Drinking Water Disinfection Byproducts: Review and Approach to Toxicity Evaluation

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There is widespread potential for human exposure to disinfection byproducts (DBPs) in drinking water because everyone drinks, bathes, cooks, and cleans with water. The need for clean and safe water led the U.S. Congress to pass the Safe Drinking Water Act more than 20 years ago in 1974. In 1976, chloroform, a trihalomethane (THM) and a principal DBP, was shown to be carcinogenic in rodents. This prompted the U.S. Environmental Protection Agency (U.S. EPA) in 1979 to develop a drinking water rule that would provide guidance on the levels of THMs allowed in drinking water. Further concern was raised by epidemiology studies suggesting a weak association between the consumption of chlorinated drinking water and the occurrence of bladder, colon, and rectal cancer. In 1992 the U.S. EPA initiated a negotiated rulemaking to evaluate the need for additional controls for microbial pathogens and DBPs. The goal was to develop an approach that would reduce the level of exposure from disinfectants and DBPs without undermining the control of microbial pathogens. The product of these deliberations was a proposed stage 1 DBP rule. It was agreed that additional information was necessary on how to optimize the use of disinfectants while maintaining control of pathogens before further controls to reduce exposure beyond stage 1 were warranted. In response to this need, the U.S. EPA developed a 5-year research plan to support the development of the longer term rules to control microbial pathogens and DBPs. A considerable body of toxicologic data has been developed on DBPs that occur in the drinking water, but the main emphasis has been on THMs. Given the complexity of the problem and the need for additional data to support the drinking water DBP rules, the U.S. EPA, the National Institute of Environmental Health Sciences, and the U.S. Army are working together to develop a comprehensive biologic and mechanistic DBP database. Selected DBPs will be tested using 2-year toxicity and carcinogenicity studies in standard rodent models; transgenic mouse models and small fish models; *in vitro* mechanistic and toxicokinetic studies; and reproductive, immunotoxicity, and developmental studies. The goal is to create a toxicity database that reflects a wide range of DBPs resulting from different disinfection practices. This paper describes the approach developed by these agencies to provide the information needed to make scientifically based regulatory decisions. — *Environ Health Perspect* 107(Suppl 1): 207–217 (1999). *http://ehpnet1.niehs.nih.gov/docs/1999/Suppl-1/207-217boorman/abstract.html*

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The availability of safe drinking water is a substantive health concern. The introduction of water chlorination as a standard treatment technique caused a large drop in mortality from infectious disease (*1*) and is considered one of the major public health advances in this century. In 1976, the U.S. National Cancer Institute published results showing that chloroform, one of the trihalomethanes (THMs) that occurs as a byproduct of drinking water disinfection, was carcinogenic in rodents (*2*). Since that time there has been a concern that disinfection of water, while providing protection against microbial risks, could also pose chemically induced cancer risks for humans (*3*). We now know that other THMs [e.g., bromodichloromethane (*4*), chlorodibromomethane (*5*), and bromoform (*6*)] and other disinfection byproducts (DBPs), such as dichloroacetic acid (DCA), are carcinogenic in rodent bioassays (*7,8*). Furthermore, several epidemiology studies have suggested a weak association (odds ratios of generally less than 2) between drinking chlorinated water and the occurrence of bladder, rectal, and colon cancer (*9–14*). In addition, disinfection alternatives to chlorine, such as ozonation, produce byproducts (e.g., bromate) that are carcinogenic to rodents (*15,16*). Recently, consumption of drinking water with high THM levels has been associated with adverse reproductive outcomes (*17–20*). Thus, one of the most complex issues facing water utilities and the U.S. Environmental Protection Agency (U.S. EPA) is how to minimize the potential DBP health effects yet maintain effective control of waterborne microbial pathogens.

In July 1994 the U.S. EPA proposed the stage 1 DBP rule (*21*) in conjunction with the Interim Enhanced Surface Water Treatment Rule (IESWTR) (*21*). The rules stemmed from a regulatory negotiation process that began in November 1992. Because of the lack of data, the negotiators agreed that there should be a 2-stage DBP rule and the Enhanced Surface Water Treatment Rule (*21*). The stage 1 DBP rule would be proposed, promulgated, and implemented concurrently with the IESWTR to ensure that microbial risk did not increase as the stage 1 DBP rule was implemented. The stage 2 DBP rule would follow after additional information was obtained on health risk, occurrence, treatment technologies, and analytical methods to better understand the risk–risk tradeoffs

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Abbreviations used: APC, adenomatosis polyposis coli; AWWARF, American Water Works Association Research Foundation; BDCM, bromodichloromethane; CDBM, chlorodibromomethane; DBPs, disinfection byproducts; DCA, dichloroacetic acid; DOD, Department of Defense; FETAX, frog embryo teratogenesis assay *Xenopus*; HAAs, haloacetic acids; IESWTR, Interim Enhanced Surface Water Treatment Rule; MCL, maximum contaminant level; MX, 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5*H*)-furanone; NHEERL, National Health and Environmental Effects Research Laboratory; NIEHS, National Institute of Environmental Health Sciences; SDWA, Safe Drinking Water Act; TCA, trichloroacetic acid; THMs, trihalomethanes; U.S. EPA, U.S. Environmental Protection Agency; NTP, National Toxicology Program; USACEHR, U.S. Army Center for Environmental Health Research.

between microbial pathogens and those from DBPs.

The proposed stage 1 DBP rule included maximum contaminant levels of 80 µg/liter for total THMs, 60 µg/liter for five haloacetic acids (HAAs), 10 µg/liter for bromate, and 1 µg/liter for chlorite along with the best available technologies to control for these DBPs (*21*). For the stage 2 DBP rule, the negotiators agreed that the U.S. EPA would collect data on the parameters that influence DBP formation and the occurrence of DBPs in drinking water through the information collection rule. Based on this information and on new data generated through research, the U.S. EPA would develop a stage 2 DBP rule (*21*). The Safe Drinking Water Act (SDWA) (*22*) was reauthorized in 1996 (*23*) and established new regulatory deadlines of November 1998 for the final stage 1 DBP rule and May 2002 for the final stage 2 DBP rule.

Animal toxicity studies have been one of the key sources of data for policy makers in setting DBP standards. These animal toxicity studies have generally focused on single chemical byproducts at high concentrations. These studies have been useful in identifying potential human reproductive and carcinogenic risks for several byproducts; however, human exposures are to mixtures of DBPs. Tables 1 and 2 illustrate several of the DBPs that occur from different treatment processes. Most health and occurrence information is on chlorination byproducts because this has been the predominant water disinfection method in the United States. However, water utilities are considering a shift to alternative disinfectants. Thus, there is a need to better understand the relative risks from DBPs that occur from other disinfection processes as well as a need for information on DBP mixtures. In addition, information on dose–response relationships and data on DBPs at concentration levels approaching human exposures will be important for making policy decisions. Currently much of those data are lacking.

Given the complexity of the issues, an integrative and multidisciplinary approach is necessary for developing information needed to develop future DBP rules. It is also critical to have an understanding of the occurrence, human exposure, and analytical techniques, as well as the toxicity of the DBPs. The purpose of this paper is to briefly review the existing cancer data on DBPs and outline a collaborative approach for cancer hazard assessment for the many

Table 1. Halogenated byproducts formed by chlorination.[a]

Oxidation byproducts	Concentrations,[b] µg/liter Median	Maximum	Reference
THMs			
Chloroform	25	240	ILSI (*25*)
Bromodichloromethane	9.5	90	ILSI (*25*)
Chlorodibromomethane	1.6	36	ILSI (*25*)
Bromoform	<0.2	7.1	ILSI (*25*)
HAAs			
Dichloroacetic acid	15	74	ILSI (*25*)
Trichloroacetic acid	11	85	ILSI (*25*)
Bromochloroacetic acid	3.2	49	ILSI (*25*)
Monochloroacetic acid	1.3	5.8	ILSI (*25*)
Dibromoacetic acid	<0.5	7.4	ILSI (*25*)
Monobromoacetic acid	<0.5	1.7	ILSI (*25*)
Tribromoacetic acid	—	—	—
Bromodichloroacetic acid	—	—	—
Chlorodibromoacetic acid	—	—	—
HANs			
Dichloroacetonitrile	2.1	10	ILSI (*25*)
Bromoacetonitrile	0.7	4.6	ILSI (*25*)
Bromochloroacetonitrile	0.6	1.1	Cumming and Jolley (*59*)
Dibromoacetonitrile	<0.5	9.4	Cumming and Jolley (*59*)
Trichloroacetonitrile	<0.02	0.02	Cumming and Jolley (*59*)
Tribromoacetonitrile	—	—	—
Haloketones			
1,1,1-Trichloropropanone	1.0	8.3	ILSI (*25*)
1,1-Dichloropropanone	0.4	2.5	ILSI (*25*)
1,3-Dichloropropanone	—	—	—
Others			
Chlorate	161	9180	ILSI (*25*)
Chloral hydrate	2.1	25	ILSI (*25*)
Chloropicrin	0.4	3.7	ILSI (*25*)
MX	0.005	0.067	Bull (*7*)
Cyanogen chloride	0.62	—	Cumming and Jolley (*59*)
Cyanogen bromide	—	—	—
Halonitriles	0.4	3.7	Richardson (*49*)

Abbreviations: HAAs, haloacetic acids; HANs, haloacetonitriles; ILSI, International Life Sciences Institute; MX, 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5*H*)-furanone. [a]Studies have primarily focused on surface water systems where high DBPs would be expected. [b]Median and maximum concentrations vary widely depending on the chemical/time/source of sampling.

Table 2. Principal byproducts formed by ozonation, chlorine dioxide, and chloramination.

Oxidation byproducts	Range of concentrations, µg/liter	Reference
Principal products formed by use of ozonation		
Aldehydes		
Formaldehyde	1–50	Reckhow and Singer (*94*)
Acetaldehyde	1–50	Reckhow and Singer (*94*)
Glyoxal	ND–15	Ferguson et al. (*95*)
Dimethyl glyoxal	0.005–3	Richardson (*49*)
Methyl glyoxal	ND–8	Richardson (*49*)
Benzaldehyde	0.005–3	Richardson (*49*)
Brominated byproducts		
Bromate	1–50	Reckhow and Singer (*94*)
Bromoform	1–50	Reckhow and Singer (*94*)
Brominated acetic acids	1–50	Reckhow and Singer (*94*)
Bromopicrin	1–50	Reckhow and Singer (*94*)
Principal products formed by use of chlorine dioxide		
Chlorite	0.01–5.36	U.S. EPA (*96*)
Chlorate	0.01–4.42	U.S. EPA (*96*)
Plus similar oxidation byproducts as ozonation	—	—
Principal products formed by chloramination		
Similar to chlorination but lower THMs, lower cyanogen bromide	—	—
Enhanced levels of cyanogen chloride	—	—
Enhanced levels of larger hydrophilic organic halides not further defined	—	—

ND, not detectable.

chemicals that appear in combinations in the U.S. drinking water. It is hoped that this paper will stimulate discussion and suggestions on the proposed approach. Because this paper does not provide an extensive discussion on the health effects data, the reader may wish to refer to reviews by the International Agency for Research on Cancer (*24*), the International Life Sciences Institute (*25*), Bove et al. (*26*), and Reif et al. (*27*). DBPs may pose other health risks (e.g., reproductive and developmental, immunotoxicity, neurotoxicity).

Animal Cancer Bioassays

Byproducts from Chlorination

Trihalomethanes, which generally occur in the greatest concentrations, were the first family of organic compounds identified as byproducts of chlorination (*28*). Of the four THMs, chloroform was the first to be evaluated because it often occurs in the highest concentration (Table 1). Chloroform was tested in a rodent bioassay by corn oil gavage and was carcinogenic (*2*). The corn oil gavage route of administration raised some concern as to whether these results in rats and mice were relevant for human exposures. Subsequent studies with drinking water exposure found renal tumors in rodents consistent with the corn oil studies but did not find mouse liver tumors (*29*). Chloroform is one of the best studied DBPs, and extensive research has been conducted to understand its carcinogenic potential. A convincing body of evidence has emerged that indicates chloroform's carcinogenic activity is secondary to events associated with induced cell injury and regenerative cell proliferation (*30,31*). Other investigators have questioned these conclusions (*32*).

The other three THMs have also been evaluated for carcinogenicity in rodents by the corn oil gavage route (Table 3). Chlorodibromomethane (CDBM) was not associated with increased tumors in rats, but a small increase in liver tumors in female mice was found (*5,33*). Bromodichloromethane (BDCM) caused a dramatic increase in colon cancer in male and female rats (*34*). When BDCM was evaluated by the drinking water route, liver tumors were not found in male mice or male rats. In both studies a low incidence of renal cancer was seen (*35*). Tribromomethane (bromoform) caused a low incidence of colon tumors in female rats (*6,36*).

The HAAs (Table 1) are another family of organic chemicals that occur frequently in drinking water (*28*). DCA causes liver tumors in mice and rats (*8,37–40*). TCA induces liver tumors in mice but not rats (*41*). A recent study also found that DCA caused testicular toxicity (*42*). The mechanism of DCA and trichloroacetic acid (TCA) carcinogenicity is not clearly understood, but DCA and TCA may act by different mechanisms (*43–46*). DCA's mode of carcinogenic activity may be related to modification of intracellular signaling pathways (*31*). The carcinogenic activity of TCA may be related to peroxisome proliferation (*8,44,47*).

A variety of halogenated acetonitriles have been found in chlorinated drinking water (*28,48*). Concentrations of halogenated acetonitriles typically range from 0.01 to 3 µg/liter (*49*) but have been reported as high as 42 µg/liter in Florida (*50*). Carcinogenicity of the haloacetonitriles has not been determined (*24*). The halogenated acetonitriles cause DNA strand breaks in cultured human lymphocytes (*51*), induce DNA damage in bacteria (*52*), and induce sister chromatid exchanges (*53*). Dibromoacetonitrile is considered weakly mutagenic in *Salmonella* species (*54*).

Several chlorinated ketones are produced during chlorination and may be detected in drinking water (*24*). These chemicals generally occur at concentrations lower than 5 µg/l and there is little toxicity information available. Halogenated phenols may be formed but rarely exceed 0.1 µg/l. There is limited evidence of the carcinogenicity of chlorophenols for humans with occupational exposures (*55*).

Chlorinated furanones have received attention because one member of this family, 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX), accounts for most of the mutagenicity found in chlorinated drinking water (*24*). Recent

Table 3. Summary of major NTP/U.S. EPA studies of disinfection byproduct chemicals.

	Research design		Positive sites for carcinogenic effects			
D/DBPs	Dose	Route	Colon	Kidney	Liver	Reference
THMs						
BDCM	Rat 50–100 mg/kg	Oil	MR, FR	MR	–	NTP (*4*)
	Mouse 24, 50 mg/kg female	Gavage	–	–	FM	
	75, 150 mg/kg male	Oil	–	MM	–	
	Mouse 0.05–0.5 g/liter	Gavage	–	–	MR[b]	
	Mouse 0.07–0.7 g/liter	Water	–	–	–	
Chloroform	Rat 90, 200 mg/kg	Oil	–	MR	MM, FM	National Cancer Institute (*2*)
	Mouse 138,477 mg/kg	Gavage	–	–	–	
Chloroform	Rat 19, 160 mg/kg	Water	–	MR	–	Jorgenson et al. (*29*)
	Mouse 34,263 mg/kg	Water	–	–	–	
Bromoform		Oil	MR, FR	-	-	NTP (*6*)
		Gavage				
CDBM	Rat 40, 80 mg/kg	Oil	–	–	–	NTP (*33*)
	Mouse 50, 100 mg/kg	Gavage	–	–	–	
HAAs						
DCA	0.05–3.5 g/liter	Water	–	–	MR, FM	Bull et al. (*8*)
		Water	–	–	MM	
TCA	0.05–5 g/liter	Water	–	–	Negative[b]	Bull et al. (*8*)
	0.05–4.5 g/liter	Water	–	–	MM, FM	
Disinfectants						
Chlorine	70–275 ppm	Water	–	–	Negative	NTP (*97*)
Chloramine	50–200 ppm	Water	–	–	Negative	NTP (*97*)

Abbreviations: BDCM, bromodichloromethane; CDBM, chlorodibromomethane; FM, female mouse; FR, female rat; MM, male mouse; MR, male rat; TCA, trichloroacetic acid.
[a]Screening study with limited number of animals and sex species combinations. [b]Tested in MR only.

Finnish studies have shown that this chemical is a multisite carcinogen in rats (*56*). This chemical has not been evaluated in mice. MX occurs at low levels in some chlorinated drinking water but is more potent than other DBPs.

A variety of other chemicals such as cyanogen halides and chloral hydrate are found in chlorinated drinking water. Chlorate hydrate has been found at levels up to 25 µg/liter (Table 1), whereas the cyanogen halides are often at 1 µg/liter or less. Although several THMs and HAAs have been evaluated in animal studies and a few have been studied for their mechanisms of toxicity, many families of chlorinated byproducts lack complete toxicology or carcinogenicity data.

Byproducts from Ozonation

Ozone treatment is more effective than chlorine in killing or inactivating harmful organisms, particularly *Cryptosporidium* sp. (*57*) but provides no residual disinfection. Ozone alone does not produce chlorinated byproducts (*58*), but if followed by chlorination or chloramination, many of the same byproducts as with chlorination are found, albeit at lower concentrations. Ozonation disinfection byproducts include aldehydes and glyoxals (*59*). In addition, ozonation of water containing bromine may produce DBPs such as bromoform, dibromoacetic acid, cyanogen bromide, and bromate (*59*). Formaldehyde is also produced, but drinking water and oral studies of formaldehyde have generally been negative (*60,61*). Potassium bromate, administered via drinking water, caused renal cancer (*15*), mesotheliomas, and thyroid follicular cell tumors (*62*) in rats. It is generally accepted that potassium bromate will cause renal cancer in rats (*63*). In addition to renal cancer, thyroid, mesothelial, and intestinal neoplasms occur in male rats with bromate administration (*64*). A significant increase of 8-hydroxydeoxyguanosine was observed in DNA following oral administration of potassium bromate and is thought to be one of the DNA lesions involved in potassium bromate carcinogenesis (*64*). Several investigators have studied the role of oxidative damage in bromate-induced carcinogenesis and have suggested that the induced tumors may be due to lipid peroxidation and subsequent DNA damage (*65–68*). There is essentially no long-term toxicity data on cyanogen bromide. Sodium bromite was negative in short-term reproductive studies in rats, but sodium bromate produced reproductive effects in male rats at a concentration of 20 ppm in drinking water.

Products from Chlorine Dioxide

Chlorine dioxide is an effective drinking water disinfectant (*57*). The inorganic species chlorite and chlorate have been identified as significant byproducts (*57,69*). Chlorine dioxide and its degradation products cause neurodevelopmental effects in animals (*31*). There is essentially no information on the long-term carcinogenicity effects of chlorate. There may be a need to evaluate chlorate because of its high occurrence and limited toxicity database. Chlorine dioxide will produce low levels of organic byproducts as compared to chlorine disinfection but similar organic byproducts (e.g., aldehydes, ketones) as compared to ozonation (*69*).

Products from Chloramination

Chloramination is of interest because treatment of water with chloramine results in lower concentrations of chlorinated DBPs than with the use of chlorine (*69*). However, significant amounts of chlorinated organic material can form. Little is known about these byproducts except that they are more hydrophilic and larger than organic halides produced from free chlorine (*69*). Chloropicrin, cyanogen chloride, 1,1-dichloropropanone, and chloramines are some of the byproducts of concern (*57*). Chloropicrin did not produce evidence of carcinogenicity in long-term rodent studies. Much less is known about the haloketones.

Questions to Be Answered

To provide scientific data for policy decisions, it is necessary to have toxicity data on relationships between exposure, internal dose, and toxicity. Although there are several vigorous research programs on DBPs, this is a complex area with many unanswered questions. For example, it is necessary to account for sensitive human subpopulations and the occurrence of chemical mixtures with potential interactions, and numerous DBPs have only begun to be studied. We are initiating a series of chronic studies to be followed by shorter-term mechanistic studies. A systematic approach also requires evaluating a series of hypotheses, answers to which can contribute to the regulatory process. It is also important to realize that drinking water standards are based both on potential health effects and what is technologically and economically feasible.

Informational Needs

- Additional information on the comparative toxicity and relative potency of families of DBPs is needed to assist in determining whether classes of DBPs should be regulated as individual chemicals or as a group.
- Additional information is needed on the relative potency of brominated versus chlorinated DBP species because there is some evidence that the brominated species may be more potent than the chlorinated species.
- Additional information is needed on the health effects of the major byproducts from the use of alternative disinfectants such as chlorine dioxide and ozone.
- Additional mechanistic information is necessary to assist in providing better characterizations and estimates of potential human risk for DBPs.
- There is a need to understand the toxicity of DBP mixtures and how source-water variables (natural organic matter as measured by total organic carbon, bromide ions, and pH) influence the toxicity of DBP mixtures.
- There is a need to evaluate alternate models, such as transgenic mice and fish models, that will reduce the time and cost for evaluating the carcinogenicity of DBPs and that will provide more sensitive methods for evaluating DBP mixtures. This is a critical need given the regulatory deadlines established in the 1996 SDWA amendments (*23*).
- There is a need for screening data on the potential immunotoxicity and neurotoxicity of DBPs.

Approach

An approach for evaluating the potential health effects for DBPs (Figure 1) starts with identifying the data deficiencies for the most commonly occurring DBPs and filling these data gaps. The next step is developing a strategy for prioritizing other DBPs for further research. The following describes the primary questions that need to be answered on the potential toxicity of DBPs.

The first step in answering the questions posed previously will be to evaluate selected DBP chemicals in standard rodent studies. We have selected chemicals that represent the important families of DBPs (Figures 1, 2) and chemicals that represent the various disinfection processes (Table 4). Not all DBPs will be tested in all assays. BDCM has been selected as the THM of interest because of the colon cancer found

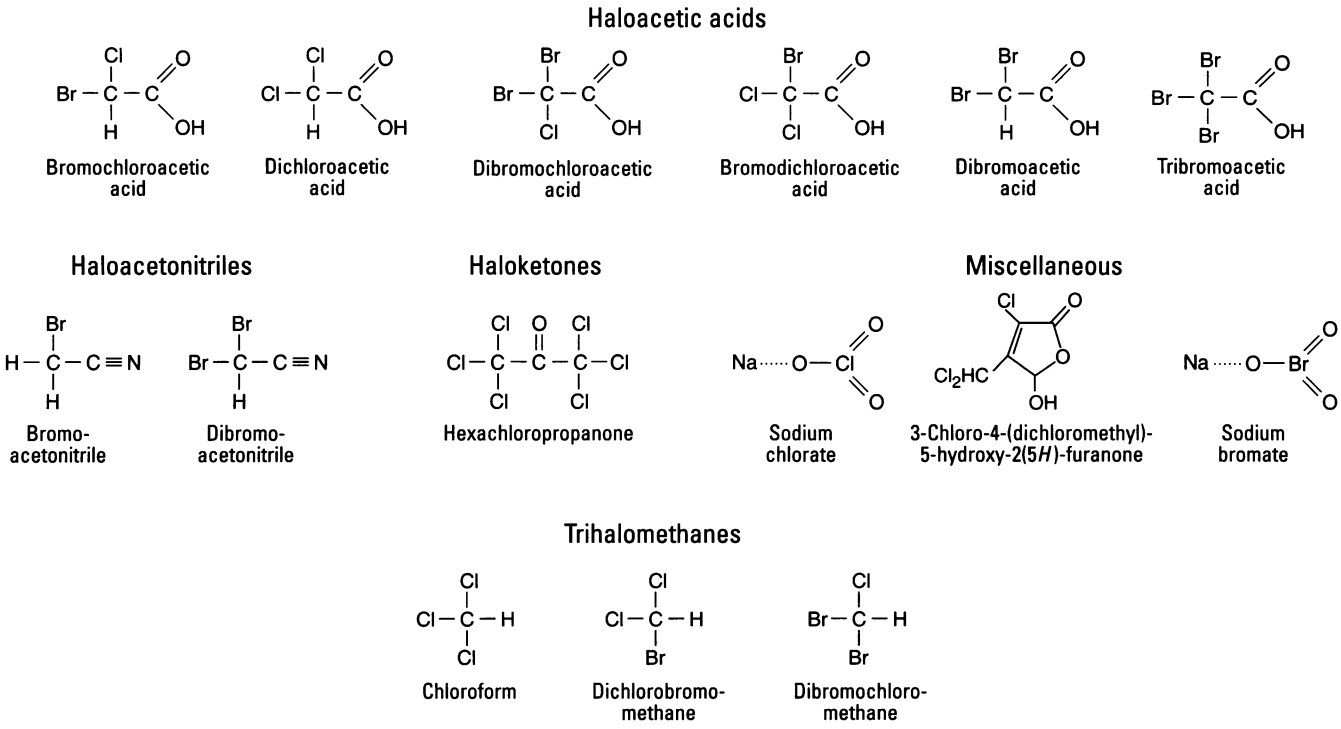

Figure 1. Structures of the disinfection byproducts selected for evaluation. The largest family of chemicals is the HAAs. These chemicals are being evaluated in rodent, transgenic mouse, frog, and fish models. Understanding comparative toxicity and toxicokinetics will allow extrapolation between models and eventually to humans.

in previous corn oil gavage studies. We feel that the data on the most prevalent THM, chloroform, are sufficient (*2,3,24,29,55, 70,71*). DCA, bromochloroacetic acid, and bromodichloroacetic acid have been selected as representative of the brominated HAAs. Both dihalogenated and trihalogenated acids were selected because the degree of halogenation appears to affect the mechanism of toxicity (*7*).

Another family of chemicals found after chlorination is the haloacetonitriles. There is essentially no carcinogenicity information on this family of chemicals. Therefore, dibromoacetonitrile was selected as a representative member of this family of chemicals for long-term rodent studies.

A carcinogenic evaluation of the major mutagen found in drinking water [3-chloro-4(dichloromethyl)-5-hydroxy-2(5*H*)-furanone (MX); also known as mutagen X] is also scheduled for evaluation in long-term rodent studies. This is of special interest because MX has recently been reported to be carcinogenic in rats (*56*). Finally, chlorate was selected as a nonorganic chemical that may be formed as a result of inefficient generation of chlorine dioxide or from the use of hypochlorite solution and gaseous chlorine for disinfection. These eight chemicals (Table 4) will be evaluated using drinking water as the route of administration and will include 14- or 21-day studies, 90-day studies, and 2-year carcinogenicity studies. There will be an emphasis on including exposure groups close to human exposures in all studies. Toxicokinetic studies are planned for all compounds to include the parent compound and potentially toxic/carcinogenic metabolites following exposure to the different concentrations. These studies will help determine whether the blood levels and target tissue concentrations are linear with exposure concentrations. This information can also be used for dose–response modeling. Tissues and animals will also be made available to U.S. EPA and other scientists for investigator-initiated studies. The National Institute of Environmental Health Sciences (NIEHS) plans to use small grant awards to support independent investigators in exploring the potential mechanisms of toxicity for the various DBPs under test. This will allow comparisons between specific cellular and molecular alterations and the carcinogenicity end points in the chronic studies in the same animals and tissues.

Cost and time will limit the number of chemicals that can be evaluated in long-term rodent models. Therefore, there will be an emphasis on selecting short-term models that can provide comparative toxicity for more members of each family class. Currently, there is great interest in the use of Tg.AC and *p53*^{def} transgenic mouse models for predicting carcinogenic risk (*72*). The National Toxicology Program (NTP), in collaboration with the

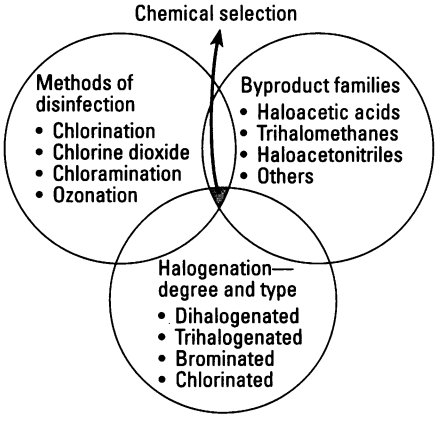

Figure 2. Chemical selection is a balance of selecting disinfection byproducts formed by the various methods of disinfection, selecting from the different families of DBPs, and looking at degree and type of halogenation. Underlying the selection process is the level and rate of occurrence of the various DBPs in drinking water.

Table 4. Initial byproducts to be evaluated in toxicity studies.

Oxidation byproducts	Rats/Mice	Transgenic mice	Fish	Reproductive toxicity
THMs				
Chloroform			X	
Bromodichloromethane	X	X	X	X
Chlorodibromomethane				X
HAAs				
DCA	X	X		
Dibromoacetic acid	X		X	
Bromodichloroacetic acid	X			
Bromochloroacetic acid				X
Dibromochloroacetic acid				X
Tribromoacetic acid				X
HANs				
Bromoacetonitrile				X
Dibromoacetonitrile	X			X
MX (–1)	X			
Ketones				
Hexachloropropanone				X
Inorganic chemicals				
Bromate		X		X
Chlorate	X		X	

International Life Sciences Institute, U.S. pharmaceutical firms, and the Japanese government, is evaluating more than 40 chemicals including some DBPs in the Tg.AC and *p53*^{def} transgenic mouse models. We have selected three DBPs for initial evaluation in these models (Table 4). In the Tg.AC mouse these chemicals will be evaluated both by the skin-painting route of exposure and by drinking water exposure. Because Tg.AC gene expression is found in both the skin and the forestomach, there is a possibility for good correlation between the two routes of exposure and with other target organs such as liver and kidney. Should this correlation prove accurate, it would create a model that could be used to screen and rank numerous DBPs for relative carcinogenic potency. It could also provide a useful tool for evaluating chemical mixtures and may prove a sensitive indicator of tumor response at doses lower than those used in the 2-year bioassays. The Tg.AC model appears most useful for identifying nonmutagenic carcinogens, whereas the *p53*^{def} transgenic mouse model is considered more useful for identifying mutagenic carcinogens (*72*). DCA, BDCM, and bromate will also be evaluated in the *p53*^{def} transgenic mouse model. These models provide results at a fraction of the cost of long-term studies. Because new data can be obtained in 6 months, additional data can be obtained for U.S. EPA stage 2 policy decisions. Blood samples will be collected from both transgenic mouse models to provide some indication of parent compound and metabolite levels following exposure to the different concentrations of the DBP. This will allow comparison between the transgenic and standard models and may provide an understanding of any differences seen between models. A potential advantage of these models is that they will provide an opportunity for evaluating more DBPs and for predicting comparative toxicity of DBPs and other chemical families found in the drinking water. These models also should be useful for setting priorities for further research and may guide effective treatment and regulatory strategies. However, the use of transgenic mouse and small fish models is relatively new and their utility in health risk assessment remains to be determined. Because we are testing some DBPs in more than one model, these data may be useful in extrapolating across these newer research models.

Epidemiology studies have suggested that bladder, rectal, and colon cancer are potentially associated with drinking chlorinated water (*9,12,73,74*). The brominated THMs also cause colon cancer in rats (*34,64*). NIEHS investigators will evaluate the feasibility of using an animal model of colon cancer for the evaluation of disinfection byproducts. Humans with familial adenomatous polyposis carry mutations on an adenomatosis polyposis coli (*APC*) gene that is related to inherited colon cancer. Transgenic mice with mutations in the murine gene analogous to the human *APC* gene have been developed and have a high rate of colon cancer (*75,76*). Studying the effects of individual DBPs and combinations of DBPs and their interactions with a gene that is known to modify intestinal neoplasia within a few months may be a powerful tool for ranking the comparative toxicity of DBPs and DBP combinations. Further, if these transgenic mice are particularly sensitive to low concentrations of DBPs, it may be possible to study DBPs at concentrations similar to those found in drinking water.

In addition to evaluating interactions between DBPs and relevant genes in transgenic murine models, tumors from the various models will be analyzed for genetic alterations in the genes most commonly associated with human cancer (such as *ras* and *p53*). The evaluation of genetic alterations in rodent tumors may help to provide a better understanding of molecular mechanisms underlying chemical carcinogenesis and consequently it is important for the accurate extrapolation of the cancer models to humans.

Small fish models are considered short-term models that can be used to provide comparative toxicity information for the members of each family of chemicals and chemical mixtures (*77–80*). Several advantages of the Japanese medaka (*Oryzias latipes*), a small fish species, include shorter time to tumor, less expense than rodent models, and the ability to use large group sizes for greater sensitivity to detect a subtle effect. Small fish models have previously been used to examine chlorination byproducts (*81,82*). The medaka has been used extensively in carcinogenicity research and has many of the characteristics of a good cancer model (*78,83*). The NTP is currently evaluating three rodent carcinogens in the medaka (*84*). The U.S. Army Center for Environmental Health Research (USACEHR) is evaluating the use of the medaka model in assessing water supplies potentially impacted by Army-related functions. Currently, the USACEHR is collaborating with the NIEHS and the U.S. EPA in studies to evaluate the efficacy of the medaka model in providing comparative toxicity data for four of the DBP chemicals. Both short-term acute toxicity tests and longer-term (9-month) carcinogenicity assays are being conducted (Table 4). Incorporated into these assays are experiments designed to provide information on the role induced hepatocellular proliferation may play in any neoplastic response observed. The U.S. EPA has a large medaka study in progress to establish low-dose effects of the chemical *N*-nitrosodiethylamine, a well-known hepatic carcinogen in mammals (*85,86*) and medaka (*87,88*). In addition, the U.S. EPA has also

initiated THM mixture studies using the medaka (*89*). Large numbers of medaka can be exposed at relatively modest cost, thus allowing evaluation of multiple DBP concentrations. As with the transgenic models, blood or liver samples will be collected from the medaka in these dose–response studies to provide preliminary indications of the tissue-specific concentrations of the DBPs and the levels of the formation of any metabolic byproducts. These data should help provide information for comparison between tissue DBP or metabolite concentrations and organ toxicity for fish, transgenic, and standard rodent models. If there is close consistency between exposure concentrations across several animal models, lower uncertainty factors could be used in risk assessment, whereas large discrepancies across species would suggest that larger uncertainty factors be used.

THMs may cause colon cancer in humans and bromodichloromethane causes a high incidence of colon cancer in rats (*3,34,36*). Loss of function of both alleles of the *APC* gene results in colorectal cancer in humans (*90*). There are mouse models with the mutated *APC* gene, including the Apc^{Min} model (*75*), a relatively recent mouse model that develops a high incidence of colon cancer in just a few months. These mice develop multiple tumors in both the small and large intestine. Thus, there is the potential for a relevant mouse model that has genetic alterations similar to those in humans with colon cancer. Because the utility of this mouse is not well understood for comparative toxicity studies, efforts will be made to adapt the model for DBP studies. One of the first chemicals to be chosen will be BDCM because it has already been shown to cause colon cancer in rats and is under study in fish and other transgenic mouse models. This model has the potential to increase our understanding of DBP carcinogenicity at the gene level.

A series of DBPs were identified by the U.S. EPA as being of special concern for reproductive/developmental toxicity. These DBPs are being tested using the design method of Harris et al. (*91*), which tests the components of reproduction in parallel (e.g., spermatogenesis, ovulation, implantation, fetal growth, etc.) in rodents. Additionally, clinical chemistry, hematology, and cell turnover data are collected by the NTP reproductive toxicity program. These additional data may be useful to help set doses and identify target organs for planned carcinogenicity studies. An amphibian assay is also being used. The frog embryo teratogenesis assay-*Xenopus* (FETAX) is a 96-hr assay that utilizes the embryos of the South African clawed frog, *Xenopus laevis*, to test for the potential teratogenicity of single compounds. The assay can be used to assess both single compounds and complex mixtures and is applicable in the weight-of-evidence approach for establishing water quality criteria, biomonitoring, toxicity screening, and hazard assessment. The assay has been used extensively in interlaboratory validation studies, including the testing of 12 NTP-recommended compounds. The FETAX model will be used to assess the developmental toxicity of DBP compounds by the NTP reproductive toxicity program and by the U.S. EPA Office of Water, which is sponsoring a pilot study with the USACEHR to evaluate chlorinated drinking water mixtures. A more detailed summary of this work is in preparation.

If the *in vivo* studies identify biomarkers of exposure, the epidemiology studies can be evaluated to provide much better indications of a potential association between exposure to DBPs and the occurrence of toxicity and cancer (Figure 3). Biomarkers of exposure may be useful in identifying a population at risk for toxicity. To date, epidemiology studies suffer from the difficulty of establishing sound exposure measures for people who are exposed to low concentrations of DBPs that vary widely over the years. Evaluation of rodents tested at much higher concentrations, if they lead to unique adducts, may provide some clues for evaluation of humans with different histories of drinking water exposure. The *in vivo* studies are an iterative process and as information is gained from one study it can be used to alter or improve other studies. Close collaboration is necessary between scientists who are conducting human epidemiology studies and those conducting animal studies so that when new data become available they can be applied to ongoing studies or used to design new studies.

Mechanisms to Accomplish the Studies

The magnitude and complexity of the problem of safe drinking water far exceeds the expertise and resources of any one agency. Because the NIEHS has been responsible for many of the chemical evaluations in the NTP, the NTP has developed expertise in the design and conduct of rodent studies and will be responsible for contracting the short-term rodent studies, the 2-year rodent studies, and the transgenic studies. The U.S EPA, through the National Health and Environmental Effects Research Laboratory (NHEERL), has established a

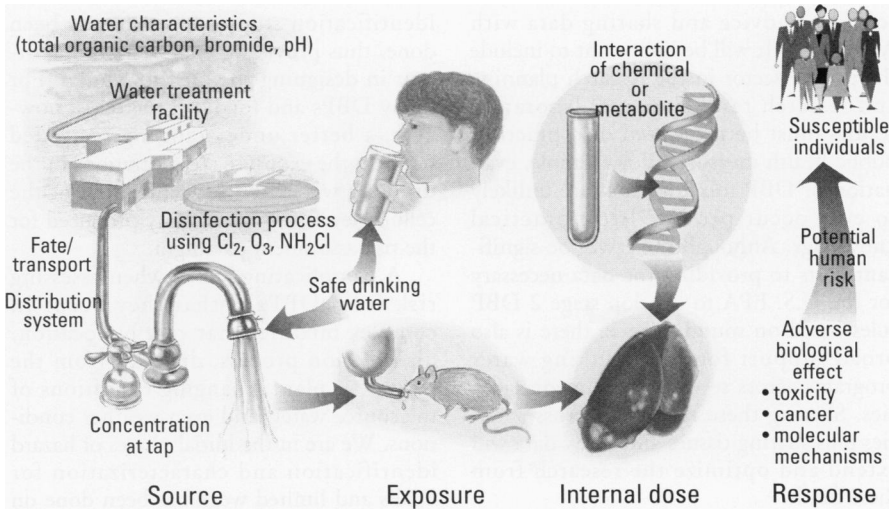

Figure 3. Evaluation of disinfection byproducts. The overall evaluation of drinking water for safety is complex, involving water engineering, distribution, study of occurrence, and animal and molecular studies with a sensitivity to the populations that may be affected from children to individuals with immune deficiency diseases. It is critical to evaluate chemicals at or near levels that occur in drinking water. When effects are found at higher concentrations, it will be important to determine what changes are occurring at the molecular levels and whether these effects are also found at or near drinking water concentrations.

research program for DBPs. Many of the NTP studies will include extra animals for mechanistic studies to be carried out at the NHEERL. The NHEERL has an active program exploring oxidative damage, DNA adducts, and cell replication following exposure to various DBPs. This collaborative effort has been used by the NTP for other chemicals and offers the advantage of having the mechanistic studies done in the same animal model that provides the evidence of toxicity and cancer in the long-term studies. This helps policymakers put the rodent study results in perspective. The U.S. EPA has also established an interagency agreement with the NIEHS for immunotoxicity, reproductive toxicity, and neurotoxicity studies. This will allow an evaluation of noncancer end points, an area that often receives insufficient attention in hazard evaluation. The Department of Defense (DOD) has expertise and years of experience with small fish models and the NIEHS has established an interagency agreement with DOD for studies with the medaka and FETAX. It is anticipated that through the grant process, the NIEHS will support investigators who want to contribute scientific information to the area of safe drinking water. Private water utilities and the American Water Works Association Research Foundation (AWWARF) have engineering, water chemistry, and analytical techniques that supplement the expertise found within government agencies. NIEHS scientists in collaboration with the U.S. EPA are soliciting advice and sharing data with AWWARF. It will be important to include the private sector in the research planning and research results because laboratory results must be translated into practical public health measures. For example, evaluation of DBP mixtures that are unlikely to ever occur provide little practical knowledge. Although there will be significant costs to providing the data necessary for the U.S. EPA to develop stage 2 DBP rules based on sound science, there is also broad support for safe drinking water programs across several government agencies. Sharing these resources across agencies plus sharing tissues and study data will extend and optimize the research from these funds.

Conclusions

Disinfection of drinking water has been one of the greatest public health advances in this century. However, animal studies have suggested that some DBPs in drinking water may cause an increased incidence of cancer and reproductive effects when tested at high concentrations. Some epidemiology studies have suggested that drinking chlorinated drinking water may be associated with increased incidences of bladder, rectal, and colon cancer (*9,11,13,14*) and adverse reproductive effects (*17*). The U.S. EPA has a mandate to ensure that disinfection of drinking water controls pathogens but also that the disinfection process does not increase other health risks (*21–23*). Presently, there are limited data to make sound scientific judgments on the next generation of drinking water standards. It is important that the DBPs are evaluated in a systematic manner to provide comparative toxicity data on both HAAs and THMs to determine the most effective means of water disinfection.

Health risk assessment is a rapidly developing science that is emphasizing our understanding of the mechanisms of toxicity (*92*). It consists of four components including hazard identification, dose-response assessment, exposure assessment, and risk characterization (*93*). Hazard identification has been the focus of many of the early NTP studies, but both the NTP and the U.S. EPA recognize that hazard identification is only one step in the risk assessment process. The U.S. EPA is initiating a new paradigm of risk assessment with a larger focus on understanding the mechanism of toxicity for each DBP. For some of the DBPs, important hazard identification studies have already been done, thus providing data that are important in designing the present studies. For many DBPs and for DBP mixtures, however, a better understanding is needed between the exposure concentrations in the drinking water, the concentrations at the cellular level, and the toxicity produced for the risk assessment paradigm.

A complicating factor when assessing risk from DBPs is that they occur in complex mixtures that vary by location, disinfection process, distance from the treatment plant, changing conditions of the source water, and even weather conditions. We are in the initial phases of hazard identification and characterization for DBPs and limited work has been done on chemical mixtures. One approach is to study mixtures with known toxicants and use hypothesis-driven research to explore the relationship of toxicity when two or more chemicals are present. We anticipate that studies of DBP mixtures and mixtures of other contaminants will receive increased attention in the future.

The standard rodent studies supplemented with toxicokinetic data and coupled with information on end points such as cell proliferation, DNA adducts, DNA repair alterations, and mutagenic events at the different cellular concentrations will provide useful data for policy makers. Our approach also includes the use of transgenic mouse models and small fish models. These data will be less familiar, with little precedent for using the data to set regulatory standards. Evaluating several DBPs in multiple models and having toxicokinetic data for all sex/species combinations evaluated may be useful for assessing the utility of alternative test species for screening and ranking DBPs.

Although much remains to be learned, the DBP studies to date are already shaping the research approach for the second generation of hazard characterization and dose–response studies. It is anticipated that there will be major additions to the DBP toxicity database in the next several years. Safe drinking water is a critical resource that affects everyone. Providing this safe resource is a complex issue involving engineers, toxicologists, epidemiologists, chemists, and policy-makers—expertise that is not contained within a single agency or research group. A coordinated approach with input from a wide variety of stakeholders and researchers offers the best chance to continue to provide safe cost-effective drinking water.

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The views, opinions, and/or findings contained in this paper are those of the authors and should not be construed as official Department of the Army position, policy, or decision unless so designated by other official documentation.

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