 0.024 \text{ mg/liter, or } 24 \text{ µg/liter.}$$

A SNARL may also be estimated for a 10-kg child consuming 1 liter of water daily, which contributes 20% of total intake:

$$\frac{0.034 \text{ mg/kg/day} \times 10 \text{ kg} \times 0.2}{10 \times 1 \text{ liter}} = 0.007 \text{ mg/liter, or } 7 \text{ } \mu\text{g/liter.}$$

TRIHALOMETHANES

Chloroform

CAS No. 67-66-3

$$CHCl_3$$

Dibromochloromethane

CAS No. 124-48-1

$$CHClBr_2$$

Chloroform (trichloromethane) is a colorless, highly refractive, heavy, sweet-tasting liquid that has a light, crisp odor. Its vapor pressure is 100 mm of mercury at 104°C, and it is very slightly soluble in water (0.8 g/g of water at 20° C). Liquid chloroform is very volatile but nonflammable; its gaseous form is capable of burning.

Chloroform is used as a grain fumigant and as a general solvent for adhesives, pesticides, fats, oils, rubbers, alkaloids, and resins. It is also registered for use in the United States as an insecticidal fumigant on stored barley, corn, oats, popcorn, rice, rye, sorghum, and wheat; as a dry-cleaning agent; as an extraction and purification solvent for penicillin; as a soil fumigant and insecticide; and as a mildew preventive for tobacco seedlings. It was used in the past as a component of cough syrups, toothpastes, liniments, and toothache compounds. Chloroform was reviewed in Volumes 1, 3, and 4 of *Drinking Water and Health* (NRC, 1977, pp. 713-717; NRC, 1980, pp. 203-204; NRC, 1982, pp. 206-209); the following is principally an examination of data that were not considered in the earlier volumes. However, some data reviewed in earlier volumes that are relevant to the current review are included here for completeness.

Dibromochloromethane is a heavy, colorless-to-pale-yellow liquid used as a chemical intermediate in the manufacture of fire extinguishing agents, aerosol propellants, refrigerants, and pesticides. Its boiling point is about 118ºC; its specific gravity is 2.38 and it has a density of 2.451 at 20°C. It is soluble in alcohol, ether, acetone, benzene, and organic solvents. Dibromochloromethane is formed during chlorination from naturally occurring

humic substances in raw water. The health effects of this by-product of drinking water disinfection were reviewed in Volume 3 of *Drinking Water and Health* (NRC, 1980, pp. 205-206); the following material updates and reevaluates that information.

Metabolism

In a paired t-test, Withey et al. (1983) compared gastrointestinal absorption of chloroform administered by gavage in a mixture with either water or corn oil. Fifteen male Wistar rats weighing about 400 g were given doses of chloroform in corn oil at 75 mg/kg bw, and 15 additional rats of comparable weight and identical sex and strain were given the same dose in an aqueous solution. The peak concentration of chloroform in blood was reached at about the same time with either solvent (5.6 minutes for water, 6.0 minutes for corn oil), but because the bioavailability of chloroform was 8.7-fold higher when given in the aqueous solution, much more chloroform reached the blood after administration in an aqueous medium (39.3 µg/ml of water and 5.9 µg/ml of corn oil).

Pohl et al. (1977) demonstrated the formation of 2-oxothiazolidine-4-carboxylic acid (OTZ) during incubation of chloroform with a liver microsome preparation. Cysteine inhibited the binding of labeled chloroform to microsomal protein *in vitro* and apparently reacted with phosgene to form OTZ. When the incubation was performed in an atmosphere of labeled oxygen, oxygen-labeled phosgene was formed. Pohl et al. (1977) suggested that the formation of unstable trichloromethanol through the action of a cytochrome P450 monooxygenase yields phosgene with spontaneous elimination of hydrochloric acid. That compound can react with cysteine and macromolecules. Pohl and Krishna (1978) developed data suggesting that deuterium-labeled chloroform was less hepatotoxic and less readily metabolized than unlabeled chloroform, indicating that cleavage of the C-H bond is the rate-limiting step in the process that renders chloroform hepatotoxic.

Additional studies have examined renal metabolism of chloroform and have found nephrotoxicity to be linked to chloroform metabolism, as is hepatotoxicity. Renal homogenate subfractions form OTZ and carbon monoxide when incubated with chloroform (Branchflower et al., 1984). Formation of reactive intermediates is decreased by manipulations that inhibit the cytochrome P450-mediated metabolism, such as incubation in the presence of CO, or addition of inhibitors such as SKF-525A, piperonyl butoxide, or metyrapone to the incubation mixture (Smith and Hook, 1984). These authors showed renal bioactivation of chloroform to be greater than that of liver, when activity is expressed relative to cytochrome

P450; emphasizing that while metabolism in the kidney may be of lesser importance for overall deactivation of compounds, the kidneys possess adequate enzyme activity to produce sufficient amounts of reactive metabolites to result in an adverse effect locally—either toxicity, as demonstrated, or possibly carcinogenicity. A deuterium isotope effect for nephrotoxicity has also been reported (Ahmadizadeh et al., 1981).

In an abstract of a study of possible free-radical formation by chloroform (Ruch et al., 1986), the effects of several substances on the toxicity of chloroform to hepatocytes isolated from mice were reported. The possible modifiers investigated were SKF-525A, an inhibitor of mixed-function oxidase; the antioxidants vitamin E and *N*, *N'*-diphenyl-*p*-phenylenediamine (DPPD); and a depletor of cellular glutathione, diethylmaleate. Chloroform by itself had a dose-related toxicity for hepatocytes from male $B6C3F_1$ mice at 1 and 5 m*M*. The toxicity of chloroform for the hepatocytes was decreased at 2, 4, and 20 hours after a dose of SKF-525A. Vitamin E and DPPD did not affect the toxicity of chloroform. Diethylmaleate potentiated its toxicity at the three time periods mentioned above. *In vivo* studies have shown that diethylmaleate potentiates toxic effects of chloroform on liver (Stevens and Anders, 1981) and kidney (Kluwe and Hook, 1981).

Hewitt et al. (1980) presented evidence from organ weights, biochemical changes, and both qualitative and quantitative microscopic studies of tissue changes that *n*-hexane, 2-hexanone, 2, 5-hexanedione (the metabolite of hexane and 2-hexanone), as well as acetone, can markedly increase the toxicity of chloroform to the liver and kidney. These potentiators by themselves did not produce marked liver injury, but when administered by gavage to male rats 18 hours before intraperitoneal administration of chloroform (0.5 ml/kg bw), they markedly increased the hepatotoxicity and nephrotoxicity of chloroform in rats. The authors hypothesized that prior exposure to ketones or substances which are metabolized to ketones enhanced the susceptibility of the liver and kidney to toxic actions of haloalkanes.

Cresteil and co-workers (1979) compared metabolism of chloroform by microsomes prepared from rat or human liver tissue (human livers were obtained from donors for renal transplantation). Binding of radioactivity from $[^{14}C]$ $CHCl_3$ was used as an index of reactive metabolite formation. Human microsomes catalyzed binding at a lesser rate than did rats (0.20.-7 nmol/mg microsomal protein/5 min for human and 1.1 ± 0.3 for rats); both the apparent spectral dissociation constant (Ks) and Michaelis-Menten constant (Km) were less for human tissue. Irreversible binding was decreased by addition of cysteine to the incubation mixture. This is an indication of phosgene formation during the metabolism of chloroform.

This study is important because it indicates that chloroform is metabolized by humans along a pathway similar to that studied in rodents and that human liver activity is comparable with that of rats.

Fry et al. (1972) studied the elimination of ingested chloroform in expired air. Eight normal subjects (five men, three women), 18 to 50 years of age and weighing 60 to 80 kg, took gelatin capsules containing 500 mg of chloroform dissolved in 1 ml of olive oil about 1.5 hours after eating breakfast. Expired air was collected during the succeeding 8 hours and was analyzed for chloroform content. Between 17.8% and 66.6% of the ingested compound was recovered (mean recovery, 40.3%). Two subjects (a man and a woman) took similar capsules containing [^{13}C]-chloroform, and their expired air was examined for its content of $13CO_2$. During 8 hours the man excreted 50.6% of the ingested label as $13CO_2$; the woman excreted 48.5%. Ingested chloroform appears, therefore, to be excreted principally in the breath (a mean of about 90% during the 8 hours after ingestion). About 45% is excreted as unchanged chloroform and about 55% as carbon dioxide.

The concentration of chloroform in the blood reached a maximum about 45 minutes after ingestion of the capsules and then decreased in a biphasic manner. The initial part of the biphasic elimination curve had a mean half-time (four subjects) of about 14 minutes after attaining the peak concentration; the mean half-time for the second portion of the elimination curve was attained about 90 minutes after the peak concentration. The rate of respiratory excretion of chloroform as such was a linear function of the concentration of that substance in the blood and was an inverse linear function of the deviation of the body weight of the subject from their ideal weight. Thus, obese subjects excrete a smaller proportion of chloroform through their lungs than subjects of normal weight. The slope of the line relating pulmonary excretion of chloroform to the deviation of the subject's body weight from the ideal was greater for men than for women, probably reflecting the greater reservoir of lipoid in the female body.

Health Effects

Observations in Humans

Because chlorination can lead to the formation of chloroform or other chlorocarbons in drinking water, there has been interest in the cancer incidence among persons whose drinking water has been chlorinated as compared with those whose drinking water has not been so treated. Several epidemiological studies have indicated an association between water chlorination and increased mortality rates from cancer (Cantor et al., 1977, 1978, 1985; Cragle et al., 1985; Kuzma et al., 1977; Page et al., 1976).

The contribution of this information to an evaluation of the ability of chloroform to induce neoplasms is made uncertain by the presence of confounding variables.

In a case-control study of the relationship between the incidence of colon cancer and water chlorination in North Carolina, Cragle et al. (1985) estimated exposure to chlorinated water through 25-year residence histories. They found a statistically significant relationship between chlorination and colon cancer above age 60 but not below that age.

Cantor and coworkers (1978) studied the association of the use of drinking water containing trihalomethanes (THMs) with cancer mortality in 923 U.S. counties, more than half of which were urban. Mortality data were classified by county of usual residence and compared with THM data obtained from the Environmental Protection Agency (EPA) surveys of water supplies in those counties. Concentrations of chlorinated THMs were subtracted from total THMs to obtain brominated THM data. Using a weighted linear-regression model to predict sex-and site-specific cancer rates, they found positive correlations between THM levels and mortality from cancers of certain sites. Bladder cancer rates in both sexes showed the strongest and most consistent correlation with an index of THM exposure. After establishing controls for differences in social class, ethnic group, and residence (urban or rural, degree of industrialization of the area, and section of the United States), the authors also found correlations with brain cancers in both sexes and with non-Hodgkin's lymphoma and kidney cancer in males. Deaths from brain cancer were associated with brominated THMs in both sexes and were correlated with magnitude of exposure. There was a suggested association between chloroform levels and kidney cancer in males.

On the other hand, Cantor et al. (1985) found no increased risk of bladder cancer among people living in areas with chlorinated surface water above that of those living in areas with unchlorinated groundwater. Moreover, among smokers, there was a negative association of the incidence of bladder cancer with the number of years of drinking chlorinated surface water. The pattern among ex-smokers was variable.

Young et al. (in press) described a population-based case-control study of possible association between colon cancer and exposure to THMs. They compared 372 cases of colon cancer and 1,451 controls with respect to estimated exposure to chloroform and other THMs from 1951 to 1981. Working from questionnaires, they used data on water sources and residences to estimate THM exposure. No association between exposure to THMs and the occurrence of colon cancer was found.

Two earlier studies recounted the effects of chloroform on human health. Challen et al. (1958) studied employees of a confectionery company that also manufactured medicinal lozenges. The process involved the use of

chloroform as a component of the dough from which the lozenges were made. The dough was mixed in closed mixers, but there was major escape of vaporized chloroform for 1.5 to 2.0 minutes during removal of the dough from the mixer, which was said to occur no more frequently than four times per day. When it occurred, the concentration of chloroform in the air of the workplace may have reached levels as high as 1,163 ppm (5,582 mg/m^3). Workers involved in manufacture of the lozenges complained of lassitude during the latter part of their workday—an effect persisting after they returned to their homes and in some cases even throughout weekends. Improved ventilation of the work area lowered the concentration of chloroform in the ambient air to a mean of 28% of that existing before the improvement and enabled employees to perform their duties comfortably.

Physical examinations of 10 employees believed to have been exposed to concentrations of chloroform vapor ranging from 77 to 237 ppm (370 to 1,138 mg/m^3) in the air revealed that 9 of the 10 complained of severe symptoms: a sensation of having a ball in the stomach, nausea, anorexia, loss of ability to concentrate, staggering, depression, irritability, and several other bothersome and in some cases painful effects. Among 10 employees who had worked only after improvement of the ventilation of the work area and who had been exposed to concentrations of vaporized chloroform ranging from 22 to 71 ppm (106 to 341 mg/m^3), 8 complained of less severe symptoms: dry mouth, borborygmi, and frequent micturition (probably due at least in part to hyperhydration). Despite the symptoms listed, no evidence of definite liver damage was found by either physical examination or tests of liver function (e.g., concentration of bilirubin in serum and thymol turbidity). Challen et al. concluded that the complaints by the workers whom they studied indicated that the maximum allowable concentration of chloroform in the air of the workplace should be no more than 50 ppm (240 mg/m^3).

Bomski et al. (1967) studied from 62 to 68 employees of a pharmaceutical company who had worked in an environment containing 0.01 to 1.0 mg/liter (2 to 205 ppm) of chloroform in the air for 1 to 4 years. The researchers examined the incidence of hepatomegaly, mean concentration of albumin in the blood, and mean levels of serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvate transaminase (SGPT) in four groups. A separate group of employees was identified who had had less contact with chloroform (19 to 39 people) than the previous group, and an additional group was identified who had had viral hepatitis but had not worked with toxic substances (19 to 23 people). Finally, a control group was identified who had no history of jaundice and had not worked with toxic substances (86 to 164 individuals). (Ranges of numbers of people in the various groups are given in parentheses because different

numbers of people in the groups volunteered to be tested for the separate parameters listed.)

Hepatomegaly was found in 25% of the employees exposed to chloroform for a comparatively long time, in 12.8% of the employees exposed to chloroform for a shorter time, in 8.7% of the people who had had viral hepatitis, and in 3.7% of the control group. The lowest mean concentration of albumin in blood was found in employees with comparatively great exposures to chloroform, but the differences from the mean values for the other groups were not significant. The mean levels of the two transaminases in the sera of the heavily exposed group of employees were lower than those of any of the other three groups. Electrophoretic fractionation of sera of the four experimental groups and three reactions that detect abnormal proteins in sera indicated that there was no significant change in the composition of the blood of the most heavily exposed group. This study suggests that exposure to chloroform may induce hepatic enlargement without persistence of other indices of injury to the liver. However, administration of the bromsulfalein excretion test revealed that 61% of the 60 people exposed to chloroform for a comparatively long time excreted bromsulfalein at rates more than 5% lower than normal value.

The relationship of these findings to the conclusions of Challen et al. (1958) is unclear, except for the premise that occupational exposure to chloroform may increase hepatomegaly with no evidence of continuing damage to the liver other than decreased biliary excretion of bromsulfalein.

Observations in Other Species

Acute Effects

Earlier work established the susceptibility of the liver and kidney to chloroform, and more recent work has elaborated on these effects.

Hill (1977) studied the effect of genetic and sex differences on the toxicity of chloroform in mice. He reported that many laboratory accidents involving the discharge of chloroform into quarters housing animals had been described in the literature and that males of some strains (but not females) had been found to die after exposure, whereas males and females of other strains survived. This suggestion of marked variations in susceptibility of specific strains led him to choose male mice of two inbred strains from the Jackson Laboratory as examples of two extremes of sensitivity to chloroform. DBA/2J was chosen as the sensitive strain and C57BL/6J as the resistant strain.

The LD_{50} of chloroform administered orally in oil to DBA/2J mice (0.08 ml/kg bw) was about one-fourth that for C57BL/6J mice (0.33 ml/kg bw). Male F_1 hybrids ($B6D_2F_1/J$) had an LD_{50} midway between those

of the two parent strains (0.20 ml/kg bw). No genotypic differences were found in the threshold or time courses of elevated enzyme activities following administration of chloroform at several dose levels, nor were there different histological changes; each strain had centrilobular necrosis in the liver. However, the dose causing excessive loss of urinary glucose or protein in the DBA mice was about 60% of that causing the changes in C57BL mice. Again, the F_1 hybrids were intermediate in their response.

Twelve hours after oral administration of carbon-14-labeled chloroform, kidney homogenates from DBA mice contained significantly more radioactive carbon than did those of the C57BL mice; kidney homogenates from the hybrids were again intermediate. Hill (1977) referred to other studies indicating that such differences had not been found in whole-blood or liver samples and commented that female mice had survived in the accidental exposures that he had reviewed, suggesting a sex difference in addition to the genotypic difference. On the basis of his investigation as well as various studies described by others, Hill concluded that sensitivity to renal damage is the main factor in the sex and strain differences observed after administration of chloroform and that androgens play an important role in modulating at least some of these differences.

Subchronic Effects

Chu et al. (1982a) gave rats drinking water containing chloroform concentrations of 5, 50, or 500 ppm for 28 days. Water intake was measured, and doses were calculated to be 0.13, 1.3, or 11 mg/rat/day. After the administration was ended, the rats were killed and examined grossly and microscopically; blood counts and analyses of serum were made, and hepatic microsomal and soluble enzymes were assayed. The only change seen was a decreased neutrophil count in rats given the highest dose.

Chu et al. (1982b) gave weanling (94 to 100 g) Sprague-Dawley rats drinking water containing chloroform concentrations of 0, 5, 50, 500, or 2,500 ppm. Half of the animals in each group were killed at 90 days; the others were given tap water for another 90 days before being killed. At the highest dose, there were many deaths, decreased growth rate, and decreased food intake. Chu et al. also noted mild to moderate liver lesions that were not significantly different from those in controls and mild to moderate thyroid lesions that were significantly different from those in controls. The significantly different thyroid lesions were seen only in males at the highest dose, however, and there was some recovery within 90 days. There were no significant dose-related changes in biochemical or hematological elements.

Munson et al. (1982) intubated groups of 14 to 24 CD-1 mice with chloroform and other halocarbons at 50, 125, or 250 mg/kg bw for 14 or

90 days. Possible effects that were investigated included changes in body weight, organ weight, blood count, bone marrow, clinical chemistry, hepatic microsomal enzyme assays, hexobarbital sleeping time, cell-mediated and humoral immunity, and morbid anatomy. Increased liver weights, expressed both as absolute weights and as organ-to-body-weight ratios, were seen in males given 125 and 250 mg/kg bw that were killed on the fourteenth day. Liver-to-body-weight ratios in all female groups killed at 14 days were increased but were related to dose only at the two higher doses. There were increases of SGPT in high-dose males and females and increases of SGOT in high-dose females. There was a decrease in antibodyforming cells of the spleen in both males and females at all doses ($p < 0.05$), but hemagglutination titers were not affected and no alterations in cellular immunity were seen. Among rats treated for the full 90 days, there was a dose-related increase in liver weights (both absolute and organ-to-body-weight ratios) in high-dose males and in all female groups. Hepatic microsomal activities were decreased ($p < 0.05$) among high-dose males and in all treated groups of females; hexobarbital sleeping times were increased in all groups and significantly so in mid-and high-dose females. High-dose males had increased blood glucose and decreased humoral immunity. High-dose females had increased glucose and, together with mid-dose females, decreased humoral immunity. Cellular immunity was significantly decreased only in high-dose females. In the 90-day study, the absence of an increase in SGOT and SGPT activity, as seen in the 14-day study, suggested to the investigators that long exposure to chloroform may result in recovery from—or development of a tolerance for—the hepatotoxic action of the chemical.

Jorgenson and Rushbrook (1980) gave 6-week-old Osborne-Mendel male rats, weighing 190 g, chloroform in drinking water at concentrations of 200, 400, 600, 900, or 1,800 ppm. There were 30 animals per dose, and exposures were for 30, 60, or 90 days. One control group of 40 rats received water ad libitum, and a second control group of 30 rats had its water consumption matched with that of the group receiving the highest dose. From water intake data, dose levels of chloroform were calculated to be 0, 20, 38, 57, 81, or 160 mg/kg bw per day. Changes in body weights, ratios of kidney fat to kidney weight, serum biochemical components, and macroscopic and microscopic anatomy were evaluated. The mean body weight of the highest-dose rats was decreased by 15%, and their mean body weight gain was decreased by 26.6%. The control rats, whose water intake was matched with that of the highest-dose rats, gained only 5.1% more weight than the highest-dose rats. A few biochemical changes were observed in rats given 400-ppm concentrations and higher, and these were attributed to reduced water intake. No effect on kidney

fat was found. Pathological changes were slight or mild in severity, were not dose related, and were either sporadic or judged to be adaptive (appearing in rats killed at 30 or 60 days but not in those killed at 90 days).

Jorgenson and Rushbrook also performed a similar experiment with 6-week-old B6C3F$_1$ female mice weighing an average of 19 g. Groups of 30 mice were given water containing 200-, 400-, 600-, 900-, 1,800-, or 2,700-ppm concentrations of chloroform. Two control groups similar to those in the rat experiment were included. From water intake data, doses were calculated to be 0, 32, 64, 97, 145, 290, or 436 mg/kg bw per day. Changes in body weights, ratios of organ fat to organ weight, and gross and microscopic anatomy were sought. Mice receiving 900 ppm and higher and those whose water intake was adjusted to that of the highest-dose group lost mean body weight during the first week, but thereafter their mean body weights were similar to those of controls. Several deaths occurred in some groups during the 13-week period of observation: 1 at 600 ppm, 2 at 900 ppm, and 4 at 2,700 ppm. There was much variation in water consumption. Ratios of liver fat to liver weight were significantly increased at 2,700 ppm. Macroscopically, there were occasional, very slight hemorrhages in mouse lungs at all doses. Microscopically, there were centrilobular fatty changes in mouse livers at the two highest doses, but they were mild and judged to be reversible. Extramedullary hematopoiesis in the liver and lymphoid atrophy in the spleen were also observed but were judged to be unrelated to treatment.

Chronic Effects

Several investigations of chronic toxicity of chloroform have been conducted (Heywood et al., 1979; Jorgenson et al., 1982; Palmer et al., 1979).

Heywood et al. (1979) administered chloroform to beagle hounds. The chloroform was mixed into a toothpaste base and administered in gelatin capsules. The administration continued for 7.5 years and was followed by a 20- to 24-week recovery period. A group of 16 males and females received toothpaste base without chloroform in doses of 0.5 ml/kg bw per day; another group of 8 of each sex was given another toothpaste base, also free of chloroform; and 8 more of each sex were untreated. Groups of 8 of each sex received chloroform doses of 15 or 30 mg/kg bw per day in 0.5 ml of toothpaste. Eleven of the 64 dogs died during the study; two of them were chloroform-treated dogs. The only significant toxic change was a moderate rise in the activity in serum of such enzymes as SGPT in the high-dose group; this peaked in the sixth year and was believed to represent minimal liver damage. Fatty cysts were seen in the livers of several dogs. Although these could have been induced by chloroform, the incidence of the nodules did not seem to be dose related.

Palmer et al. (1979) administered chloroform in a toothpaste base by gavage to Sprague-Dawley rats, 50/sex/dose level, at 0 or 60 mg/kg bw per day, for 6 days a week for 80 weeks. There was a marginal but consistent and progressive retardation in weight gain in rats of both sexes. The only significant ($p < 0.01$) change in organ weights was a decrease in relative liver weights of treated female rats. There was a decrease, reaching a maximum at week 52, in the cholinesterase activity of plasma but not in cholinesterase activity in erythrocytes among treated females. Differences in numbers or timing of deaths between control and treated rats were not significant. There were many minor microscopic hepatic changes but no severe fatty infiltration, fibrosis, or marked bile duct abnormality. There were slight increases in the incidence of moderately severe glomerulonephritis, but the significance of this effect was uncertain. Macroscopic or microscopic changes of a treatment-related nature were not seen in the brains. Tumor incidences among groups were not significantly different.

Jorgenson et al. (1982) gave chloroform in drinking water to male Osborne-Mendel rats at concentrations of 0, 200, 400, 900, or 1,800 ppm for 23 months. From data on body weights and water intake, which were monitored throughout the experiment, mean daily doses were calculated to be 0, 34, 66, 143, or 305 mg/kg bw per day. Body weight gains were inversely proportional to dose, but survival during 96 weeks was proportional to dose; that is, survival was lowest among the negative controls and highest in high-dose animals. It has been suggested that this result could reflect a beneficial effect of reduced body weight on survival; it could also reflect a beneficial effect of relative dehydration in view of the considerably greater survival among the matched controls than among the negative controls. Ten rats at each dose level were killed at 3 or 6 months, and liver triglyceride levels were measured. There was no increase in the mean percent of fat in the liver at any dose level except for an increase of 12% at the 1,800-ppm concentration at 6 months ($p < 0.05$). Groups of 20 rats/dose, killed at 6, 12, or 18 months, were examined for possible changes in blood elements. White-blood-cell (WBC) counts were decreased in the high-dose group and in the matched controls at 6 and 12 months. Significant increases in red blood cells (RBCs) and hemoglobin at 12 months among the rats exposed to chloroform concentrations of 200, 900, and 1,800 ppm indicated some hemoconcentration, but significant differences were not seen among these same groups at 18 months. Serum chloride, potassium, phosphorus, bilirubin, alkaline phosphatase, total iron, albumin, and albumin/globulin ratio tended to be higher in treated than in control groups, whereas cholesterol, triglycerides, lactic dehydrogenase, and globulin tended to be lower in treated than in control groups.

The investigators attributed the changes to reduced consumption of food and water in the control groups.

Mutagenicity

Rapson et al. (1980) studied the mutagenicity of various compounds known or believed to be produced by treatment with chlorine or chlorine dioxide. They used the Ames mutagenicity assay with the TA100 strain of *Salmonella typhimurium*. Chloroform was tested at levels of 100 µg per plate; 1, 10, and 100 µg per plate; and 1 mg per plate and was not found to be mutagenic in this assay. On the other hand, Callen et al. (1980) showed that chloroform, as well as six other halogenated hydrocarbons (dichloromethane, halothane, carbon tetrachloride, trichloroethylene and tetrachloroethylene, and *syn*-tetrachloroethane) induced genetic effects in yeast. Chloroform induced increased numbers of mitotic recombinants and of other genetic alterations in the *ade*2 locus of *Saccharomyces cerevisiae* strain D7. Frequencies of gene convertants at the *trp*5 locus and of reverse mutations of the *ilv*1 marker also were increased, but without absolute increases in the numbers of induced events.

Carcinogenicity

Eschenbrenner and Miller (1945) administered chloroform by gavage at 0.15, 0.3, 0.6, 1.2, and 2.4 g/kg bw in olive oil and induced hepatomas in male mice. They used 3-month-old strain A mice from the National Cancer Institute, with a historical incidence of spontaneous hepatomas of less than 1% at 16 months of age. Dose groups included five animals of each sex. Chemical analysis of the chloroform was not indicated, but the compound was described as chemically pure. Doses were given every 4 days over 120 days for a total of 30 doses. The mice were killed for postmortem examination at 8 months of age—30 days after the last dose. They were given an additional dose of chloroform 24 hours before necropsy.

None of the males of the three highest-dose groups (0.6, 1.2, and 2.4 g/kg bw) and none of the females of the highest-dose group survived; all deaths occurred 24 to 48 hours after the first or second administration. Liver necrosis was observed in both sexes at the three highest doses. Males in all groups developed dose-related renal necrosis, but this effect was not seen in females. All surviving females given doses of 0.6 or 1.2 g/kg bw developed hepatomas.

Necrosis was not seen in tumor cells; hepatomas contained cords of enlarged liverlike cells that formed disorganized, anastomosed columns. The hepatomas were not judged to be invasive, and metastases were not found. The renal necrosis found in males was localized to the proximal and distal tubules; glomeruli and collecting tubules appeared not to be affected. These investigators confirmed the previously reported observations that the Bowman's capsule of female mice is lined with squamous

epithelium, whereas that of male mice is lined, at least partially, with cuboidal epithelium similar to that of the proximal convoluted tubule. The researchers also stated, however, that further research is necessary to determine whether this difference between the sexes has any relation to the tubular necrosis found in male mice gavaged with chloroform.

Five other groups of mice, with one male and two females in each group, were given a single dose of chloroform at the same dose levels. There was a sharp distinction between normal and necrotic liver cells. At doses of 1.2 and 2.4 g/kg bw, there was extensive necrosis in all liver lobules, whereas at 0.6 g/kg bw there was necrosis in some lobes.

Another bioassay of possible carcinogenicity was performed by the National Cancer Institute (NCI, 1976). Osborne-Mendel rats and $B6C3F_1$ mice were intubated with chloroform in corn oil at two dose levels five times a week for 78 weeks. Male rats, 52 days old, were given 90 or 180 mg/kg bw. Female rats, 52 days of age, were given 125 or 250 mg/kg bw for the first 22 weeks; their doses were then reduced to 90 or 180 mg/ kg bw, so that their average doses were 105 or 171 mg/kg bw. Mice, 35 days old, were given 100 or 200 mg/kg bw (males) or 200 and 400 mg/ kg bw (females); these doses were increased after 18 weeks to 150 or 300 mg/kg bw (males) and 250 or 500 mg/kg bw (females), so that the average doses were 133 or 265 mg/kg bw for males and 233 or 465 mg/kg bw for females.

Rats were killed at 111 weeks. There was a decreased survival rate and weight gain in all treated groups. There were no renal tumors in control rats; but the low-dose group had an 8% incidence, and the high-dose group had a 24% incidence ($p = 0.0016$). An increase in thyroid tumors, judged not to be biologically significant, was observed in female rats that had been given chloroform. Tumors were found mainly in the digestive, urinary, and endocrine systems; only a few tumors were found in other systems.

Mice were killed after 92 or 93 weeks. Except for high-dose females, survival rates and weight gains were similar in all groups. Significant increases ($p < 0.001$) in hepatocellular carcinomas were observed in all treated groups; an incidence of 98% for males and 95% for females given the high dose and 36% for males and 80% for females given the low dose, compared with a 6% incidence in matched and in colony control males, 0 in matched control females, and 1% in colony control females. Many of the low-dose male mice that did not develop hepatocellular carcinoma had nodular hyperplasia in the liver.

Roe et al. (1979) also investigated the carcinogenicity of chloroform, administered in toothpaste base or in arachis oil, to four strains of mice (C57BL, CBA, CF/1, and ICI). Ten-week-old mice were given chloroform by stomach tube 6 days a week for 80 weeks, followed by a 16- to 24

week rest period. The investigation was conducted in several parts: First, ICI mice, 52 of each sex per dose level, were given chloroform in toothpaste at doses of 17 or 60 mg/kg bw per day; controls (104 of each sex) were given toothpaste without chloroform. Second, toothpaste without chloroform, peppermint oil, or eucalyptol was given to 260 male ICI mice; toothpaste with chloroform at levels equivalent to 60 mg/kg bw per day was given to a group of 52 mice; and a third group of 52 mice of each sex was untreated. Other groups of 52 males were given toothpaste augmented with peppermint oil or eucalyptol, or both, but not chloroform. In a third part of the investigation, four groups of 52 male mice, two groups/strain, were given chloroform in toothpaste at doses of 0 or 60 mg/kg bw per day, while a fifth group of 52 male ICI mice was given the same dose of chloroform in arachis oil. There were three control groups in this third phase: an untreated group of 100 ICI mice, another group of 52 ICI mice given toothpaste without chloroform, and a group of 52 ICI mice given arachis oil only. Body weights were recorded in each study, and food consumption was estimated in the second and third study phases. Adrenals, kidneys, livers, lungs, and spleens were weighed at the end of the study, and selected tissues and organs were examined microscopically.

Liver and kidney weights were reported to be slightly lower in the chloroform-treated male ICI mice than in controls, and there were other significant organ weight changes that did not appear to fit any pattern; however, details were not provided in the report. Tumor incidences in control and chloroform-treated mice were not significantly different in male C57BL, CBA, CF/1, and female ICI mice. In male ICI mice treated with high doses of chloroform, there was an increased frequency of tumors of the renal epithelium, along with a greater incidence and severity of nonneoplastic renal disease. This effect was more pronounced in mice given chloroform in arachis oil in amounts equivalent to 60 mg/kg bw per day than in controls fed arachis oil. Malignant tumors were identified as hypernephromas and benign tumors as cortical adenomas. There was a significantly higher incidence ($p < 0.001$) of moderate to severe kidney lesions in CBA and CF/I male mice and of moderate to severe kidney disease ($p < 0.05$) in male ICI mice treated with chloroform in arachis oil than that found in controls fed arachis oil. In addition, moderate to severe fatty degeneration of the liver was slightly more frequent among chloroform-treated mice than in controls.

Jorgenson and coworkers (1985) administered chloroform in drinking water to male Osborne-Mendel rats and female B6C3F_1 mice for 104 weeks at doses of 0, 200, 400, 900, or 1,800 mg/liter. Based on measured water intakes and body weights, the average doses were 0, 19, 38, 81, or 160 mg/kg bw per day for rats and 0, 34, 65, 130, or 263 mg/kg bw per day for mice. A second control group was paired for water intake with

the high-dose groups; that is, their water intake was restricted to that of the companion test group. The administration was continued for 104 weeks. Group sizes were adjusted to achieve a detectable tumor response at low doses on the assumption that there was a linear relationship between tumor incidence and dose; thus, group sizes varied from 330 rats and 430 mice at doses of 0 and 200 mg/liter to 150 of each species at doses of 400 mg/ liter and 50 of each species at doses of 900 and 1,800 mg/liter.

Monitoring of room air and animal feed showed that room air chloroform was consistently below 6 ppb and that there was no detectable amount in feed. The total amount of chlorinated hydrocarbon pesticides in feed was about 90 ppb. Malathion was also present at levels commonly below 50 ppb but sometimes as high as 220 ppb.

High-dose male rats had a 14% incidence of adenomas and adenocarcinomas of the renal tubules, compared with a 1% incidence in controls; this is consistent with, although slightly lower than, findings from a previous investigation (NCI, 1976). In contrast to results of the previous investigation, however, an excess incidence of hepatocellular adenomas and carcinomas in mice did not occur. Jorgenson and coworkers (1985) suggested as an explanation that there may have been some interaction with the mode of administration (corn oil gavage) in the NCI study.

Jorgenson et al. (1982) also reported that water consumption was decreased in rats in a dose-related manner, but water intake tended toward normal at the two lower doses near the end of the study. There were decreases in body weight, from which it was inferred that food consumption had decreased; however, food consumption was not measured. Survival of rats was inversely related to dosage level of chloroform, and it was suggested that this might, in turn, be the result of the lower body weights of the rats given the higher doses. The pattern in mice was different, inasmuch as some mice at the two highest doses refused to drink water during the first week, and about 25% of each group died, as did 6% of those given doses of 400 mg/liter. After this initial period, survival did not differ significantly among groups.

Neoplastic lesions other than the renal tumors in rats included increased incidences (as compared with controls) of neurofibromas, leukemias, lymphomas, and circulatory system tumors and decreased incidences of renal tumors and thyroid C-cell adenomas. However, these were not believed to be the result of chloroform administration because they were not dose related or statistically significant; nor did they represent a natural progression of tumors, such as from C-cell adenoma to carcinoma, which might have been expected because of the longer survival of animals to which chloroform was administered.

Nonneoplastic lesions were frequent in all groups of rats. For example, there was a 91% incidence of nephropathy in the negative control rats and

90% in the matched controls versus 92% to 100% in the rats exposed to chloroform, there was a 92% to 100% incidence.

Pereira et al. (1985) administered ethylnitrosourea (ENU) intraperitoneally in doses of 0, 5, or 20 mg/kg bw to 15-day-old outbred CD-1 Swiss mice. At weaning, at 5 weeks of age, 23 to 29 mice at each dose level were given drinking water containing 1,800-ppm concentrations of chloroform. Other groups of 25 to 36 of the ENU-treated mice were given sodium phenobarbital in water at concentrations of 500 ppm. After 46 weeks of exposure, that is, at 51 weeks of age, the mice were killed for necropsy. There was dose-related development of liver adenomas and hepatocellular carcinomas in males and of lung tumors (mostly adenomas) in both sexes that were attributable to ENU alone. The administration of chloroform seemed to inhibit tumor development in male mice that had received ENU. Phenobarbital seems to have increased the incidence of liver tumors among ENU-treated mice. A significant effect of chloroform or phenobarbital on lung-tumor incidence was not evident.

As Jorgenson and his coworkers (1985) had done, Pereira et al. (1985) suggested that the differences between the results of their study, in which chloroform was found to inhibit the development of hepatic tumors, and those of the NCI (1976) study might be due to the different vehicles used: water versus corn oil. They speculated that toxicokinetic differences might have been engendered by the administration of chloroform as a bolus in corn oil or, alternatively, that there might be a synergistic interaction of chloroform and corn oil. In the study of Pereira and associates (1985), however, the only groups whose body weight gains were reduced were male mice given chloroform.

Further pursuing this hypothesis, Bull et al. (1986) examined more specifically the effect of the vehicle on chloroform toxicity. Male and female $B6C3F_1$ mice were given chloroform either in corn oil or in 2% Emulphor—an emulsifying agent used to produce aqueous emulsions of lipophilic chemicals in water—by gavage at doses of 0, 60, 130, or 270 mg/kg bw per day; 10 mice of each sex were tested at each dose level in each vehicle, for a total of 80 mice of each sex. Administration was continued for 91 to 94 days, after which all animals were killed for necropsy.

Urine was collected from metabolism cages the day before the mice were killed, and blood was obtained by cardiac puncture at necropsy. Brains, livers, spleens, lungs, thymus glands, kidneys, hearts, and gonads were removed and weighed, after which liver tissue sections were prepared and a liver lobe was processed for lipid analyses.

At 270 mg/kg bw per day, chloroform caused an increase in SGOT when given in corn oil but not when given in Emulphor. There was no effect on lactic dehydrogenase (LDH). There was a small increase in blood

urea nitrogen (BUN) in those given the corn oil solutions but not in those receiving the Emulphor suspensions. There was a decrease in body weight and an increase in liver weight from chloroform treatment regardless of the vehicle, but the effects were greater in those given the corn oil. Chloroform in corn oil caused a significant amount of diffuse parenchymal degeneration in the liver and mild to moderate early cirrhosis. Corn oil alone or chloroform in Emulphor did not cause significant pathological changes.

Thus, chloroform in corn oil caused more marked hepatotoxic effects in mice than did chloroform in Emulphor or, in comparison with other investigations (Jorgenson et al., 1985), than did chloroform in water. This seems to suggest that the discrepancy between the incidences of liver tumors in the investigations of NCI (1976) and of Jorgenson et al. (1985) can be accounted for by the vehicle of administration, chloroform in corn oil causing more marked hepatic effects than chloroform in other vehicles.

Moore et al. (1982) also developed data on some possible effects of vehicle. Toothpaste containing 0.0325%, 0.94%, or 3.59% of chloroform and corn oil solutions containing 0.045%, 1.8%, or 7.2% chloroform were given to male mice by gavage in amounts to yield chloroform doses of about 15, 60, and 240 mg/kg bw. Three days later, the investigators measured the incorporation of [6-^{3}H]-labeled thymidine into liver and kidney during 24 hours and examined plasma heparinized to determine its urea concentration and glutamic oxalacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) activities. Control groups received only toothpaste without chloroform or plain corn oil but otherwise were given everything that the experimental groups received.

The mean concentration of urea in plasma was increased to 5 to 8 times the mean control value after the highest doses of the chloroform-containing preparations were administered; the smaller doses had no definite effect on urea in plasma. The only clearly significant effect on urea levels in the plasma was observed among the mice given chloroform in corn oil. This group also had the only clearly significant increases (to 2.2 times the control value) in the activity of SGPT.

The uptake of labeled thymidine by kidney and the weight of the kidney increased significantly after the largest dose of chloroform was given in either vehicle. The intermediate dose of chloroform in corn oil also resulted in a significant increase in kidney uptake of labeled thymidine, whereas the largest dose of chloroform in corn oil resulted in significant increases in the uptake of thymidine by the liver. No dose of chloroform in the toothpaste altered significantly the uptake of labeled thymidine into the liver.

The largest doses of chloroform in either vehicle-induced tubular basophilia and necrosis in the kidneys. The livers of mice that had received

the largest dose of chloroform in corn oil had undergone centrilobular enlargement and some necrosis, whereas the largest dose of chloroform in toothpaste resulted in about 0.6 as much centrilobular enlargement and no evidence of necrosis of liver cells. The intermediate dose of chloroform in corn oil resulted in basophilia and necrosis of the tubules of the kidneys of some mice; the intermediate dose in toothpaste produced little evidence of liver or kidney toxicity.

The agreement between the effects of chloroform administration on the concentration of urea in plasma and on renal integrity is striking. Similarly, the agreement between the effects of chloroform on the activity of GPT in the plasma and on the morphology of the liver is impressive. Furthermore, the uptake of thymidine into the kidney corroborated the histological finding of kidney necrosis.

Moore et al. (1982) discussed the results obtained in an earlier study (Roe et al., 1979), in which a daily chloroform dose of 60 mg/kg bw had been found to induce the formation of renal tumors but not hepatic tumors and a smaller daily dose of chloroform (17 mg/kg bw) had not resulted in renal tumors. Moore and his colleagues concluded that early acute cellular damage and subsequent repair are requisite for the production of tumors in target organs for this trihalogenated hydrocarbon.

Herren-Freund and Pereira (1986) administered diethylnitrosamine (DENA) to male rats 18 hours after partial hepatectomy and 7 days before they were given drinking water containing 1,800-ppm concentrations of chloroform for 10 weeks. No evidence of the ability to promote carcinogenesis, such as an increased incidence of γ-glutamyl transpeptidase (GGT) foci, occurred. However, when chloroform was given in the drinking water at the same time as weekly doses of DENA, there was an increased incidence of liver tumors. In a further attempt to study tumor promotion by chloroform, 15-day-old Swiss mice were given ENU; at weaning, they were given either chloroform (1,800 ppm) or sodium phenobarbital in drinking water (500 ppm), the latter compound being used as a positive control. The chloroform and barbiturate were given until the mice were 51 weeks old, when they were necropsied. Administration of ENU at 5 and 20 mg/kg bw caused a dose-related increase in liver tumors. Chloroform inhibited the development of spontaneous and ENU-induced liver tumors in male mice, but phenobarbital enhanced this development in mice of both sexes.

Klaunig et al. (1986) also investigated tumor promotion by chloroform. Thirty-five 4-week-old male $B6C3F_1$ mice imbibed drinking water containing DENA in doses of 10 mg/liter for 4 weeks, after which they were given various chlorinated hydrocarbons, including chloroform, in their drinking water at the maximum tolerated dose (MTD) or at one-third of the MTD (1,800 or 600 mg of $CHCl_3$/liter of water). One group of controls

received DENA followed by untreated drinking water. Another positive control group received drinking water containing phenobarbital in doses of 500 mg/liter. Mice were killed at 6 months (10/group) and at 12 months (25/group) and were examined grossly and microscopically for liver tumors. There was no increased incidence of tumors among mice given chloroform after the course of DENA exposure, but phenobarbital enhanced the production of liver tumors when given after exposure to DENA.

The carcinogenicity of dibromochloromethane was assessed by the National Toxicology Program in a 2-year rodent bioassay (Dunnick et al., 1985; NTP, 1985). Both sexes of Fischer 344 rats and B6C3F_1 mice were gavaged 5 days per week for 104 weeks (rats) or 105 weeks (mice). Solutions were made up with corn oil and administered in doses equivalent to 5 ml/kg bw. The doses used were 0, 40, and 80 mg/kg bw for rats and 0, 50, and 100 mg/kg bw for mice. Mice on the dose equivalent to 50 mg/kg bw were given approximately 7 times the target dose at week 58. All females survived and were retained in the study; 35 of the males died, leaving an insufficient number (15) for analysis. There were no statistically significant increased incidences of neoplastic lesions in either male or female rats. Both sexes of rats showed dose-related liver toxicity, as fatty change, a ground-glass appearance of hepatocytes, and decreased cytoplasmic basophilia. Both sexes of mice showed increased incidence of nonneoplastic liver lesions and the males a greater incidence of nephrosis. The significant liver lesions in males were hepatocytomegaly (12/50 versus 0/50 in controls) and necrosis (9/50 versus 2/50 in controls), and in females were calcification (7/50 versus 0/50 in controls) and fatty metamorphosis (28/50 versus 7/50 in controls). Liver carcinomas were significantly increased in males (19/50 versus 10/50 in controls) but not in females, whereas adenomas were increased in females (11/50 at the dose equivalent to 100 mg/kg bw versus 2/50 in controls) but not in males. The combined incidence of adenoma or carcinoma was significant for females by incidental tumor test and life-table analysis, but for males the combined incidence was significant only by life-table analysis.

Conclusions and Recommendations

The evidence that THMs are capable of inducing cancer gives rise to a complex set of issues in risk assessment. Convincing evidence that the THMs, per se, are carcinogenic to humans is lacking. There is some evidence that associates increased cancer risk with the chlorination of drinking water (see "Epidemiological Studies" in Chapter 3), but THMs are not the only mutagenic and potentially carcinogenic by-products that are formed (see "Observations in Humans" above). Therefore, calculation of the levels of risk to humans who consume drinking water containing

these compounds must rely on data that demonstrate the carcinogenicity of the individual THMs in experimental animals.

Outlined above are studies that have described increased renal tumors in male Osborne-Mendel rats (Tables 4-1 and 4-2), liver tumors in female $B6C3F_1$ mice (Tables 4-3 and 4-4), and renal tumors in male $B6C3F_1$ (Table 4-5) and ICI mice (Table 4-6). The subcommittee considered that the data obtained in the study of Osborne-Mendel rats given chloroform in drinking water (Jorgenson et al., 1985) provided the most appropriate basis for estimating the carcinogenic risk to humans (Table 4-2). Although a relatively low response, the observation was reproducible in two different studies with varying experimental designs (Jorgensen et al., 1985; NCI, 1976). Second, the mode of administration (drinking water) in this study was the most appropriate. Finally, the relatively large numbers of animals used provide a basis for estimates of risk with smaller confidence intervals.

The increased incidence of liver tumors in $B6C3F_1$ mice was examined, and the extrapolation calculations were completed (Tables 4-3 and 4-4). The committee does not believe, however, that this study provides useful data for estimating risk to humans because the induction of these tumors by chloroform appears to be dependent on the use of a vehicle that contains large amounts of polyunsaturated fat (i.e., corn oil or olive oil). The liver tumors could not be reproduced in these animals at similar doses of chloroform in drinking water (Jorgenson et al., 1985). As discussed above, the use of such an oil vehicle appears to increase the toxicity of chloroform to the liver, as evidenced both by pathological examination and by indications of regenerative hyperplasia. Data from Roe et al. (1979) suggest that renal tumors produced in male ICI mice may involve a similar interaction with the oil vehicle (Table 4-7). The estimation of carcinogenic risk calculated in Table 4-8 takes these factors into consideration.

In the National Toxicology Program study of dibromochloromethane, results in $B6C3F_1$ male mice show some—or equivocal—evidence, but not clear evidence, of carcinogenicity. Tumor incidence is shown in Table 4-9. The protocol for these studies was the same as that used for the NCI bioassay of chloroform and suffered from the same limitations as described for the chloroform study, i.e., extrapolation to exposure in water with consumption occurring over a period of time (rather than a single bolus) and the use of corn oil as the vehicle. For dibromochloromethane the problem is compounded by loss of the low-dose group of male mice (dose relatedness cannot be determined). Further studies are needed, and a protocol relevant to consumption in drinking water should be employed. The carcinogenic risk to humans of ingesting this compound is estimated in Table 4-10.

TABLE 4-1 Tumor Incidence in Male Rats Fed Chloroform in Drinking Water[a]

Animal	Sex	Tumor[b] Site	Dose, mg/kg bw/day	Tumor Rates
Osborne-Mendel rats	Male	Kidney	0	4/301
			19	4/313
			38	4/148
			81	3/48
			160	7/50

[a] Based on data from Jorgenson et al. (1985).
[b] Tubular cell adenomas and adenocarcinomas.

TABLE 4-2 Carcinogenic Risk for Chloroform[a] Estimated for Humans with the Linearized Multistage Model

Animal	Sex	Estimated Human Lifetime Risk[b]	Cancer Risk, Upper 95% Confidence Level[b]
Osborne-Mendel rats	Male	5.16×10^{-8}	8.9×10^{-8}

[a] Based on data from Jorgenson et al. (1985).
[b] Assuming daily consumption of 1 liter of water containing the compound in a concentration of 1 µg/liter.

TABLE 4-3 Tumor Incidence in Female Mice Fed Chloroform in Corn Oil by Gavage[a]

Animal	Sex	Tumor Site	Dose, mg/kg bw/day	Tumor Rates
$B6C3F_1$ mice	Female	Liver	0	0/80
			138	36/45
			277	39/41

[a] Based on data from NCI (1976).

TABLE 4-4 Carcinogenic Risk for Chloroform[a] Estimated for Humans with the Linearized Multistage Model

Animal	Sex	Estimated Human Lifetime Risk[b]	Cancer Risk. Upper 95% Confidence Level[b]
$B6C3F_1$ mice	Female	1.5×10^{-6}	1.9×10^{-6}

[a] Based on data from NCI (1976).
[b] Assuming daily consumption of 1 liter of water containing the compound in a concentration of 1 µg/liter.

TABLE 4-5 Tumor Incidence in Male Mice Fed Chloroform in Corn Oil by Gavage[a]

Animal	Sex	Tumor Site	Dose, mg/kg bw/day	Tumor Rates
$B6C3F_1$ mice	Male	Kidney	0	1/77
			138	1/50
			277	2/45

[a] Based on data from NCI (1976).

TABLE 4-6 Carcinogenic Risk for Chloroform[a] Estimated for Humans with the Linearized Multistage Model

Animal	Sex	Estimated Human Lifetime Risk[b]	Cancer Risk, Upper 95% Confidence Level[b]
$B6C3F_1$ mice	Male	1.7×10^{-8}	4.7×10^{-8}

[a] Based on data from NCI (1976).
[b] Assuming daily consumption of 1 liter of water containing the compound in a concentration of 1 µg/liter.

TABLE 4-7 Tumor Incidence in Male Mice Fed Chloroform in Toothpaste Base[a]

Animal	Sex	Tumor Site	Dose, mg/kg bw/day	Tumor Rates
ICI mice	Male	Kidney	0	0/72
			17	0/37
			60	8/38

[a] Based on data from Roe et al. (1979).

TABLE 4-8 Carcinogenic Risk for Chloroform[a] Estimated for Humans with the Linearized Multistage Model

Animal	Sex	Estimated Human Lifetime Risk[b]	Cancer Risk, Upper 95% Confidence Level[b]
ICI mice	Male	1.605×10^{-10}	3.6716×10^{-7}

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

TABLE 4-9 Tumor Incidence in Female Mice Fed Dibromochloromethane in Corn Oil by Gavage[a]

Animal	Sex	Tumor Site	Dose, mg/kg bw/day	Tumor Rates
$B6C3F_1$ Mice	Female	Liver	0	6/50
			50	10/49
			100	19/50

[a] Based on data from NTP (1985).

TABLE 4-10 Carcinogenic Risk for Dibromochloromethane[a] Estimated for Humans with the Linearized Multistage Model

Animal	Sex	Estimated Human Lifetime Risk[b]	Cancer Risk, Upper 95% Confidence Level[b]
$B6C3F_1$ Mice	Female	5.7×10^{-7}	8.3×10^{-7}

[a] Based on data from NTP (1985).

[b] Assuming daily consumption of 1 liter of water containing the compound in a concentration of 1 µg/liter.

HALOACIDS

Dichloroacetic Acid

CAS No. 79-43-6

$CHCl_2COOH$

Trichloroacetic Acid

CAS No. 76-03-9

CCl_3COOH

Dichloroacetic acid (DCA), a colorless liquid with a pungent odor, is soluble in water. It boils at 193-194°C and has a density of 1.563. Its two crystalline forms melt at 9.7°C and -4°C (Windholz et al., 1983, p. 3,038). Dichloroacetic acid is used as a chemical intermediate and in pharmaceuticals and medicine (Hawley, 1981, p. 332).

Trichloroacetic acid (TCA) takes the form of nonflammable, deliquescent colorless crystals, also having a sharp pungent odor. The crystals melt at 57.5°C and boil at 197.5°C. At 25°C, 1.2 kg of TCA crystals is soluble in 1 liter of water. The compound is used in organic synthesis, as a reagent for detection of albumin, in medicine for the removal of warts and as an astringent, in pharmacy, and in herbicides.

Metabolism

Harris and associates (1978) developed evidence that dichloroacetate (DCA) promotes leucine catabolism by the formation of glyoxylate, which acts as a substrate for the transamination of leucine by glyoxylate aminotransferase (GAT), an enzyme found in peroxisomes. Liver cells from male rats were incubated with [2-^{14}C] DCA, and labeled amino acids were assayed, as were such enzymes as α-ketoisocaproate dehydrogenase and pyruvate dehydrogenase (PDH).

The increased formation of [1-^{14}C] α-ketoisocaproate from L[1-^{14}C] leucine induced by DCA was not affected by valine, isoleucine, glutamate, lysine, tryptophan, proline, arginine, or threonine but was suppressed by serine, methionine, asparagine, glutamine, phenylalanine, and glycine. Asparagine was the most potent of the inhibitors, followed by phenylalanine and methionine. The effects of these amino acids were attributed largely to their influence on the accumulation of α-ketoisocaproate. This accumulation, in turn, was attributed to activation of the transamination of leucine rather than an effect of DCA on α-ketoisocaproate dehydrogenase.

It was concluded that DCA enhances leucine catabolism by the formation of glyoxylate, which acts as a substrate for the transamination of leucine by GAT. In support of this conclusion, it was pointed out that (a) the amino acids that act as substrates for GAT block the stimulatory effect of DCA on leucine oxidation; (b) glyoxylate stimulates this oxidation; (c) both DCA and glyoxylate increase the glycine content of isolated hepatocytes; (d) labeled DCA is converted by isolated hepatocytes to labeled glycine, oxalate, and CO_2; (e) 2-chloropropionate (2CP) also activates PDH but is not converted to glyoxylate and does not stimulate the oxidation of leucine; and (f) ethylene glycol, which is also converted to glyoxylate by the liver, also stimulates leucine oxidation.

Dierickx (1984) studied the interaction of acetic acid (AA), monochloroacetic acid (MCA), dichloroacetic acid (DCA), and trichloroacetic acid (TCA) with rat liver glutathione *S*-transferase (GST) in vitro. Initially, the reaction of these acids was studied in crude rat liver supernatants. The inhibition of GST activity, with glutathione (GS) and 1-chloro-2, 4-dinitrobenzene (DNCB) as substrates, was dose-dependent but not linear. He speculated that the ability of TCA to precipitate proteins might account for the alterations in GST activity but concluded this was not the case under his experimental conditions after trying to induce such protein precipitation at concentrations of 50 m*M*.

These acids inhibited six of the seven GST isoenzymes from rat liver, but to greatly differing degrees; for example, GST A was much more inhibited by AA and MCA than was GST B. The seventh (GST D) was present in too small a quantity for the study. Kinetic experiments did not

reveal competitive inhibition kinetics, indicating an attack by the acids at the same site as that of the substrates, tripeptide glutathione (GSH) and DCNB. Titration of remaining GSH in incubation mixtures did not indicate a catalytic function of GST in the conjugation of GSH with AA, MCA, DCA, or TCA, so it was concluded that the major mechanism of interaction involves the binding to GST. This suggested to the investigator that GST could have a protective function against these compounds.

Blackshear et al. (1974) infused fasted male rats with aqueous solutions of sodium dichloroacetate (300 mg/kg bw per hour for 1 to 4 hours at a rate of 1.2 ml/hour). Other animals were similarly infused with NaCl solutions. The rats were anesthetized with sodium pentobarbitone for catheterization of the femoral artery and vein, the artery for withdrawal of arterial blood for analyses at various times during the infusion and the vein for infusions of the DCA or NaCl. There was a significant and rapid decrease in blood glucose, lactate, and pyruvate in rats treated with DCA in comparison with those treated with NaCl; there was also an unaccounted-for rise in blood glucose in the control (NaCl-treated) rats. Plasma insulin decreased. 3-Hydroxybutyrate and acetoacetate increased significantly also.

Some animals were functionally hepatectomized after the infusion; that is, they were anesthetized with sodium pentobarbitone by vein, and ligatures were placed around the celiac and superior mesenteric arteries and the hepatic portal vein, thereby preventing the pooling of blood in the viscera and producing the functional hepatectomy. The infusion of DCA caused significant decreases in hepatic glucose, glucose-6-phosphate, 2-phosphoglycerate, lactate/pyruvate ratio, citrate, malate, alanine, and glutamate and glutamine, indicative of a restriction in substrates for gluconeogenesis. Rats so hepatectomized did not have a different disappearance of blood glucose following the 2-hour infusions, but they did have very significant decreases in the rate of accumulation of lactate, pyruvate, glycerol, and alanine compared with those of control animals. The increased accumulation of glutamine after functional hepatectomy seemed to compensate for the decreased accumulation of alanine. DCA caused a decrease in the clearance of ketone bodies. The authors concluded that DCA-induced hypoglycemia results from a decrease in the net release of extrahepatic precursors of gluconeogenesis, along with the inhibition of peripheral uptake of ketone bodies. Thus, their findings are consistent with previous indications that DCA activates PDH.

DCA decreased glucose synthesis from lactate, pyruvate, and alanine in isolated rat hepatocytes, but it did not decrease such synthesis from substrates that do not involve pyruvate carboxylase, viz., propionate or glycerol (Demaugre et al., 1978). The DCA also inhibited pyruvate carboxylation in isolated mitochondria, but only after a period of preincubation.

Further, there was no effect on partially purified pyruvate carboxylase. DCA labeled with carbon-14 was incubated with hepatocytes or mitochondria with or without exogenous substrate. The investigators interpreted that the labeled DCA was biotransformed to labeled oxalate, which inhibited pyruvate carboxylase and mimicked the effects of DCA on mitochondrial pyruvate carboxylation. They concluded that DCA is transformed in isolated hepatocytes and mitochondria to oxalate, which, in turn, inhibits gluconeogenesis and pyruvate carboxylation.

Crabb et al. (1981) have reviewed and summarized the effects of DCA on metabolic processes. DCA activates PDH in many tissues by inhibiting PDH kinase; similarly, DCA activates myocardial branched-chain α-ketoacid dehydrogenase by inhibiting branched-chain α-ketoacid dehydrogenase kinase (Paxton and Harris, 1984). Some of the effects of DCA *in vitro* are the result of biotransformation products of DCA, viz., oxalate and glyoxylate. DCA inhibited lactate gluconeogenesis by hepatocytes in an *in vitro* preparation because of the inhibition by oxalate of pyruvic carboxylase and stimulated the oxidation of leucine because of the transamination of leucine by glyoxylate. *In vivo*, DCA decreases blood glucose by restricting the supply in the liver of precursors of gluconeogenesis, an effect that is consequent to activation in peripheral tissues of pyruvic dehydrogenase. Crabb et al. pointed out that DCA lowers blood cholesterol in hyperlipidemic patients but did not offer a conclusion on the mechanism of this action. In their summary of toxic effects, they pointed out that DCA is neurotoxic, can cause cataracts, and may be mutagenic.

Inasmuch as DCA was believed to activate PDH by inhibition of PDH kinase, O. B. Evans (1982) assayed PDH activity in muscle and liver tissues from rats that were administered DCA. The compound was given by intragastric intubation at 100 mg/kg bw either as a single dose or as a repeated dose given daily for 7 days.

Three hours after a single dose of DCA, hepatic tissue concentrations of DCA increased to a maximum. Following the seven repeated doses of 100 mg/kg bw each, PDH activation was maximal 3 hours after and returned to basal activity 24 hours after the final dose. Hepatic tissue concentrations of DCA were maximal 3 hours after the last of the seven doses, and DCA was eliminated slowly over the next 3 days, with a half-life of 9.74 hours. DCA concentrations in liver and muscle were similar after this repeated administration.

Health Effects

Observations in Humans

In a study of the metabolic effects of DCA in man (Stacpoole et al., 1978), daily oral doses of 3-4 g (43-57 mg/kg bw per day if body weights

were 70 kg) of sodium dichloroacetate were administered for 6-7 days to patients ranging in age from 42 to 71 years and having diabetes mellitus or hyperlipoproteinemia or both. None had received any other treatment for 10 days before getting dichloroacetate. Seven women were studied for 7 additional days after treatment stopped, while three women and one man were studied in more detail for a 15-day period after treatment. DCA significantly reduced fasting blood glucose by an average of 24% (from hyperglycemic levels) and produced a marked, concomitant fall in plasma lactate (73%) and alanine (82%). It also produced a significant decrease in plasma cholesterol (22%) and triglyceride (61%) and a significant increase in plasma ketone body levels (71%). Urinary uric acid excretion and urate excretion were reduced, with a concomitant elevation of serum uric acid. Maximum effects were seen at the end of the 6-7-day treatment period, and levels subsequently returned to pretreatment levels. Levels of plasma insulin, free fatty acid, and glycerol were unaffected. Plasma cholesterol levels were not affected by treatment in one patient, and, in the other patients, depressed cholesterol levels returned toward pretreatment levels after treatment stopped. Other laboratory tests showed that liver and kidney function were unaltered during or after DCA administration. A few patients felt mildly sedated, but no other laboratory or clinical evidence of adverse effects was noted during or soon after the treatments.

Stacpoole and coworkers (1979) orally administered DCA at about 50 mg/kg bw to a 21-year-old man with severe receptor-negative homozygous familial hypercholesterolemia, which had proved refractory to conventional dietary or pharmacological management. After 16 weeks of DCA, during which time his concentrations of total and low-density lipoprotein in the plasma fell markedly, he developed a polyneuropathy, characterized by weakness of facial, finger, and lower-extremity muscles, diminished deep tendon reflexes, and slowing of nerve conduction velocity. The neuropathy improved after the DCA therapy was stopped. No eye changes were found. There were no other adverse changes attributable to DCA therapy.

Stacpoole and colleagues (1983) also administered sodium dichloroacetate (NaDCA) to 13 patients hospitalized in an intensive-care unit. They ranged in age from 16 to 72 years. All had lactic acidosis, defined as a level of lactate in arterial blood of 5 m*M*/liter or higher, and their acidosis had been refractory to sodium bicarbonate treatment. Arterial blood pH was usually less than 7.30, and they were hypotensive. Two of the patients were inadvertently given sodium bicarbonate after initiation of DCA therapy, in violation of the intended protocol, so pharmacokinetic data from them were excluded in the analysis of the results; hence, conclusions are based on the other 11 patients. DCA doses were 35-50 mg/

kg bw, given by vein for 30 minutes, with a second dose being given 2 or more hours after the first if plasma lactate levels exceeded 5 m*M*/liter and plasma DCA was less than 130 g/ml.

Arterial plasma lactate decreased in all 11 patients (average decrease, 29%), and there was a slight rise in bicarbonate level and in pH. The DCA-induced reduction in lactate was significant (i.e., greater than a 20% reduction) in 7 individual patients. Arterial alanine levels were normal or elevated prior to treatment and fell an average of 54% in the 7 responding patients. β-Hydroxybutyrate concentrations increased an average of 34% after DCA treatment.

In all but 3 of the 13 originally selected patients, there was an increased arterial systolic blood pressure, usually occurring within a few minutes of infusion and lasting from several minutes to several hours. In 4 subjects whose cardiac output was monitored, the rise in systolic pressure was accompanied by a mean increase in cardiac output of 21%.

The investigators concluded that their results were sufficiently encouraging to warrant further studies of DCA in the treatment of lactic acidosis. They pointed out that, although the DCA therapy caused a marked improvement in overall morbidity, only one of their patients survived to leave the hospital; previously, however, none of their patients with hyperlactatemia of this magnitude had survived. Further, the patients whose hyperlactatemia was reversed by DCA died because of the failure to reverse their primary, life-threatening illnesses. It was thought that the DCA stimulated PDH activity, leading to accelerated oxidation of pyruvate, lactate, and alanine, as well as to increased bicarbonate formation and, consequently, to a rise in arterial pH.

Lukas and coworkers (1980) administered sodium dichloroacetate to humans, rats, and dogs in a study of pharmacokinetic characteristics of this potential antidote for lactic acidosis. Labeled (^{14}C) or unlabeled sodium dichloroacetate was given by vein to four human subjects (two at 10 and two at 20 mg/kg bw in 100 ml of saline). Maximal plasma concentrations in the two subjects given 10 mg/kg bw were 19.9-24.7 µg/ml and in those given 20 mg/kg bw, 57.3-74.9 µg/ml. Half-lives for the four subjects varied between 20 and 36 minutes.

Observations in Other Species

Acute Effects

Lukas and coworkers (1980) administered labeled (^{14}C) or unlabeled sodium dichloroacetate by vein to three rats (100 mg/kg bw as 10% aqueous solution) and to two beagle hounds (100 mg/kg bw as 20% aqueous solution) in addition to the humans mentioned above. In the rat, maximal plasma DCA concentrations of 120 and 164 µg/ml were

obtained; subsequent declines were with half-lives of 2.1 and 4.4 hours. The same dose in dogs yielded maximal plasma concentrations of 447 to 508 μg/ml, and the decline was slower, with half-lives of 17.1 to 24.6 hours. As mentioned earlier, the half-lives for the four human subjects varied between 20 and 36 minutes.

Subchronic Effects

Katz et al. (1981) administered sodium dichloroacetate daily for 3 months to CD rats and beagle hounds. Ten rats of each sex (131 to 156 g bw) were intubated with an aqueous solution at 125 and 500 mg/kg bw per day, and 15 of each sex were intubated with 0 or 2,000 mg/kg bw per day. Dogs received 0, 50, 75, and 100 mg/kg bw orally as a solid contained in gelatin capsules (4 of each sex at 0 and 100 mg/kg bw, 3 of each sex at 50 and 75 mg/kg bw). In rats, 2,000 mg/ kg bw proved lethal and 500 mg/kg bw nonlethal; in dogs, the lethal dosage was 75 mg/kg bw with 50 mg/kg bw nonlethal. Both species experienced reduced food consumption and body weight gain; hind limb weakness; frequent urination; progressive reduction in erythrocyte counts, hematocrit, and hemoglobin levels; reduced blood levels of glucose, lactate, and pyruvate; vacuolation of myelinated white tracts in the cerebrum and, to a lesser extent, in the cerebellum; and degeneration of germinal epithelium of the testes, with syncytial giant cell formation. Additionally, rats often had aspermatogenesis, whereas dogs had atrophy of the prostate gland, cystic mucosal hyperplasia in the gall bladder, hemosiderin-laden Kupffer cells, and eye lesions consisting of bilateral lenticular opacities, injected bulbar conjunctivae, and superficial corneal vascularization, with a tendency toward keratoconjunctivitis sicca. Some animals of each species were not killed at the end of the experiment, but were allowed a 1-month recovery period before being necropsied. During this period, there was some recovery; however, the lenticular opacities and gall bladder anomalies in dogs, the brain lesions in both species, and the aspermatogenesis and loss of testicular germinal epithelium in rats persisted or improved only minimally.

Organs were weighed at the time of necropsy. Livers of female rats at 500 and 2,000 mg/kg bw were significantly heavier than those of controls. Relative liver weights (not defined, but probably ratios of liver weights to body weights) were significantly increased at all doses among both sexes, in a dose-dependent manner. There were also significant increases in relative weights of kidneys (females at all doses) and adrenals (males at 500 mg/kg bw and females at 2,000 mg/kg bw). Absolute and relative organ weights of intoxicated rats approached those of controls at the end of the month's recovery period.

The brain lesions in dogs were thought to be characteristic of edema, but their persistence in hounds permitted to recover for 5 weeks indicated

to the investigators that more than simple edema was involved in the development of the lesions. Moreover, the lesions were not seen in the optic or sciatic nerves.

The high dose of 2,000 mg/kg bw resulted in a 13% mortality rate in both male and female rats. Piloerection, tactile-induced vocalization, low body position, and unthriftiness occurred prior to death in male rats. Female rats that died first exhibited cachexia and unthriftiness. Dogs were dramatically more sensitive: one of three females died at a dosage level of 75 mg/kg bw and one of four males died at 100 mg/kg bw. In contrast to the changes observed after oral administration of DCA, the administration by vein to dogs (up to 100 mg/kg bw for 30 days) was without evident testicular, prostate, or central nervous system effects.

Yount and coworkers (1982) compared the metabolic and toxic effects of DCA and 2-chloropropionate (2-CP) as activators of PDH in rats. In suckling rats, both compounds were effective in causing a lowered blood lactate and glucose and increased blood ketone bodies. The effects were similar except that DCA caused a greater increase in blood ketone bodies. In a longer-term study, young male rats were given 2-CP or DCA in their feed (0.04 *M* of either compound/kg of feed) for 12 weeks in a study of the effects produced by 2-CP or DCA. Rats given 2-CP and DCA ate less than controls. Initially, the reduction in feed consumption by rats and the consequent weight changes were more marked with 2-CP than with DCA; however, at 9 weeks, there was little difference in the mean body weights of the 2-CP and the DCA groups. Within 2-4 weeks, hind-limb weakness and abnormal gait were seen in both intoxicated groups. DCA-treated animals showed significantly reduced organ weights of spleen, lungs, heart, testes plus epididymides, and brain. In the 2-CP-treated group, the weights of the liver, kidneys, spleen, heart, testes plus epididymides, and brain were significantly less than in control animals. The DCA-treated rats had increased organ-to-body-weight ratios for adrenal glands, brain, and kidneys.

There were consistent anatomic abnormalities seen in the testes of 2CP-treated rats, and similar abnormalities were seen in DCA-treated rats. There was an arrest of testicular maturation and degeneration of germ cells, some of which contained enlarged or multiple nuclei. Ketone body levels were markedly increased in DCA-treated rats, and glucose levels were practically unchanged. Triacylglycerol levels were unchanged in DCA-intoxicated rats (although significantly decreased in 2-CP rats). Free glycerol was not affected. There were also changes in function and morphology of nerves; these are discussed in the later section on neurotoxic effects.

Davis (1986), in a study of renal toxicity of chloroacetic acids, reported that DCA and TCA at lower doses did not affect food consumption,

growth, or urine output, whereas higher doses caused decreased food consumption and lowered body weight gain. These effects were observed in male rats from daily gavage of TCA at 300 mg/kg bw or administration through drinking water at 3 g/liter, equivalent to about 250 mg/kg bw per day. No effects were observed at 0.3 g/liter, equivalent to about 25 mg/ kg bw per day. Impaired growth in male rats from DCA occurred only at the highest concentration in drinking water, 7.5 g/liter; however, in female rats, there was weight loss after exposure to drinking water containing 1.875 g/liter. No effects were observed in either males or females exposed to DCA in drinking water at 0.5 g/liter, equivalent to about 60 mg/kg bw per day. Exposures at the higher concentrations caused lower outputs of urine and elevated urine osmolalities. Hippurate excretion was not increased in the groups given water containing DCA; results from groups given TCA were not described.

Mutagenicity

Crabb et al. (1981) reviewed the evidence of mutagenicity of DCA in bacterial systems, showing that there is some reason to believe that such mutagenicity stems from an impurity in the DCA, not from DCA itself. One impurity suspected of causing mutagenicity is dichloroacetaldehyde.

In an investigation of several compounds produced by chlorination, Rapson and associates (1980) found no evidence of a mutagenic effect by TCA at levels of 100 ng, 1 µg, 10 µg, 100 µg, and 1 mg per plate in *S. typhimurium* strain TA100.

Waskell (1978) tested 0.45 mg of TCA in the TA100 strain of *S. typhimurium* . No evidence of mutagenicity was found at that level in the presence or absence of S9 liver homogenate.

Carcinogenicity

In a study by Herren-Freund et al. (Sydna Herren-Freund, Health Effects Research Laboratory, U.S. Environmental Protection Agency, Cincinnati, Ohio, personal communication, 1986), both DCA and TCA administered to male $B6C3F_1$ mice intraperitoneally at a dose of 5 g/liter of drinking water produced hepatocellular carcinomas in 61 weeks. Although these data were derived from an initiation-promotion study, the effect was seen in 81% of the animals receiving DCA and in 32% of those receiving TCA without prior initiation with ethylnitrosourea. These results should be confirmed by other studies, as no background incidence of hepatocarcinomas occurred in controls.

Neurotoxic Effects

Reversible polyneuropathy developed in a 21-year-old man who was administered DCA at about 50 mg/kg bw for 16 weeks (Stacpoole et al., 1979).

Katz et al. (1981) described brain lesions and reversible hind-limb

weakness or paralysis in rats dosed orally with DCA for 90 consecutive days to rats at 125, 500, and 2,000 mg/kg bw per day. In dogs paralysis occurred at 100 mg/kg bw, and even dogs treated at 50 mg/kg bw suffered vacuolation of white myelinated tracts in the brain. White tract changes of the brain were still present in animals of both species after a 4-week recovery period. This study is described in greater detail earlier in this section.

In the subchronic study of Yount et al. (1982) described earlier, young male rats were administered 2-CP or DCA in their feed at 0.04 *M* of either compound/kg feed for 12 weeks. In both groups of rats, there were significant reductions in nerve conduction velocities. At least some of the hind-limb weakness, abnormal gait, and smaller morphometric measurements of tibial nerves seen in rats intoxicated with DCA or 2-CP in this study were thought to have stemmed from impairment of maturation of nerves, rather than from a direct neurotoxic effect, although both of these effects could have occurred. In DCA-treated and in 2-CP-treated rats, there were significant reductions in nerve conduction velocities. Sciatic nerve lipid and protein contents (myelin phospholipid, myelin cholesterol, myelin protein, or total protein) were equivalent in both treated groups and controls.

Reproductive Effects

Katz et al. (1981) reported that the brain and testes appeared to be the principal target organs of DCA intoxication in the rat. All high-dose male rats had small testes, even after a 4-week recovery period. At 500 mg/kg bw, 40% suffered testicular germinal epithelial degeneration; at 2,000 mg/kg bw all animals were affected. Male dogs also experienced dose-dependent testicular changes and prostate glandular atrophy. More details of this study were described earlier in this section.

After administering 0.04 *M* of DCA or 2-CP per kg of feed to rats for 12 weeks, Yount et al. (1982) consistently found arrested testicular maturation and degeneration of germ cells, some of which contained enlarged or multiple nuclei, in the testes of these animals.

Testicular changes noted by Katz et al. (1981) were still present in some animals during a 4-week recovery period (see above).

Other Effects

Dogs receiving oral DCA at 50, 75, or 100 mg/kg bw per day for 13 weeks showed eye changes consisting of bilateral lenticular opacities, bulbar conjunctivitis, superficial corneal vascularization, and a tendency toward keratoconjunctivitis sicca; of these, the lenticular opacities were found to be irreversible (Katz et al., 1981).

On the other hand, after 16 weeks of administering 50 mg/kg bw of DCA to a 21-year-old man, Stacpoole et al. (1979) found no eye changes.

Conclusions And Recommendations

DCA produces neurological, reproductive, and ocular effects. The neurological effects are seen in both the central and peripheral nervous systems. Reproductive effects are seen in the testes, and ocular effects are mainly changes in the lenticular tissue. Less is known concerning the toxic effects of TCA. Davis (1986) has examined the effects of both DCA and TCA on renal function. The principal changes seen were decreased food consumption and lower body weight gain. No-observed-effect levels (NOELs) were 60 mg/kg bw per day and 25 mg/kg bw per day for DCA and TCA, respectively. Using these NOELs, suggested no-adverse-effect levels (SNARLs) may be calculated for DCA and TCA assuming that a 70-kg human consumes 2 liters of water daily, which contributes 20% of total intake. The committee chose uncertainty factors of 1,000 for these two substances because of the severity of the neurological and ocular effects found for DCA and because of the general lack of toxicity information, especially for TCA. The committee estimated SNARLs as:

$$\frac{60 \text{ mg/kg/day} \times 70 \text{ kg} \times 0.2}{1{,}000 \times 2 \text{ liters}} = 0.420 \text{ mg DCA/liter, or } 420 \text{ µg DCA/liter;}$$

$$\frac{25 \text{ mg/kg/day} \times 70 \text{ kg} \times 0.2}{1{,}000 \times 2 \text{ liters}} = 0.175 \text{ mg TCA/liter, or } 175 \text{ µg TCA/liter.}$$

SNARLS may also be estimated for a 10-kg child consuming 1 liter of water daily, which contributes 20% of total intake:

$$\frac{60 \text{ mg/kg/day} \times 10 \text{ kg} \times 0.2}{1{,}000 \times 1 \text{ liter}} = 0.120 \text{ mg DCA/liter, or } 120 \text{ µg DCA/liter;}$$

$$\frac{25 \text{ mg/kg/day} \times 10 \text{ kg} \times 0.2}{1{,}000 \times 1 \text{ liter}} = 0.050 \text{ mg TCA/liter, or } 50 \text{ µg TCA/liter.}$$

HALOALDEHYDES

Chloroacetaldehyde

CAS No. 107-20-0

CH_2ClCHO

Dichloroacetaldehyde

CAS No. 79-02-7

$CHCl_2CHO$

Trichloroacetaldehyde, Chloral

CAS No. 75-87-6

Ccl_3CHO

Trichloroacetaldehyde, one of the best known of the haloaldehydes, has a long history of use as a hypnotic agent in human medicine in its monohydrate form (chloral hydrate) but is no longer widely used clinically because it is habit forming with prolonged use. The oral hypnotic dose of chloral hydrate for humans is 0.3 to 1.0 g. A summary of the physical properties and solubility for three haloaldehydes is shown in Table 4-11.

Metabolism

No specific data on the absorption rates of the haloaldehydes were found. Acute animal toxicity data available on chloroacetaldehyde, dichloroacetaldehyde, and trichloroacetaldehyde suggested absorption of these compounds from the intestinal tract since effects were seen after oral administration. Chloral hydrate produces rapid anesthesia in man, and thus its reaction product, trichloroethanol, must be rapidly absorbed from the gastrointestinal tract (Gilman et al., 1985, pp. 360-362).

Because the haloaldehydes are intermediate metabolites of the haloethylenes, the majority of metabolism data for the chloroacetaldehydes comes from the haloethylene literature. Vinyl chloride is metabolized to chloroethylene oxide and 2-chloroacetaldehyde by microsomal cytochrome P450 (Guengerich et al., 1979). The 2-chloroacetaldehyde was thought initially to result from rearrangement of the epoxide. However, Miller and Guengerich (1982) found that the epoxide is not an obligate intermediate in the oxidative metabolism of trichloroethylene but is produced along with chloroacetaldehydes and chloroacyl chlorides by various

TABLE 4-11 Physical Properties and Solubility of Haloaldehydes

Compound	Molecular Weight	Boiling Point	Melting Point	Specific Gravity	Solubility[a]
Chloroacetaldehyde	78.5	85	—	—	3
Dichloroacetaldehyde	112.9	90	—	—	2
Trichloroacetaldehyde	147.4	97.8	-57.5	1.51	1, 2, 3

[a] In medium 1, water; 2, alcohol; 3, ether.

transformations of a common intermediate product. Similarly, Liebler et al. (1985) found that vinylidene chloride is oxidized by microsomal cytochrome P450 to 2,2-dichloroacetaldehyde, 2-chloracetyl chloride, 2-chloroacetic acid, and 1,1-dichloroethylene oxide. These compounds were isolated from rat liver microsomes and hepatocytes. Because both vinyl chloride and vinylidene chloride are considered carcinogenic or potentially carcinogenic, respectively, the oxides were considered initially to be the most reactive metabolic products from their parent compounds and were postulated to react with deoxyribonucleic acid, whereas the haloaldehydes reacted with protein. In fact, the haloaldehydes were found bound to glutathione and microsomal protein in these *in vitro* experiments. Covalent binding of [^{14}C] vinylidene chloride to microsomes was inhibited by glutathione but not by lysine, suggesting that thiol groups rather than amino groups are the major targets for the reactive metabolic products (Liebler et al., 1985).

Chloral is an intermediate in the catabolism of trichloroethylene (TCE), together with trichloroethylene oxide and other active intermediates. This was found in experiments *in vitro* with hepatocytes and P450 prepared from rat liver. In the studies, formation of the chloral appeared to be in a separate metabolic pathway than the epoxide intermediates (Miller and Guengerich, 1982, 1983). Chloral is metabolized further to trichloroethanol (TCEA) and trichloroacetic acid (TCAA); however, other intermediates may yield the same products. Humans exposed to 170 ppm TCE for 7 hours excreted 44% of the dose as TCEA and 18% of the dose as TCAA (Ogata et al., 1971).

The metabolism of trichloroethylene and monochlorobenzene was studied in mouse, rat, rabbit, and man *in vitro* and *in vivo*. TCE was metabolized to chloral by the hepatic microsomal drug-metabolizing enzyme system and then to trichloroethanol by nicotinamide adenine dinucleotide phosphate (NADPH)-alcohol dehydrogenase and NADPH-dependent unspecified enzyme *in vitro*. The enzyme metabolizing chloral to trichloroacetic acid was localized in mitochondria and supernatant fractions. TCE was metabolized mainly to trichloroethanol in rabbits and to trichloroethanol and trichloroacetic acid in rats, mice, and man *in vivo*. Within 5 days total excretion was 13-22% of the dose administered in all species. The ratio of mercapturic acid conjugates to 4-chlorocatechol conjugates after oral and subcutaneous administration of monochlorobenzene was 3-9 in rats and after oral administration in man was >0.4 (Ogata, 1982).

Butler (1948) injected dogs intravenously with chloral hydrate and found that the animals excreted TCEA and TCAA in urine. Dogs given doses of either chloral hydrate or TCE excreted most of the TCEA produced within their bodies as TCEA glucuronide in urine. The concentration of chloral in venous blood plasma fell as that of TCEA rose

to its peak at about 30 minutes after intravenous injection of chloral hydrate. The concentration of TCAA in venous blood plasma rose more slowly and to only about one-third the value to which the TCEA rose; it remained at much more of a plateau, however, than that of TCEA. Consequently, 120 minutes after the injections of chloral hydrate, the concentration of TCEA in the plasma was slightly lower than that of TCAA at the same time.

Ikeda and Ohtsuji (1972) administered chloral hydrate in concentrations of 2.78 *M*/kg bw to rats by intraperitoneal injection and quantified the metabolites 48 hours later. In the urine, they found 198 mg/kg bw of TCEA, 29.2 mg/kg bw of TCAA, and 236.3 mg/kg bw total trichlorocompounds. Quantification of these metabolites was performed using the Fujiwara reaction, a colorimetric reaction for TCAA. The values for trichloroethanol were obtained by difference after oxidizing all metabolites to TCAA.

Sellers et al. (1972) concluded that reduction of chloral to TCEA by reductases of erythrocytes is more important than that by enzymes of the hepatocytes. Müller et al. (1974) gave three human subjects oral doses of chloral hydrate of 15 mg/kg bw and found that the concentrations of TCEA in blood during 100 hours thereafter were practically identical with those established by oral doses of TCEA of 10 mg/kg bw. The concentrations of TCAA in plasma after the dose of chloral hydrate were about 24.6% above those established by the dose of TCEA, but the time relations of these two curves were similar, both reaching peaks at 25 to 35 minutes after ingestion of the compounds and declining to about one-half the peak value by 60 to 70 hours after the doses.

Ikeda et al. (1980) stated that the conversion of chloral hydrate to chloroethanol was apparently carried out in rat liver cytosol by at least two NADPH-dependent enzymes other than alcohol dehydrogenase. The formation of TCAA from chloral hydrate was not so well characterized. Earlier studies reported that chloral hydrate is not a substrate for human aldehyde dehydrogenase; however, Cooper and Friedman (1958) found that an aldehyde dehydrogenase prepared from rabbit liver mitochondria has converted chloral hydrate to TCAA.

Observations in Humans

Toxic doses of chloral hydrate (approximately 10 g in humans) produce severe respiratory depression and hypotension. Liver and kidney damage may also be induced.

Observations in Other Species

Acute Effects

The oral LD_{50} values for chloroacetaldehyde in rats and mice range from 0.069 to 0.087 ml/kg bw (approximately 82 and 104

mg/kg bw) (Lawrence et al., 1972). These authors indicated also that chloroacetaldehyde was highly irritating to the eye (3 on a scale of 0 to 3 at a concentration of a 0.25%) and less so to the skin (3 on a scale of 0 to 3 at a concentration of 7.5%). Others have reported similar findings; that chloroacetaldehyde is corrosive and destructive to lipids and membrane structures, causing irritation of the eyes, mucous membranes, respiratory tract, and skin was reported by the U.S. Department of Health and Human Services (NIOSH/OSHA, 1981).

These investigators (Lawrence et al., 1972) also found chloroacetaldehyde to cause a dose-related increase in pentobarbital sleeping time, apparently indicating a decrease in liver enzyme activity. Mice were given chloroacetaldehyde by intraperitoneal injection or by inhalation for 3 days prior to pentabarbital administration. Doses or concentrations were 0. 1, 0.2, or 0.5 times the LD_{50} or the LT_{50} (median survival time). The increases in sleeping times were statistically significant in all cases except at the shortest inhalation exposure, 15 seconds.

Acute oral LD_{50} values for trichloroacetaldehyde monohydrate in rats and mice have been reported to be 285 and 1,100 mg/kg bw, respectively (NIOSH, 1974, p. 194).

In two abstracts, Saunders and Harper (1980, 1981) reported that trichloroethylene and trichloroacetaldehyde produced damage to lungs, but not to livers, of rats given daily injections intraperitoneally of 0.2 to 2.0 g/kg bw for 5 days. This procedure reduced the mixed function oxidase activity of pulmonary microsomes by 25% to 50%. Trichloroacetaldehyde reduced pulmonary mixed function oxidase activity but did not cause any histological changes.

In a behavorial study that will be described in more detail later, chloral given by oral gavage to mice in doses less than 0.1 LD_{50} was found to cause acute motor incoordination 5 minutes to 2 hours after dosing. The ED_{50} for this effect was 84.2 mg/kg bw.

Subchronic Effects

Lawrence et al. (1972) administered chloroacetaldehyde for 30 consecutive days to male Sprague-Dawley rats by intraperitoneal injection. Dose levels were 1.88 and 3.76 µl (approximately 2.2 and 4.5 mg/kg bw) of chloroacetaldehyde/kg bw (0.3 and 0.6 times the intraperitoneal LD_{50}). Mortality at the end of the study was 25% and 67%, respectively. Weight gains were significantly depressed ($p < 0.05$) in both dose groups. At the end of the 30-day period, hematocrit, hemoglobin, and erythrocyte counts were significantly depressed ($p < 0.05$) in rats receiving the high dose. The low-dose group had an increased count of segmented neutrophils and monocytes and a decreased lymphocyte count. Results from sulfobromophthalein (BSP) tests of liver function in both test groups were similar to those of controls. Significant differences in ratios of organ to body weights were observed in the case of several

organs. The weight ratios of brain and lungs were significantly increased in both low-and high-dose groups, and gonad, heart, liver, kidney, and spleen organ weight ratios were significantly increased in the high-dose group. The body weight gains of these two groups of animals decreased significantly, such that their weights were approximately half (at the high dose) to three-quarters (at the low dose) of control weights.

These investigators (Lawrence et al., 1972) also performed a 12-week study of chloroacetaldehyde, in which male Sprague-Dawley rats were administered 0.32, 0.8, 1.6, or 3.2 µl/kg bw (approximately 0.4, 1.0, 1.9, or 3.8 mg/kg bw) of chloroacetaldehyde. The compound was injected as a 0.5% aqueous solution into the peritoneal cavity three times a week for 12 weeks. Controls were injected with saline solution. Each group consisted of 12 rats, except for the highest-dose group, which consisted of 8 rats. In the highest-dose group, mortality was 5/8, while only two deaths occurred in other groups, one in the lowest-dose group and another in a mid-dose group. Weight gain was reduced significantly in the two highest-dose groups, and the reduction in weight gain was dose-related. Significant decreases in erythrocyte count and elevations of segmented neutrophils occurred in the two highest-dose groups, together with significantly increased hemoglobin at the highest dose. Clotting time was also prolonged in rats administered the highest dose.

Organ-to-body-weight ratios of adrenals, brain, gonads, heart, kidneys, liver, lungs, and spleen were determined at the end of the experiment. At the highest dose, brain and liver weight ratios were markedly elevated, which might have reflected the marked reductions in weight gains. There were other statistically significant changes, seemingly scattered and not dose-related.

Lungs of animals receiving the two highest doses had focal, chronic bronchopneumonia and changes in the respiratory tract suggested to be premalignant; however, these changes were not described. Similar changes in lower-dose groups were said to be of a lesser degree and not accompanied by evidence of atypia of the respiratory epithelium.

Sanders and coworkers (1982) investigated acute toxicity characteristics of chloral hydrate in male and female CD-1 mice. Mice were given the chloral hydrate by intragastric catheter, after which they were observed for 14 days and then necropsied. Urine and blood samples were taken at that time. LD_{50}s were 1.44 g/kg bw for males and 1.26 g/kg bw for females. Within 10 minutes of administration of low doses, mice became sedated, whereas at intermediate and high doses the mice became lethargic and lost the righting reflex. At the high doses, respiration was markedly depressed, and this depression was apparently responsible for the ensuing deaths. Gastric hyperemia was the only significant pathological change.

Sanders and associates (1982) administered chloral hydrate to CD-1 mice in the drinking water for 90 days. A 14-day range-finding study had

been conducted in male mice at doses of 14.4 and 144 mg/kg bw, which were 0.01 and 0.1 times the LD_{50}. Blood counts and coagulation values were normal. Serum glutamic pyruvate transaminase (SGPT) and blood urea nitrogen (BUN) were normal, but lactic dehydrogenase (LDH) was depressed in mice given 144 mg/kg bw. The concentrations of chloral hydrate in the drinking water (0.07 and 0.7 mg of chloral hydrate/ml) in the 90-day study were estimated to yield the same doses as those used in the 14-day, range-finding study. The control group consisted of 260 mice/sex, and each test group had 140/sex/group. Forty-eight mice of each sex of the controls and 32 mice of each sex in each treatment group were randomly selected for measurement of body weights and fluid consumption, recorded twice weekly. Average doses were calculated to be 18 and 173 mg/kg bw per day for female mice and 16 and 160 mg/kg bw per day for males.

Males gained weight in a dose-related manner over the 90-day period; no effect on weights of females was apparent. Macroscopic lesions related to exposure were not seen. However, ratios of liver weights to body weights and absolute liver weights were increased in male mice. Lung weights of males were decreased, but the effect was significant only when expressed as a ratio to body weight and was not dose-related. Decreased RBCs in high-dose females, increased fibrinogen levels in high-dose males and low-dose females, and slightly increased activated partial thromboplastin times in both sexes were seen but were not statistically significant.

Microsomal protein was increased 10% over control in females exposed to 0.7 mg/ml, and cytochrome b_5 levels were increased 26% in males given 0.07 mg/ml and 40% in males at 0.7 mg/ml, contrasted with a 12% decrease in the cytochrome b_5 levels in females on the higher dose. At least one dose induced an increase in aminopyrine *N*-demethylase and an increase in aniline hydroxylase in one or the other sex. Males receiving 0.7 mg/ml had decreased serum calcium and phosphorus and increased LDH and serum glutamic oxaloacetic transaminase (SGOT); BUN decreased in both groups of male mice. The pattern was different in females: At 0.7 mg/ml there were increases in potassium, glucose, cholesterol, and phosphorus, with a decrease in nonprotein sulfhydryl. Only a few of these changes (potassium, cholesterol, and phosphorus in females and calcium, phosphorus, LDH, and SGOT in males) were significantly different from those of corresponding controls ($p < 0.05$). To the extent that a pattern is evident from these results, it seems that the liver was most affected by this repeated exposure to chloral hydrate, with males being more affected than females.

These mice were also examined with respect to humoral and cell-mediated immunity in an investigation described by Kauffmann and coworkers (1982). In the study of humoral immunity, IgM response to sheep erythrocytes was estimated. In the study of cell-mediated immunity, delayed-type

hypersensitivity to sheep erythrocytes, lymphocyte responsiveness, bone marrow DNA synthesis, and functional ability of the reticuloendothelial system were evaluated. Neither sex exhibited any change in cell-mediated immune status, and males did not demonstrate any alteration in humoral immune function. However, the humoral immune function of female mice exposed to chloral hydrate for 90 days was depressed. There were fewer splenic antibody-forming cells produced against sheep erythrocytes, 36% fewer in females given 0.07 mg/ml and 40% fewer in those given 0.7 mg/ml.

The effects of repeated doses of trichloroacetaldehyde on behavior were measured in a study by Kallman et al. (1984). Adult male CD-1 mice were administered doses of 14.4 or 144 mg/kg bw orally for 14 days. No effects on body weight, behavioral performance, locomotor activity, motor coordination, or swimming endurance were found 24-48 hours after the exposure was terminated.

Concentrations of chloral of 0.70 or 0.07 mg/ml in drinking water (equivalent to 15.7 and 159.8 mg/kg bw per day) resulted in a slower weight gain in mice during 90 days as compared with that of controls (Kallman et al., 1984). Animals treated with the higher dose showed a decrease in body temperature at 45 days, and both groups showed a decrease at 91 days. No changes were observed in the behavioral tests used.

Chronic Effects

See "Carcinogenicity" below.

Mutagenicity

All the chloroacetaldehydes were found to be mutagenic in one or more strains of *Salmonella typhimurium* (Bignami et al., 1980a, b; Bruce and Heddle, 1979). In addition, all chloroacetaldehydes showed activity in a forward and back mutation system in *S. coelicolor* and in two forward mutation systems in *Aspergillus nidulans* (Bignami et al., 1980b). The mutagenic activity of the metabolites of vinyl chloride was assessed in the Ames TA 1535 test. Chloroacetaldehyde was found to be 1/190 as potent as chloroethylene oxide when the contact time for the bacteria with chloroethylene oxide was 6 minutes and that with chloroacetaldehyde was 60 minutes (Perrard, 1985).

Carcinogenicity

No recent carcinogenicity data were found in the literature. Prior references tested chloroacetaldehyde for carcinogenicity by repeated skin applications in mouse skin initiation-promotion studies, by repeated subcutaneous injections, and after intragastric feeding in mice and found it inactive (Van Duuren et al., 1979). The mouse skin bioassays were performed on 30 female Ha: ICR Swiss mice and used a dose of 1.0 mg/application/mouse three times weekly for 581 days. Benign lung papillomas

were observed in 14 mice, and a squamous cell carcinoma was found in 1 mouse. Subcutaneous injections of 0.25 mg/injection/mouse in 30 mice showed 1 mouse with local sarcomas after 630 days on test. The same dose given to 30 males and 30 females resulted in 3 males and 1 female with forestomach tumors after 630 to 636 days on test. The incidence of tumors was not considered significantly different than controls.

A related compound, 2-chloropropanal, induced the formation of injection-site sarcomas in female Swiss mice after subcutaneous injection and of tumors of the stomach in both male and female mice after intragastric delivery (Van Duuren et al., 1979). Four of 30 female mice injected with vehicle (trioctanoin) and none of 30 injected with water on similar schedules developed such tumors. Three of 30 male mice, one of which had a squamous carcinoma of the forestomach, and 6 of 30 female mice had tumors of the stomach after gavage once per week of 1 mg of 2-chloropropanal for about 82 weeks; whereas none of the 30 male or 30 female mice gavaged on similar schedules with the vehicle (trioctanoin) developed tumors of the stomach.

Because chloroacetaldehyde is a metabolite of the carcinogen vinyl chloride, it was tested for carcinogenic activity along with chloroethylene oxide in an initiation-promotion mouse skin experiment (Zajdela et al., 1980). Groups of 20 to 28 mice were given a single dose on the skin of 0.05, 0.1, 1.0, or 2.5 mg of chloroacetaldehyde dissolved in 80 µl of acetone followed by applications three times a week of 12-*O*-*n*-tetra-decanoylphorbol-13-acetate for 42 weeks. Chloroethylene oxide produced skin tumors, but chloroacetaldehyde did not.

In vitro experiments have shown that chloroacetaldehyde can react with calf-thymus DNA to form products that are found in liver DNA isolated from rats exposed to 250 ppm vinyl chloride in their drinking water for 2 years (Green and Hathway, 1978). Etheno-deoxyadenosine and etheno-deoxycytidine were identified in the enzyme hydrolysates from these two experiments. In addition, chloroacetaldehyde has been identified as an important alkylating agent when purified rat P450 systems were examined (Guengerich et al., 1979). Whether chloroacetaldehyde reaches the sensitive tissues to react with them is unknown.

Teratogenicity

In the study by Kallman et al. (1984), mice were perinatally exposed to 21.3 and 204.8 mg/kg bw per day of trichloroacetaldehyde in the drinking water. The pups had normal body weight, normal development of the neurobehavioral reflexes, and normal motor coordination. Retention of passive avoidance learning was reduced at 1 and 24 hours among mice exposed to the high dose, but the significance of this

finding in the absence of other findings was unclear. No reproductive or teratogenic studies with other haloaldehydes were found.

Other Related Substances

Small amounts of several aldehydes have been identified in drinking water but will not be reviewed in this report. One of the most common is acrolein, known to many as an irritant compound found in many kitchens, especially during the heating of lipids as in the frying of food. Investigations of the toxic features of acrolein have been critically reviewed by Beauchamp and associates (1985) and by the NRC (1981, pp. 234-241).

Beauchamp et al. (1985) pointed out the strongly irritant properties and reviewed the absorption of the inhaled acrolein and its metabolism, as well as many experimental investigations, mostly of its toxic effects on the respiratory tract. They commented on a bioassay of possible carcinogenicity, then under way, involving administration of acrolein in drinking water (625 ppm) to Fischer 344 rats; there had been a seemingly significant increase in adenomas and of neoplastic nodules in the adrenal cortex of female rats. Conclusions will undoubtedly await completion of the study and a thorough evaluation of the data. In a 52-week inhalation study of Syrian golden hamsters, acrolein was not found to be carcinogenic (Feron and Kruysse, 1977).

A group of halogenated acroleins (propenals) are formed in the chlorination of water containing humic acids and wood pulp. Some of these chemicals are potent mutagens (Meier et al., 1985). There are little other data with respect to their potential health hazards. They are potentially important disinfection by-products that require further toxicological characterization.

Haloaldehydes are derivatives of acetaldehyde, a material that has been extensively studied because it is thought to be the toxic metabolite of ethanol. Liver damage, central nervous system (CNS) toxicity, and fetal alcohol syndrome are well-recognized effects of alcohol abuse.

One hypothesis to explain liver toxicity is that hepatic metabolism forms the active intermediate, acetaldehyde, which covalently binds to hepatocellular macromolecules (especially proteins) and alters hepatocellular structure and function. This binding results in injury and eventually to irreversible disease (Tuma and Sorrell, 1985). The electrophilic nature of the carbonyl carbon of acetaldehyde is susceptible to attack by a variety of nucleophilic agents including amino acids, peptides, proteins, lipids, nucleic acids, and other endogenous materials. The reaction product between proteins and acetaldehyde, a Schiff base, is chemically unstable, reforming the protein and aldehyde. The Schiff base is probably converted to more stable products by reduction with ascorbic acid. These products have been found *in vitro* with liver homogenates and liver slices, and the

amount of adduct found is reduced by increased concentrations of cysteine and glutathione. Adducts with acetaldehyde have also been found with phospholipids in rat liver microsome preparations (Kenney, 1984) and with tetrahydrofolate in liver *in vitro* incubations (LaBaume and Guynn, 1985).

A recent paper described an alteration in acetaldehyde metabolism after carbon tetrachloride treatment and suggested that toxicant-toxicant interactions should also be considered in a description of aldehyde toxicity (Yuki et al., 1984). Specifically, rats were treated with carbon tetrachloride at 2.08 *M*/kg bw intraperitoneally twice a week for 8 to 12 weeks. The changes in acetaldehyde metabolism were examined in a perfused liver system. The researchers found that the activity of aldehyde dehydrogenase in hepatic mitochondria was decreased by about 75%, that the mitochondrial nicotinamide adenine dinucleotide (NADH) oxidation was reduced by approximately 35% of the control level, that the basal level of hepatic oxygen uptake was reduced by 50% and that this was decreased further by acetaldehyde, and that hepatic acetaldehyde metabolism was decreased.

The findings described for acetaldehyde should be included in considerations of the toxicity of haloacetaldehydes because the reactions described should be the same. This is particularly true of adduct formation, in which the presence of the halogen groups should make the initial nucleophilic attack even more favorable and rapid. Whether successive reactions will proceed with haloacetaldehydes must be examined on an individual basis and should probably be more carefully researched.

Summary and Conclusions

Haloaldehydes are rapidly metabolized to alcohols and acids, and they probably react with proteins, lipids, and nucleic acids like acetaldehyde. If sites of unsaturation are present as in chloropropenals, there will be additional reactions with sulfhydryl-containing proteins like glutathione in a manner analogous to the metabolism of acrolein. The haloaldehydes are one of the intermediates found in the metabolism of the haloethylenes, compounds that have been identified as carcinogens.

Toxicity data are lacking for these compounds. The acute oral LD_{50} values for the chloroacetaldehydes in rats are approximately 100 to 300 mg/kg bw; and effects on weight gain, organ-to-body-weight ratios, and lung pathology were found after 12 weeks of study with doses in the approximate range of 2-5 mg/kg bw of monochloroacetaldehyde. Trichloroacetaldehyde showed liver effects and body weight changes in mice after 90 days of 16-18 mg/kg bw. These compounds are mutagens, but very little work has been done to determine their carcinogenic activity. Teratology data are also lacking.

The relative importance of the haloaldehydes as a hazard in drinking water cannot be evaluated. There are insufficient data to assess their toxicity, and there are no good measures of their concentration in drinking water because they are difficult to analyze. They are direct-acting mutagens and are postulated intermediates in the metabolism of chloroethylenes, compounds that are known or suspected carcinogens. The compounds are important for future study.

There are not sufficient data available for the calculation of suggested no-adverse-effect levels (SNARLS) for the haloaldehydes described in this section.

HALOKETONES

1, 1, 1-Trichloroacetone

CAS No. 918-00-3

Cl_3CCOCH_3

1, 1, 3, 3-Tetrachloroacetone

CAS No. 632-21-3

$Cl_2CHCOCHCl_2$

Hexachloroacetone

CAS No. 116-16-5

$Cl_3CCOCCl_3$

The haloketones are principally found as chemical intermediates in industrial processes. A summary of their physical and chemical properties is shown in Table 4-12.

Metabolism

No data are available.

Health Aspects

Observations in Humans

No data are available.

TABLE 4-12 Physical and Chemical Properties of Selected Haloketones[a]

Compound	Molecular Weight	Boiling Point,°C	Melting Point, °C	Specific Gravity	Solubility[b]
1, 1-Dichloroacetone	127	120	NA	1.31	2, 3
1, 1, 1-Trichloroacetone	161	149	NA	1.44	2, 3
1, 1, 3, 3-Tetrachloroacetone	196	180	NA	NA	2, 3, 4, 5
Pentachloro-2-acetone	230	192	2.1	1.69	NA
Hexachloro-2-acetone	265	202	-2	1.44	5
1, 3-Dichloro-2-butanone	141	166	NA	1.31	2, 3, 4, 5

[a] Modified from Weast (1983), pp. C-74, C-75, and C-193.
[b] In medium 1, water; 2, alcohol; 3, ether; 4, acetone; 5, benzene; NA, not available.

Observations in Other Species

Acute Effects

Borzelleca and Lester (1965) determined the oral LD_{50} in male Wistar rats for hexachloroacetone to be 1,550 mg/kg bw. They also determined the dermal LD_{50} in male albino rabbits to be 2,980 mg/ kg bw and the LC_{50} in albino rats to be 660 ppm for a 3-hour and 360 ppm for a 6-hour inhalation exposure. Examination of the lungs immediately after death showed extensive hemorrhage. Those animals that survived showed edema, hemorrhagic changes, and congestion in the lungs 15 days after exposure.

Chronic Effects

No data are available.

Mutagenicity

A comprehensive investigation of the mutagenicity in *Salmonella* bacteria induced by four haloketones (1,1,3-trichloroacetones, 1,1,3,3-tetrachloroacetones, pentachloroacetones, and hexachloroacetones) was performed by Nestmann et al. (1985) in a study that confirmed the initial report by Zochlinski and Mower (1981) concerning solvent effects in tests with hexachloroacetone. Trichloroacetone-induced dose-related increases of mutants were found in strains TA1535, TA97, TA98, and TA100, but not in strains TA1537 or TA1538; addition of an S9 activation mixture had no activating effect. Tetrachloroacetone was mutagenic in strains TA98 and TA100, with S9 enhancing the effects in TA98 only; negative results were found with strains TA1535, TA1537, TA1538,

and TA97. Pentachloroacetone was mutagenic in strains (TA97, TA98, and TA100) containing plasmid pKM101 but was nonmutagenic in the strains without the plasmid (TA1535, TA1537, and TA1538); S9 slightly increased the effects in strains TA97 and TA98 but not in strain TA100. Hexachloroacetone was mutagenic in strains TA98 and TA100 and was shown previously to be nonmutagenic in strains TA1535, TA1537, and TA 1538 (Zochlinski and Mower, 1981). Nestmann et al. (1985) dissolved the haloketones in either acetone or dimethyl sulfoxide (DMSO), and all compounds except for hexachloroacetone, which was negative when dissolved in acetone, were mutagenic in either solvent. Hexachloroacetone and DMSO interacted, resulting in a chemical reaction and the formation of a mutagen more potent than was hexachloroacetone, a liquid, by itself. This and certain previous studies on solvent effects and interactions led Nestmann et al. (1985) to propose recommendations for choosing solvents and performing tests to prevent artificial results from appearing in the literature.

Carcinogenicity

No data are available.

Reproductive Effects

No data are available.

Conclusions and Recommendations

There are few toxicological data available for the haloketones. Nevertheless, trichloroacetone, tetrachloroacetone, pentachloroacetone, and hexachloroacetone were shown to be mutagenic in *Salmonella* bacteria. There are insufficient data available for the committee to estimate suggested no-adverse-effect levels (SNARLs) or to perform other risk assessments.

HALOACETONITRILES

Dichloroacetonitrile

CAS No. 3018-12-0

$CHCl_2CN$

Trichloroacetonitrile, Trichloromethylnitrile, Tritox, Trichloromethyl cyanide

CAS No. 545-06-2

CCl_3CN

Bromochloroacetonitrile

CAS No. 83463-62-1

$CHBrClCN$

Dibromoacetonitrile

CAS No. 3253-43-5

$CHBr_2CN$

The haloacetonitriles are colorless to yellow, volatile liquids. The chlorinated acetonitriles are used as insecticides and fungicides. A summary of their chemical and physical properties is shown in Table 4-13.

Metabolism

Data from Pereira et al. (1984) suggest that absorption of the haloacetonitriles (HANs) from the gastrointestinal tract is limited. No other absorption data were found.

Roby et al. (1986) studied the pharmacokinetics of dichloroacetonitrile in male Fischer 344 rats and male $B6C3F_1$ mice. Aqueous solutions of [2-14C] dichloroacetonitrile (DCAN) were administered at 0.2, 2.0, and 15 mg/kg bw; the highest dose level represented 5-10% of the oral LD_{50} of 2 mM/kg bw determined for rats. The rats eliminated 84% of the administered dose in 48 hours; total recoveries from tissue and excreta were greater than 95%. Tissues contained about 15% of the amount administered after 48 hours, with the liver (5%), muscle (4%), and blood (3%) retaining most of the dose. In another experiment, rats that received 0.1% of the LD_{50} of

TABLE 4-13 Chemical and Physical Properties of Haloacetonitriles

	Dichloro-acetonitrile $CHCl_2CN$	Trichloro-acetonitrile CCl_3CN	Bromochloro-acetonitrile $CHBrClCN$	Dibromo-acetonitrile (DBAN) $CHBr_2CN$
Molecular weight	109.94	144.39	154.4	198.9
Appearance	Liquid	Colorless, volatile liquid	Liquid	Liquid
Density (g/ml)	1.37	1.44	1.68	2.30
Melting point (°C)	[a]	−42	[a]	[a]
Boiling point (°C)	112.3	85.7	125–130	67–69

[a]Not available.

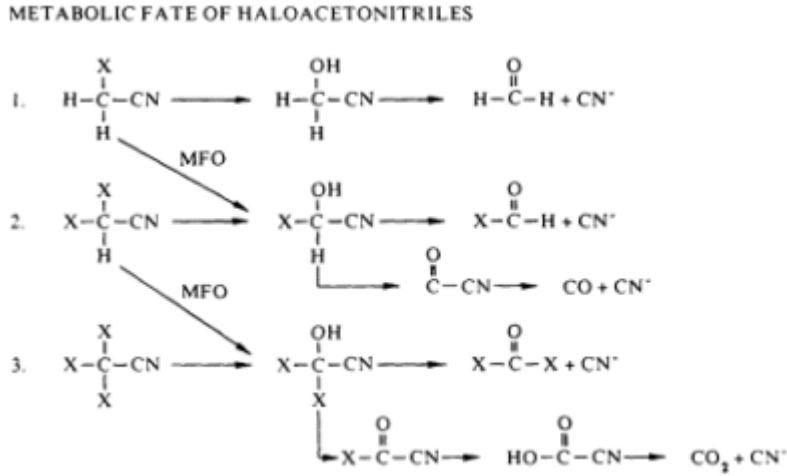

Figure 4-1 Proposed metabolic pathways for cyanide release from haloacetonitriles. MFO is mixed function oxidase. X is ClBr. From Pereira et al. (1984) with permission.

[1-^{14}C] DCAN by gavage took 6 days to eliminate 70% of the dose. Total recoveries in tissues and excreta were more than 89%. Urinary excretion (32%) was similar to that found in the 2-position-labeled compound, but the amount expired as CO_2 was much lower (9%). Tissues contained about 19% of the dose after 6 days, with blood (7%), muscle (4%), skin (3%), and liver (2%) containing most of the retained dose. These findings support that the C-C bond of DCAN seems to be split during biotransformation.

Pereira et al. (1984) reported that 7.7% (DBAN) to 12.8% (BCAN) of orally administered single doses of DCAN (82 mg/kg bw), DBAN (149 mg/kg bw), BCAN (115.8 mg/kg bw), and TCAN (108 mg/kg bw) to male Sprague-Dawley rats was converted to thiocyanate and excreted in the urine and that some HANs inhibited hepatic manganese demethylase activity. Levels of HANs or metabolites in feces and exhaled air were not reported. They concluded that haloacetonitriles are converted to highly toxic metabolites. A proposed metabolic pathway appears in Figure 4-1. Daniel et al. (1983) reported in an abstract that haloacetonitriles are direct-acting alkylating agents, and this could be the mechanism for organ toxicity and carcinogenicity. Bull (1982), Meier et al. (1983), and Simmon et al. (1977) all suggested that the haloacetonitriles may be biologically reactive, acting directly or by conversion to toxic products.

Health Effects

Observations in Humans

Data are not available.

Observations in Other Species

Acute Effects

Hayes et al. (1986) reported the following oral LD_{50}s for DCAN—in male CD-1 mice, 270 mg/kg bw; in CD-1 females, 279 mg/kg bw; in male Charles River CD rats, 339 mg/kg bw; and in Charles River CD females, 330 mg/kg bw. Death was preceded by slowed respiration, decreased activity, ataxia, and coma. No consistent compound-related gross pathological effects were noted at necropsy.

Hayes et al. (1986) also reported the following oral LD_{50}s for DBAN: in male CD-1 mice, 289 mg/kg bw; in CD-1 females, 303 mg/kg bw; in male Charles River CD rats, 245 mg/kg bw; and in Charles River CD females, 361 mg/kg bw. Death was preceded by slowed respiration, ataxia, depressed activity, and coma. No consistent compound-related gross pathological effects were noted at necropsy. Meier et al. (1985) reported an oral LD_{50} of 295 mg/kg bw for rats.

Subchronic Effects

Hayes and associates (1986) administered a solution of DCAN in corn oil to male and female CD rats by gavage at doses of 12, 23, 45, or 90 mg/kg bw per day for 14 days or at 8, 33, or 65 mg/kg bw per day for 90 days. The compound-related mortality at 65 mg/kg bw was 50% of males and 25% of females; at 33 mg/kg bw it was 10% of males and 5% of females at 8 mg/kg bw. Two percent of males had died. The apparent NOEL was determined to be 8 mg/kg bw per day. There were no deaths in either study. Administration of 65 mg/kg bw per day caused a depression of weight gain; reduction of weights and ratios of liver, spleen, thymus, lungs, kidneys, and gonads in the male rats; and a depression of weight gain and reduction in weight and ratio of the spleen in female rats. Similar findings were observed at 90 mg/kg bw per day after 14 days of dosing. Cholesterol levels were significantly lower at 65 and 90 mg/kg bw per day. There were no significant or consistent compound-related effects on formed elements in blood; on serum calcium, chloride, phosphorous, glutamic pyruvic transaminase (SGPT), glutamic oxaloacetic transaminase (SGOT), alkaline phosphatase, 5'-nucleotidase, protein (albumin, globulin), or creatinine; on blood urea nitrogen; on urinary pH, protein, glucose, ketone, or blood; or on macroscopic anatomy.

Hayes and associates (1986) also administered DBAN in corn oil to male and female CD rats by gavage at doses of 23, 45, 90, or 180 mg/ kg bw per day for 14 days or 6, 23, or 45 mg/kg bw per day for 90 days. All the animals that received 180 mg/kg bw per day died within 7 days. At 90 mg/kg bw per day, 40% of males and 20% of females were dead by day 14. The following were reported for the 14-day repeated dosing study: a dose-dependent depression of weight gain; decreases in serum

proteins albumin and globulin, alkaline phosphatase (ALP), 5'-nucleotidase, glucose, and phosphorous; and increases in cholesterol and blood urea nitrogen (BUN)/creatinine ratio at 90 mg/kg bw per day only; decrease in weight and ratio of spleen and thymus in males at 90 mg/kg bw per day and increase in liver and decrease in lung and thymus weight and ratios in females at the 90 mg/kg bw per day dose only. The reported no-observed-effect level was 23 mg/kg bw per day in the 14-day study. The following were noted after 90 days of dosing: dose-dependent depression of body weight gain in males; decreased thymus weight and ratios in both males and females at 45 mg/kg bw per day. Compound-related mortality was 5% for males at 45 mg/kg bw and 0% for males and 10% for females at 23 mg/kg bw. There were no significant and consistent compound-related effects on formed elements in blood; on serum calcium chloride, phosphorus, SGPT, SGOT, alkaline phosphatase, 5'-nucleotidase, protein (albumin, globulin), or creatinine; on blood urea nitrogen; on urinary pH, protein, glucose, ketone, or blood; or on macroscopic anatomy.

Reproductive Effects

Smith and coworkers (1986) studied reproductive effects of HANs. Pregnant female Long-Evans rats were administered by gavage a single high dose, the maximum tolerated dose (MTD, 50-55 mg/kg bw) during gestation, and litters were born normally. Evaluation of offspring for weight and number was performed on days I and 4. Maternally toxic doses of HANs (55 mg/kg bw) were fetotoxic, as indicated by reduced weights of pups at birth and by postnatal growth. This toxicity study compared chloroacetonitrile (CAN), DCAN, and TCAN as well as other HANs, and it was concluded that the toxicity was increased with increasing substitution of the alpha carbon. The investigators cited the findings of Pereira et al. (1984) on the reduction in urinary excretion of cyanide with increasing chloride substitution of acetonitrile.

Mutagenicity

Bull et al. (1985) evaluated the mutagenic and carcinogenic properties of CAN, DCAN, TCAN, BCAN, and DBAN. They reported that DCAN and BCAN were direct-acting mutagens in *Salmonella*; all five HANs-induced sister chromatid exchanges in Chinese hamster ovary cells *in vitro* ; all five compounds were without activity in the mouse micronucleus test; DCAN, DBAN, and CAN applied topically initiated tumors in mouse skin. DCAN was reported by Valencia et al. (1985) to induce sex-linked recessive lethals in *Drosophila*. The activity of DCAN, DBAN, TCAN, and BCAN in the mouse sperm-head abnormality assay was evaluated by Meier et al. (1985). All the compounds were negative in this test. Meier et al. (1983) and Bull (1982) reported

that DCAN was positive in *Salmonella* tester strains TA98, TA100, and TA1535. Kraybill (1980, 1983) identified DCAN as a mutagen or suspected mutagen in chlorinated drinking water in the United States. Simmon et al. (1977) reported that DCAN was positive in the *Salmonella* TA100 assay but was negative in *Saccharomyces cerevisiae*. These data suggest that DCAN is mutagenic and possibly carcinogenic.

Carcinogenicity

Bull and Robinson (1985) evaluated the oncogenic effect (lung tumors) of DCAN, DBAN, TCAN, and BCAN in female A/J mice. The HANs were administered orally, three times weekly for 8 weeks and the animals sacrificed at 9 months. TCAN and BCAN significantly increased the incidence of lung tumors; DCAN and DBAN elicited marginal increases in incidence (not statistically significant). The authors assessed the ability of DCAN, DBAN, TCAN, or BCAN to act as tumor initiators in Sencar mice. The animals received six topical applications over a 2-week period at total doses of 1,200, 2,400, or 4,800 mg/kg bw. 12-*O*-Tetra-decanoylphorbol-13-acetate (TPA) was then applied three times per week for 20 weeks. Mice were observed for 1 year and sacrificed. Squamous-cell tumors of skin were increased significantly only in the BCAN group that received 4,800 mg/kg bw and in the groups given DBAN at 1,200 and 2,400 mg/kg bw.

Conclusions and Recommendations

There are few toxicological data available in the HANs. However, it has been shown that DCAN and DBAN are absorbed from the gastrointestinal tract and are biotransformed to thiocyanate and excreted in the urine, and small percentages of administered DCAN and DBAN are stored in tissues. The committee noted that available evidence suggests that DCAN is mutagenic and potentially carcinogenic. The conservative no-observed-effect level (NOEL) in rats exposed by gavage for 90 consecutive days is 8 mg/kg bw per day. Available evidence does not support the mutagenicity or carcinogenicity of DBAN, although DBAN was positive in a Sencar mouse-skin initiation model. The NOEL in rats exposed (by gavage) for 90 consecutive days is 23 mg/kg bw per day.

A suggested no-adverse-effect level (SNARL) for DCAN may be calculated based on the NOEL of 8 mg/kg bw per day assuming that a 70-kg human consumes 2 liters of water daily, which contributes 20% of the total intake:

$$\frac{8 \text{ mg/kg bw/day} \times 70 \text{ kg} \times 0.2}{1{,}000 \times 2 \text{ liters}} = 0.056 \text{ mg/liter, or } 56 \text{ µg/liter.}$$

Even using a conservative 1,000 uncertainty factor, the committee does not recommend this SNARL because of the concern that DCAN may be carcinogenic. The committee recommends that further data be developed.

A SNARL for DBAN may also be calculated based on the NOEL of 23 mg/kg bw per day using the same assumptions as above and a conservative uncertainty factor of 1,000 because of the general lack of toxicological data and the positive finding in the Sencar mouse-skin initiation model for this substance:

$$\frac{23 \text{ mg/kg bw/day} \times 70 \text{ kg} \times 0.2}{1{,}000 \times 2 \text{ liters}} = \begin{array}{l}0.161 \text{ mg/liter, or} \\ 161 \ \mu\text{g/liter.}\end{array}$$

A SNARL may be estimated for a 10-kg child consuming 1 liter of water daily:

$$\frac{23 \text{ mg/kg bw/day} \times 10 \text{ kg} \times 0.2}{1{,}000 \times 1 \text{ liter}} = \begin{array}{l}0.046 \text{ mg/liter, or} \\ 46 \ \mu\text{g/liter.}\end{array}$$

The committee recommends these two SNARLs for DBAN but also notes that further toxicological data should be developed.

CHLOROPICRIN

Nitrotrichloromethane, Trichloronitromethane, Nitrochloroform

CAS No. 76-06-2

CCl_3NO_2

Chloropicrin is a slightly oily, colorless, refractive liquid that is relatively stable, nonflammable, and is not decomposed by water or mineral acids. It has a boiling point of 112°C and a freezing point of -69.2°C; its specific gravity is 1.692 at 0°C; and it is soluble in alcohol, benzene, ether, and carbon disulfide and slightly soluble in water (0.17 g/100 g water at 18°C). A strong irritant that is toxic when ingested or inhaled, chloropicrin is used in organic synthesis, dyestuffs (crystal violet), fumigants, fungicides, insecticides, rat extermination, and tear gas. The American Conference of Governmental Industrial Hygienists has set 0.1 ppm (≈0.7 mg/m^3) as the threshold limit value (TLV) and 0.3 ppm (≈2.0 mg/m3) as the short-term exposure limit (STEL; 15 minutes) for chloropicrin in air (ACGIH, 1986).

Chemistry and Environmental Fate

In 1980, de Greef et al. reported that tap water from 13 major water supplies of The Netherlands contained chloropicrin in concentrations of about 0.01 μg/liter, whereas tap water from 7 water supplies that had undergone chlorination contained up to 3.0 μg/liter. Sayato et al. (1982) in Japan and Bruchet et al. (1985) in France also reported chloropicrin in surface waters that had been chlorinated. Duguet et al. (1985), using waters from several French water systems, found that the presence of nitrate in water increases only slightly (3.4%) the production of chloropicrin during chlorination. In contrast, the addition of nitrite to the water increased the production of chloropicrin by more than 3,300 times during chlorination. The production of chloropicrin during chlorination passed through a maximum at a concentration of 100 mg of nitrite/liter in the water; with this concentration of nitrite the production of chloropicrin was nearly 3,800 times that measured in the original water. A concentration of 200 mg of nitrite/liter added to the water, however, resulted in the production of less chloropicrin following chlorination than in the original water.

Duguet et al. (1985) reported that the initial reaction between nitrite and chlorine in water loaded with natural organic matter is very rapid, with nearly 51% of the chloropicrin produced during a contact time of 120 hours being formed during the first 46 minutes. The presence of organic matter in the water had an important modifying action on the production of chloropicrin. When nitrite in a dilution of 20 mg/liter was added to a sample of water with a low content of organic matter and to another sample of the same water to which fulvic acid in a dilution of 50 mg/liter had also been added, and both samples were chlorinated for 120 hours, the latter sample contained 15.6 times as much chloropicrin as the former. When ammonia was present in water, the production of chloropicrin during chlorination was found to depend on the ratio of chlorine to nitrogen, the ratio for maximal production of chloropicrin in such water being 8.

Chloropicrin in water hydrolyzes very slowly: after 10 days in darkness no detectable loss of chloropicrin had occurred (Castro and Belser, 1981). These investigators found that exposure of the contaminated water to sunlight or to light from an incandescent lamp for 72 hours resulted in loss of nearly one-half of the chloropicrin. However, exposure of the mixture to ultraviolet light for about 9 hours resulted in loss of more than 95% of the chloropicrin. The stoichiometry was represented as follows:

$$Cl_3CNO_2 \xrightarrow[H_2O]{h\nu} 3Cl^- + NO_3^- + 4H^+ + CO_2.$$

In the vapor phase, Moilanen et al. (1978) found that chloropicrin is quite stable in the dark. During 70 days of storage, 7.9% of the chloropicrin was lost. When the vapor was exposed to radiation above 290 μm, 75.2% of the chloropicrin was lost during 70 days. The products of degradation both in the dark and in the light were $COCl_2$ (phosgene), NOCl, NO_x, and Cl_2. The formation of phosgene, a potent lung irritant that may induce pulmonary edema, provides a secondary hazard of exposure to vaporized chloropicrin that has existed in a closed space for a protracted period of time.

Castro et al. (1983) reported the isolation from soil of four species of *Pseudomonas* that were capable of degrading chloropicrin:

$$Cl_3CNO_2 \rightarrow Cl_2CHNO_2 \rightarrow ClCH_2NO_2 \rightarrow CH_3NO_2.$$

In the degradation, about 4% of the chloropicrin is degraded completely to CO_2.

Metabolism

Castro et al. (1985) found that a preparation of cytochrome P450 prepared from *Pseudomonas putida* reacted with chloropicrin in solution approximately as well as did the intact organism and with essentially the same stoichiometry. Each step in the sequence of dechlorination reactions required two hemes. Ghosal et al. (1985) pointed out that the genes for many degradative enzymes active against halogenated compounds are associated with bacterial plasmids and are clustered. This allows cloning of the gene clusters in broad-host-range vectors for dissemination to a large number of gram-negative bacteria, as has been done for 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4, 5-trichlorophenoxyacetic acid (2,4,5-T). The researchers raised the possibility of cloning the gene clusters responsible for the organisms degradative abilities. They suggested that the naturally existing population of organisms capable of degrading chloropicrin could be increased by creating new organisms with this capacity.

Although no actual study of metabolism of chloropicrin by mammals has been found, the report by Castro et al. (1985) summarized above suggests that the direct metabolic products, produced primarily in the liver, probably would be the same as those produced by *P. putida* . Modification of some of these direct products may occur through other enzyme systems. Also, the direct products and any secondary derivations may modify normal enzymatic and other functions in such a way as to alter the simple degradative chain of reactions that is now known. Therefore, study of the catabolism of chloropicrin in mammalian organisms seems to be of considerable importance to understanding and accurately assessing the hazard of exposure to this substance.

Health Effects

Observations in Humans

Little information on human health effects has been reported. Flury and Zernik (1931), using data accumulated during World War I, when chloropicrin was used as a toxic antipersonnel agent, reported that a concentration of 0.3 to 3.7 ppm of chloropicrin in air induced closing of the eyelids, and a concentration of 4 ppm incapacitated men sufficiently to render them unfit for combat. Sufficient irritation of nasal and tracheobronchial mucosa to be unbearable for longer than 1 minute resulted from exposure to 15 ppm, and that concentration could induce damage within the respiratory system. In addition, Prentiss (1937) reported that 0.3 ppm induced lachrimation and that exposures to concentrations of 119 ppm for 30 minutes or of 298 ppm for 10 minutes were lethal.

Kvetensky et al. (1979) described a mass poisoning by chloropicrin without documenting the number of persons involved. Irritation of the trachea and bronchi resulted in frequent coughing (up to 35 times per minute) and feelings of nausea. Blood pressure initially remained reasonably steady at 110/80, but the pulse rate rose to 110 per minute. Later, as pulmonary edema developed, the blood pressure fell to shock levels in some patients. For treatment of severe intoxication by chloropicrin, these investigators recommended cardiotonics, forced inhalation of 5 to 7 liters per minute of 50% oxygen that had been bubbled through 95% ethanol, infusion of hypertonic glucose (40%) that may contain calcium gluconate, codeine, vitamin C, aminophyllin-like drugs, furosemide, and corticoids. Exposed individuals may become sensitized to chloropicrin, so that subsequent exposures may induce more serious changes than the initial one.

Pitt (1982) has devised a "vapor hazard index" to attempt to express the hazard implicit in working with volatile chemicals that may be released accidentally into a workplace. The index was defined as the quotient of the concentration of a saturated vapor divided by 1,000 times the TLV for that chemical. Chloropicrin has an index of 260. For comparison, methylhydrazine has an index of 240, and the index for 1, 1-dimethylhydrazine is 270. Obviously, a change in the value of the TLV for a chemical will result in a change in the vapor hazard index. The value of this index for safeguarding human health remains to be established.

Observations in Other Species

Acute Effects

From data provided by the Dow Chemical Company in 1972, Tatken and Lewis (1983, p. 687) determined the oral LD_{50} of

chloropicrin to be 250 mg/kg bw in the rat. Stokinger (1982, pp. 4,1644,166) reported the acute lethality of chloropicrin in mice, cats, and dogs from inhalation exposures. In mice, 25 ppm for 15 minutes was not lethal, while 50 ppm for 15 minutes resulted in death within 10 days. Mice exposed to 125 ppm for 15 minutes died within 1 day. In cats, doses of 38-76 ppm for 20-25 minutes were lethal doses in cats. Death occurred in 1-12 days. Dogs exposed to 48 ppm for 15 minutes tolerated the exposure, 43% of those exposed to 117-140 ppm for 30 minutes died, and those exposed to 155 ppm for 12 minutes became ill, but the mortality experience was not reported.

Kane et al. (1979) exposed rats to chloropicrin at various concentrations to determine the concentration that increased significantly the rate of breathing of one-half the group of animals. This was designated the RD_{50} and was found to be 7.98 ppm with 95% confidence limits of 6.22 to 10.6 ppm. The effect of various concentrations of chloropicrin on the rate of breathing was found to be represented by the expression

$$y = 9.54 + 44.87 \log x,$$

where y is the percentage of decrease in the rate of breathing and x is the concentration of chloropicrin in air expressed in parts per million.

Subchronic Effects

Buckley et al. (1984) reported exposing mice for 6 hours/day during 5 days to chloropicrin at 7.98 ppm. This exposure resulted in exfoliation, erosion, ulceration, and necrosis of the respiratory epithelium, in ulceration and necrosis of the olfactory epithelium, especially in the dorsal meatus of the nostrils (of marked severity), and in inflammation and squamous metaplasia of the respiratory epithelium (of moderate severity). In the lung, chloropicrin induced serious exudation and severe fibrosing peribronchitis and peribronchiolitis. In a study undertaken for the National Cancer Institute (NCI, 1978), the mean daily dose of chloropicrin that resulted in no significant decrease in growth of male and female mice or in their probability of survival was 32 mg/kg bw. Mice were given chloropicrin by gavage 5 days/week at daily doses of 25 mg/kg bw for 13 weeks and 35 mg/kg bw for 65 weeks.

Chronic Effects

Male and female $B6C3F_1$ mice and Osborne-Mendel rats were exposed to mean daily doses of chloropicrin at 24.5 and 25.7 mg/kg bw (male rats), 20.3 and 21.5 mg/kg bw (female rats), and 33.3 and 66.7 mg/kg bw (male and female mice) gavage for 5 days/week during 78 weeks. The exposures resulted in dose-related loss of weight by both sexes of both species and in significant and dose-related mortality among rats. The mice given the larger mean daily dose had significantly decreased probabilities of survival (NCI, 1978).

Carcinogenicity

In the study performed for the National Cancer Institute (NCI, 1978) and outlined above under the heading "Chronic Effects," it was found that the incidence of tumors in rats given chloropicrin was lower than that in the control rats. When the summed incidences of tumors among the control animals and among those given chloropicrin were adjusted to the same-sized populations, the control rats had 16, whereas the treated rats had only 5, of the same types of tumors: chromophobe adenoma and mammary gland adenocarcinoma or fibroadenoma. In mice, after the same sort of adjustment as for the rats, the control animals had 10.2, versus 7 in the treated mice, of these types of tumors: alveolar or bronchiolar adenoma and hepatocellular carcinoma. Of possible significance are the findings that 1 of 48 female mice given the low dose of chloropicrin had a squamous-cell papilloma of the stomach and that 2 of 48 male mice given the high dose of chloropicrin had squamous-cell carcinomas of the stomach. No such lesions of the stomach were found in control mice. Despite that fact, the report concluded that the occurrence of these tumors of the stomach was not significant under the Bonferroni criterion.

In 1980, 2 years after publication of the results of the study performed for the National Cancer Institute, Griesemer and Cueto (1980) placed chloropicrin in a group of "chemicals with equivocal evidence for carcinogenicity in animals" on the basis of the tumors of the stomach found in mice given chloropicrin in that study.

Mutagenicity

Moriya et al. (1983) tested chloropicrin as a mutagen in six bacteria: one strain of *Escherichia coli* (WPZ hcr) and five strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537, TA1538). Chloropicrin was without effect in TA 1535, TA 1537, and TA 1538. Chloropicrin induced dose-related reversions in WPZ hcr and TA98, but they were not numerous enough to satisfy the investigators' criterion for positive mutagenic action of an excess of 100 beyond the number of spontaneous revertants. Chloropicrin was positively mutagenic for TA100 when it was applied with S9 activation. Shirasu et al. (1982, 1984) reported that chloropicrin is an indirect mutagen on TA100 with S9 activation when applied along with the S9 mix.

Valencia et al. (1985) examined the mutagenic potential of chloropicrin on *Drosophila*, applying the chemical either by ingestion or by intracoelomic injection. The investigators concluded that chloropicrin given by feeding is questionably inductive of sex-linked recessive lethal mutations in the fruit fly but is not mutagenic at all by injection. This finding suggests that any mutational activity possessed by chloropicrin in this organism arises from a derivative formed in the digestive tract of the fly rather than from the compound itself.

No information on the mutagenic activity of chloropicrin in vertebrate species was found.

Reproductive Effects

No studies of effects on reproduction have been found.

Conclusions and Recommendations

Chloropicrin, formed during the chlorination of nitrite-containing and heavily organically contaminated waters, is acutely lethal and toxic by either inhalation or ingestion in mammals. Ingestion of the compound may lead to formation within the body of phosgene, dechlorinated derivatives of the parent compound, and nitrogen oxides. However, no metabolism studies have been conducted in mammals to see if these metabolites are actually produced by metabolism. Some of the apparent toxicity of chloropicrin may be due to these derivatives instead of to the original material. Chloropicrin has lacrimatory activity, but its major effects are on the olfactory and respiratory epithelia. Inhalation exposure to chloropicrin particularly injures medium and small bronchi, inducing extensive coughing. Injury to the alveoli is followed by pulmonary edema, which may appear relatively soon after an exposure. Death due to bronchopneumonia, bronchiolitis obliterans, or secondary infections may occur days or even weeks after an acute exposure. An exposed individual may become sensitized to subsequent exposures to chloropicrin.

Chloropicrin had questionable carcinogenic activity in the mouse and none in the rat in the one available study. Chloropicrin had indirect mutagenic activity in the TA100 strain of *Salmonella typhimurium* and possibly had weak direct mutagenic activity in the WPZ hcr strain of *Escherichia coli* and the TA98 strain of *S. typhimurium*. It had no mutational activity in the TA1535, TA1537, and TA1538 strains of *S. typhimurium*. Chloropicrin had mild mutagenic activity in the fruit fly when ingested. No information on mutagenic and carcinogenic activities of chloropicrin in vertebrate or mammalian species seems to exist other than one assay of its carcinogenic activity in the mouse and the rat performed for NCI. No research on its effects on reproductive processes was found. No information on its catabolism within the mammalian body has been located. Additional toxicity and carcinogenicity data in mammals are required to assess adequately potential risks posed by the chloropicrin concentrations present in finished waters. For this reason, the committee decided not to calculate a suggested no-adverse-effect level (SNARL).

CHLOROPHENOLS

Monochlorophenols

2-Chlorophenol, o-Chlorophenol

CAS No. 95-57-8

C_6H_5ClO

Dichlorophenols

2, 4-Dichlorophenol

CAS No. 120-83-2

$C_6H_4Cl_2O$

Trichlorophenols

2,4,6-Trichlorophenol, Dowicide 2S

CAS No. 88-06-2

$C_6H_3Cl_3O$

2-Chlorophenol is a light amber liquid with an unpleasant penetrating odor. It is very soluble in water with a melting point of 9.3°C and a boiling point of 174.9°C; its density is 1.2634. 2-Chlorophenol (2-CP) is a soil sterilant and is also used for organic synthesis (dyes) and as a chemical intermediate, e.g., for higher chlorinated phenols.

2,4-Dichlorophenol (2,4-DCP) is a white, low-melting-point solid that is slightly soluble in water with a melting point of 45°C and a boiling point of 210° C. Its density is 1.383, and the vapor pressure is 1 mm of mercury at 53.0°C.

2,4-DCP is used in organic synthesis, in synthesis of the anthelmintic bithionol sulfoxide, and as a chemical intermediate, e.g., for 2,4-dichlorophenoxyacetate, Bifenox, Dichlorprop, and 4-(2,4-dichlorophenoxy) butyrate herbicides. 2,4-DCP was reviewed in Volume 1 of *Drinking Water and Health* (NRC, 1977, pp. 725-726). Principally, studies completed since that review are described here.

2,4,6-Trichlorophenol (2,4,6-TCP), Dowicide 2S, is in a yellow flake or needle form with a strong phenolic odor. Its solubility is <0.1 g/100 g of water with a boiling point of 246°C and a melting point of 69°C. Its density is 1.4901. 2,4,6-TCP is used as a fungicide, bactericide, preservative, and disinfectant; as an isomeric mixture, herbicide, and defoliant; and as a sanitizer. 2,4,6-TCP was reviewed in Volume 4 of *Drinking Water and Health* (NRC, 1982, pp. 264-268).

Metabolism

Data are not available.

Health Effects

Observations in Humans

Data are not available.

Observations in Other Species

Acute Effects

Borzelleca et al. (1985a) reported acute oral toxicity data in CD-1 ICR mice from a series of chlorinated phenols. The data are summarized in Table 4-14.

Acute oral toxicity data for 2,4-DCP are summarized in Table 4-15.

Subchronic Effects

Borzelleca et al. (1985a) exposed male and female CD-1 mice to 2,4-DCP dissolved in drinking solutions (10% Emulphor) for 90 days. The concentrations of solutions and actual doses are shown in Table 4-16. There were no consistently significant compound-related adverse effects on any of the parameters evaluated (behavior, body weight, organ weights and ratios, gross pathology, hematological parameters, serum chemistries, urinalyses).

Kobayashi et al. (1972) exposed male mice to 2,4-DCP as a dietary mixture for 6 months at doses of 45, 100, and 230 mg/kg bw per day. The authors reported a slight abnormality in liver histopathology at the highest dose.

Chronic Effects

Data are not available.

Reproductive Effects

Seyler and associates (1984) evaluated the effects of a series of dichlorophenols on a number of *in vitro* parameters designed to assess potential reproductive effects and reported that none of the DCPs

TABLE 4-14 Acute Oral Toxicity Data of Chlorinated Phenols in CD Mice and in Rats[a]

		Mouse Study				Rat Studies	
				Acute Oral LD_{50}, mg/kg bw			Acute Oral
Compound (CAS No.)	Molecular Weight	Vehicle/Solvent	Purity	Male	Female	Vehicle/Solvent	LD_{50}, mg/kg bw
3-Chlorophenol (108-43-0)	128.56	Deionized water	99%	521 (463-586)[b]	530 (468-601)[b]	Olive oil	570
4-Chlorophenol (106-48-9)	128.56	Corn oil	99 + %	1,373 (1,191-1,583)[b]	1,422 (1,333-1,518)[b]	Olive oil	670
2, 3-Dichlorophenol (576-24-9)	163.00	Corn oil	98%	2,585 (2,046-3,266)[b]	2,376 (2,186-2,585)[b]		
2, 4-Dichlorophenol (120-83-2)	163.00	Corn oil	99%	1,276 (982-1,569)[b]	1,352 (1,094-1,670)[b]		
2, 5-Dichlorophenol (583-78-8)	163.00	Corn oil	98%	1,600 (1,233-2,075)[b]	946 (633-1,438)[b]		
2, 6-Dichlorophenol (87-65-0)	163.00	Corn oil	99%	2,198 (1,727-2,797)[b]	2,120 (1,799-2,498)[b]		
3, 4-Dichlorophenol (95-77-2)	163.00	Corn oil	99%	1,685 (1,504-1,887)[b]	2,046 (1,472-2,846)[b]		
3, 5-Dichlorophenol (591-35-5)	163.00	Corn oil	99%	2,643 (2,269-3,078)[b]	2,389 (1,829-3,120)[b]		
Pentachlorophenol (87-86-5)	226.34	10% Emulphor	99%	177 (125-252)[b]	117 (65-212)[b]	Fuel oil Peanut oil Peanut oil	27 146 175

[a] Adapted from Borzelleca et al. (1985b).
[b] Confidence limits.

TABLE 4-15 Acute Oral Toxicity Data for 2, 4-Dichlorophenol

Species	Strain/Sex	Acute Oral LD50, mg/kg bw	Reference
Rat		1,600	Kobayashi et al., 1972
		580	Deichmann, 1943
		2,830	Vernot et al., 1977
Mouse		1,630	Vernot et al., 1977
	CD-1/M	1,276	Borzelleca et al., 1985a
	F	1,352	Borzelleca et al., 1985a

affected sperm motility; 2,5-DCP, 3,4-DCP, and 3,5-DCP depressed sperm penetration of mouse ova; and 3,4-DCP and 3,5-DCP disrupted the sperm acrosome. The levels that the animals received were not provided.

Mutagenicity

Rapson et al. (1980) investigated a number of compounds believed to be produced by chlorination, including 2-CP, 2,4-DCP, and 2,3,6-TCP, in an Ames assay (TA100). None of the compounds evaluated was found to be mutagenic in this test system. Probst et al. (1981) reported that 2,4-DCP was negative in the hepatocyte unscheduled DNA synthesis and in a modified Ames assay.

Carcinogenicity

No data are available except for 2,4,6-TCP, which was reviewed in Volume 4 of this series (NRC, 1982). Technical-grade 2,4,6-TCP was shown to be positive in animal carcinogenicity bioassays. The role of dioxins or other impurities in the technical material in producing this effect was not established.

Summary and Conclusions

There are relatively few toxicological data available for the chlorophenols, with the exception of 2,4-DCP. Nonetheless data have yet to be

TABLE 4-16 Dosing of CD-1 Mice with 2, 4-DCP in Drinking Solutions

		Actual Doses mg/kg bw/day	
Concentrations of 2, 4-DCP in Drinking Solution, mg/ml	Theoretical Doses, mg/kg bw/day	Females	Males
0.0	0	0	0
0.2	50	50	40
0.6	150	143	114
2.0	1,500	491	383

developed assessing the chronic effects of 2,4-DCP. Kobayashi et al. (1972) had shown a possible no-observed-effect level (NOEL) of 100 mg/ kg bw per day during a 6-month dietary mouse study for 2,4-DCP.

More recently, Borzelleca et al. (1985a) were able to show no effects at levels of 383 and 491 mg/kg bw per day in male and female mice, respectively, exposed to 2,4-DCP in drinking water for 90 days. To be conservative, the committee chose to use 100 mg/kg bw per day as the NOEL for estimating a suggested no-adverse-effect level (SNARL). Assuming that a 70-kg human consumes 2 liters of water daily, which contributes 20% of total intake, a SNARL may be calculated using an uncertainty factor of 100 as:

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

A SNARL may also be estimated for a 10-kg child consuming 1 liter of water daily, which contributes 20% of total intake:

$$\frac{100 \text{ mg/kg bw/day} \times 10 \text{ kg} \times 0.2}{100 \times 1 \text{ liter}} = 2 \text{ mg/liter.}$$

However, these levels are above the odor threshold for 2,4-DCP, so that esthetic factors would make human consumption of this amount highly unlikely.

The committee recommends that further toxicological data be developed for this class of compounds.

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5

Conclusions and Recommendations

DISINFECTION METHODS AND EFFICACY

Waterborne disease outbreaks continue to occur, not only in developing nations, but also in the United States, where almost 70,000 cases were reported in 235 disease outbreaks during the period 1978-1983. The etiology of waterborne disease in the United States has changed dramatically since the early 1900s. Recent outbreaks have generally been dominated by gastrointestinal illness associated with viruses and protozoan cysts. These pathogens are generally more resistant to disinfection than the kinds of pathogenic bacteria that formerly caused most outbreaks. Problems also continue to occur in cases of consumption of untreated water, errors of insufficient or interrupted disinfection, failures to maintain adequate levels of residual disinfectant in potable water distribution systems, and breaches in the systems.

Although chlorination continues to be the predominant method of drinking water disinfection in the United States, the use of alternative methods is increasing. Treatment facilities in several states have recently increased and/or switched to chloramination for primary disinfection, largely in response to the maximum contaminant level of 100 µg of total trihalomethanes per liter set by the Environmental Protection Agency under the Safe Drinking Water Act. Kansas, which formerly prohibited chloramination, now requires the use of ammonia to convert all free chlorine residual to chloramines following 30 minutes of chlorination. The Metropolitan Water District of Southern California now uses chloramination for distribution system residual maintenance. Other alternatives, such as

ozone disinfection, are being used increasingly in Europe and Canada. Potentially substantial increases in ozone use in the United States also appear possible in view of recent improvements in the reliability and efficiency of ozone disinfection technology, together with the higher efficacy of ozone (compared with chlorine) against resistant protozoan cysts and viruses.

Research is needed to improve understanding of the comparative efficacy of major drinking water disinfection practices (especially chlorination, chloramination, ozone, and chlorine dioxide) against the currently most important, resistant protozoan cysts and viruses. Studies of the major factors affecting such efficacy under treatment plant operating conditions are also important. Without such studies, it is possible that many future drinking water treatment operations, decisions about alternative methods, and trade-offs regarding toxic by-products of chlorination may be inordinately influenced by the preponderance of existing knowledge about types of bacterial pathogens that pose less of a public health problem in the United States today than several decades ago.

"Life-cycle" studies of disinfectants are also needed for comprehensive examination of the direct and indirect implications of potential widespread local conversions to alternative disinfection practices. For example, the reliability of ozone disinfection technology and the tropospheric impact of potentially large increases in ozone generation that might result from widespread application should be investigated more thoroughly, before national and local decisions regarding conversion become a matter of fact.

CHEMISTRY AND TOXICITY OF DISINFECTION

Reactions and By-Products

Studies of chlorination of model compounds and isolated humic and fulvic acid precursors during the past few years have improved understanding of the reaction mechanisms and types of by-products formed during chlorine disinfection of drinking water. Although many of the specific by-products are not yet well characterized, they appear to vary according to the structures of the humic and fulvic acid molecules being chlorinated, the chlorine-to-carbon (Cl/C) ratio, the pH, the time of reaction, and other factors. The principal by-products, especially at high Cl/C molar ratios of 3:1 or 4:1, are volatile, hydrophobic compounds (mainly chloroform). However, a large variety of nonvolatile, hydrophilic compounds are also produced. These nonvolatile products include both chlorinated and unchlorinated aromatic and aliphatic compounds. The production of these smaller, hydrophilic molecules appears to increase at lower pH and Cl/C molar ratios (less than 1:1), while higher ratios favor the formation of chloroform and other volatile by-products. At the lower

Cl/C ratios, which more closely approximate typical drinking water disinfection conditions, the humic acid precursors appear to support the formation of unchlorinated by-products (e.g., monobasic and dibasic aliphatic acids) to a greater extent than do fulvic acid precursors. Increasing the Cl/C ratio appears to drive both types of precursors toward chloroform production and a larger fraction of identifiable products, which nevertheless represent only a small fraction of the initial organic material.

It is clear from the studies described in Chapter 4 that the importance of trihalomethanes may be overestimated from experiments involving Cl/C ratios and chlorination times that greatly exceed typical drinking water disinfection conditions. Nonvolatile by-products of humic and fulvic acid chlorination may be more important than previously believed. Further studies of reaction mechanisms, controlling factors, and by-product identification are needed. Improved methods for characterizing the nonvolatile products are also needed to support such studies.

Toxicity

Both chlorinated and untreated drinking water contain genotoxic compounds identified in concentrated residues by short-term assays. Chlorination tends to destroy some of these compounds, as well as produce new ones. Short-term animal skin tests, although not conclusive, provide indications that organic concentrates from chlorinated water are tumorigenic under some experimental conditions. Studies by routes other than dermal application have not shown such an effect. Based on the available data, the recommended SNARLs and risk assessments developed by the committee are shown in Table 5-1.

One finding common to most studies performed throughout the world is that chlorination as a means of disinfection introduces mutagens that are not present, or that are present in lower amounts, in raw, untreated water. Recent studies indicate that most of the mutagenic activity in treated water may be due to nonvolatile (rather than volatile) compounds that are produced from chlorination of humic and fulvic acids.

The upper 95% lifetime cancer risk for humans based on drinking water studies in laboratory animals show a risk of 8.9×10^{-8}, or approximately 1 chance in 10 million of cancer for the consumption of 1 µg/liter of chloroform in water. This and other risk assessment calculations are shown in the section on trihalomethanes. The committee in reviewing the results of these calculations recommends that the current level of total trihalomethanes (THMs), regulated at 100 µg/liter in finished drinking water, be reduced. This level is unsupportable on the basis of the risk values for chloroform developed in this review, noting that chloroform is the principal THM. Nonetheless, the committee is concerned about the toxicity of the

TABLE 5-1 Summation of Suggested No-Adverse-Response Levels (SNARLs) and Risk Estimates for Chemicals Reviewed in This Volume

	Estimated SNARLs		
Compound	Adult	Child	Upper 95% Confidence Estimate of Lifetime Cancer Risk
Disinfectants			
Chlorine	[a]	a	
Ozone	[a]	a	
Chlorine dioxide	210 µg/liter	60 µg/liter	
Chloramine	581 µg/liter	166 µg/liter	
Disinfectant by-products			
Chlorate	24 µg/liter	7 µg/liter	
Chlorite	24 µg/liter	7 µg/liter	
Trihalomethanes			
Chloroform			8.9 x 10-8[d] 1.9 x 10-6[e]
Chlorodibromomethane			8.3 x 10-7[e]
Haloacids			
Dichloroacetic acid	420 µg/liter	175 µg/liter	
Trichloroacetic acid	120 µg/liter	50 µg/liter	
Haloaldehydes			
Chloroacetaldehyde	[b]	b	
Dichloroacetaldehyde	[b]	b	
Trichloroacetaldehyde	[b]	b	
Haloketones			
1, 1, 1-Trichloroacetone	[b]	b	
1, 1, 3, 3-Tetrachloroacetone	[b]	b	
Hexachloroacetone	[b]	b	
Haloacetonitriles			
Dichloroacetonitrile	56 µg/liter[c]		
Dibromoacetonitrile	161 µg/liter	23 µg/liter	
Bromochloroacetonitrile	[b]	b	
Trichloroacetonitrile	[b]	b	
Chloropicrin	40 µg/liter	12 µg/liter	
Chlorophenols			
2, 4-Dichlorophenol	7,000 µg/liter	2,000 µg/liter	
2, 4, 6-Trichlorophenol	[b]	b	
2-Hydroxylchlorophenol	[b]	b	

[a] Not calculated.
[b] Insufficient data for calculation.
[c] Not calculated; the adult value was calculated for comparison purposes; it is not recommended by the committee.
[d] Tumor data for risk assessment calculation from drinking water animal study.
[e] Tumor data for risk assessment calculation from corn oil gavage animal study.

other individual by-products produced by the reactions of alternative chemical disinfectants in common use in water supplies because their toxicity is essentially unknown and their potential health impacts cannot be adequately assessed.

There is a larger risk associated with the unidentifiable by-products of water disinfection, especially with chlorine. The magnitude of this risk is not quantifiable by the studies done to date, but it is high enough to warrant additional effort to determine its qualitative source and quantitative magnitude. Associated with this effort, methods should be sought to follow the risk associated with chlorination by-products even in the absence of individually quantifiable compound risks. Correlation of health risks with surrogate parameters is a classic method, but unfortunately the parameters measured to date, those of THMs and total organic halogen, do not appear to correlate well. Also many of the risk studies with humic material, the principal source of precursor, have been done with commercial humic acid. It is recommended that future studies focus on humic material concentrated from aquatic sources. A large variety of such materials has been collected by the USCS laboratory in Denver, Colorado, under the auspices of the International Humic Substances Society, and methods have been developed for the concentration of large quantities of aquatic humic materials from waters rich in such materials.

Chloramination is becoming widely practiced as a method of disinfection because of the regulation of THM levels and the low capacity of chloramine to form THM. Because it is a much weaker disinfectant than chlorine, chloramine must be used at higher concentrations and for longer periods of contact to achieve sufficient disinfection. Even with extended contact time and higher concentrations, however, chloramination is not recommended as a primary disinfectant, especially where virus or parasitic cyst contamination is potentially present. Preformed monochloramine is undesirable as a primary disinfectant. The use of marginal chlorination as a method of introducing chloramines into a water supply system is specifically not recommended because, along with the depletion of chlorine to produce inorganic monochloramine, organic chloramines that have even lower efficacy as disinfectants are formed. Organic chloramines have also been implicated as major contributors to the mutagenicity of chlorinated drinking and natural waters. For these reasons, chloramine treatment is used to minimize by-product formation. When free chlorine is used as the primary disinfectant, an amount should be used that is sufficient to produce a slight residual of free chlorine above that required to oxidize nitrogen, followed by the addition of ammonia to form monochloramine and limit THM formation.

Chloramine, as inorganic monochloramine, is only weakly mutagenic. Organic concentrates from chloraminated water exhibited half the mutagenic

response of those from chlorinated water. Even though it does not form significant levels of THM, chloramine is nevertheless capable of producing halogen substitution to form organic compounds, and thus may produce important levels of total organic halogen (TOX).

Little is known about the oxidant residuals formed in drinking water because of the general lack of accuracy in measuring them. There is also a great need to define more accurately the nature of the oxidant residuals referred to as total chlorine, by which chloramines are measured by difference. The determination of adequate water disinfection has traditionally relied on accurate measurement of the concentration of residual disinfectant. There are currently no suitable methods for fully quantifying the organic chloramine fraction in the presence of inorganic monochloramine. Until such methods are developed, utilities that handle water supplies containing high concentrations of organic nitrogen run the risk of overestimating the ability of their systems to maintain adequate disinfection.

Additional work is required on organic base precursor fractions, primarily on the organic nitrogen precursors. Neither the toxicity nor products formed from these precursors have been well documented. This is especially true for the organic chloramine portion of the chlorine residual. These compounds appear to be candidates for significant health concern because of their potential to interfere with judgments made on the basis of chlorine residual as to the degree of disinfection achieved, as well as their potential for increasing mutagenicity.

When possible, organic precursors should be removed prior to the disinfection process. This can be achieved by changing the order of the procedures of conventional treatment. A better approach, however, is to improve specific conventional water treatment processes to remove organic compounds and to add processes such as carbon absorption and preoxidation. Initial removal of organic by-product precursors precludes the need for reducing contact time, thus improving the efficacy of the disinfection processes and minimizing formation of organic chlorine by-products.

The use of alternative oxidants, especially ozone and chlorine dioxide, will increase in the United States in the coming decades. Little is known about the types of by-products produced by ozonation of natural organics. Well-conceived studies need to be conducted that will focus on the stable compounds expected from ozone reactions with humic material. But these studies must recognize that the analytical methods used for nonpolar chloroorganics (extraction, GC/MS) may not be successful for the more polar, more labile compounds expected from ozonation processes. Particular attention should be given to the search for unsaturated aldehydes and the hydroxy-hydroperoxides.

Following these studies, further health effect studies are needed to determine whether ozone by-products are mutagenic or carcinogenic or

produce other adverse effects. These studies should take into account variations that are likely to occur when the oxidation process is carried out in different matrices (pH, O_3/TOC ratio, alkalinity).

Notwithstanding the fact that these studies need to be carried out, drinking water suppliers should not dismiss the possibility of using ozone as an alternative to chlorine and chloramines in water treatment. Ozone is an excellent disinfectant (although it must be used in combination with a secondary disinfectant to maintain a residual in the distribution system); ozone is an excellent oxidant for the various needs of water treatment; it does not form chlorinated by-products; and the admittedly inadequate studies now available point to lower toxicities of ozonated water than of chlorinated water.

Epidemiology

The studies reviewed in this volume present progressive improvement in design and in suggestive evidence that by-products induced by chlorination, or some other water parameter, may be causally related to some internal cancers of the epithelial tissue of the digestive organs and the lower urinary tract. Confidence in the demonstration of causal relationships would be increased if well-designed studies could be replicated in other populations.

There have been many differences in research design among studies by various investigators. These differences can greatly influence observed risk ratios. No epidemiological study has measured actual levels of THMs or other potentially carcinogenic materials over periods of time in drinking water. Many have relied on dichotomous coding of chlorination as a yes-or-no variable. Few have considered population migration.

It is possible that true geographic differences are involved in exposure to chlorination by-products, with rural areas having generally higher levels. The National Bladder Cancer Study (see Chapter 3) demonstrated distinct geographic variations in risk with the use of a common methodology. Geographic differences in available substrate may lead to varying amounts of chlorination by-products, or there may be other carcinogens, such as pesticides, in rural drinking water. This latter factor may be important for two reasons: (1) the presence of other causal factors in the drinking water that are randomly distributed with respect to chlorination but unaccounted for in the analysis would produce a lower observed risk ratio than the true risk ratio for chlorination; and (2) other unknown causal variables whose distribution parallels that of chlorination could act as classical confounders. Surface waters are the most frequently chlorinated source of drinking water, followed by shallow alluvial wells; the least frequently chlorinated sources are deep wells. The same distributional

pattern would be expected for many runoff contaminants. A major fault of virtually all previous studies has been the failure to obtain exposure data on carcinogens unrelated to chlorination in finished water supplies. In addition, by-products of chlorination other than the trihalomethanes may be of importance in the genesis of human cancer.

Even in the studies showing statistically significant risk ratios, the magnitude of the ratios has been relatively small but of major public health importance. For example, if the risk ratio for rectal cancer associated with drinking chlorinated water is 1.5 and 50% of the persons in the United States drink chlorinated water, about 6,400 new cases of rectal cancer might be caused each year by chlorinated drinking water. Given the frequency of water consumption, even a small excess risk (less than 10%) may account for a lot of disease. The entire issue of drinking water and cancer deserves continued investigation.

The following are recommendations of approaches to the assessment of human health risks to water disinfectants. One role of epidemiological studies is to provide qualitative descriptions of the relation of disease to the various methods of disinfection. Current evidence suggests that the relative risk to human health from exposure to disinfectants is probably small in settings with effective control of treatment levels in distribution systems. Routine monitoring of disease occurrence in populations with different treatment methods may provide crude qualitative descriptions of the patterns of disease in populations using different treatment strategies. The expense of such studies can be kept relatively low, as they can use existing data bases maintained by state health departments, cancer registries, and lists of residents. A major limitation to this approach is that it is difficult to control for important confounders, such as smoking history.

The recommended design for descriptive studies, aimed toward generating hypotheses about the health effects of drinking water disinfectants, is a retrospective cohort study. The health outcomes typically would be prevalent cases of disease (mortality is an exception), and exposure classification would be the type of disinfectant in drinking water in the residence at the time of ascertainment of health status (for example, if cause of death is the outcome of interest, then residence at death would determine the exposure classification). Public health department statistics routinely include information on some important covariates of disease that are potential confounders, such as socioeconomic status, last occupation, race, age, and gender. These qualitative descriptions would be based on a range of point estimates (usually rate ratios of standardized mortality ratios) that offer evidence about the nature of an association. There is often little quantitative meaning to these estimates. To suggest that rate ratios ranging from 1.13 to 1.39, which represent low to high doses of chlorine, respectively, are evidence of a dose-response relationship of chlorine to a

particular cancer is an inappropriate interpretation of results based on crude and indirect measures of human exposure. The implication of such crude analyses is that one must report results that are consistent with the accuracy of the exposure measures. For descriptive studies such as these, estimates of effect (e.g., rate ratios) should not be reported beyond two significant digits. Point estimates would best be described within a range of confidence intervals, rather than a *p*-value, since it is easy to achieve "statistical significance" levels when there is a lot of information. Statistical differences may not have bearing on biological differences.

After studies have been undertaken to generate hypotheses about the health effects of water disinfectants, the role for epidemiological studies is to quantify associations when data are available. For these studies, data on confounding factors and accurate historical information on the duration and type of disinfectant exposures should be required. Information on the timing of the onset of disease should also be confirmed if there truly were a health hazard associated with a particular disinfectant.

Design options for these analytical studies depend on the frequency of occurrence of the health effects of interest and the true relationship of a particular disinfectant to the occurrence of these health effects. One approach is a prospective cohort study, in which members of a healthy population are chosen as samples according to the type of disinfectant in their drinking water supply. Over time, the cohort is monitored for disease occurrence, and at the termination of follow-up, the rates of disease are compared. The advantages of this design are (1) direct measures of individual water consumption can be obtained; (2) by-products can be measured from periodic tap samples; (3) incident cases of disease are measured (as opposed to prevalent cases); and (4) a variety of outcomes can be investigated. The major disadvantages of this design are the expense and administrative tedium in carrying out these studies. However, if large cohorts have already been assembled for other investigations, then it may be feasible to add a component to the study objectives to investigate potential adverse human health effects of water disinfectants. Usually a prospective cohort design is not feasible, particularly if the etiologic period is many years or decades, as it may well be for the chemicals under discussion, and if the disease occurrence due to disinfection by-products is rare. The occasion of a change in water disinfection treatment is an ideal time to begin such a study.

A more suitable approach is to sample people according to their health status and historically assess their usual source of drinking water. This design is a case-control sampling strategy. The advantages to this design are (1) a large series can be identified; (2) the cost, usually, is lower than a prospective sample; and (3) if data are available on lifetime drinking water history, it may be possible to identify the etiologic period during

which these by-products have an effect on human health. To this end, both the age of exposure and duration, as well as the lag time from exposure to disease onset, could be investigated. The disadvantages are (1) information on the duration and type of disinfectant is difficult to obtain, and direct measures of chemical levels are not possible unless routine testing of water has occurred in the past; (2) the study cost can increase dramatically if data collection includes personal interviews to learn about exposure to potential confounders. Despite these advantages, case-control designs are the most effective in meeting the objective of quantifying the risk of a particular disinfectant strategy on human health. Incident cases of disease can be identified from existing registries (e.g., cancer or birth defects records) or pathology logs.

A third role for epidemiological studies of the health effects of drinking water disinfectant methods is that these studies can contribute to experimental models of chemical carcinogenesis, atherogenesis, and teratogenesis. As noted above in the list of advantages of case-control studies, it is possible, although difficult, to identify the relevant etiologic period when exposure has the greatest impact on disease occurrence. Usual lifetime exposure will dilute the measure of effect, if there truly is an adverse effect of a particular treatment strategy. In addition, exposure measured only at the time of disease onset or death may not accurately reflect the relevant exposures that occurred or began to occur 10, 20, or 30 years earlier, during childhood or early adulthood.

Finally, a fourth contribution is to describe the effect of disinfectant by-products in conjunction with other risk factors for disease. For example, epidemiological studies can evaluate interaction between smoking and drinking water or concurrent chemical contamination and disinfectant content on the risk to human health. Epidemiological studies undertaken to learn about effect modification require stratified analysis or a sampling approach that selects individuals according to their exposure to a particular covariate so that the distribution of exposure is balanced for efficient contrasts of the disease rates according to categories of the covariates. For example, if the interest were to study the interaction of smoking and chlorination on bladder cancer risk, then the study population should be selected expressly in terms of their smoking experience. A 50% split between current smokers and nonsmokers would provide an efficient experience to examine the additional risk of chlorination in the presence of smoking relative to chlorination exposure in the absence of smoking and no exposure to either smoking or chlorination.

Clearly there are limitations to epidemiological research in clarifying the risks of environmental exposures to human health. Human studies are unlikely to contribute knowledge about dose effects because the levels of exposure to hazardous by-products of chlorination hardly vary. In addition,

the weaker the effect of a particular chemical on disease occurrence, the more difficult (in terms of measurement accuracy, cost, and administration of the study) it is to detect this association in an epidemiological study. In light of current knowledge, it seems probable that the by-products of concern incur a relatively low risk on human health, regardless of the treatment strategy. One would therefore want to be particularly conscious of locating a study base that is potentially informative about the relation under study. In principle, the population experience should be quite homogenous with respect to covariates of the diseases of interest and, in addition, at low risk for the same diseases so that a small excess risk could be detected.

Four potential contributions from epidemiological observational studies have been discussed: (1) descriptive research, primarily aimed at generating hypotheses; (2) analytic research, aimed at quantifying risk with proper control for confounding; (3) evidence for improving models of chemical carcinogenesis, teratogenesis, and atherogenesis; and (4) effect modification by covariates of disease, such as smoking and occupation. Results from epidemiological studies that are properly conceptualized and employ valid methodologies can offer important information for policymakers on disinfectant treatment strategies.

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