Disinfection By-products in Drinking Water:

Critical Issues in Health Effects Research

Workshop Report

Chapel Hill, North Carolina

October 23-25, 1995

International Life Sciences Institute
Health and Environmental Sciences Institute

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FOREWORD

Disinfection of drinking water supplies results in a number of by-products, the profiles of which are dependent upon the methods of disinfection used, the source water quality, and the technology applied. Chlorine, chlorine dioxide, chloramines, and ozone are the most common disinfectants, each of which results in various disinfection by-products. Results from laboratory animal studies have suggested that exposure to certain disinfection by-products may be associated with adverse health effects, including cancer, reproductive, developmental, and other types of non-caner toxicity. The potential for these effects varies with the specific compound and the level of exposure, although there are a number of by-products for which there is inadequate information.

The most frequently found by-products in drinking water include trihalomethanes and halogenated acetic acids. The drinking water standards for these compounds are based in part on health risk assessments performed by the U.S. Environmental Protection Agency, utilizing the available toxicologic databases for each compound. For most by-products, insufficient data have been available to conduct biologically based risk assessments; therefore, conservative and/or default assumptions have been used to estimate risk. It is recognized that these practices may result in significant overestimation of the risk from disinfection by-products.

New studies on the key disinfection by-products have been initiated or recently completed that may provide additional data for assessing risks from exposure to these compounds through drinking water. These have an important bearing on the understanding of the toxicology of disinfection by-products and, thus, the risk assessment process. As these new data become available, biologically based models can play an increasingly important role in risk assessment by utilizing mechanistic and pharmacokinetic data to replace conservative default assumptions.

This workshop has been organized to discuss the emerging data and address the critical issues in health effects research for disinfection by-products in drinking water.

INTRODUCTION

Denise E. Robinson
Health and Environmental Sciences Institute,
International Life Sciences Institute

Drinking water disinfection has been one of the major public health success stories of the past century. The incidence of microbial infection, disease, and mortality has dramatically declined as a direct result of the use of disinfectants. The reminder that microbial risks can still pose a health concern has been brought to light by a number of incidents over the past few years, including the outbreak of cholera in Latin America and *cryptosporidiosis* in Milwaukee and elsewhere, as well as the recent identification of previously unknown pathogens. Technology to deal with these risks continues to evolve; however, questions continue to be raised regarding how best to ensure the safety of our drinking water, both from microbial as well as chemical contaminants.

The 1992 workshop sponsored by the International Life Sciences Institute (ILSI), U.S. Environmental Protection Agency (U.S. EPA), World Health Organization (WHO), Pan American Health Organization (PAHO), U.S. Food and Drug Administration (U.S. FDA), and American Water Works Association (AWWA) brought together toxicologists, microbiologists, water treatment professionals, and public health officials to discuss the various perspectives on water safety. This workshop represents a continuation of the dialogue on the chemical risk side of the equation as scientists seek to understand the mechanisms of action and interaction among the chemicals that we use to treat our water and to make decisions based on that knowledge.

All currently used disinfectants are highly reactive compounds and it is this reactivity that imparts to these chemicals their efficacy against microbes. Another consequence of these chemical properties is that a number of reaction products are formed with naturally occurring humic substances in the source water. Which by-products and their relative levels are dependent upon the types and combinations of disinfectants used, the quality of the source water, as well as technology applied.

Questions regarding the potential health effects of human exposures to the various by-products have been raised by the results of both epidemiologic and laboratory animal studies. The toxicology database is far from complete and work continues to be conducted to evaluate the significance of these effects for human health. While it is recognized that the choice of disinfectant and the recommended safe levels of disinfection by-products in finished drinking water are currently based upon a combination of scientific, technological, and economic considerations, it is the goal of this workshop to help to understand the information needed to improve the scientific basis for human health risk assessment of these compounds. Furthermore, it is the intent of the workshop to encourage more cross-disciplinary interactions, particularly among the epidemiology and toxicology communities as well as those related to exposure assessment.

The workshop program is structured to provide a forum for presentation and discussion of the latest health effects research on disinfectants and their by-products, focusing on both cancer and non-cancer endpoints. The program is structured to help provide a comparative evaluation of the by-products resulting from the different drinking water disinfectant technologies. Evolving risk assessment approaches such as biologically based modeling and issues related to evaluating drinking water as a complex mixture will be discussed.

The ultimate charge of the workshop rests with the final panel on the last day of the workshop. Their important and difficult task is to evaluate the currently available information in light of what needs to be known about the health effects of the various by-products and to make recommendations that go beyond a listing of research needs into what priorities should be assigned to specific research. Given that there are limited resources, difficult decisions must be made as to the most important areas toward which to allocate those resources. The safety of drinking water is a central issue throughout the world, as evidences by the number of national and supranational governmental bodies currently grappling with the establishment of new guidelines and standards for drinking water, including the U.S. EPA, Canada, the European Union, and the WHO. It is hoped that the product of this workshop will provide important input to each of these groups.

Fred S. Hauchman
National Health and Environmental Effects Research Laboratory,
U.S. Environmental Protection Agency

Several events over the past few years have brought together experts from a variety of scientific disciplines to identify priorities for research to address drinking water health concerns. In 1991, the U.S. EPA and the AWWA Research Foundation sponsored a landmark workshop, *Drinking Water and Health in the Year 2000*, in which toxicologists, epidemiologists, and microbiologists were successful in developing a consensus view of health research priorities for chemical and microbial contaminants in drinking water. The 1992 International Conference on the *Safety of Water Disinfection: Balancing Chemical and Microbial Risks*, sponsored by ILSI and several other organizations, provided a unique forum for scientists, engineers, regulators, and industry representatives from around the world to discuss critical knowledge gaps, research needs, and approaches for comparing chemical and microbial risks. Other conferences and scientific advisory meetings in the past three years have provided additional guidance on health research needs and priorities for drinking water. These activities have all contributed to the development of the U.S. EPA's comprehensive new research strategy for chemicals and microbes in drinking water, which supports the U.S. EPA's recently proposed rulemaking on disinfectants and disinfection by-products (DBPs).

This workshop provides a timely opportunity to discuss the latest findings in health research on DBPs and to develop recommendations for future health research. Several central themes will be emphasized throughout this workshop. One relates to the need to better understand the toxicity of the individual DBPs that are the most important from a risk perspective. There are hundreds of DBPs, many of which are poorly characterized with respect to toxicity, but it is clear that it is neither practical nor necessary to conduct toxicity studies on all of them. Beyond the need for screening-level data for hazard identification purposes, an improved understanding of the chemical, physical, and biological processes involved in the toxic response is critical to evaluating the human health risk associated with exposure to the highest priority DBPs of concern. Research on pharmacokinetics and mechanisms of action not only provides the foundation for conducting scientifically defensible extrapolations of toxicity data from animals to humans, but it can also help to validate the findings of epidemiology studies by determining if there is a biological explanation for the reported associations. Prioritization of contaminants for further study is essential, since this type of research is time consuming and resource intensive to conduct.

While much of the discussion at this workshop will be focused on individual DBPs, the importance of understanding the potential toxicity of the complex mixture of by-products in drinking water should be emphasized. Consideration should be given to research that will lead to better understanding of the relative toxicity of different complex mixtures of by-products, and of the potential for DBP interactions to significantly influence the overall toxicity of the mixtures. Research on mixtures as well as on individual DBPs is particularly important in assessing the potential cancer risks associated with the by-products of alternative disinfectants, since limited historical exposure data will make it difficult to conduct cancer epidemiology studies in communities served by water treated with disinfectants other than chlorine or chloramine.

Another important issue relates to the linkages that should be made between the disciplines of toxicology and epidemiology. Although epidemiology is not a primary focus of this conference, we need to consider how toxicologists can support the work of epidemiologists by developing improved tools for assessing exposure and effect (i.e., biomarkers) in the field. Epidemiology and toxicology research may each provide guidance as to which contaminants, toxic endpoints, and host factors should be further investigated in both the laboratory and the field.

By addressing these themes during the workshop, it should be possible to develop a set of conclusions and recommendations that will have a significant impact on research approaches and priorities in the years to come. We will also achieve the equally important goal of promoting communication and coordination of drinking water research between government, industry, and academia.

Stuart Krasner
Metropolitan Water District of Southern California

Maximum contaminant levels (MCLs) and maximum residual disinfectant levels (MRDLs) are set as close to the MCL goals (MCLGs) and MRDL goals (MRDLGs) as feasible, with treatment techniques, costs, and analytical detection limits as additional considerations. Because the control of disinfectants and disinfection by-products (D/DBPs) must be balanced with disinfection requirements to control microbial pathogens, it is critical that the health effects of D/DBPs are better understood. Additional sound, scientific research is needed in the areas of health effects and epidemiology. This research needs to assess acute and chronic health effects associated with the DBPs produced by chlorine as well as those of alternative disinfectants. Such health effects information will be essential in ensuring that future MDLs and MRDLs provide consistent and appropriate levels of public health protection rather than establishing MCLs and MRDLs based solely on occurrence data and treatment technology performance.

Assessment of carcinogenic risk relies on two major sources: epidemiological studies and animal studies. The degree of resolution in epidemiological studies conducted to date has been insufficient for providing definitive information for regulation. Significant uncertainties exist in applying the results of animal studies to human health risk assessments. Uncertainties include: Is there a threshold? Are people as sensitive as the most sensitive sex of the most sensitive species in the most sensitive study? Can we meaningfully extrapolate from high to low doses?

Ultimately, these uncertainties have a large impact on the MCLs and MRDLs. Depending on the nature of these uncertainties, carcinogenic risk may be overestimated. Conversely, the uncertainties

associated with animal testing, exposure assessment, and human variability may cause carcinogenic risk to be underestimated. Based on advantages in understandings about cancer mechanisms and the availability of new molecular biological tools, risk assessment methodologies need to better address some of these uncertainties.

SESSION 1
DISINFECTION BY-PRODUCTS: CRITICAL ISSUES

Session Introduction

Denise E. Robinson
Health and Environmental Sciences Institute,
International Life Sciences Institute

This workshop session provides a context for the detailed toxicological and methodologic presentations of the subsequent sessions. The speakers will focus on the critical regulatory, water treatment, and risk assessment issues that stimulated the organization of the workshop. Given the diverse audience, the presentations will review the basis for disinfection of drinking water, highlight current water treatment practices, discuss the public health aspects of human exposures to by-products from drinking water disinfection processes, and examine current and evolving risk assessment methodologies for evaluating the potential of the various by-products to pose a risk to public health.

The first speaker, Mr. Regli, will discuss the regulatory issues to be considered in developing specific guidelines for both chemical and microbial contaminants. He will also examine the impacts of various regulatory options. Dr. Singer will give an overview of the various water treatment methods, including the factors influencing the formation and occurrence of disinfection by-products and the difficulties associated with assessing human exposure.

The final two presentations will focus on the types of information available to assess the potential chemical risks of disinfection by-products. Dr. Krewski will focus on methodologies that have been developed to incorporate the available data into both quantitative and qualitative estimates of risk for cancer and noncancer endpoints. He will also speak about evolving methodologies and their data requirements and provide a brief overview of the current process in Canada for revising the drinking water guidelines. Dr. Hoel will review the currently available epidemiological studies addressing both cancer and reproductive and developmental endpoints.

Research Needs: Regulatory Perspective

Stig Regli
Office of Ground Water and Drinking Water,
U.S. Environmental Protection Agency

From 1992 to 1994, the U.S. Environmental Protection Agency (U.S. EPA) participated in a regulatory negotiation process concerning how best to regulate disinfection by-products. Key regulatory issues addressed during the negotiation included: What is the magnitude of risks (cancer, developmental, reproductive, neurotoxic) from different types of disinfected water? How stringent should the limits be for disinfection by-products, given the large uncertainties in associated risk and potentially high costs of reducing such risks? How can we ensure microbiologically safe water while

controlling disinfection by-products? To what extent should alternative disinfectants to chlorine or precursor removal technologies be used to limit disinfection by-products? Should maximum contaminant levels (MCLs) target individual disinfection by-products, groups of disinfection by-products such as total trihalomethanes, or other surrogate parameters (total organic carbon, total organic halogens)? While these issues are not yet resolved, participants agreed on a process to resolve them over time.

During the regulatory negotiations, participants claimed that the national baseline incidence of cancer attributed to disinfection by-products in drinking water could range from less than 1 case per year to over 10,000 cases per year. Considering this range of baseline cancer risk, the U.S. EPA estimated that, under the Stage 1 disinfection by-product rule, the cost for avoiding one case of cancer could range from several hundred thousand dollars to several billion dollars per year.

Concern was also raised about the significance of reproductive and developmental risks from chlorinated waters, although no data were considered conclusive. This issue is particularly important from a regulatory perspective. If reproductive or developmental effects are of concern, then standards might be set to prevent the threshold risk level from occurring anywhere in the distribution system. This is unlike the existing total trihalomethanes (TTHMs) MCL or the newly proposed Stage 1 MCLs for TTHMs and haloacetic acids, where compliance is based on an annual average of concentrations measured throughout the distribution system.

Despite enormous uncertainties in risk from disinfection by-products, the Negotiating Committee recognized that the existing risks could be large, and therefore should be reduced. They agreed that the Stage 1 requirements were of sufficient benefit to be proposed for all system sizes. More stringent requirements, under a tentatively proposed Stage 2 rule, could not be agreed to until more conclusive health effects data became available. The Committee also agreed that the U.S. EPA should propose an enhanced surface water treatment rule (ESWTR) to prevent increases in microbial risk while systems complied with the disinfection by-product rules, and an information collection rule to gather additional occurrence and treatment data to support development of the ESWTR and Stage 2 disinfection by-product rule.

As part of its regulatory impact analysis (RIA) to predict national and local impacts from the ESWTR and disinfection by-product rules, the U.S. EPA is developing models for predicting possible changes in risk and costs that could result from different regulatory options. The U.S. EPA's current approach to the RIA is to tackle the problem from two fronts: (1) develop models that predict changes in exposure, risks, and costs resulting from different regulatory options intended to control for pathogens (e.g., different ESWTR rule options) while keeping constant the regulatory criteria that control for disinfection by-products (i.e., the Stage 1 disinfection by-product rule), and (2) develop models that predict possible changes in exposure, risk, and costs resulting from different regulatory options intended to control for disinfection by-products (i.e., Stage 2 rule options) while keeping the regulatory criteria constant that control for pathogens (e.g., a particular ESWTR rule option). Through iterative consideration of the outputs from these models, preferred regulatory strategies should become more apparent and the appropriate balance of risk should be achieved. However, good models cannot be established without much more exposure, health effects, and treatment information for disinfection by-products and pathogens.

During the negotiations the U.S. EPA estimated the compliance costs for systems to meet the disinfection by-product rule options while also being required to achieve less than one infection of giardia per 10,000 people per year. It was estimated that, if all systems in the United States were required to achieve the levels proposed under the Stage 2 disinfection by-product rule of 40 μg/L for TTHMs and 30 μg/L for haloacetic acids without restricting use of alternative disinfectants to chlorine, treatment systems would incur annual costs of $1.5 billion per year above those anticipated for Stage 1. Requiring systems to use precursor removal technologies, such as granular activated carbon (GAC) and membrane technology, to meet a 40/30 μg/L rule was projected to cost systems several more billion dollars per year than if they could meet such a standard using alternative disinfectants to chlorine. In systems using chlorine and serving more than 100,000 people, household water bills could increase by about $10 per year if the systems switched to ozone/chloramination and by about $50 per year if the systems switched to GAC to meet a 40/30 μg/L standard.

Important decisions on the Stage 2 disinfection/disinfection by-product rule will be difficult without establishing the magnitude and relative differences in health risks (acute, short-term, and chronic) from exposures to different types of waters (e.g., water with high bromide and high organic disinfection by-product precursors, water with low bromide and low organic disinfection by-product precursors, and water with high bromide and low organic disinfection by-product precursors) subjected to different types of disinfection (chlorine/chloramines, ozone/chloramines, and chlorine dioxide).

Disinfection By-products: From Source to Tap

Philip C. Singer
Department of Environmental Sciences and Engineering,
University of North Carolina

The objectives of municipal drinking water treatment are to provide a water that is free of harmful contaminants and that is aesthetically pleasing. Accordingly, water treatment facilities are designed to remove harmful impurities such as pesticides, radon, and pathogenic microorganisms, as well as aesthetically undesirable impurities such as taste and odor-causing substances, color, and turbidity. Additionally, the water must be treated in such a manner that it maintains its integrity during transport through the distribution system to the consumer's tap; corrosion control chemicals and a residual disinfectant are added for this purpose.

Typical processes used for treating drinking water include: coagulation, flocculation, sedimentation, and filtration to remove particulate material (turbidity) and color-causing organic material (humic substances); chemical oxidation processes for oxidation of reduced iron and manganese, taste and odor-causing organic material, and removal of color; chemical disinfection processes to inactivate pathogenic microorganisms, including bacteria, viruses, and protozoan cysts; and activated carbon adsorption to remove synthetic organic chemicals and taste and odor-causing organics.

The most common oxidants and disinfectants used are free chlorine, combined chlorine (monochloramine), ozone, and chlorine dioxide. Each of these tends to react with natural organic material (NOM) in the water to produce a variety of disinfection by-products, only a fraction of which have been identified and quantified. Examples of disinfection by-products include the trihalomethanes, haloacetic acids, haloacetonitriles, and chloral hydrate from chlorine; cyanogen chloride from

monochloramine; bromate, aldehydes, and organic acids from ozone; and chlorite and chlorate from chlorine dioxide. In the case of chlorine and ozone, the presence of bromide in the water leads to the formation of brominated by-products. In the case of ozone, a number of the by-products are biodegradable and, if not properly controlled, contribute to the potential formation of microbial biofilms in the distribution system.

The formation of disinfection by-products is influenced by the type, concentration, and point of application of the disinfectant, the type and concentration of the organic precursors (NOM), pH, bromide concentration, temperature, and contact time. Because of diurnal and seasonal variations in source water quality, variations in reaction kinetics with temperature, variations in distribution system contact time due to location relative to the treatment plant, and variations in contact time as a result of variable water quantity demands over time, the distribution of disinfection by-product concentrations at the consumers' taps may vary appreciably both spatially and temporally. Attempts have been made to model the formation of disinfection by-products as a function of these variables and to estimate consumer exposure to these disinfection by-products by coupling disinfection by-product formation models with distribution system residence time models. Coupling of these models shows great promise in being able to accurately predict disinfection by-product concentrations at the consumer's tap. Alternatively, consumer exposure to chlorinated disinfection by-products can be predicted with reasonable accuracy using chlorine demand (consumption) as a surrogate for disinfection by-product formation.

An Integrated View of Toxicology and Epidemiology

David G. Hoel
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Medical University of South Carolina

Concern about potential health risks of chlorination by-products in drinking water was first raised in 1974 by a study of the correlation of various types of cancers and consumption of drinking water supplied by the Mississippi River in Louisiana. A number of epidemiology studies followed which compared surface water with ground water as a drinking water source. This approach was later refined by correlating measured trihalomethane levels with age-standardized cancer mortality rates. Correlations between source of drinking water with water and cancer of the lung, gastrointestinal tract, and urinary bladder were found.

Because of the uncertainties associated with the correlation studies, case-control studies were conducted. These studies generally are better able to estimate individual exposures, as compared to correlation studies which use community exposure levels. Of the seven case-control studies, four used community exposures and three used individual exposure estimates. Of the four community exposure studies, one found only colon cancer, another found only rectal cancer, and the other two found no excess cancer. Of the three individual exposure studies, two reported increases in urinary bladder cancer while the third reported combined increases in colon and rectal cancers.

Overall, the studies are difficult to interpret, although the evidence points most strongly to cancer of the urinary bladder. Meta-analysis techniques have been applied to the data by Morris et al. (1992) who

reported significantly increased risks for bladder and rectal cancers associated with consumption of chlorinated drinking water.

A more recent area of concern has to do with the possible relationship between tap water consumption and pregnancy outcome. Studies in California originally suggested that abstaining from tap water during the first trimester of pregnancy reduced the risk of spontaneous abortion, although subsequent analyses attributed the association to recall bias.

Toxicology studies provide conflicting support for the epidemiological findings. For example, fetal resorption was slightly increased in a tap-water versus bottled water rat study. Carcinogenesis studies, on the other hand, show liver tumors and not bladder/colon tumors. Further, the quantitative risk estimated based on the animal studies is much less than the risk predicted by meta-analysis of human studies.

These studies have been reviewed by the U.S. EPA (1994) and the International Agency for Research on Cancer (IARC, 1991). IARC concluded that "there is inadequate evidence for the carcinogenicity of chlorinated drinking water in humans" and also gave the same statement with regard to experimental animals.

A panel of epidemiologists was added to the program to provide a more in-depth discussion of the available epidemiologic data and to provide recommendations on future research and study design issues. The abstract and edited transcript of the panel are provided later in this report.

Assessing the Health Risks of Drinking Water

Daniel Krewski
Environmental Health Directorate,
Health Protection Branch,
Health Canada

Although the general public perceives the potential health risks of drinking water contaminants to be low relative to other environmental risks, concerns about drinking water safety remain. In a recent national survey of health risk perception in Canada, 93% of respondents felt that the land, air, and water around us are, in general, more contaminated than ever before. Canadians expressed high expectations for environmental safety, with 62% of respondents believing that a risk-free environment is an attainable goal for Canada.

Toxicological and epidemiological studies continue to serve as the primary sources of information on the potential health risks of drinking water contaminants. Toxicological studies are able to identify potential hazards in controlled laboratory studies, although the application of toxicological data to human risk assessment can be subject to considerable uncertainty. Epidemiological studies avoid the need to extrapolate from nonhuman test systems to humans, but are generally less sensitive and are often subject to other uncertainties, including uncertainty about past exposures to the agent of interest as well as potential confounders.

Risk assessment methodologies developed for environmental hazards are used to evaluate the potential risks associated with drinking water contaminants. In the absence of evidence to the contrary, carcinogenic risk assessment is generally done under the assumption of low-dose linearity. This assumption is supported by theoretical considerations such as additivity to background, as well as experimental evidence indicating that the formation of DNA adducts by genotoxic carcinogens is linearly related to dose, even at very low dose levels. This assumption may be avoided if information on the mechanism of carcinogenesis suggests a nonlinear dose-response relationship in the low-dose region.

Risk assessment methods for most noncarcinogenic effects are based on the application of one or more uncertainty factors to a no-observed-adverse-effect level (NOAEL) observed in toxicological experiments. Another approach is based on the benchmark dose (BMD), defined as a 95% lower confidence limit on the dose associated with an excess risk of 5%. The BMD is a more precisely defined quantity than the NOAEL, and may be considered as an alternative to the NOAEL for risk assessment purposes. The BMD may also provide a means of integrating cancer and noncancer risk assessments, by serving as a reference dose either for the application of a suitable uncertainty factor or for linear extrapolation to lower doses.

Recent results have shown that carcinogenic potency expressed in rodent bioassays is highly correlated with the maximum tolerated dose used in such experiments. This correlation may be exploited to obtain preliminary estimates of cancer risk in advance of conducting a bioassay. The distribution of carcinogenic potency, which varies over some seven orders of magnitude, may also be used to define a threshold of regulation for chemical carcinogens. Although the threshold of regulation concept has been considered in dealing with trace levels of indirect food additives that migrate from packaging materials into food, it may also be of value in evaluating the risks of trace levels of contaminants in drinking water.

Risk assessment methods for time-dependent exposure patterns developed in recent years may be useful in evaluating the risk of drinking water contaminants that demonstrate substantial temporal variation. For agents with carcinogenic potential, the lifetime average daily dose (LADD) is often used to estimate potential lifetime risk. Time-dependent risk assessment methods can be used to obtain more accurate estimates of risk using the lifetime equivalent constant dose, or to place bounds on the magnitude of the error associated with the LADD. Although the LADD can lead to substantial overestimates of risk under certain conditions, it is not likely to underestimate risk by more than a factor of two- to five-fold under a range of plausible assumptions.

Pharmacokinetic models may be used to obtain estimates of the dose of the reactive metabolite reaching target tissues. In the presence of nonlinear pharmacokinetic processes, tissue doses obtained using physiologically based pharmacokinetic models may be expected to lead to more accurate risk estimates than external exposure measurements. In applying such models, however, it is important to take into account uncertainty in tissue dose predictions induced by uncertainty in the model's physiologic, biochemical, and metabolic parameters.

More generally, methods for uncertainty analysis may be used to provide a more complete characterization of the uncertainties associated with estimates of potential health risks. Using prior information on the uncertainties associated with exposure and pharmacokinetic and pharmacodynamic parameters, a distribution of risk estimates can be generated. This distribution provides an indication

of both the most likely risk, as well as likelihood of higher or lower risks occurring. Variability in exposure within the population of interest can also be taken into account with such distributional approaches to risk assessment.

In Canada, drinking water guidelines are established by the Drinking Water Subcommittee of the Committee on Environmental and Occupational Health. This Committee involves representatives from both the federal and provincial governments, and is the forum within which national drinking water quality objectives are set. In addition to risk, the Committee takes into account the availability and cost of water treatment technologies in establishing drinking water guidelines. In selecting contaminants for evaluation or re-evaluation, the Committee considers the frequency with which the agent is detected in drinking water supplies, the level at which the agent is present in water, and the potential for toxicity.

Estimates of risk are based on established risk assessment methodologies, including those described above. Current trends in drinking water risk assessment include a renewed emphasis on evaluation of the quality of data on which drinking water guidelines are based, a proposed six-category carcinogen classification scheme corresponding to that used in conjunction with the Canadian Environmental Protection Act (CEPA), and consideration of alternatives to the NOAEL such as the BMD. (The BMD is used in denominator of the exposure-potency index used to rank toxic substances in order of priority for risk management actions under CEPA.) The use of formal methods for uncertainty analysis is also being explored.

A new Drinking Water Safety Act is currently under consideration by the Canadian federal government. This proposed legislation would establish standards for water treatment devices used by homeowners, additives used by municipal drinking water authorities (including disinfectants, dechlorination agents, fluoride compounds, coagulants, water softening agents, and anti-corrosive or anti-scaling agents), and the various components used in different water systems from the water treatment facility to the end user.

Drinking water disinfectants such as chlorine and chloramine are known to produce a number of disinfection by-products, including trihalomethanes, haloacetic acids, haloacetonitriles, haloketones, chlorophenols, aldehydes, and others. Although every effort is made to reduce disinfection by-product concentrations to as low a level as possible, methods for controlling disinfection by-products must not compromise the effectiveness of water disinfection. Recent surveys have found that trihalomethanes and haloacetic acids are present at low levels in treated water samples in Canada, with higher levels found in the summer than in the winter.

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Session Discussion

Chaired by Denise E. Robinson
Health and Environmental Sciences Institute,
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DR. CONOLLY: Is the change from 100 μg/L to 80 μg/L in the trihalomethanes maximum contaminant level based on health effects data?

MR. REGLI: Health effects went into the decision, but it was more influenced by potential risks, cost considerations, and occurrence information. The negotiated rulemaking people considered various options and evaluated potential risk reductions, what utilities would have to do to meet different occurrence levels in the distribution system, and what the cost would be.

DR. HAUCHMAN: Dr. Singer, some of the toxicology data suggest that there are specific trihalomethanes and, perhaps even more importantly for some endpoints, haloacids that may be the most important from a health perspective. How difficult would it be to extend your analysis to some of the other types of by-products—for example, the haloacids?

DR. SINGER: If we believe, as I do, that there are meaningful correlations between the haloacetic acids as a group and the trihalomethanes as a group (particularly in low-bromide waters where you are not confounded by the bromide influence), then if we can model trihalomethanes effectively, we can use the correlations to make a reasonably good estimate of the di- and trichloroacetic acid levels at those same locations. We would like to try to make some predictions of this type and then make measurements to verify that this is indeed the case. However, developing a simple model when bromide species are involved would be much more difficult.

DR. BIRNBAUM: No one has yet mentioned the issue of susceptible populations. What may be an insignificant exposure to one group may be very significant to another. With drinking water, we may have populations who are at enhanced risk because of age, disease status, or whatever.

DR. KREWSKI: I agree. In Canada, we try to take this concern into account to a limited extent through uncertainty factors, through looking at markers of susceptibility. But we do not go nearly as far as we could. This is a concern that we should keep in mind during subsequent discussions.

SESSION 2
CHLORINATION

Session Introduction

Michael Cox
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The disinfection of public drinking water supplies with chlorine has been an important component of the public health strategy for preventing the transmission of waterborne infectious disease. The use of chlorine has dramatically reduced the waterborne disease rate in the U.S. and has provided safe drinking water to individuals. However, since the mid-1970s, there has been growing concern about the possible health effects from a number of by-products of chlorination, including trihalomethanes, haloacetic acids, chloral hydrate, haloacetonitriles, haloketones, and chloropicrin. These potential health effects from chlorinated waters are of special concern because of the large number of exposed individuals. An estimated 75% of all public water systems use chlorine as their primary disinfectant. Collectively, these systems service more than 150 million people, all of whom are exposed to chlorination by-products.

While there is little debate concerning the benefits of chlorination in reducing the rate of waterborne disease, there is a growing public perception that the drinking water delivered by public water supplies may not be safe. This was recently reinforced by the outbreak of *Cryptosporidium* in Milwaukee and several epidemiology studies concerning the potentially large number of cancer cases linked to the consumption of chlorinated surface waters. As public health professionals, can we say with certainty that, based on the current data, the drinking water delivered to the public is safe?

To answer this question, we need to review the data from the toxicological and epidemiology studies on chlorinated waters. Ideally, the adverse effects from the toxicity and epidemiology studies would be similar. This is, however, not the case. The findings of cancer from the toxicological studies show impacts in the liver, kidney, and large intestines of laboratory animals exposed to individual trihalomethanes (THMs) or haloacids, while the findings from epidemiology studies indicate a slight increased risk for bladder, colon, and rectal cancers in individuals exposed to chlorinated surface water for many years. Most toxicological research concerning disinfection by-products (DBPs) has focused on the trihalomethanes, especially chloroform. The trihalomethanes have been found to produce liver, kidney, and large intestinal cancers in rodent studies. In the mid-1980s, other groups of chlorinated by-products, including chlorinated acetic acids, chlorinated furanones, and haloacetonitriles, were found to exhibit a wide spectrum of effects, including cancer, neurotoxicity, mutagenicity, and reproductive and developmental effects, in laboratory animal studies.

Epidemiology research has attempted to evaluate the association of chlorinated water with several adverse outcomes, including cancer, cardiovascular disease, and adverse reproductive outcomes. Several of studies have found a small increase in bladder cancer, and rectal cancers. In some cases, associations were linked with duration of exposure and volume of water consumed. Based on a review of all the data, the U.S EPA and the International Agency for Research on Cancer (IARC) have concluded that, due to problems with study design and characterization of exposures, the data are

inadequate to link cancer in humans with consumption of chlorinated water at this time. Other scientists, however, have concluded that the epidemiology data, when combined with the toxicological data, is compelling and that consumption of chlorinated water potentially increases the cancer risk to individuals. With regard to cardiovascular disease, animal studies in the mid-1980s indicated a potential increase in the serum lipid levels in animals exposed to chlorinated water. However, in a cross-sectional study in humans that compared chlorinated and unchlorinated water supplies with varying water hardness, no adverse effects on serum lipid levels were found. Finally, recent studies have associated increased incidence of decreased birth weight, prematurity, intrauterine growth retardation, and neural tube defects with chlorinated water. As with the other adverse outcomes from the epidemiology studies, there is considerable debate in the scientific and legal community on the significance of these findings.

Based on these findings, what can we conclude about the safety of our water supply? Can we ensure members of the public that the water they drink is safe? How do we reconcile the differences in the findings from the toxicological and epidemiology studies? Should we focus our research efforts on individual chemicals or mixtures? And on which compounds should we focus our research (THMs, haloacetic acids, brominated species)?

We hope to begin to answer some of these questions in this session on chlorination. The first speaker will be Ms. Pat Fair who will provide an overview of the factors that influence the formation of chlorination by-products. Dr. Gary Klinefelter will provide an overview of the current research on the reproductive effects from several of the most prevalent chlorination by-products. Dr. Sid Hunter will discuss the effects of haloacetic acids on embryonic development. Dr. Byron Butterworth will present findings from his laboratory on the cancer risk posed by chloroform. Dr. Anthony DeAngelo will discuss the Water Carcinogenesis Research Program at the U.S. EPA. Dr. Richard Bull will discuss his current work on elucidating the carcinogenic properties of the brominated haloacetates. Finally, Dr. Jouko Tuomisto will discuss his work in the area of genotoxicity and carcinogenicity of MX and other chlorinated furanones.

Influence of Water Quality on Formation of Chlorination By-products

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Chlorine reacts with naturally occurring organic/inorganic material in water to produce hundreds of (DBPs). Halogenated organic compounds are most frequently associated with chlorine, but nonhalogenated compounds (e.g., aldehydes) are also formed as the result of oxidation reactions. The availability of analytical techniques to detect and quantitate specific DBPs has limited our knowledge of formation to a few classes of halogenated compounds. Most research has focused on THMs, haloacetic acids (HAAs), chloral hydrate (CH), haloacetonitriles (HANs), haloketones (HKs), and chloropicrin (CP) (See Table 1.).

Several factors influence the type and quantity of DBPs formed in chlorinated drinking water, but the ones of most significance from a predictive perspective are: concentration of organic precursor material, pH during and after chlorine application, and bromide ion concentration. Using these factors,

one can make general predictions concerning the distribution and concentration of the DBPs listed above.

Total organic carbon (TOC) is the measurement currently used to indicate the amount of organic precursor material present in water. In general, as the concentration of TOC increases, the concentrations of DBPs also increase, because there is more organic material to react to form by-products.

While the quantity of TOC available for reaction determines the total quantity of DBPs formed, the pH at which the reaction occurs influences the distribution of the various DBP classes. The formation of THMs increases as the pH increases, while the formation of some HAAs decreases as pH increases. Thus, the predominant class of DBPs shifts from HAAs to THMs as the pH increases (e.g., see Table 2).

The stability of some DBPs is also affected by pH. For example, HANs and CH undergo base-catalyzed hydrolysis, which means the half-life decreases as the pH increases above 7 (e.g., see Table 2). However, prediction of concentrations within a distribution system is complicated by both formation and decomposition reactions occurring when a free chlorine residual is also present. Fortunately these classes of compounds occur at relatively low concentrations relative to THMs and HAAs, so the range of concentrations found in drinking water is narrow.

The distribution of halogenated compounds within a class is determined by the amount of bromide ion in the water. Chlorine oxidizes the bromide ion to hypobromous acid, which quickly reacts with organic precursor material to form brominated DBPs. When bromide ion is present, formation of the brominated and mixed bromochloro analogs is favored over formation of the chlorinated species.

Table 1: Occurrence of Major Chlorination By-products in Drinking Water Samples[1]

DBP	N	MEDIAN (ug/L)	MAXIMUM (ug/L)
TRIHALOMETHANES			
Chloroform	87	25.0	240.0
Bromodichloromethane	87	9.5	90.0
Dibromochloromethane	87	1.6	36.0
Bromoform	87	<0.2	7.1
HALOACETIC ACIDS			
Dichloroacetic acid	85	15.0	74.0
Trichloroacetic acid	86	11.0	85.0
Bromochloroacetic acid	43	3.2	49.0
Monochloroacetic acid	87	1.3	5.8
Dibromoacetic acid	84	<0.5	7.4
Monobromoacetic acid	87	<0.5	1.7
HALOACETONITRILES			
Dichloroacetonitrile	84	2.1	10.0
Bromoacetonitrile	83	0.7	4.6
Dibromoacetonitrile	80	<0.2	3.2
Trichloroacetonitrile	83	<0.2	0.3
HALOKETONES			
Dichloropropanone	83	0.4	2.5
Trichloropropanone	83	1.0	8.3
OTHER			
Chloropicrin	83	0.4	3.7
Chloral Hydrate	84	2.1	25.0
Chlorate Ion[2]	111	161.0	9,180.0

DBP: Disinfection By-product
N: Number of water systems sampled

[1] Data from U.S. Environmental Protection Agency studies in drinking water samples collected at the treatment plants (1987-1993). Samples are biased to systems expected to have high DBP concentrations.

[2] Data from American Water Works Association Research Foundation project entitled "Minimizing Chlorate Ion Formation in Drinking Water When Hypochlorite Ion is the Chlorination Agent" by Gilbert Gordon, Luke Adam, and Bernard Bubnis. Samples of finished water were collected at treatment plants using hypochlorite solutions for drinking water disinfection.

Table 2[3]: Conditions of formation of DBPs

By-product	Conditions of Formation		
	Chlorination at pH 5	**Chlorination at pH 7**	**Chlorination at pH 9.4**
TTHM	Lower formation		Higher formation
TCAA	Similar formation		Lower formation
DCAA	Similar formation - perhaps slightly higher at pH 7		
MCAA	At concentrations <5 μg/L, trends not discernible		
DBAA	At concentrations <1 μg/L, trends not discernible		
CH	Similar formation		Forms within 4 h; decays over time to <5 μg/L
CP	At concentrations <1 μg/L, trends not discernible		
DCAN	Higher formation	Forms within 4 h; then decays over time to <5 μg/L	At concentrations <2 μg/L, trends not discernible
BCAN	At concentrations <2 μg/L, trends not discernible		
DBAN	At concentrations <0.5 μg/L, trends not discernible		
TCAN	Not detected		
111-TCP	Higher formation	At concentrations <2 μg/L, trends not discernible	Not detected

[3] Reprinted from "Formation and Control of Non-Trihalomethane Disinfection By-products," published in *Journal AWWA*, Vol. 81, No. 8 (August 1989), by permission. Copyright © 1989, American Water Works Association.

Recent Reproductive Effects Associated with Disinfection By-products

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In the last several years an effort has been made to provide reproductive toxicity data for priority chemicals in the growing list of DBPs. The chemicals for which at least limited reproductive data have been gathered include chloral hydrate (CH), bromodichloromethane (BDCM), trichloroacetic acid (TCAA), dichloroacetic acid (DCAA), dibromoacetic acid (DBAA), bromoacetic acid (BAA), and sodium bromate (B) (See following table.). Only epididymal sperm motion has been evaluated following exposure to CH, BDCM, and TCAA and the observed effects were modest. Sodium bromate decreases in number of sperm stored in the epididymis. Finally, while no reproductive effects are seen after BAA exposure, a multitude of reproductive endpoints are compromised following exposure to the haloacetic acids, DBAA and DCAA.

To focus our research on elucidating the mechanisms of action of DBAA, DCAA, and other haloacetic acids in the male reproductive system, and to continue to provide reproductive data on high priority DBPs, we have recently implemented a tiered-testing strategy in collaboration with the National Toxicology Program (NTP). A prioritized list of DBPs including sodium bromate, chlorodibromomethane, dibromoacetonitrile, bromochloroacetic acid, and dibromochloroacetic acid was established based on occurrence data. These chemicals are currently being screened in NTP's 28-day protocol which evaluates the ability of suspected toxicants to alter reproductive competence in the adult male, adult female, and pregnant female. Any DBP that produces a dose-related reproductive effect will either be studied more extensively within the intramural program at the U.S Environmental Protection Agency's National Health and Environmental Effects Research Laboratory (NHEERL) or in NTP's continuous breeding protocol.

Toth et al. (1992) found that subchronic exposure to DCAA results in a significant reduction in fertility at 125 mg/kg, and alterations in spermatogenesis as well as significant decreases in the number, morphology, and motility of epididymal sperm at 62.5 mg/kg. Recently, Linder et al. (1995) demonstrated that subchronic exposure to DBAA produces remarkably similar effects. While only the highest dose tested (250 mg/kg) caused a significant reduction in fertility, there was a significant dose-related decline in the number of males that could produce two liters successfully with a decrease evident even at the lowest dose level (10 mg/kg). Moreover, there was a dose-related decrease in the number of copulatory plays. This, together with the fact that artificial insemination revealed that sperm stored in the epididymis were fertile while marked infertility was observed in males that mated naturally, suggests that DBAA compromises the normal reproductive behavior of adult males and that this effect may be manifest at rather low exposures.

The DBAA study also describes effects on spermatogenesis as well as on epididymal sperm number, morphology, and motility at 50 mg/kg. Of particular interest are the histological effects that DBAA induces in the testis, as these are effects that undoubtedly can also play a role in reducing fertility. Interestingly, it appears that decreases in sperm quality preceding decreases in sperm quantity. The process of spermatogenesis in inherently inefficient in the human male, with sperm production per gram testis being only 20% of that in the rat, and with up to 50% of the sperm that are produced being

qualitatively abnormal. Thus, it stands to reason that any toxic insult which compromises spermatogenesis in the rat, either quantitatively and/or qualitatively, might exert a similar and more consequential effect in man.

The DBAA-induced effects on spermatogenesis are rather unique. In the rat, exposure to DBAA results not only in delayed spermiation (the release of mature spermatids), but also in the formation of atypical residual bodies and fused sperm. These manifestations suggest that the Sertoli cell in the testis is compromised, as the Sertoli cell is responsible for the normal release of spermatids into the lumen of the seminiferous tubule, the phagocytosis of residual germ cell cytoplasm, and the separation of mature sperm prior to their release. Most recently, using parallel 14-day exposures to either DCAA or DBAA, we have found that DCAA produces similar spermatogenic effects as well as decreases in epididymal sperm motility. However, on a molar basis, the effects of DCAA are less severe than those of DBAA.

In an attempt to uncover the mechanism by which DBAA and DCAA compromise spermatogenesis, we have begun to isolate those stages of seminiferous tubules that are intimately involved in late spermatid maturation and release and presumably contain compromised Sertoli cells. Tubules are isolated from *in vivo* exposed animals and subsequently evaluated *in vitro*. Thus far, electron microscopic and immunocytochemical evaluations have not yet provided any additional information. We now are beginning to evaluate proteins that are synthesized and secreted by tubules isolated from control and DBAA-treated animals. A DBP-induced increase or decrease in the expression of specific proteins may provide us with insight into the cause of the defect in spermatogenesis. For instance, it is possible that particular germ cell adhesion molecules are over-expressed or that proteases requisite for spermatid release are under-expressed. Such changes would prompt the use of specific proteins as biomarkers for DBP-induced testicular toxicity.

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Status of Reproductive Toxicity Research for Disinfection By-products[1]

	REPRODUCTIVE TOXICITY		
	MALE	FEMALE	COUPLE
DISINFECTANTS			
CHLORINE			
CHLORINE DIOXIDE			
CHLORITE			
CHLORAMINE			
TRIHALOMETHANES			
CHLOROFORM			
BROMOFORM			
BROMODICHLOROMETHANE			
CHLORODIBROMOMETHANE			
HALOGENATED ACIDS			
CHLOROACETIC ACID			
DICHLOROACETIC ACID			
TRICHLOROACETIC ACID			
BROMOACETIC ACID			
DIBROMOACETIC ACID			
TRIBROMOACETIC ACID			
BROMOCHLOROACETIC ACID			
DIBROMOCHLOROACETIC ACID			
DICHLOROBROMOACETIC ACID			
HALONITRILES & ALDEHYDES			
CHLOROACETALDEHYDE			
CHLOROACETONITRILE			
DICHLOROACETONITRILE			
BROMOACETONITRILE			
DIBROMOACETONITRILE			
TRIBROMOACETONITRILE			
BROMOCHLOROACETONITRILE			

[1] Shaded areas indicate where research has been conducted.

	REPRODUCTIVE TOXICITY		
	MALE	FEMALE	COUPLE
KETONES			
1,1-DICHLOROPROPANONE			
1,3-DICHLOROPROPANONE			
1,1,3,3-TETRACHLOROPROPANONE			
PENTACHLOROPROPANONE			
HEXACHLOROPROPANONE			
MISCELLANEOUS			
SODIUM BROMATE			
CHLORAL HYDRATE			
CHLOROPICRIN			
1,2-DICHLOROPROPANE			
1,2,3-TRICHLOROPROPANE			

[1] Shaded areas indicate where research has been conducted.

Effects of Haloacetic Acid Water Disinfection By-products on Embryonic Development

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Many DBPs have been shown to be developmental toxicants. Among DBPs, the haloacetic acids (HAAs), trichloroacetic acid (TCAA) and dichloroacetic acid (DCAA), are teratogenic when administered to rats during organogenesis (Smith et al., 1989; Smith et al., 1992; Epstein et al., 1992). Although structurally related, the relative toxicities of the HAAs may be different as indicated by the observation that dibromoacetic acid (DBAA) is a more potent toxicant than DCAA in the male rat reproductive system (Toth et al., 1992; Linder et al., 1994). To determine if there are differences in potencies in developmental endpoints, the effects of chloro- and bromo-substituted acetic acids on embryonic development were assessed.

Direct effects of these toxicants on mouse conceptuses were evaluated using the whole embryo culture system. Advantages of this approach include a precise control of the embryonic age at exposure, as well as the concentration and duration of exposure to the toxicant. Neurulation staged CD-1 mouse conceptuses with 3-6 pairs of somites were placed into culture medium containing the xenobiotics, and embryonic development was evaluated following a 26-hour period. Over this interval, growth and development of embryos *in vitro* mimics that *in vivo* (Sadler, 1979). The HAAs evaluated were TCAA, DCAA, chloroacetic acid (MCAA), tribromoacetic acid (TBAA), DBAA, bromoacetic acid (MBAA), bromochloroacetic acid (BCAA) and acetic acid (AA). All of the HAAs evaluated affected development and produced neural tube defects (NTDs). Many of the HAAs also produced dysmorphogenesis of the heart, as well as the craniofacial (e.g., eye and pharyngeal arch) region (Hunter et al., 1996). Using NTDs as the morphological endpoint, the concentration required to produce a 5% increase in abnormalities was estimated using a log-logistic model (Allen et al., 1990). The concentration of toxicant producing a 5% increase in the incidence of NTDs ranged from 4.2μM for MBAA to 323μM for DCAA. The ranking of the toxicants in order of increasing potency is DCAA < AA < TBAA < TCAA < DBAA < BCAA < MCAA < MBAA. Although the monosubstituted haloacetic acids are most potent, there is no consistent relationship between the degree of halogenation and potency as monitored by the concentration calculated to induce a 5% increase in the incidence of NTDs. The two toxicants with known teratogenic effects *in vivo* (DCAA and TCAA) are among the least potent of the HAAs with regard to induction of neural tube defects *in vitro*.

The developmental toxicities of dibromoacetic and dichloroacetic acids were evaluated using an *in vivo* screening protocol developed by Chernoff and Kavlock (1982). Each chemical was administered by gavage to CD-1 mice on gestation days 6-15. Dibromoacetic acid, administered at 25-800 mg/kg/day, produced signs of maternal motor depression at 800 mg/kg/day. There was a dose-dependent delay in parturition at all levels of treatment. There was a trend towards an increase in prenatal mortality at 600 mg/kg/day and an increase at 800 mg/kg/day. Postnatal mortality was increased at both 600 and 800 mg/kg/day. Gross external examination of pups revealed tail defects (kinked, short, or absent) at both 600 and 800 mg/kg/day. In contrast, DCAA administered at 30-900/mg/kg/day produced no maternal effects. A trend towards delayed parturition and increased perinatal loss were present at 720 and 960/mg/kg/day. These doses of DCAA are higher than that required to alter growth and development

in rats (140/mg/kg/day) reported by Smith et al., 1992. Although the preliminary evaluation of DCAA-induced effects in mice remains inconclusive, DBAA appears to be at least twice as potent as DCAA (2.8/mmol/kg/day DBAA compared to 5.6/mmol/kg/day for DCAA) in mice when the lowest doses to induce effects are compared.

Preliminary work on a possible mechanism of DCAA-induced developmental toxicity has focused on the generation of reactive oxygen species (ROS) produced by perturbation in embryonic metabolism. Unlike adult tissues, early organogenesis-stage mouse embryonic carbohydrate metabolism is characterized by high rates of glycolytic metabolism with very little utilization of the Krebs cycle (Hunter and Sadler, 1988). Since exposure to DCAA up-regulates pyruvate dehydrogenase activity (Crabb et al., 1987) this would suggest that generation of ROS may be responsible for DCAA-induced embryonic effects. To test this hypothesis, Cu,Zn-superoxide dismutase (SOD) was added to culture medium in combination with DCAA. SOD did not induce malformations when added alone. When embryos were exposed to DCAA in combination with SOD, the incidence of malformations was the same as that produced by DCAA alone (Hunter, unpublished result). Since addition of exogenous SOD prevents pyruvate-induced malformations (Hunter et al., unpublished result), as well as abnormalities induced by a wide variety of toxicants [e.g. ethanol (Kotch et al: 1995), arsenic (Tabacova et al., 1996), hyperglycemia (Eriksson and Borg, 1991)], this suggests that DCAA-induced defects are not primarily due to generation of superoxides.

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Assessing the Cancer Risk of Chloroform

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Chloroform is one of the best-studied examples of a nongenotoxic-cytotoxic carcinogen (Butterworth et al., 1992). Chloroform is a common environmental pollutant produced by some industrial processes and in trace amounts from the chlorination of drinking water. Inhalation and ingestion are the most important routes of exposure. Chloroform induces liver cancer in male and female $B6C3F_1$ mice when administered by gavage but not when given in the drinking water, kidney cancer in male but not female Osborne-Mendel rats when given by gavage or in the drinking water, and kidney cancer in male but not female BDF_1 mice when administered by inhalation (NCI, 1976; Jorgensen et al., 1985; Yamamoto et al., 1994). No cancer was induced in either male or female F-344 rats exposed to chloroform by inhalation (Yamamoto et al., 1994).

The doses employed in these cancer studies have been criticized as being excessive and not relevant to common environmental exposures. The weight of evidence indicates that tumor formation was secondary to induced target cell killing and regenerative cell proliferation in every case. Cytotoxicity and regenerative cell proliferation are seen in the mouse liver when chloroform is given by gavage but not when given in the drinking water, suggesting that tumor formation is secondary to these effects (Larson et al., 1994). Dosimetry studies suggest that the reason toxicity is not observed when chloroform is given in drinking water is because the compound is not delivered or converted to toxic metabolites at a rate sufficient to kill hepatocytes.

Carcinogenic doses of chloroform given to male Osborne-Mendel rats by gavage also induce kidney toxicity and regenerative cell proliferation (Templin et al., 1996b). An Osborne-Mendel male rat kidney tumor response was seen when chloroform was given in the drinking water but not only at the

highest concentration of 1,800 ppm, which also resulted in a 60% decrease in water consumption and a 25% loss in body weight (Jorgensen et al., 1985). The nutritional status of this high-dose group was uncertain, and the validity of extrapolation of the tumor response to lower doses is questionable.

Chloroform administered to F-344 rats by inhalation for up to 90 days produced responses for toxicity and regenerative cell proliferation that are consistent with the lack of induced tumors in a recently completed two-year chloroform inhalation study (Yamamoto et al., 1994; Templin et al., 1996c). Chloroform also produces toxicity and cell proliferation in the nasal turbinates of rats and mice, but these lesions do not progress to cancer (Yamamoto et al., 1994; Méry et al., 1994).

Chloroform given by inhalation to BDF_1 mice yields severe kidney toxicity in the male mice (unpublished observation), consistent with the suggestion that the kidney tumors formed at 30 and 90 ppm were secondary to chloroform-induced cytolethality and regenerative cell proliferation in that target organ (Yamamoto et al., 1994). In fact, in that cancer study, the male BDF_1 mice had to have exposure concentrations increased slowly over a period of weeks to adapt to a concentration high enough to produce cancer. Exposing male BDF_1 mice directly to atmospheres of 30 or 90 ppm of chloroform results in significant deaths from acute kidney failure (Templin et al., 1996a), and calls into question the validity of cancer data produced at these doses.

When applied to the mouse liver tumor data from the chloroform gavage study, the linearized multistage model estimates a virtually safe dose (VSD) as an increased lifetime risk of cancer of one in a million at an airborne exposure concentration of 0.000008 ppm (U.S. EPA, 1985; 1994) . Assuming that chloroform-induced female mouse liver cancer is secondary to events associated with necrosis and regenerative cell proliferation, then no increases in liver cancer in female mice would be predicted at the No-Observed-Effect Level of 10 ppm or below, based on the inhalation toxicity and cell proliferation study (Larson et al., 1996). Applying an uncertainty factor of 1,000 yields an estimate of a VSD at an airborne concentration of 0.01 ppm. This estimate relies on inhalation data and is more consistent with the mode of action of chloroform.

Predictions of cancer risk from chloroform based on these mechanistic data are lower by orders of magnitude than predictions based on the default risk assessment procedures. No increased cancer risk would be expected with the trace amounts of chloroform commonly found in air or drinking water. Risk assessments are the basis of regulatory standards and should be based on the best science available.

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The Carcinogenicity of Disinfection By-Products in Drinking Water: Results from the Water Carcinogenesis Research Program at the U.S. Environmental Protection Agency's National Health and Environmental Effects Research Laboratory

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The Safe Drinking Water Act (SDWA) of 1974 required the U.S. EPA to establish national interim primary drinking water regulations that applied to public drinking water systems and that specified "contaminants which, in the judgement of the Administrator, may have adverse effects on the health of persons." Amendments to the SDWA in 1986 mandated EPA to establish maximum contaminant level goals (MCLGs) for 83 specific contaminants and for other drinking water contaminants that might have adverse effects on public health. Additionally, the Agency was mandated to establish a drinking water priority list of contaminants for which maximum contaminant levels (MCLs) and MCLGs were to be set. Disinfection by-products were prominent on the list.

The Water Carcinogenesis Research Program was established to respond to the regulatory needs of the EPA Office of Water (OW) by developing a smaller-scale cancer bioassay program. The goals of the program are to (1) identify drinking water chemicals that are carcinogenic in rodent bioassays, (2) provide statistically valid data on cancer potency, (3) provide information on the mechanisms(s) of chemical carcinogenesis, and (4) develop biologically based dose-response (BBDR) risk assessment models. Meeting these goals will allow EPA's National Health and Environmental Effects Research Laboratory (NHEERL) to respond in a timely manner to specific needs of the Office of Water that may occur because of (1) large numbers of toxic disinfection by-products, (2) mandates of the SDWA that require compound-by-compound regulation of drinking water contaminants, (3) Agency risk policy requiring specific assessment procedures for carcinogens, and (4) long lead times to nominate and place chemicals of interest in the National Toxicology Program's Cancer Bioassay Program.

In collaboration with the Office of Water, the most important disinfection by-products were selected from the chemical priority list for investigation. These include the (1) haloacetic acids (i.e., dichloroacetic and trichloroacetic acids and their brominated analogues), (2) haloacetaldehydes (i.e., chloral hydrate and chloroacetaldehyde), (3) trihalomethanes (i.e., chloroform, bromodichloromethane, and bromoform), and (4) inorganic by-products (i.e., bromate and chlorate).

The results from studies on the priority disinfection by-products carried out in the NHEERL Water Carcinogenesis Program are summarized in the following table. These results were presented, together with results from investigations into underlying mechanism(s) of action. The data were discussed within the framework of current knowledge about the carcinogenicity of these disinfection by-products and how they might be used for risk assessment.

STATUS/RESULTS OF EPA WATER CARCINOGENESIS
RESEARCH PROGRAM FOR DISINFECTION BY-PRODUCTS

DBPs	SPECIES	COMPLETION DATE	COMMENTS
Chloroacetic acid	Rat, Mouse	Completed	(-) both species
Dichloroacetic acid	Rat, Mouse	Completed	(+) both species in liver
Trichloroacetic acid	Rat, Mouse	Completed	(+) mouse liver, (-) rat
Bromochloroacetic acid	Mouse	1996	Screening study only; main focus liver and kidney
Bromodichloroacetic acid	Mouse	1996	Same as above
Dibromoacetic acid	Mouse	1996	Same as above
Chloral hydrate	Rat, Mouse	Completed	(+) mouse in liver, (-) rat
Chloroform	Rat	Completed	Limited bioassay; part of comparative kidney tumor study
Bromodichloro-methane	Rat, Mouse	Completed	(-) mouse and weak response in rat liver
Bromate	Rat, Mouse	1997	In-life complete Oct. 1995
Bromoform	Male Rat, Mouse	1999	Start FY96
Chlorate	Male Rat, Mouse	1999	Start FY96

Carcinogenic Properties of Brominated Haloacetates

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Haloacids are common by-products of drinking water disinfection processes. The most prevalent group are the haloacetates (HAs). Brominated HAs are found when the applied oxidant is capable of activating bromide ion present in the treated water to hypobromous acid.

Work in our laboratory has focused on how bromine substitution modifies responses of the liver to the haloacids. We have also conducted limited evaluations of the carcinogenic potential of three brominated HAs [dibromoacetate (DBA), bromochloroacetate (BCA), and bromodichloroacetate (BDCA)] in B6C3F$_1$ mice and F344 rats.

Dichloroacetate (DCA) has induced hepatic tumors in mice and rats whereas trichloroacetate (TCA) is only active in mice. The carcinogenic activity of TCA was associated with peroxisome proliferation, while the carcinogenic activity of DCA appeared related to changes in glycogen metabolism. The effects of DCA and TCA on the replication of normal hepatocytes were similar; an initial stimulation that eventually became inhibitory. DCA stimulated cell division within *c-jun*-expressing areas of DCA-induced lesions, but replication within TCA-induced lesions appear independent of continued treatment./

BDCA, DBA and BCA increased hepatic tumors in mice. Similar doses to those required of DCA were involved, but the latent period was longer and the tumor multiplicity reduced with brominated HAs. Increased numbers of neoplastic lesions were found in the mouse lung with brominated haloacetates. Histological classification of these lesions has not been completed, but those produced by DBA appeared to include a significant number of adenocarcinomas. DBA also increased the numbers of aberrant crypt foci in the colon of male F344 rats.

Only DBA consistently increased cyanide-insensitive acyl-CoA oxidase activity in the liver of mice. These increases were not, however, maintained with chronic treatment. While histochemical evidence of glycogen accumulation was a consistent finding in later stages of haloacetate treatment, chemically measured hepatic glycogen levels with the brominated haloacetates at four weeks of treatment were not significantly greater than in controls. This is in sharp contrast with DCA which induces maximal increases in hepatic glycogen concentrations with as little as two weeks of treatment. There was a tendency towards increased rates of replication of normal hepatocytes with all of the haloacetates, but only DBA produced a consistent response that was maintained through 28 days of treatment.

Substantial quantitative differences in the activity of different pathways of haloacetate metabolism were evident in mice and rats. In addition, the activity of certain of the metabolic pathways in rats is substantially modified by subcarcinogenic treatment regimens with DCA. Bromine substitution considerably increases the extent of trihaloacetate metabolism in both mice and rats. The more rapid rate of metabolism of brominated haloacetates appears to be associated with significant evidence of oxidative damage to DNA (i.e., formation of 8-OH-deoxyguanosine), an effect that was only marginally apparent with either TCA or DCA.

These data indicate some similarities in the toxicologic and carcinogenic effects of brominated and chlorinated haloacetates. However, it appeared unlikely that these chemicals were acting by a single mechanism. Moreover, the predominant mechanism appeared to vary with individual haloacetates. This apparently contributed to a greater diversity of target organs and the potential for genotoxic effects with brominated than chlorinated haloacetates. Thus, the carcinogenic risks attributable to the individual members of this class are likely to vary significantly at low doses. (Supported by NIEHS Grant No. R01-ES04648, AWWARF/NWRI No. 701-92/699-516-92, and EPA CR-819562.)

Genotoxicity and Carcinogenicity of MX and Other Chlorinated Furanones

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In the mid-1980s, Finnish drinking waters were found to be highly mutagenic after chlorination of surface water (Vartiainen and Liimatainen, 1986). About one-third of the mutagenicity was explained by a furanone compound, 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (Kronberg and Vartiainen, 1988). The mutagenicity of this furanone, called "MX," was exclusively high in *Salmonella typhimurium* TA100. In fact, it is one of the most potent mutagens known for this tester strain. These findings have initiated two overlapping lines of research: correlation studies of mutagenicity with several types of cancer in epidemiological studies, and laboratory studies on the genotoxic and carcinogenic properties of MX, one of the key compounds in the acidic nonvolatile fraction of chlorination products.

In so called ecological epidemiological studies, increased risk ratios have been found for six cancers: bladder, kidney, stomach, pancreatic cancer, Hodgkin's disease, and nonHodgkin's lymphoma (Koivusalo et al., 1994, 1995). Since these studies used aggregate data, both a cohort study analyzing a number of different malignancies and a case-control study on kidney and bladder cancers are being conducted to further assess the risk.

MX has been screened for general toxicity and pharmacokinetic profile. This compound seems to be a reactive, rapidly disintegrating compound that binds avidly to plasma albumin and may thus be partially protected from metabolism (Komulainen et al., 1992; Haataja et al., 1991). Acute toxicity is moderate (Komulainen et al., 1994), and there is little indication of delayed toxic damage in rats (Vaittinen et al., 1995).

Genotoxicity studies also suggest that various factors may inhibit the mutagenic activity of MX in cultured cells. MX was much more potent in causing ouabain resistance mutation, sister-chromatid exchanges, and chromosome aberrations in CHO cells *in vitro* when the cells were treated in buffer compared with treatment in McCoy's 5A with or without fetal calf serum in the exposure medium (Mäki-Paakkanen et al., 1994). The results obtained by Watanabe et al. (1994) show that the mutagenicity of MX is strongly inhibited by L-cysteine and glutathione; McCoy's 5A medium contains both these components.

Analyses of micronuclei in the bone marrow of mice (acutely) exposed to MX have yielded negative results (Meier et al., 1987; Tikkanen and Kronberg, 1990). However, clearly significant increases in

sister-chromatid exchanges were observed in rat peripheral lymphocytes after subchronic administrations of MX (Jansson et at., 1993). Also, administration of a high dose of MX on three consecutive days caused sister chromatid exchanges and micronuclei in peripheral lymphocytes and sister chromatid exchanges in the kidney cells of rats (Mäki-Paakkanen and Jansson, 1995). We believe that the metabolism of MX and/or its avid binding to proteins or other macromolecules suppresses its inherent genotoxic activity.

The mechanism by which MX causes genotoxic effects is not known. Studies done in our laboratory (Hyttinen et al., 1995) show that MX induces predominantly G:C→T:A transversions in TA100 cells (87% with a 3:1 preference for the second position of the *hisG46* [CCC] target codon). Another mutagenic chlorohydroxyfuranone (less potent than MX), 3,4-dichloro-5-hydroxy-2(5H)-furanone (mucochloric acid, MA), causes a different mutational spectra in these bacterial cells, showing that at least these two compounds do not share a common mechanism of mutagenicity even though they are structurally similar. MA has been reported to cause adducts in the DNA (Kronberg et al., 1992, 1993). No induction of DNA adducts by MX has been reported.

One feature which may explain the high activity of MX as a *Salmonella typhimurium* (TA100 strain) mutagen and the lesser genotoxic potency of MX in mammalian cells *in vitro* or *in vivo* is the presence in TA100 of plasmid pKM101, which enhances error-prone DNA repair. MX has been shown to be much less mutagenic in the parental strain TA1535, which lacks the pKM101 plasmid, than in the strain TA100 (Meier et al., 1987).

A two-year carcinogenicity bioassay in the Han-Wistar rat has been completed (Tuomisto et al., 1993), and the results are being calculated. Preliminary screening of the results clearly indicates that MX is carcinogenic, but detailed results are not yet available.

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Session Summary

Michael Cox
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The following is a brief summary of the key points made by each speaker.

Ms. Patricia Snyder Fair: Influence of Water Quality on Formation of Chlorination By-products.

- Knowledge of DBPs is influenced by the availability of analytical techniques.
- Major DBPs include: THMs and HAAs. Others include CH, haloacetonitriles, haloketones, and chloropicrin.
- Three major water quality parameters influence DBP formation: total organic carbon (TOC), pH, and the presence of bromide ion.
- General trend: High TOC indicates high levels of DBPs. High pH favors THM formation and decreased formation of HAAs.

Dr. Gary R. Klinefelter: Recent Reproductive Effects Associated with Disinfection By-products.

- Recent data indicate numerous reproductive effects in animal studies following exposures to DCAA, DBAA. Only limited reproductive effects have been seen from CH, BDCM, TCAA, and BAA.
- The U.S. EPA, in conjunction with the National Toxicology Program, has initiated a tiered-testing strategy to provide reproductive data for several high-priority DBPs. The DBPs include sodium bromate, chlorodibromomethane, dibromoacetonitrile, bromochloroacetic acid, and dibromochloroacetic acid. Any DBP that produces a dose-related reproductive effect in the screening studies will be studied more extensively at the U.S. EPA.
- Recent studies suggest that DBAA compromises the normal reproductive behavior of adult males rats and that this effect may be manifest at reasonably low exposures. DCAA has been found to produce spermatogenic effects in rats, as well as decreases in epididymal sperm motility, that are similar to those produced by DBAA, but on a molar basis the effects of DCAA are less severe than those of DBAA.

Dr. Sid Hunter: Effects of Haloacetic Acid Water Disinfection By-products on Embryonic Development.

- Several of the haloacetic acids have been shown to be teratogenic when administered to rats during organogenesis.

- Direct effects of several HAAs on mouse conceptuses were evaluated using the whole embryo culture system. Using neural tube defects as the morphological endpoint, the most potent HAA was bromoacetic acid, followed by chloroacetic acid, bromochloroacetic acid, dibromoacetic acid, trichloroacetic acid, tribromoacetic acid, acetic acid, and finally dichloroacetic acid.

- Preliminary evaluation of an *in vivo* screening test for developmental effects of DBAA and DCAA indicate that DBAA is twice as potent in mice as DCAA when the lowest doses that induce effects are compared.

- Preliminary work on a possible mechanism of DCAA-induced developmental toxicity is underway. Results suggest that DCAA does not induce dysmorphogenesis through generation of reactive oxygen species (free radicals).

Dr. Byron E. Butterworth: Assessing the Cancer Risk of Chloroform.

- Weight of evidence is that chloroform is acting through a nongenotoxic/cytotoxic mode of action.

- To the extent measured, the dose-response patterns for chloroform-induced cytolethality and regenerative cell proliferation account for the patterns of tumor formation.

- The risk assessment for chloroform and other similar nongenotoxic/cytotoxic carcinogens should apply appropriate safety or uncertainty factors to the NOEL for induced cytolethality/cell proliferation.

Dr. Anthony B. DeAngelo: The Carcinogenicity of Disinfection By-products in Drinking Water: Results from the Water Carcinogenesis Research Program at the U.S. EPA's National Health and Environmental Effects Research Laboratory.

- The Water Carcinogenesis Research Program was established to respond to the regulatory needs of the U.S. EPA's Office of Water.

- Work has been completed on chloroacetic acid (negative for rat/mouse), dichloroacetic acid (positive for rat/mouse in liver), trichloroacetic acid (positive for mouse in liver, negative for rat), chloral hydrate (positive for mouse in liver, negative for rat), and bromodichloromethane (negative or weak response in rat/mouse in liver).

- Work is currently underway for bromate, bromoform, and chlorate. Results are expected in 1998.

Dr. Richard J. Bull: Carcinogenic Properties of Brominated Haloacetates.

- All haloacids, with the probable exception of trichloroacetic acid, are important DBPs to study.

- Brominated haloacids have effects which suggest they may be more important than their chlorinated analogs at low doses. Brominated haloacids produce oxidative damage to hepatic DNA and target the lung in mice; dibromoacetic acid targets the colon in rats.

- The haloacids contribute a classic tumor-promoting mode of action that would be predictably synergistic with those of other classes of DBP at high doses.

- The critical question of what happens at low exposures still needs to be addressed.

Dr. Jouko Tuomisto: Genotoxicity and Carcinogenicity of MX and Other Chlorinated Furanones.

- Finnish drinking waters were found to be highly mutagenic, with MX contributing about one-third of the mutagenicity.

- Ecological epidemiology studies have shown increased risk ratios for six cancers in towns with mutagenic waters. Since these studies used aggregate data, both a cohort study and a case-control study are being conducted to further assess the risk from these waters.

- MX is mutagenic in several *in vitro* genotoxicity studies, but *in vivo* genotoxicity has been more difficult to establish.

- A 2-year carcinogenicity bioassay in rats for MX has been completed and the results are being compiled. Preliminary results indicate that MX is a rodent carcinogen.

Session Discussion

Chaired by Michael Cox
Office of Water,
U.S. Environmental Protection Agency

MR. COX: I would like to pose some questions to stimulate discussion: First, considering the available epidemiology and toxicology studies, what are the most important compounds? Is further research on chlorination by-products necessary? If so, what are the critical research needs in this particular area? Have we too narrowly focused our research attention on trihalomethanes? Should we expand our research capabilities to other areas?

Second, do levels of disinfection by-products in drinking water present real or trivial human health risks? Third, how do we reconcile the results of the epidemiology studies with those from animal studies, and how can we better integrate human and animal studies in the future? Finally, are current methods of risk assessment appropriate?

DR. BULL: As far as which are the most important compounds from a human health standpoint, that is a question of prioritization. EPA's margin-of-exposure approach to cancer risk assessment for things other than genotoxic compounds bears some resemblance to what Dr. Krewski talked about in his presentation during Session 1. In my opinion, the correlation he suggested—that all compounds with a maximum tolerated dose were assumed to be carcinogenic—probably is a little dangerous. I think you

have to back away from that in the absolute sense because there are serious exceptions that are meaningful in specific applications. But the fact that this correlation does exist provides justification to a selection process based on concentration to prioritize chemicals for further study. For example, you could decide forget about chemicals for which exposure is far less than the maximum tolerated dose.

If you do not have biological data, how do you decide which are the most important by-products? As Dr. Krewski pointed out, the potencies of carcinogens and toxins differ by seven to nine orders of magnitude. So, it is not just a matter of concentration; it is a combination of concentration and potency. But clearly, concentration can be justified as an initial screen to decide whether to focus on characterizing the toxicology of a particular chemical.

MR. COX: Do any other panel members have a thought on Dr. Bull's suggestion that we need to prioritize and whether the maximum tolerated dose is one way to do that?

DR. DeANGELO: Regarding the acids, we know a great deal about how widespread the exposure to them is, so we now need to decide how important they are in terms of the problem they might be causing. Regarding trihalomethanes, we not only drink them, we take them in when we shower. We have a good grasp on their carcinogenic effects but we need a better understanding of the mechanisms. Research on bromate and other compounds that have several sites of effect in several animals and species is very important.

MR. COX: So, you are suggesting that exposure should be a prioritization factor?

DR. DeANGELO: Yes. Since exposure to chlorinated drinking water is so widespread, maybe we should be looking at chlorinated by-products first before we go on to other disinfection by-products.

DR. BULL: The ratio of the level of a by-product that induces tumors versus its concentration in drinking water gives you a margin of exposure that shows how close the exposure is to the dose of concern. This could be use as a tool for prioritization. Let me just give one example. If my back-of-the-envelope calculations are correct, the ratio for MX might be as much as 10^8 to 10^9, whereas the haloacetic acids might be more like 10^4 to 10^5. So, at first blush, the haloacetic acids seem more important than MX. I think chloroform is very far from being a concentration that would cause concern. For compounds that lack a good database, I suggest we use something like the approach Dr. Krewski proposed for prioritization.

MR. COX: I believe that many of the epidemiology studies show weak associations of exposure to chlorination by-products with bladder, colon, and rectal cancer, whereas the animal studies show liver, kidney, and possibly intestinal cancer sites. How do we reconcile the differences between the epidemiological and toxicological studies? Is this important?

DR. BULL: It is extremely important. That discrepancy is telling you something, though not necessarily something very precise. I would argue it might mean there is something wrong with our extrapolation model, and that we have to study the mechanism of action, as has been suggested by Dr. Butterworth's approach to chloroform, and some of Dr. DeAngelo's and my activities with the haloacetic acids. They are very interesting compounds, but at low doses, they may not be important. However, we need a scientific basis for coming to that conclusion.

The reason why bioassays have not shown the same kinds of cancer effects that have been found in some epidemiological studies may be that we have not looked at the entire spectrum of compounds produced with disinfection. For example, we are just beginning to move to the brominated compounds. Some of these seem to be producing colon cancer in animals. Dr. Cantor will tell you that this is not the right site; it should be the rectum, but nevertheless the colon is more like the rectum than the liver is like the rectum.

It is important to play epidemiology and toxicology back and forth against one another, because each tells you something different. Epidemiology deals with the whole mixture. However, the epidemiology needs to be much more sophisticated in looking at how response varies with geographic location, because, as Ms. Snyder Fair's presentation made clear, factors such as differences in pH and bromide concentration introduce a great deal of variability. I think there is enough information to suggest that these types of factors might have something to do with the variation in the results between epidemiological and toxicological studies and some of the inconsistencies found in the epidemiology data as one moves from one geographical area to the other. This is a hypothesis, of course, but you have to drive these things by hypotheses or we will never find the end of this.

DR. TUOMISTO: We have been trying to do epidemiological and toxicological studies side by side, because it brings some realism to the whole business, and there is feedback between them. So, I suggest that epidemiology and toxicology should be done in conjunction with one another more so than they are now.

MR. COX: Would anyone in the audience like to respond to this question? Considering all the epidemiology and toxicology data, what are the most important compounds that we should be focusing our research on in trying to elucidate the human health effects?

DR. BIRNBAUM: I do not necessarily want to address the issue of specific compounds, because I am not sure it would be helpful. I wonder whether, if we use the animal cancer data to back-predict the human risk, we will ever be able to detect that kind of response in a population unless it is a rare tumor. I think the answer is probably no. That makes me wonder about all the epidemiological data, and whether it is going to tell us anything. In fact, if we did see a response in epidemiological studies, would that indicate that we, as public health professionals, had failed to protect the human population?

DR. CANTOR: For better or for worse, the toxicological approach has been a reductionist approach that tries to work out the metabolic details or the effects of a necessarily limited number of individual chemicals. What about synergistic and antagonistic interactions, and the other compounds that we probably will never be able to understand from a classical toxicologic standpoint?

I would like to ask the toxicologists here: What should we measure in the human population that might provide early measures of effect from exposure to the whole mixture? Also, can you make four or five standard mixtures that might represent much of what we see in chlorinated drinking water or other disinfected water, and use these as the basis for exposures in toxicological studies of drinking water?

DR. BULL: In response to Dr. Birnbaum's question, liver cancer is a low incidence cancer, but it comes close to a level you might be able to detect in human populations. It approaches 10^{-3} if you sum up the average concentrations of liver carcinogens in chlorinated water. That is why I do not believe that liver carcinogens are important.

Regarding Dr. Cantor's question, we did attempt to go in the direction you are suggesting. We did at least one experiment in that direction. But when you chlorinate humic acid, you eventually have a salt problem. The addition of chlorine leads to formation of hydrochloric acid, which must be neutralized with sodium hydroxide. The high sodium chloride concentrations that result limit the dose you can administer to the animals.

We did an experiment like that, where we expected lots of dichloroacetic acids and trichloroacetic acids and so on. Dr. van Duuren of New York University did that experiment and came up with negative results. That discouraged us from further work of this nature because we could not reach a real maximum tolerated dose as it related to the by-product. However, we only looked at chlorinated humics. Other variations, which include bromide for example, could be examined.

MR. FAWELL: One of the important points we have been missing all along with the epidemiology studies is that we are looking for cause. We are trying to measure something which is a clear cause so that we can allocate so many cancers to drinking water. However, we know that, in reality, this is multifactorial. Many things are happening and it will not be very easy or perhaps biologically sensible to attempt that sort of allocation. It is a theoretical statistical approach, and it is not very biological.

So we may not be able to disentangle from this background of activity. If you took drinking water away, you would still find a substantial number of these tumors. That is the background noise. We are trying to allocate drinking water responsibility within that background noise. That is going to be very difficult to disentangle.

One thing to remember about the mechanistic data from toxicological studies is that we are working constantly at high doses. At those very high doses, we may be in danger of identifying the wrong mechanisms, because high doses affect so many other things.

I come back to this question: How do we examine what is going on at much lower doses? Do we have the techniques and the technology?

DR. BUTTERWORTH: No, we do not.

DR. BULL: There is an exercise you can do in your mind, which comes back to something I did not like what about Dr. Krewski said. If you do everything worst case, as we have done historically in this area, each carcinogenesis bioassay that comes out positive is going to add to the risk and give you an additive risk at low dose.

I would hate to see us apply the same margin of safety to the maximum tolerated dose that we apply to cancer. That is an extension of that logic that some policymakers might want to make, which is clearly not justifiable on scientific grounds.

I would like to switch back to cancer risk for by-products to illustrate the problem. The calculation of mean risks from chlorinated water based on extrapolation of results from those compounds that have been studied produces relatively high risk numbers. If you assume additivity, you can push the average risk of liver cancer from chlorinated water to somewhere between 10^{-4} to 10^{-3} without the brominated acetic acids. So when our results are reported, the calculated risk will probably increase by a factor of two or three, just by adding the additional risks from these three compounds.

So, there is a real reason for doing what you are saying, Mr. Fawell. I think we have to convince ourselves that chlorination is really that dangerous. Chlorination has some real benefits and is certainly needed. We should not discard chlorine lightly. I do not like to jump into other alternatives before convincing myself the current method is not safe.

DR. REIF: I would like to comment on the toxicology-epidemiology interface from the point of view of reproductive epidemiology. At least two of the reproductive studies performed to date, the Kramer study in Iowa and the Bovet et al. study in New Jersey, found an association between low or decreased birth weight and exposure to chlorinated surface waters.

That is whole different kind of epidemiology. Every baby has a birth weight, and the numbers of births are adequate in any population to provide the power to see effects. If chlorinated water by-products are related to birth weight and one only has to look at exposures within a year of birth, we will have a much easier time finding effects than we do when we conduct epidemiological studies that look for effects such as cancer.

Dr. Klinefelter, it was interesting to me that, in the slides you showed this morning, the birth weights did not appear to be manifestly different. Two out of four of the epidemiological studies that have been reported in the literature do find a statistically significant decrease in mean birth weights in women who deliver in areas served by chlorinated surface systems. So, decreased birth weight may be a very sensitive endpoint to use in epidemiological studies. This endpoint has the potential to drive the regulatory process, since we are more likely to get results sooner than waiting for data on rare cancers in human populations.

DR. KLINEFELTER: My thinking on this point is rather simple. Clearly, reproductive endpoints often are as sensitive as, if not more sensitive than, cancer endpoints, so the reproductive data can be more easily bridged with the epidemiological data.

To get back to the subject of mixtures, I would think we could create a mixture of around five haloacetic acids, each at the lowest dose that produces a reproductive effect in the rat. We could then test that mixture in rats to determine whether there is synergism or antagonism among the components. If we see synergism, we could then try to screen a human population that is exposed to some reasonable elevation of those haloacetic acids.

MR. COX: Let us move on to the second question: Do the levels of disinfection by-products in drinking water present real or trivial human health risks?

DR. BUTTERWORTH: We have to keep a perspective. There are far more mutagens and carcinogens in the food we eat than in the water we drink. So we must ask: Have we focused far too narrowly? Are we really justified in expending so much energy on drinking water and ignoring other sources of exposure like food? Personally, I do not think we are justified. I think that the drinking water supply is very safe, and the toxicological risks are trivial.

We also need to consider the cost-benefit ratio. For example, if we are considering switching from chlorination to ozonation, we should ask how much that will cost, and could we better spend the money elsewhere. For example, someone talked earlier about reducing trihalomethanes from 100 parts per

billion to 80 parts per billion. That is a minor change that falls within the noise that we have in our biological data. Are we justified in doing this if it costs millions of dollars?

DR. BULL: In my opinion, this is still an open question. It relates in part to the question of what constitutes acceptable risk. There are many reasons for switching to ozone besides ensuring the microbiological safety of drinking water. Many water treatment experts would like to see this happen for entirely different reasons. So, the cost of ozonation is not necessarily what holds you back. I am more concerned about the fact that ozonation likely is not an option for communities with limited resources. For third-world countries, chlorination probably is the only feasible method. The risks from disinfection by-products are small by comparison to the risks of not disinfecting.

DR. TARDIFF: I tend to agree with Dr. Butterworth's comment that cost-benefit analysis is important. My main point is more technological. Mr. Cox framed the two main questions to this panel with the presumption that we want to identify those compounds that are most important toxicologically. Therefore, we must know everything about the chemistry of the substances we are concerned with. I think that is simply untrue. It ought to get us back to first principles.

There are several technologies out there—ozone, chlorine dioxide, and chlorine itself—that can control or, in some cases, eliminate serious diseases to varying degrees. The EPA's job is to make sure that the disinfection technology does not impose risks that are greater than the benefits of disinfection. To do that we need a better understanding of all the chemistry associated with those alternative technologies. We ought to examine the totality of the risk associated with any technology we might be considering.

I do not think that toxicologists can help epidemiologists determine which biomarkers they ought to look at, because we have not even identified many of the compounds in these mixtures and the toxicological information is limited for many compounds that have been identified. We need more fundamental research to identify what is there and to get baseline toxicological information on compounds on an individual and combined basis.

DR. KREWSKI: I am very interested in developmental toxicity as an endpoint for quantitative risk assessment from a couple of viewpoints. There has been some nice work lately in developing very elaborate models for estimating the benchmark dose, for example, looking at joint analyses of prenatal mortality and postnatal teratogenicity, using changes in body weight as covariants to adjust the benchmark doses, and so on. Dr. Hunter, in doing the kind of modeling you talked about in your presentation, do you see any opportunities for putting a little bit more biology into the dose-response models that we have been using for characterizing dose-response relationships? Are there some common pathways that might be limiting effects in developmental toxicity that we can try to model?

DR. HUNTER: The easy answer is yes. There will be some determining events, whether it is expression of a specific gene or whether it is a specific biochemical pathway. But we do not necessarily have adequate information about what these are. For example, in the transgenic animal models that are now being generated where you have the gene knockouts, if you do not see a malformation when you knock out a specific gene, that suggests that there is a compensatory mechanism available to the embryo so that it can undergo normal morphogenesis. That would then tell us that looking for the effect of a toxicant only on that specific gene is not the appropriate endpoint because you can compensate for the loss of that function. But with developmental HOX knockouts, for

example, we see developmental effects. That means there is no mechanism to compensate for the loss of that gene, which makes that a very interesting target for toxicants that also produce similar defects. That is the kind of approach that we are going into now, trying to look for genes related to apoptosis. Many toxicants induce cell death; now we want to find out whether the same regulatory genes are involved, and whether they and their gene products are perturbed by a toxicant.

DR. KREWSKI: In carcinogenesis, we have identified mutation and cell proliferation as two of the key factors. If in developmental toxicity you could identify a small number of critical elements that we could work with, it would really help move risk assessment forward.

DR. BIRNBAUM: Dr. Butterworth, earlier we heard a lot of discussion about moving away from a no-observed-adverse-effect level safety factor approach and toward a benchmark approach or some alternative. Is the Chemical Industry Institute of Toxicology taking that approach? You have a lot of dose-response information.

DR. BUTTERWORTH: Our dose-response curves are very thorough because we spent a lot of time and money developing them. They really do lend themselves quite nicely to the benchmark approach, and we think that is a reasonable way to go. It would alter things a bit, but we have no problem with that.

MR. FAWELL: Dr. Bull, in your presentation you showed some data from studies conducted at low doses. It is very important to investigate at these low doses. Do you have any thoughts about how we can do that effectively within the constraints of our experimental techniques?

DR. BULL: There are two types of interactions: 1) pharmacodynamic, or toxicodynamic if you prefer, and 2) interactions involving metabolism and pharmacokinetics. We have to carefully segregate these two because the latter deals with the dose that is delivered, and the former with the response that is produced. They can and do vary independently between species. More important is that the two types of interactions will not necessarily occur in the same dose ranges. Dr. Butterworth's point about dose-response is the key question with dichloroacetate. The depression of cell division that we see at the low dose of 0.5 g/L may be extended to somewhat lower doses. However, the selective stimulation of division within the tumor at higher doses is being seen in a range where the blood levels are skyrocketing, thus the effective dose is much greater than would appear from examining external dose. This, I am sure, is what drives the very rapid development of tumors observed at these concentrations of dichloroacetic acid. Thus, it is highly unlikely that this effect will be observed at concentrations that occur in drinking water.

DR. RICHARD: Have you done any work on dibromoacetate or bromochloroacetate? With bromodichloroacetate it seems that what you are seeing is some increased metabolism and then effects very similar to dichloroacetate, which you are postulating is produced by the metabolic pathway anyway. But with dibromoacetate, you could be seeing an entirely different beast. It is not clear whether that is going to act more like dichloroacetate or something entirely different because of the different nature of the bromine and the metabolic pathways. Also with bromochloroacetate, it again is questionable whether that is going to be something entirely different or very similar to dichloroacetate.

DR. BULL: To the extent we have looked, dibromoacetate, bromochloroacetate, and bromodichloroacetate produce the same effects as dichloroacetate in the liver. Thus, at high doses, the

effects of bromodichloroacetate and dibromochloroacetate in the liver are likely to be attributable in large part to their conversion to the corresponding dihaloacetates. What is different seems to be the oxidative stress that is produced by bromine substitution to both the dihalo- and trihaloacetates. Accentuation of this mechanism may add an additional dimension to liver cancer induction at low doses or to the induction of lung tumors that have been seen at high doses with these compounds, but not with dichloroacetate. The induction of oxidative damage to DNA suggests that they may also be mutagenic, which I think plays little if any role in dichloroacetate-induced tumorigenesis. Thus, I think the brominated compounds are likely to be more important than chlorinated compounds at low doses, even though at high doses dichloroacetate is much more effective at producing liver tumors than the brominated compounds. This clearly makes dichloroacetate very interesting from a mechanistic standpoint, but the effects of the brominated compounds may actually be more critical because they have the potential to extend further into the low-dose region.

The glycogen accumulation in the liver that you see with dichloroacetic acid, but not with trichloroacetic acid, is similar with all three of the other brominated haloacetic acids. That would hint that they produce some of the same kind of liver pathology as does dichloroacetic acid.

DR. KLAASSEN: Dr. Tuomisto, what is the major target organ for acute doses of MX and is there target organ toxicity?

DR. TUOMISTO: The animals seem to die of breathing difficulties. Beyond that, I do not know.

DR. KLAASSEN: In the studies you presented earlier, how did the MX concentrations that you administered to the animals in the drinking water compare to the maximum tolerated dose? Was the highest dose about as much as you could give?

DR. TUOMISTO: In Finland, we have had up to 100 nanograms per liter of MX. That is five to six orders of magnitude less than what we have been using in drinking water in the two-year rat study.

DR. KLAASSEN: What I really mean is, how much could you give to the rats before they died or lost tremendous weight? Are you close to those levels in this study?

DR. TUOMISTO: Our subchronic study was close to the maximum tolerated dose, which is something like five-fold more than our highest dose for the two-year study.

DR. DeMARINI: Dr. Tuomisto, your presentation raised an issue that has not yet been addressed at this workshop. That is the issue of the nonvolatile organics in drinking water. Could you compare and contrast the nonvolatile organics with the volatile or semi-volatile fraction in terms of the most important health effects? Do you have a sense of which fraction might be more important in terms of human health effects. The EPA regulates on the volatile or semi-volatile fraction, and not on the nonvolatile fraction. Yet your work and that of many others suggest there is lots of mutagenicity in the nonvolatile fraction. I am curious what you think about the importance of one versus the other.

DR. TUOMISTO: We have only been working with MX, so we are able to raise such questions, but not able to answer them.

DR. BREACH: My company did some research on mutagenic activity in drinking water some years ago and found that there was some correlation with MX, as you suggest. We found that we could reduce the mutagenic activity in drinking water by the same treatment techniques as you use for reducing trihalomethanes—activated carbon, reducing chlorine contact, and so on—even though there was no direct correlation between mutagenic activity and trihalomethanes. What techniques has your waterworks used to reduce mutagenic activity?

DR. TUOMISTO: Of course, you reduce both when you reduce carbon or chlorine, but there is no direct linear correlation. There is a correlation between trihalomethanes and MX on mutagenicity, but it is not a direct one, because they separate, especially at higher dose ranges.

SESSION 3
ALTERNATIVE DISINFECTANTS

Session Introduction

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This session provides an overview of alternative disinfectants and focuses on the toxicology of several by-products of greatest interest. The use of chloramine, chlorine dioxide, ozone, and combinations of disinfectants has increased in recent years as utilities have sought to reduce levels of potentially harmful disinfection by-products without sacrificing biocidal effectiveness. In general, however, the by-products of these alternative disinfectants have not been as well studied as the by-products of chlorination. Additional health and exposure information is needed to more fully characterize the potential risks associated with exposure to the by-products of alternative disinfectants. Such data are essential for comparing the relative risks of the different disinfection treatments.

Opportunities for conducting cancer epidemiology studies in the United States to evaluate the risks of alternative disinfectants are limited, since few communities have experienced the long exposure periods required for such studies. Risk assessors must therefore rely primarily on animal toxicology data to estimate cancer risks for alternative disinfectants. Laboratory data on potential noncancer effects, especially adverse reproductive outcomes, are also important for conducting risk assessments of specific disinfection by-products as well as for designing future epidemiology studies.

Toxicology research on disinfection by-products, irrespective of the disinfectants used, must address three critical questions:

1. *What is the toxicity of those by-products that are of greatest importance from a risk perspective?* Screening studies and more detailed investigations to characterize the potential cancer and noncancer effects of disinfection by-products are essential steps in hazard identification and dose-response assessment. Much of the information presented in this session and in the previous session on chlorination addresses this question.

2. *How can uncertainties in the use of animal data for human health risk assessment be reduced?* Presentations on bromate and rodent nasal tumors in this session highlight the importance of mechanistic research in facilitating extrapolation of animal data to humans. The development of biologically based risk assessment models is discussed in considerable depth in Sessions 4 and 5.

3. *What is the relative toxicity of complex mixtures of disinfection by-products?* This very important research issue is discussed in Session 5.

The first presenter for this session will be Dr. David Reckhow from the University of Massachusetts. He will provide an overview of the occurrence and levels of alternative disinfection by-products. Mr.

John Fawell from the Water Research Centre in the United Kingdom will talk next about the toxicity and risk of bromate. The third presenter is Dr. Morando Soffritti from Italy on the carcinogenicity of formaldehyde and acetaldehyde. Dr. Robert Romano from the Chemical Manufacturers Association (CMA) will talk about recent studies on chlorite and chlorate, and Dr. Kevin Morgan will discuss some of the mechanistic issues, particularly as they relate to rodent nasal tumors.

Overview of Alternative Disinfectants

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Introduction

The alternative disinfectants generally include the chloramines, chlorine dioxide, and ozone. To be complete, one must also consider bromine, iodine, ultraviolet light, and possibly electron irradiation. Most U.S. utilities use either chlorine or chloramines for providing a disinfectant residual in the distribution system. Some of these also use ozone at some earlier stage of treatment to provide additional chemical disinfection. However, a small number of other community and private water systems employ ultraviolet light, bromine, and iodine for disinfection. Organic and inorganic oxidation by-products form from all of these disinfection agents. Many of the by-products are common across several of the disinfectants. Some of the others are almost exclusively produced by only one disinfectant.

General Disinfection By-products

There are several groups of by-products that may be formed from more than one disinfectant. These include some halogenated organic compounds, inorganic halooxidants, and simple oxygen-containing organics (see the following tables). This first group may be most widely represented by dichloroacetic acid, a known by-product of chlorine, chloramines, and chlorine dioxide. Cyanogen chloride can form from both chlorine and chloramines. In addition, when chloramination is practiced so that a free chlorine residual is allowed to exist for some measurable amount of time, a wider spectrum of chlorinated organic compounds (e.g., trihalomethanes, other haloacids) may form, resembling the by-products normally associated with free chlorination. Finally, a range of brominated (and presumably iodinated) organic by-products will form from either the ozonation or chlorination of water containing bromide.

By-products from Alternative Disinfectants

Chloramines. Chloramines are known to form a wide range of oxygenated oxidation by-products. The amount of small oxygenated organics formed is probably somewhat less than found for chlorine. It has been proposed that because of chloramines' lower reactivity, it shows less of a tendency to fragment organic matter, and therefore produces a greater abundance of high-molecular-weight by-products. Many of these large compounds contain covalently bonded halogen atoms. Unlike chlorine, however, the chloramines seem not to produce the small halogenated compounds such as

trihalomethanes. One prominent exception is dichloroacetic acid. This appears to be a major by-product of chloramination.

Because of the peculiar chemistry of inorganic chloramines, there is a tendency to form high levels of cyanogen chloride. While this compound may also be produced at high levels with chlorine, it is rapidly degraded in the presence of a chlorine residual, so that it does not usually persist at high levels. The chloramines will also lead to the formation of a wide range of organic chloramine by-products (N-chloro organic compounds).

As normally practiced, chloramination causes the water to be exposed to a short-lived free chlorine residual. For this reason, many of the commonly reported chlorination by-products have also been reported in systems using chloramines.

Chlorine Dioxide. Chlorine dioxide produces many of the same general oxygenated by-products as chlorine. In addition, it also leads to the formation of chlorite and a small amount of chlorate. As for the common organic halide compounds, only dichloroacetic acid appears to be produced in large amounts. The formation of other low-molecular-weight organohalides may be attributable to the presence of some free chlorine in the chlorine dioxide generator effluent.

Ozone. Ozone produces a wide array of oxygenated organic by-products. Among these are the low molecular weight aldehydes, ketones, keto-acids, and diacids. Many of these are readily biodegradable and contribute to a water's potential to support microbial growth (e.g., assimilable organic carbon or biodegradable organic carbon). Of the alterative oxidants, probably the most is known about these ozonation by-products and the role of source water quality in their formation. Other organic ozonation by-products are represented by the reactive organic peroxides, and perhaps, epoxides.

Ozone will also oxidize inorganic bromide and iodide to hypobromous acid and iodine, respectively. The hypobromite can continue to react with ozone (or related oxidants) to form bromate, or it can react with other dissolved constituents forming brominated organic by-products. There is now a considerable body of literature on the kinetics and mechanisms of bromate formation in ozonated waters.

Others. Bromine will react with natural organic matter to form a wide range of brominated organic compounds, most of which are analogous to the by-products of free chlorination. There is a general tendency for bromine to form higher levels of trihalomethanes, and less of some other compounds. Although less is known about iodine, there are indications that this disinfectant produces many of the same types of by-products as bromine and chlorine. Ultraviolet and electron irradiation have not been well studied for disinfection by-products. The available literature suggests that these treatments will produce some low molecular weight oxygenated by-products.

Mixed Oxidant By-products

Little is known about the types of unique by-products that might form from the mixed application (simultaneous or sequential) of two or more oxidants. Recent studies have shown that some ozonation by-products (e.g., acetaldehyde) can be substituted with chlorine upon subsequent treatment with hypochlorous acid. This pathway probably produces some compounds that could not easily form from the application of any single chemical oxidant.

Chloramine By-products

By-product Class	Examples	Samples	Levels	Refs
Cyanogen Halides	Cyanogen chloride, cyanogen bromide	treated waters	high	many
Chloroacids	Dichloroacetic Acid Trichloroacetic Acid 6,6-Dichlorohexanoic Acid	fulvic acid, treated waters	high	1
Unsaturated Chloroacids	3,3-Dichloropropenoic Acid	fulvic acids		1
Chlorohydroxy acids	2-Chloro-4-hydroxybutanoic Acid 2,3-Dichloro-3,3-dihydroxypropanoic Acid	fulvic acids		1
Unsaturated Chlorohydroxy acids	4-Chloro-4-hydroxypentenoic Acid	fulvic acids		1
Chlorinated Ketones and Aldehydes	Chloroacetaldehyde Dichloroacetaldehyde Chloropropanal 3-Chlorobutanal 3,3-Dichloropropanal 1,6,6-Trichlorohexanal Chloropropanone	fulvic acid		1
Chlorodiacids	Dichlorosuccinic Acid	fulvic acids	high	1
Nitriles		model compounds		2,3
Aldehydes		model compounds		3
C-Chloro Amines		model compounds		4,9
N-Organo Chloramines		model compounds		5,7,8
Chlorohydrins		model compounds		6,9
MX and derivatives	MX, EMX, red-MX, ox-EMX	humics		1,10
Epoxides		model compounds		6

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Chlorine Dioxide By-products

Class of By-products	Examples	Samples	Refs
Oxychlorine Compounds	Chlorite, chlorate	treated waters	7,8
Aliphatic Mono- Acids	Butanoic Acid, Pentanoic Acid, Hexanoic Acid, 2-ethylhexanoic acid	full-scale plant	1,2
	acetic acid, butyric acid	model compounds	11
Di-Acids	oxalic acid	model compounds	5
	succinic acid, glutaric acid, adipic acid	aquatic fulvic acid	2
Haloacids	Dichloroacetic Acid, chloromalonic acid, chlorosuccinic acid	aquatic fulvic acid	2
Chlorohydrins	9-chloro-10-methyl stearate	model compounds	10
Aliphatic aldehydes	acetaldehyde, propanal	treated river water	3
Halogenated ketones	1,1,3,3-Tetrachloropropanone	full-scale plant	1
Unsaturated ketones	2,3,4-trimethylcyclopent-2-en-1-one 2,6,6-trimethyl-2-cyclohexene-1,4-dione	full-scale plant	1
Maleic Acid & Derivatives	maleic acid	model compounds	5
	2-tert-butylmaleic acid 2-ethyl-3-methylmaleic acid	full-scale plant	1
Styrenes	3-ethyl styrene, 4-ethyl styrene	full-scale plant	1
Aromatics	benzoic acid	full-scale plant	1
	p-benzoquinone, hydroxy-PAHs	model compounds	6,9
Naphthalene Derivatives	nephthalene 1-methylnaphthalene	full-scale plant	1
Misc. Organics	hexanedioic acid, dioctyl ester	full-scale plant	1
Furan derivatives	methylfurancarboxylic acid	aquatic fulvic acid	2
Epoxides		model compounds	4

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Ozone By-products

Class of OBPs	Examples	Samples	Levels	Refs
Oxidized Halogens	Bromate, hypobromite	drinking waters	1-50 μg/L	11,12,13
Organic Bromides	Dibromoacetic acid, bromoform	drinking waters	1-50 μg/L	9,10,12
Inorganic Peroxides	Hydrogen Peroxide			many
Organic Peroxides		model compounds		
Aliphatic Aldehydes	Formaldehyde, Acetaldehyde, Propanal	drinking waters	1-50 μg/L	many
Aromatic Aldehydes	Benzaldehyde, Ethylbenzaldehyde	surface waters		7,17
Aliphatic Ketones	Acetone, propyl ethyl ketone	natural waters		1,14
Aromatic Ketones	Acetophenone, 4-phenyl-2-butanone	surface water		7
Mono-Acids	Hexanoic acid	model compounds		16
	Acetic acid	humic acid		3,4
Di-Acids	Oxalic acid, malonic acid	humic acid	1-100 μg/L	4,8
	succinic acid	surface waters		7
Mono-acid, Mono-Carbonyls	Glyoxalic Acid, Pyruvic Acid, Ketomalonic Acid	drinking waters	1-50 μg/L	many
	Oxobutanoic Acid, 4-oxopentanoic acid, 4-oxo-2-butenoic acid	natural waters		1,7
Di-Acid, Mono-Carbonyls	Ketosuccinic Acid, Ketoglutaric Acid	lab ozonated raw water		1
Di-Carbonyls	Glyoxal, Methylglyoxal	drinking waters		many
	1,2-Dioxobutane, Dioxopentane	natural waters		1
Mono-Acid, Di-Carbonyls	Dioxopropanoic Acid, Dioxobutanoic Acid, Dioxopentanoic Acid	natural waters		1
Hydroxy Acids	Hydroxymalonic acid	humic acid		4
Epoxides		model compounds		2,15
Benzene Carboxylic Acids		humic acids		4,5,6
	Benzoic acid, 3,5-dimethylbenzoic acid	surface water		7
Miscellaneous	5-methoxy-∝-pyrone	humic acid		4

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Toxicity and Risk of Bromate

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Bromate is formed during water treatment by the action of ozone in the presence of bromide ion and in the electrolytic generation of chlorine. Bromate was, however, first studied because of its widespread use in food processing.

Limited studies on the *in vitro* metabolism of bromate showed rapid absorption of a bolus dose with increased bromide levels in serum and some tissues. Bromate was detected in urine only at doses above 2.5 mg/kg body weight. There are, however, some difficulties with analytical techniques. *In vitro* studies showed strong degradative activity by rat liver and kidney homogenates and ready degradation of bromate by cysteine and glutathione.

Potassium bromate has been shown to cause chromosome damage in mammalian cells *in vitro*. There is some evidence from studies in bacterial tests that activity may be a consequence of free radical generation.

Rats given 600 mg/L or above of bromate in drinking water for 13 weeks showed regeneration in kidney tubules with eosinophilic droplets in the tubular epithelium of males. This change was reversible. No changes were observed in mice given up to 1,000 mg/L for 10 weeks.

In long-term drinking water studies in rats, bromate caused degenerative kidney changes with an increased incidence of renal tumors in both sexes. Peritoneal mesotheliomas were also increased in males and thyroid tumors were increased in both sexes. These findings have recently been confirmed by the U.S. EPA. There was a significant increase in kidney tumors in male hamsters, but with a lower incidence than in rats. There was no significant increase in tumors in female mice, although there was an indication of a small increase in kidney tumors at the highest dose.

There appear to be no published data on the reproductive toxicity of bromate, although, during this workshop, some evidence of reproductive effects following sodium bromate exposure was presented for laboratory animals.

Interest is now centering on the mechanism of carcinogenicity of bromate and whether its demonstrated genotoxicity is relevant to the mechanism or coincidental. Bromate is a strong oxidant, but mammalian cells are well-equipped to deal with oxidants. Tissue damage and subsequent regeneration is a well-established mechanism of carcinogenicity in the rat kidney, but it is not clear how big a role this will play in these circumstances. One possible mechanism is through lipid peroxidation—oxidative damage to lipids in cell membranes producing radicals in a chain reaction.

Studies to date have concentrated on the kidney. These studies *in vitro* and *in vivo* provide some support for the view that oxidative stress, and lipid peroxidation in particular, are responsible for bromate toxicity and carcinogenicity at high doses. Confirmation of this mechanism would in turn indicate that there would be a threshold to bromate carcinogenesis and that the dose-response would be nonlinear.

There are outstanding questions regarding bromate metabolism, but these may be very difficult to resolve satisfactorily due to complications over the analysis of bromate and bromide. The most urgent questions seem to center around the mechanism of toxicity and carcinogenicity, including differing tumor types, species differences, and the shape of the dose-response curve.

Long-term Carcinogenicity Bioassays on Formaldehyde and Acetaldehyde Administered in Drinking Water: Results and Implications

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Introduction

In Italy, more than 6 billion cubic meters of drinking water are distributed annually, 65% of which are derived from ground water, 35% from surface water. Almost all the surface water treatment facilities use chlorine compounds for disinfection. Only the Bologna and Turin facilities use ozone.

Treatment of raw water with ozone produces many by-products, including aldehydes such as formaldehyde and acetaldehyde. In ozonated water, the concentration of formaldehyde usually ranges from 5 to 18 μg/L, and the concentration of acetaldehyde ranges from 2 to 9 μg/L. Formaldehyde and acetaldehyde may occur naturally or may be generated by a variety of industries for industrial applications or as pollutants. Therefore, they are widespread in our environment, and may be found in outdoor and indoor air, in soil, in surface and groundwater, in beverages and food. Because of the wide use and environmental diffusion of formaldehyde and acetaldehyde and because of their formation during some processes of drinking water disinfection, in the mid 1980s, the Cancer Research Center of the European Ramazzini Foundation, in the Bentivoglio Castle Laboratory, started long-term carcinogenicity bioassays on both compounds.

Materials and Methods

The formaldehyde used was in water solution at the concentration of 30%; methyl alcohol was present as stabilizer at the concentration of 0.3%. The acetaldehyde used had a purity of >99%.

The test animals were Sprague-Dawley rats, derived from the Bentivoglio Castle Laboratory breeding facilities. The animals of this colony have been used for long-term studies over the last 25 years, and data from more than 10,000 historical controls are available.

Groups of 50 male and 50 female rats received formaldehyde at the concentrations of 0, 10, 50, 100, 500, 1,000, and 1,500 ppm in tap water ad libitum. Two groups served as controls: one of 50 males and 50 females supplied with tap water containing 15 ppm of methanol, and other one of 100 males and 100 females receiving tap water alone. The treatment started when animals were 7 weeks old, and lasted 104 weeks, after which the surviving animals were kept under observation until spontaneous death.

Groups of 50 male and 50 female rats, 7 weeks old at the start of the experiment, received acetaldehyde at the concentrations of 0, 50, 250, 500, 1,500 or 2,500 ppm in drinking water ad libitum. The treatment lasted 104 weeks and the surviving animals were then kept under observation until spontaneous death.

Both the experiments on formaldehyde and acetaldehyde were conducted following Good Laboratory Practices (GLP). The animals were kept under highly standardized housing and diet conditions, the same followed in the Bentivoglio Castle Laboratory over the last 20 years. Mean daily feed and drinking water consumption was determined once weekly from 7 weeks of age for the first 13 weeks, and then every 2 weeks until 111 weeks of age. The weight of the individual animals was measured with the same periodicity until 111 weeks of age and thereafter every 8 weeks until the end of the experiment. Clinical examination was carefully performed until 111 weeks of age with the same periodicity as the weight measurement and thereafter every 2 weeks until the end of the experiment.

All dead animals were subjected to complete necropsy. All organs and tissues were fixed in 70% ethyl alcohol, except the bones which were fixed in 10% formalin and then decalcified. The trimmed specimens were processed as paraffin blocks and sections were routinely stained with hematoxyl//in-eosin.

Results

1. Experiment on formaldehyde A slight decrease in water consumption in males and females was observed at 500, 1,000, and 1,500 ppm dose levels. The feed consumption, the body weight and the survival in the treated groups were similar to those of controls.

   Formaldehyde was found to increase the incidence of total malignant tumors, of a variety of gastrointestinal neoplasia, both benign (acanthomas, adenomatous polyps, and leiomyomas), and malignant (squamous cell carcinomas, adenocarcinomas, and leiomyosarcomas), and of lymphomas and leukemias in males and females. The compound also caused an increase in mammary carcinomas in females and in Leydig cell testicular tumors in males.

   The incidence of gastrointestinal tumors in treated animals was low. However, these findings must be considered since these tumors were not observed in the control groups and they are extremely rare among the historical controls.

   The increase in incidence of lymphomas and leukemias was observed in male and female rats treated with formaldehyde at the concentrations of 50, 100, 500, 1,000, and 1,500 ppm. The percentage of male and female animals with leukemias and lymphomas increased from 7.5 in controls to 32.0 in animals treated at the highest dose (44% in treated males).

   On the basis of our results, formaldehyde must be considered a multipotential carcinogen.

2. Experiment on acetaldehyde. Feed and water consumption, body weight, and survival in treated groups were similar to the control group.

   Following acetaldehyde administration, a definite increase in the incidence of total malignant tumors, and of lymphomas and leukemias was found among males and females. Moreover, an increased incidence of mammary carcinomas was detected in female rats. The percentage of animals (males and females) with leukemias and lymphomas increased from 8.0 in the control group, to 15.0 in the group

treated at the highest dose. The percentage of females with mammary carcinomas increased from 8.0 in the control group to 22.0 in the group treated with the highest dose. A borderline increase in the incidence of a variety of other tumors was also found.

On the basis of our results, acetaldehyde must be considered a multipotential carcinogen, although of lower potency than formaldehyde.

Conclusion

On the basis of the results of our long-term carcinogenicity bioassays, formaldehyde and acetaldehyde must be considered potential human carcinogens. Therefore, their production in drinking water should be minimized.

Update on Chlorite Toxicology Studies

Robert Romano
Chemical Manufacturers Association

In the 1970s and 1980s, several laboratories conducted studies on sodium chlorite and chlorine dioxide. Many of these studies identified potential toxicological concerns with these chemicals. Unfortunately, most of these studies were conducted for the purpose of basic scientific research. The results are not useful for risk assessment, nor do they adequately address regulatory issues. Most of these studies were not done under good laboratory practices (GLPs). These early studies, however, did demonstrate effects of chlorite/chlorine dioxide exposure. Most of the effects of treatment were on the blood including increased erythrocyte turnover, anemia, and decreased glutathione levels. Other observations included a decrease in thyroid hormones. In studies on reproduction and development, marginal decrease in exploratory behavior in neonates and possible neurodevelopmental delays in neonatal animals were noted.

Since 1990, a series of studies have been conducted to provide regulatory agencies the toxicity data to set standards for human consumption of chlorite. They include a 90-day subchronic rat study, a developmental toxicity study in rabbits, and a two-generation reproduction/developmental neurotoxicity study in rats.

The subchronic toxicity study was conducted in rats by dosing them with sodium chlorite by gavage for 90 days. The results confirmed those of earlier studies that demonstrated that the primary toxicological effect was on the blood. Specifically, chlorite produced methemoglobin and a decreased hematocrit at higher doses. Ulcerative effects on the gastric mucosa were noted due to the corrosive nature of the test material. There were no serious effects produced by long term exposure at low or moderate doses.

To determine if there were effects on the developing fetus, a developmental toxicology study was conducted with rabbits. Rabbits were exposed to sodium chlorite in their drinking water during gestation. Initially, there was aversion to drinking the water containing the higher chlorite concentrations due to palatability, but the animals gradually adapted and drank adequate amounts of water. This study demonstrated that there were no malformations or birth defects attributed to the test material, even at concentrations bordering on maternal toxicity.

Recently, the U.S. EPA raised some concerns regarding developmental neurotoxicity to young animals based on the results of a few studies conducted several years ago. To address these concerns, CMA is currently conducting a two-generation reproductive/neurodevelopmental study. This study evaluates reproductive parameters (such as estrus cycle monitoring, fertility index, pup viability, sperm motility, reproductive organ pathology) and developmental/neurological parameters (such as eye opening, reflexes, functional observational batteries, measurement of motor activity, neuropathology). Parental animals in this study are dosed form the time they are six to eight weeks old through mating, littering, and lactation. The neurodevelopmental evaluations are conducted on the F_1 generation which are exposed to the test material in utero, through lactation, and throughout their life.

This study is ongoing and will be completed in mid-1996. At this point, observations have included a decrease in water consumption in high dose animals. There have been no effects on body weights, food consumption, fertility, or reproductive parameters in the F_0 animals. No effects on motor activity, functional observational battery evaluations, or indications of adverse developmental/neurological effects have been noted in the F_1 animals.

Chlorate ion is also produced in very small quantities in drinking water treated with chlorine dioxide, therefore, a brief summary of chlorate is presented. Chlorate ion is not as acutely toxic as chlorite ion. The oral LD_{50} for chlorite is approximately 300 mg/kg, whereas the oral LD_{50} for chlorate is over 5,000 mg/kg. In a 90-day subchronic rat study, sodium chlorate was given to rats by gavage resulting in a decreased RBC count, hematocrit, and hemoglobin. The dose of sodium chlorate required to produce these effects were much higher than sodium chlorite. A developmental reproduction study in rabbits was also conducted with sodium chlorate. As with chlorite, this study demonstrated that there were no malformations or birth defects attributed to the test material, even at concentrations bordering on maternal toxicity.

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The Need for Understanding Mechanisms When Using Data from Rodent Nasal Toxicology Studies to Develop Drinking Water Standards

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For many chemicals, data derived from rodent toxicology studies play an important role in assessing potential human health risks and developing exposure standards. The effective scrubbing ability of the nose, which is needed to protect the lungs, places this region of the respiratory tract at risk of injury from inhaled chemicals. Furthermore, the rich nasal blood supply, combined with the high metabolic capacity of the nasal mucosa, accounts for the induction of nasal lesions by certain chemicals administered by noninhalation routes, including via drinking water (Brown et al., 1991). Interpretation of such responses in rodents when undertaking human risk assessment should consider underlying mechanisms, including the potential role of species-specific dose-response relationships. In the nose, as for any other organ or organ system, *"The dose makes the poison."*

Steep dose-response (or airborne concentration-response) relationships present an important feature of chemical toxicity in the nose. Such responses may exhibit experimental response thresholds, such as those seen for a number of neoplastic and nonneoplastic lesions induced by inhaled formaldehyde (Butterworth et al., 1995). The latter chemical induces extensive epithelial destruction at 10 and 15 ppm (carcinogenic concentrations) with no evidence of significant tissue damage, or cancer, occurring at or below 2 ppm. When such threshold concentrations can be identified and their extrapolation to humans supported by an understanding of underlying mechanisms, the application of safety factors to human risk assessments might be usefully refined.

Of the growing list of chemicals found to induce nasal lesions in rodents exposed to high airborne concentrations, a number may be present in drinking water in trace amounts. These chemicals include formaldehyde, chlorine, chloroform, and ozone—agents for which there is an extensive database on toxic responses following inhalation exposure of laboratory animals. The nature of toxic responses in the nose for each of these compounds is chemical-specific with respect to (1) concentration-response curve, (2) location of lesions (Méry et al., 1994), and (3) features of the tissue responses observed. A brief summary of these changes is shown in the following table.

When extrapolating these rodent data to humans, the data must be normalized for dose to critical target sites. For instance, studies of the toxicity of airborne formaldehyde suggest that the rat may be a more sensitive species compared with primates (DeSesso, 1993) due at least in part to airflow-driven dosimetry patterns (Kimbell et al., 1993). In addition to airflow, the disposition of xenobiotics in the nose may be influenced significantly by a number of other features of nasal physiology, including mucus flow, blood flow, and local metabolism (Morgan, 1994). Nasal metabolism, for instance, should generally be considered when addressing the issue of regional distribution of nasal lesions (Méry et al., 1994) and may often account for site-specific tissue susceptibility (Reed, 1993). Clear qualitative and quantitative similarities and differences in nasal metabolism exist between rats and humans (Dahl and Hadley, 1991). These differences may result in species specificity with respect to the potential for a toxic outcome. The development of appropriate water quality standards will need to consider the role of such differences, in addition to addressing the issue of species-specific dose normalization, if mechanistic data are to be incorporated effectively into the human risk assessment process for potentially toxic chemicals in drinking water.

Selected Toxicologic Information for Chemicals Associated with Drinking Water That Are Nasal Toxicants When Delivered by the Inhalation Route[1] at Sufficiently High Concentrations

Chemical	Reported airborne LOEL[2] and concentration range studied (ppm)	Nature of principal responses	Principal target sites in the nose
Formaldehyde[3]	2.0-15	Inflammation, epithelial degeneration, cancer	Transitional epithelium of the lateral meatus and respiratory epithelium of the anterior midseptum
Chlorine[3]	0.4.-2.5	Inflammation, mucus hypersecretion, olfactory sensory cell loss.	Transitional epithelium of the lateral meatus, respiratory epithelium of the midseptum, and olfactory epithelium of the dorsal medial meatus
Chloroform	10-300	Degeneration of Bowman's glands, osseous hyperplasia	Olfactory lamina propria and adjacent bone of the lateral and ventral lateral ethmoid turbinates
Ozone[3]	0.12-1.0	Mucus hyperplasia, inflammation	Transitional epithelium of the lateral meatus

[1]Chloroform also induces similar nasal toxicity when administered by gavage.
[2]Lowest-observed-effect level reported.
[3]Data also available for nonhuman primates.

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Session Summary

Fred S. Hauchman
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Dr. Reckhow presented an overview of alternative disinfectants and their different chlorinated and brominated by-products. The nature and concentrations of these by-products are influenced by the characteristics of the source water and the treatment system.

Chloramination produces many of the same by-products as chlorination, although the levels are generally lower and the higher molecular weight compounds typically predominate. Chloramination by-products of particular interest include dichloroacetic acid, cyanogen chloride and cyanogen bromide, and MX.

Chlorine dioxide disinfection produces a wide variety of organic by-products, including chlorite, some chlorate, a number of carboxylic acids, some maleic acids, oxalic acids, and dichloroacetic acid. Ozonation produces bromate and other brominated by-products (including the brominated aldehydes, ketones, and acids), a variety of aromatics, and carbonyls. Limited data are available on the by-products of mixed treatments. Dr. Reckhow also presented information on bromine and ultraviolet treatments.

Dr. Reckhow highlighted the need for additional research to characterize the by-products of combined oxidants and to better understand what happens to the by-products as they move through the distribution system. There can be some very significant changes as the disinfectant and the by-product precursors move through the system. This is an important consideration for risk assessment and epidemiology studies.

The second presentation, by Mr. Fawell, focussed on bromate, a potent oxidant formed by ozonation in the presence of bromide. Metabolism data are somewhat limited, due in part to analytical constraints. Bromide levels have been found to increase in the blood and other tissues, but not necessarily in the liver.

The mutagenicity data are generally weak or negative in bacteria. However, mammalian cell studies *in vivo* and *in vitro* have been positive, particularly with respect to clastogenicity.

In chronic exposure studies, bromate produced renal tumors, peritoneal mesotheliomas, and thyroid tumors in rats, but not in mice. There are no published data on the reproductive toxicity of bromate, although the results of screening studies conducted by the National Toxicology Program will soon be available. Mechanistic research for bromate has largely focused on oxidative damage in the kidney.

The third speaker, Dr. Soffritti, presented data from some very recent studies conducted at the Cancer Research Center in Italy on formaldehyde and acetaldehyde, which are by-products of ozonation. There is not much information in the literature prior to the 1990s concerning chronic drinking water exposures to these compounds, particularly acetaldehyde. Dr. Soffritti's studies showed an increase in certain malignant tumor types for both acetaldehyde and formaldehyde, although these increases were not all dose-related. This information will fill some key data gaps in our understanding of these by-products.

Dr. Romano spoke primarily about studies on chlorite, which is a by-product of chlorine dioxide. Some early studies suggested a potential for oxidative damage to erythrocytes. Recent studies have confirmed the blood effects in rats at high doses. No reproductive or developmental effects have been observed in rats. Dr. Romano spoke about a very comprehensive two-generation reproductive neurodevelopmental study that is underway and should be completed in 1996. Preliminary observations show (1) no effects on fertility or reproductive parameters in the F_0 generation, (2) no effects on motor activities, and (3) no other adverse developmental or neurodevelopmental effects on the F_1 generation. More definitive conclusions will be possible after the complete study is published.

Dr. Morgan presented some very interesting information on the importance of considering the inhalation route of exposure for comprehensive risk assessments, particularly for such activities as showering and swimming in pools. He emphasized that the types of studies we conduct, regardless of the organ being studied, offer opportunities to incorporate mechanistic considerations in risk assessments if we are careful about how we select and design these studies. Dr. Morgan pointed out that, among the different disinfectants and small set of by-products he studied, the toxic response in the nose varies with respect to dose, the location of the lesions, and the features of the tissue response. This can certainly be generalizable to other types of toxic responses and the effects of agents.

For inhalation risk assessments, we need to consider both the qualitative and quantitative differences in nasal metabolism and toxicity. The issues of defining adversity and determining the importance of these endpoints from a public health perspective are both very important.

Session Discussion

Chaired by Fred S. Hauchman
National Health and Environmental Effects Research Laboratory,
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DR. HAUCHMAN: I would like to propose that we address the following questions to help focus our discussion on identifying priorities for research. These issues are relevant to alternative disinfection treatments as well as to chlorination:

- Are we focusing on the most important by-products? What key compounds have not been studied?
- What are the toxicities of greatest concern? Are we focusing on the most important ones from the standpoint of risk assessment?
- How "far" in assessing the drinking water risk can the individual chemical data take us? That is, if we have adequate data on individual by-products, what does that really tell us about the risks that might be associated with exposure to the mixture of by-products produced by a disinfection process?
- What studies are needed to compare the toxicity of drinking water mixtures produced by combinations of these alternative disinfectants?

There may be some by-products for which additional toxicity data are not considered necessary because the levels in drinking water are so low or the available data suggest toxicity is low. Some by-products may only need to be evaluated as far as screening level tests, whereas others may warrant more detailed toxicity studies. I would like to look at these issues for the different alternative disinfectants and their by-products, starting with the ozonation by-product, bromate.

MR. FAWELL: I find it very difficult to believe that the levels of bromate in drinking water are genuinely a risk to human health. But the compound is very potent in carcinogenicity studies. It produces tumors at relatively low doses. The margin between the drinking water concentrations and the levels that produce tumors is not that great. Therefore, bromate is a very important compound, because it could drive many of the regulations and could be the rate-limiting step in a whole range of water treatment processes.

Bromate will be difficult to measure and control at very low concentrations. So, now that we know bromate is carcinogenic and genotoxic, we absolutely must look much more closely at the dose-response curve. We must determine the mechanism, because we cannot do experiments involving thousands and thousands of animals to look for a classical toxicity type of dose-response curve. We must get into much more detailed mechanisms, down to the biochemical level. If we can do that, I think we will be able to demonstrate that bromate is operating through an indirect mechanism, and that the dose-response curve is sufficiently nonlinear to suggest that we have to take a different approach to developing bromate standards.

Finding the best approach for the standard is important. The politicians and regulators must be able to justify that the regulations are sufficiently protective of human health while not being excessively stringent.

DR. HAUCHMAN: What additional bromate research should we focus on?

MR. FAWELL: We need to better understand the mechanism behind the renal tumors, and then use that data to investigate the mesotheliomas. We also need to better understand the thyroid tumors, which may have more to do with the oxidation of iodine in the diet than the direct action of bromate. But this issue is important, because the preliminary data from the U.S. EPA that were shown yesterday indicate that the thyroid tumors may be occurring at around 15 milligrams per liter, which is about 1,000-fold above the proposed standard. So, we do need to see whether the thyroid tumors are actually due to the oxidation of iodine.

DR. SOFFRITTI: We must remember that, before we move from one technology to another, we should have good data on the new technology. There is a huge difference in the carcinogenic potencies of trihalomethanes and the ozone by-products.

DR. HAUCHMAN: How can we best prioritize by-products for further study?

MR. FAWELL: It is essential that we start using a little common sense in looking at these compounds. We have a lot of information on some compounds. When our general knowledge about a group of compounds indicates that we do not have to worry about them, we should drop them. Then we can start concentrating on the other ones.

The analytical chemistry is very important because we also need to consider which compounds are present at the highest concentrations. One problem we find is that the only way to generate the funding to find out what concentrations people are exposed to is by doing a regulation. But to do the regulation, you need a feel for the concentrations. We must get back to looking at that in a more sensible way.

Some compounds are present in extremely low concentrations. When there is a huge gap between the potential toxicology and the actual exposure level, we ought to forget those compounds and focus our efforts on compounds that are present in the highest concentrations.

I believe that synergism, and not just additivity, is a concern with mixtures. But the greatest concern regarding synergism is with compounds present at higher concentrations—unless it can be demonstrated that a substance present at some tiny concentration can produce a huge increase in a response.

DR. RICHARDSON: I am an analytical chemist, and work at the U.S. EPA in Athens, Georgia. Our lab has been involved in identifying new disinfection by-products from different disinfectants. We have only identified the easy chemicals so far. There are many we have not identified yet, and we are identifying new compounds every day. I am frequently asked whether these are toxic and whether they are going to be regulated. Because there are so many compounds, is there any way we can reduce the number of toxicology studies conducted on a single by-product? How many more studies do we need on trihalomethanes or formaldehyde? I would like to see some of the newer compounds addressed as well.

DR. MORGAN: There is no end to the toxicology that can be conducted on any individual chemical. One of the biggest problems for carcinogenesis is that we do not understand cancer because we do not understand normal cell development.

DR. RECKHOW: Can structure-activity relationships help us decide which types of compounds we should be looking at?

DR. BULL: That is an interesting question. Such a long list of chemicals and limited information on related chemicals creates a real difficulty for structure activity relationships. However, the cost of doing baseline toxicology on compounds that are found in infinitesimally small concentrations makes no sense.

Regarding the ozonation by-products, no sensible toxicologist would bother with many of the compounds, for example, the non-halogenated acids. There may be a little more concern about some of the aldehydes. Guessing, with clearly inadequate data, I would say there probably is not a problem with any of the ozone by-products, except for bromate and the formation of brominated organic compounds, which are likely to be aldehydes and acids.

Getting back to Dr. Richardson's question, structure-activity relationships are very nice if you know something about the class of compounds you are dealing with. It is likely that no one at this workshop would have predicted that haloacetic acids would be a problem based on structure-activity relationships. My first reaction to hearing back in the 1980s that trichloroacetic acid was a chlorination by-product was to say that we should move on to something more interesting because I did not believe that it would be easily absorbed and distributed within the body due to its high polarity. I may still be right, but for the wrong reasons.

Data on structure-activity relationships are not readily available for many by-products, particularly for the types of polar compounds that are clearly the dominant forms produced in drinking water. We need a fair amount of information even before structure-activity relationships become a particularly useful way of looking at the class.

I think we should return to what Dr. Krewski suggested yesterday: Can we use a screening technique that involves gathering a minimal amount of information on maximum tolerated dose and then looking at the concentration of the compound in drinking water? Can we cease, or at least postpone, further investigation of compounds that have a maximum tolerated dose far above that which would be derived from drinking water without collecting data to more clearly define the potential health effects these compounds could produce in humans?

MR. KRASNER: I will try to simplify the list from three pages to three compounds: bromate, chlorite, and dichloroacetic acid and its brominated analogs (dibromoacetic acid and bromochloroacetic acid). I will take a different perspective using occurrence data as a tool to prioritize by-products for further research. Let us focus on the alternative disinfectants and first consider ozone. Ozone does produce a wide variety of by-products, but if a utility uses biological filtration (and not all U.S. utilities do), then many of the by-products can be removed. Formaldehyde is a good example. A biological filter easily removes more than 90 percent of formaldehyde.

We do not yet have a regulation that requires ozone systems to do biological filtration. Maybe the type of data that Dr. Soffritti is developing provides one more impetus for biological filtration. While this type of filtration was originally designed to make water biologically stable, it also can help control potentially toxic chemicals.

This is why I am more concerned with bromate from an ozone viewpoint. Studies show that the amount of ozone needed for disinfection, or the amounts of hydroxyl radical activity required to control things such as atrazine, are also the levels that will produce bromate if bromide is present.

On the chlorine dioxide side, I suggest that chlorite is a concern because about 80% of chlorine dioxide ends up as chlorite. Chlorine dioxide disinfection produces around 1 mg/L of chlorite.

Finally, I picked dichloroacetic acid and its analogs because ozone destroys the precursors of trihalomethanes and trichloroacetic acid, but not dichloroacetic acid, as Dr. Reckhow's work has shown. And, while chloramination and chlorine dioxide treatment may control trihalomethane formation, they do not seem to control dichloroacetic acid formation. So, from the perspective of alternative disinfectants, the trihalomethanes and trichloroacetic acids may come down, but the dihalogenated acids may remain the outlier. I have oversimplified this a bit, but if I were to prioritize, I would emphasize those three.

MR. REGLI: We need to shift the primary focus of our research efforts from individual by-products to groups of by-products. There is nothing that precludes us from having treatment technique requirements to control for different classes of by-products. Regulatory control of by-products would be greatly facilitated if we can define boundary conditions that would limit exposure to by-products and if we can define the magnitude of risks associated with groups of chemicals.

With microorganisms, we have a simpler conceptual approach because we have identified key microorganisms that are much more resistant to disinfection than others. For example, by targeting *Cryptosporidium*, we think we have a high probability of reducing risks for most other pathogens. If we can create similar analogs for different groups of by-products, we could move a long way. Nothing in the Safe Drinking Water Act precludes us from setting limits for groups of DBPs.

DR. CHARLES: Mr. Fawell, do you think that we eventually might be able to use oxidatively-damaged DNA adducts as a tool to look at biomonitoring and dose-response relationships?

MR. FAWELL: I do think that DNA adducts could possibly be used to look at dose-responses, particularly in model systems. However, I am not sure that DNA adducts will be quite so useful as a biomarker in looking at human populations because they are difficult to track down. The oxidative damage markers are not a specific biomarker, so you could have lots of confounding effects.

DR. CHARLES: It has been suggested that halogenated compounds can promote or modulate oxidative damage. Mr. Fawell, do you think that chlorination in a situation that involves a lot of compounds could exacerbate the oxidative effects of bromine?

MR. FAWELL: That is a very interesting question. We saw some indication yesterday that dichloroacetic acid may modulate the lipid peroxidation and oxidative damage at very high concentrations. I think the problem really is concentration-dependent. I think synergism is the key in mixtures. At the low concentrations of by-products in drinking water, if you do not see synergism in a mixture, then additivity will not be very important. We need to identify a couple of compounds that we expect will produce a substantially greater effect as a mixture than separately. Perhaps we should do this with bromate and some of the other by-products if they are present in sufficient concentration.

DR. BULL: Dr. Morgan, I think the work you presented on the nose in this session is of interest to drinking water because people do use drinking water to shower. I would like to relate a problem we encountered when we first started to evaluate the alternative disinfectants, chlorine dioxide and chloramine.

We were attempting to conduct initiation/promotion studies and wanted to utilize realistic exposure conditions. Therefore, we attempted to get mice to swim in water containing varying concentrations of these disinfectants for 5 minutes or so. The mice survived chlorine at several pHs well up to and beyond 100 mg/L. However, with chlorine dioxide we were lucky to get them to survive even exposures as short as 5 minutes and concentrations as low as 20 mg/L because they developed severe respiratory congestion and many died. Chloramine produced similar difficulties. I bring this up to point out that concern that a chemical at a few parts per million in water could be an inhalation hazard is not irrational.

DR. MORGAN: I agree. There is nothing trivial about these questions.

DR. GATES: It is important to note that chlorine as a gas in water does not last as chlorine very long. It hydrolyzes, so you must keep those chemical components in mind. For example, it probably is not chlorine that we smell around swimming pools or in chlorinated drinking water but either chlorine monoxide or other organic chemicals. The various disinfectants all have different boiling points. Ozone and chlorine dioxide remain as gas, for example. So, it is important to understand the chemistry in order to do the toxicology properly.

DR. MORGAN: I agree. Toxicology started in chemistry and pharmacology. These issues certainly are complex. I, personally, do not feel that, apart from the chlorine issue, the parent compounds are a concern with respect to nasal toxicity.

SESSION 4
BIOLOGICALLY BASED RISK ASSESSMENT

Introduction and Overview of Approaches

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This session will focus on additional data as well as the use of the data for modelling. I will briefly review what we mean by environmental risk assessment. There are many kinds of risk assessment. For example, we all do risk assessment daily. When we choose to ride in our cars, we have made a risk assessment determination—first that the benefits of riding in the car outweigh the risks, and second that the benefits outweigh alternative approaches.

Environmental risk assessment is the process we use to evaluate the likelihood and nature of the public health effects of environmental pollution. It is important to note that much environmental risk is involuntary. It is imposed upon the public. This affects public perception of environmental risk.

The risk assessment process was first codified by the National Academy of Sciences in their 1983 "red book." The process involves four different components: hazard identification, dose-response assessment, and exposure assessment, which together feed into the fourth component, risk characterization.

Hazard Identification

Hazard identification involves identifying the potential for something to cause an effect. It relates exposure to a substance to the incidence and/or severity of an adverse health effect. Hazard identification does not determine the dose-response relationship, but rather investigates whether, under certain conditions, the chemical has the potential to cause a certain kind of response. Many screening methodologies are really hazard identification processes.

We can get hazard identification data in different ways—from human and animal toxicity data and from *in vitro* data. *In vitro* data include not only work with cells, organs, or tissues from animals or people, but also some of the computer modeling work, for example, structure-activity modelling and the kind of mathematical modelling that provides insight into understanding what may be happening in an environmental situation. Human data include epidemiological studies, case reports, field studies, and controlled clinical experiments. Animal data may be short- or long-term, and may be from specialized assays that look for a particular type of effect, such as carcinogenicity or developmental and reproductive toxicity.

It can be extremely difficult to decide which responses are adverse. Some responses, such as mortality, are obviously adverse. Effects such as morbidity or disease will usually qualify as adverse. But other effects are less clear cut, such as pathophysiologic changes that do not result in frank disease, but clearly are not a normal response. For example, we can readily identify biomarkers as physiological changes, but we often do not understand their significance. When an effect has unknown significance, that does not

mean it is not adverse. It simply means that we lack sufficient knowledge to understand its significance. Also, there may be populations of differential susceptibility, and a change of uncertain significance in one part of the population may be an important change in another part of the population.

Some physiological changes may not themselves be adverse but may signify that the susceptibility of the population has changed. With lead, for example, we cannot say that the IQ of an individual child would be a certain number of points higher if the child had blood levels of 10 μg/dL instead of 30 μg/dL. However, we can look at the population distribution and say that populations with higher blood lead levels have higher IQs overall than populations with lower blood lead levels. We should start to look for these subtle shifts in distribution, and consider populations as a whole rather than looking at control versus exposed groups.

Exposure Assessment

Exposure assessment is the qualitative or quantitative description of contact of a substance with an organism in terms of intensity, frequency, duration, and route. I used to say that you could address exposure assessment by asking the same basic questions you would ask in writing a newspaper article: Who, how, what, where, when, and why? You want to know who is exposed, how many people are exposed, how they are they exposed, and for how long.

Many problems with epidemiology studies would be less of a problem if we had better exposure information—for example, if we had better markers of exposure than simply basing exposure on where people live or whether the wells in an area have a certain level of a contaminant over a certain period of time.

Dose-Response Assessment

Dose-response assessment is the third part of the risk assessment paradigm. This is the quantitative relationship between the amount of exposure and the observed health response. I suggest that, for some of the well-studied compounds, we are past the point of needing more hazard identification and our real research need is in the area of dose-response assessment and defining the shapes of the dose-response curves.

With dose-response assessment and with risk assessment as a whole, we have to deal with extrapolation issues. Dose extrapolations are probably the easiest extrapolations. They can involve the development of physiologically based pharmacokinetic models, which provide a lot of power in doing high-to-low dose, acute-to-chronic, and route-to-route extrapolations. Response extrapolations, where we predict human response based on animal data, are much more difficult. We are starting to develop biologically based dose-response models to aid in these kinds of extrapolations.

Mechanistic studies are critical for response extrapolations. We have to know whether or not human populations have the same capability to respond as animal populations in order to extrapolate from animals to humans. In this area, modelling work can shed insight into the kind of studies that are needed and the mechanisms that are involved.

To improve risk assessment, we need to move from the default assumptions to biologically based approaches. We should incorporate the newest and best science, including experimental and modelling

work, both *in vivo* and *in vitro*. I suggest that we need iterative approaches. The people doing experimental work should be integrated with the people doing modelling work, so that you do an experiment, then you develop a model, and then you use the model to predict experiments that you can use to validate or invalidate the model.

In vitro and *in vivo* work also need to have an iterative relationship. The *in vitro* work can illuminate what is happening at the cellular level, while the *in vivo* work is essential to determine whether the *in vitro* findings have any relationship to reality in the animal. Also, iterative *in vivo* and *in vitro* work can help us answer certain mechanistic questions.

Risk Characterization

Risk characterization integrates the results of hazard identification, dose-response assessment, and exposure assessment. One of the most important aspects of risk characterization is to very explicitly define the assumptions used and the uncertainties involved in all the different steps of risk assessment.

The results of risk assessment can be used as the basis for standard-setting and regulatory decision-making or other activities to manage the risk. Risk management decisions are influenced by many factors other than the scientific risk assessment, including economic, legal, social, political, and engineering issues. Risk management decisions can range anywhere from a decision that no action is necessary to, for example, sending information to educate the public, to the setting of ambient standards, and eventually to outright bans of certain processes or chemicals.

Session Overview

In this session, we will look at a series of case studies that involve different compounds and different approaches to those compounds. These case studies will demonstrate various approaches that can be used, such as mechanistic approaches and various modelling approaches to improve our understanding of the science, as well as a database approach for risk assessment.

Dr. Nesnow will show mechanistic data for dichloroacetic acid and describe how this is being used to develop a biologically based dose-response model. Dr. Pegram will focus on bromodichloromethane. He will describe the pharmacokinetic and mechanistic data for this compound, and compare bromodichloromethane with chloroform. Dr. Conolly will talk about some mechanistic and pharmacokinetic data for chloroform and how this feeds into a biologically based dose-response model. Dr. Chiu will look at some of the uncertainty surrounding the exposure to these compounds.

Overview of the Mechanisms of Carcinogenic Action of Dichloroacetic Acid in the Male B6C3F$_1$ Mouse: Application to the Construction of a Biologically Based Dose-response Model

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The chloroacetic acids are formed when water containing organic matter is treated with chlorine. They are found in finished drinking water. Large populations are exposed to this class of disinfection by-product. Dichloroacetic acid (DCAA), the most widely distributed chloroacid in drinking water, occurs in the 45 to 160 μg/L range. In addition, DCAA has been used for therapeutic purposes and is a metabolite of several industrial chlorinated solvents, including trichloroethylene and tetrachloroethylene. DCAA is a hepatocellular carcinogen in the male and female B6C3F$_1$ mouse and in the male F344 rat.

Liver cancer, like most other forms of cancer, proceeds through a series of identifiable steps to the ultimate lesion, hepatocellular carcinoma. Intermediary lesions have been identified, such as altered foci, hyperplastic nodules, adenomas, and carcinomas. Moolgavkar, Venson, and Knudson (MVK) described a multi-stage, two-genetic event, biologically based model for cancer development. This model predicts that the carcinogenesis process proceeds as a result of an alteration in the DNA that leads to an *initiated* cell with a competitive growth advantage over the unaltered surrounding cells. Over time, a relative abundance of progeny cells is produced (i.e., promotion). These initiated cells are presumed to be predisposed to subsequent transformation(s) that induce the cell(s) to proceed to the malignant state (i.e., progression/conversion). The MVK model predicts that physical or chemical agents that increase the rates of initiation, clonal expansion of the altered phenotype, or the progression/conversion of initiated cells into malignant cells will influence the final number of carcinomas. Also, chemicals that affect cell death/differentiation rates at each stage will alter the number of tumors predicted by the model.

Mechanistic evidence was presented for the construction of a biologically based dose-response (BBDR) model for DCAA that is more extensive than the MVK model as it contains at least five stages with possible shunts. In the construction of a BBDR model for DCAA in the male B6C3F$_1$ mouse, three major categories of evidence are needed: (1) the number and identity of lesions (stages in model), (2) the effects of DCAA on cell proliferation and apoptosis (α and β), and (3) genetic toxicity and metabolism (μ) (see table).

We described the chronic effects of DCAA treatment in the drinking water of the B6C3F$_1$ mouse using pathological, immunohistopathological, and marker analysis of lesions. Histological characterization of lesions was used to provide evidence of lesion sequence. Cell proliferation studies *in vivo* and *in vitro*, and apoptosis studies, were summarized. Oxidative responses, genetic toxicity, and metabolism studies were summarized. We reported that (1) DCAA induces peroxisome proliferation and lipoperoxidation, (2) DCAA gave mixed responses for single strand breaks in male B6C3F$_1$ mouse liver, (3) DCAA induces micronuclei in B6C3F$_1$ mouse blood polychromatic erythrocytes and DNA damage in blood leukocytes *in vivo*, (4) DCAA was mutagenic in *E. coli* and *Salmonella* and a gene mutagen and clastogen in mammalian cells, (5) the H-ras codon 61 mutant spectra in tumors induced by DCAA is different than that from spontaneous tumors, and (6) metabolism studies suggest covalent DCAA-protein adducts. We concluded that DCAA has the potential to damage DNA directly and/or indirectly and to induce DNA damage, gene

mutations, and chromosomal events. A model was proposed that explained the results of these mechanism-of-action studies involving DCAA in the male B6C3F$_1$ mouse.

Components of a Biologically Based Dose-response (BBDR) Model for Dichloroacetic Acid in the Male B6C3F$_1$ Mouse

Model Parameter	Mechanistic Study
Characterization and quantitation of lesions (Number of stages in model)	• Pathological analysis of lesions • Immunohistological characterization of lesions • Marker analysis within lesions • Quantitative morphometry of lesion size and number
Effects of DCAA on cell proliferation and apoptosis (α,ß)	• Cell proliferation, apoptosis, and gap junction intercellular communication in normal tissues and lesions
Genetic toxicity *in vivo* and *in vitro* (μ)	• Metabolism • Mutation and chromosomal damage • Direct/indirect DNA damage • H-ras mutations and p21 overexpression • Oxidative properties of DCAA • Peroxisome proliferation • Lipoperoxidation

Use of Mechanistic and Pharmacokinetic Data: Bromodichloromethane as a Case Study

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Bromodichloromethane (BDCM) is generally second only to chloroform in prevalence among disinfection by-products in chlorinated drinking water, but brominated trihalomethanes (THMs) can occur at greater concentrations than chloroform when source waters with high bromide levels are disinfected. BDCM appears to be a more potent carcinogen in rodents than chloroform, producing tumors at lower daily doses in the liver, kidney, and large intestine.

Research has been undertaken to examine the pharmacokinetics and biological activities of BDCM in rats with the goal of developing a biologically based dose-response model for BDCM carcinogenesis. Comparisons to chloroform have been incorporated into experiments when possible.

A physiologically based pharmacokinetic model has been developed for BDCM using circulating bromide levels after constant concentration inhalation exposures to determine metabolic rate constants. The metabolic parameters and partition coefficients derived for the model as well as various model simulations indicate that BDCM is absorbed into tissues and metabolized more rapidly than chloroform and that metabolism other than oxidation may be important for BDCM. The cytochrome P450 isozymes, CYP2E1 and CYP2B, are involved in the metabolic activation of BDCM, which is required for hepatotoxicity, and repeated dosing can lead to a loss of hepatic CYP2B activity. Metabolic inhibition, such as that noted for CYP2B, is a possible explanation for the inverse dose-response observed for liver cancer in rats when BDCM was given in drinking water.

Two potential carcinogenic modes of action have been examined for BDCM: cytotoxicity and genotoxicity. The cytotoxicity of THMs has been attributed to macromolecular binding, and *in vitro* microsomal protein and lipid binding by metabolites of BDCM was found to exceed that of chloroform-derived intermediates. BDCM is also a more potent *in vivo* renal cytotoxicant than chloroform after gavage in an aqueous vehicle.

We have employed a strain of *S. typhimurium* TA1535 transfected with glutathione *S*-transferase (Thier et al., 1993) to demonstrate that BDCM can be activated to a mutagenic intermediate via glutathione (GSH) conjugation, while chloroform was found to be inactive in this assay system over a similar concentration range. This is the first demonstration of GSH-mediated activation of a THM and may explain the greater genotoxicity of brominated THMs compared to chloroform. Significant *in vivo* covalent binding of BDCM to DNA was observed in all cancer target tissues six hours after oral dosing, but was especially high in the liver and intestinal tract.

In conclusion, our results, in conjunction with data from other studies, demonstrate that chloroform is not the most potent THM. BDCM exhibits greater carcinogenicity, metabolic rates, macromolecular binding, and both cytotoxicity and genotoxicity. These findings further indicate that the different THMs do not act via identical mechanisms, and a biologically based dose-response model for BDCM carcinogenesis should include both cytotoxic and genotoxic modes of action.

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Use of Mechanistic Data: Chloroform as a Case Study

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Under the draft revisions to the U.S. EPA's cancer risk assessment guidelines, the development of quantitative models for low-dose extrapolation is a two-step process. First, a model is developed for the exposure range over which tumor data or data closely linked to tumors are available. Second, a model is developed for extrapolation from the exposure range used experimentally to the lower exposure levels that are typically more relevant for risk assessment. The form of the extrapolation model may be based on the mechanism of action of the carcinogen. This two-step process is different from the current default approach to risk assessment modeling for chemical carcinogens - the linearized multistage (LMS) model.

The LMS model provides no flexibility for the form of the low-dose extrapolation. The proposed new guidelines, by specifically encouraging the use of mechanistic information for low-dose extrapolation, would dramatically alter the default approach to carcinogen risk assessment.

In this presentation, we consider how the proposed new guidelines would be used for human cancer risk assessment based on chloroform hepatocarcinogenicity in mice. For the first step, modeling in the range of the data, we use dose-response data for chloroform-induced hepatocyte proliferation as a surrogate for the tumor response. This choice is based on (1) the substantial body of data showing a lack of direct *in vitro* or *in vivo* genotoxicity of chloroform, (2) the potency of chloroform in causing hepatic cytolethality with subsequent regenerative cell proliferation, and (3) the consistency with which no hepatic tumors are seen with non-cytolethal exposures, but are seen with exposures causing cytolethality and regenerative cell proliferation.

For the second step, extrapolation modeling, we assume that exposures not causing cytolethality are not associated with an increased risk of cancer. This choice is based on (1) the lack of direct genotoxicity of chloroform, and (2) the consistency of the correlation between chloroform-induced regenerative cell proliferation and cancer. Thus, for low-dose extrapolation, we assume an exposure threshold and calculate a reference dose (RfD), or reference concentration (RfC), or a margin of exposure (MOE) based on a predicted exposure-curve for hepatic cytolethality in people. Implicit in this approach is the assumption that chronically stimulated cell proliferation and other related events, such as inflammation, nuclease release, and growth stimulation, act to initiate and promote the carcinogenic process.

A biologically based dose-response (BBDR) model is used to link chloroform exposure with cytolethality and regenerative replication and to predict the exposure-cytolethality curve for people. This approach maximizes the use of mechanistic information in predicting the human exposure-response curve and thereby reduces the uncertainty of the prediction relative to predictions based on default methods. The BBDR consists of two submodels. First, a physiologically-based pharmacokinetic (PBPK) model (Corley et al., 1990) links chloroform exposure with dose to the liver. The PBPK model was developed using data from B6C3F1 mice and F344 rats. Scale-up of the model to people was also described by Corley et al. (1990), including an estimate of human metabolic capacity for chloroform based on experiments with human liver microsomes. Second, a pharmacodynamic (PD) model links liver dose predicted with the PBPK model with corresponding cytolethality and cell replication. Butterworth and colleagues (Larson et al., 1994a; Larson et al., 1994b; Larson et al., 1994c; Larson et al., 1995a; Larson et al. 1995b; Larson et al. 1996) have carried out extensive determinations of the relationships between chloroform exposure in drinking water, air, or corn oil gavage and induced cell replication measured as 3.5 day labeling index in B6C3F1 mice and F344 rats. For the purposes of constructing the PD model we assumed a one-for-one correspondence between cells killed by chloroform and new cells created by regenerative replication. A delay of 40 hours was used to describe the lag between chloroform cytolethality and the start of regenerative replication. The PD model links hepatic dose of chloroform with cell killing using an empirical function calibrated against dose surrogates predicted with the PBPK model and the cell replication data. A simulated labeling index was created by using the BBDR model to track cell replications over the interval ($t = 3.5$ days) where t is the current time. It was possible to obtain good simulations of the labeling index data for both mice and rats using a fixed form of the PD submodel and scaling the PBPK model for mice and rats as described by Corley et al. (1990). This result suggests that differences between B6C3F1 mice and F344 rats in sensitivity to the hepatotoxicity of chloroform are due to differences in chloroform pharmacokinetics between mice and rats (Figure 1). this implies that mouse and rat hepatocytes are roughly equally sensitive to killing by chloroform and supports the use of this

approach for scale-up of the BBDR model to the human case. For human scale-up, the PBPK model is scaled according to Corley et al. (1990) and the PB model is used in the fixed form developed by calibration against the mouse and rat data.

A preliminary test of the ability of the human version of the BBDR to predict chloroform-induced hepatotoxicity was conducted by analyzing a clinical case report of chloroform poisoning (Wallace, 1950). A 78 kg man addicted to a cough syrup containing chloroform was estimated to have had a chronic daily exposure of 33.4 mg chloroform/kg/day. Simulation of this exposure using the BBDR model predicted no hepatotoxicity (Figure 2). However, change in the chloroform V_{max} estimated by Corley et al. from 15.7 to 31.4 mg/kg/day predicted a hepatotoxic response (Figure 2). This adjustment of the V_{max} is not unreasonable given the uncertainties associated with estimating an *in vivo* V_{max} from data obtained in microsomes and expected interindividual variation. This result, while preliminary, shows that development to date of the BBDR model for chloroform is consistent not only with extensive experimental data obtained from laboratory rodents but also with a clinical case report of human chloroform poisoning. This BBDR for chloroform, combined with the well-supported assumption that the hepatocarcinogenicity of chloroform in rodents is secondary to cytolethality and induced regenerative replication, illustrates how mechanistic research and quantitative modeling can be used within the context of the new cancer risk assessment guidelines.

In summary, the proposed new guidelines for cancer risk assessment are compatible with the development of a new, mechanism-based cancer risk assessment for chloroform that (1) explains the hepatocarcinogenicity of chloroform in mice as being driven by chronic stimulation of cell proliferation, and (2) uses a threshold approach to low-dose extrapolation based on the exposure-response for cytolethality and the lack of direct genotoxicity.

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DOSE SURROGATE VS LABELLING INDEX: FEMALE B6C3F1 MICE, MALE F344 RATS

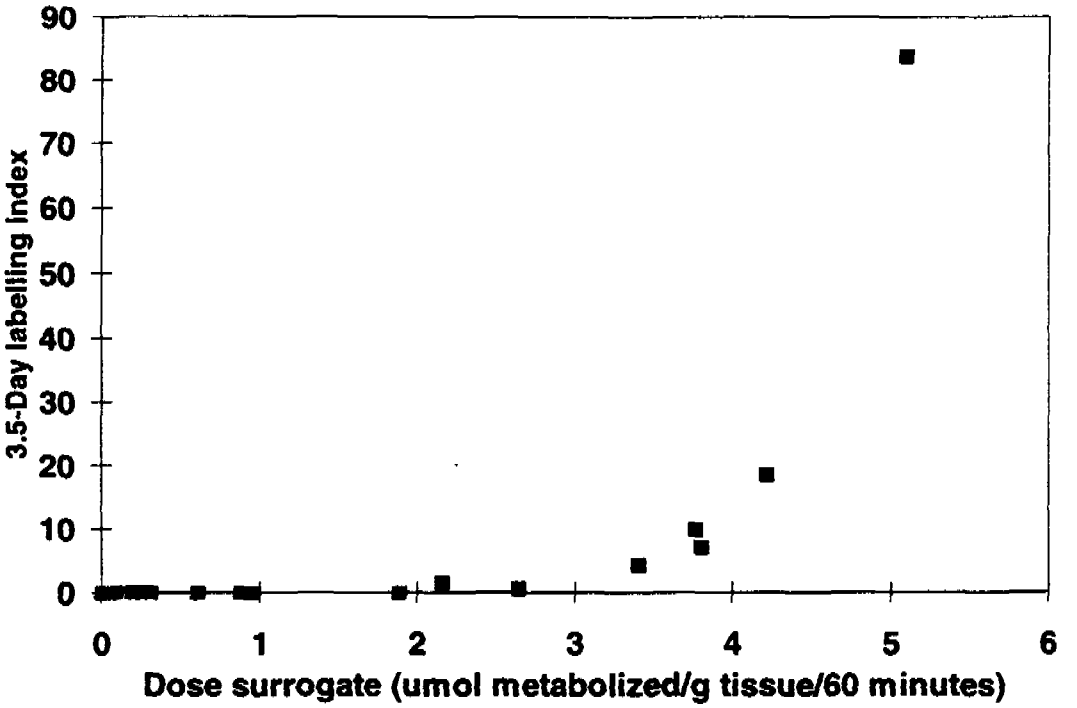

Figure 1. Correlation between chloroform dose to the liver of female B6C3F1 mice and male F344 rats predicted with a PBPK model for chloroform and hepatic 3.5 day labeling for animals given various doses of chloroform by corn-oil gavage for 4 days with sacrifice on the 5th day.

SIMULATION OF HUMAN CHLOROFORM TOXICITY: SEVEN DAYS OF COUGH SYRUP

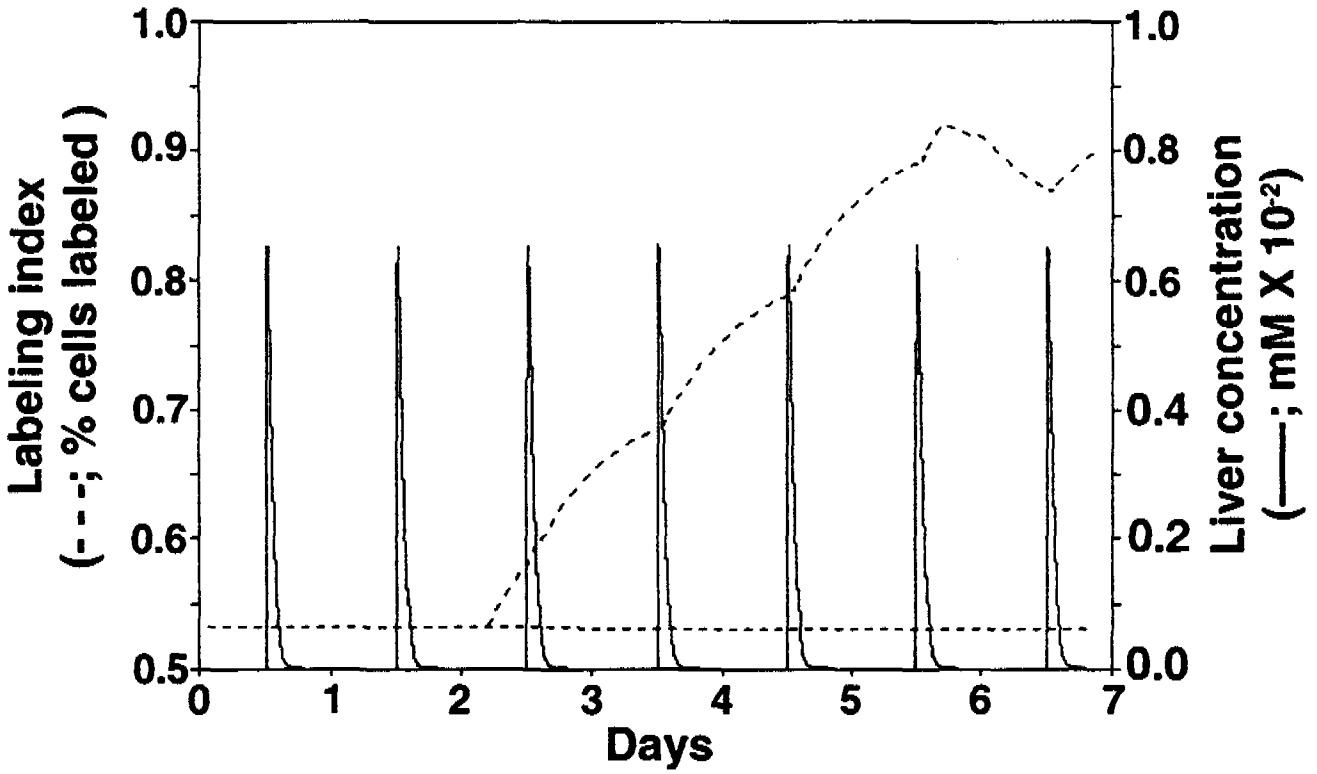

Figure 2. Simulation of hepatic concentration of chloroform (solid line) and hepatic 3.5-day labeling index (dashed line) in a 78-kg man consuming a bolus dose of 33.4 mg chloroform/kg/day for 7 days. Labeling index is increased (upper dashed line) when the capacity to metabolize chloroform (V_{max}) is set to 31.4 mg chloroform/kg BW/day. With V_{max} at 15.7, the value obtained by Corley et al. (1990), no increase in labeling index over the basal level was seen (horizontal dashed line).

Risk Characterization for Chloroform in Drinking Water: Issues and Estimates on Exposure and Cancer Risk

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U.S. Environmental Protection Agency

Human exposure to a chemical in tap water can occur by pathways other than ingestion of drinking water, including inhalation and dermal pathways. To estimate the inhalation and dermal exposures of chloroform from the drinking water, various modeling approaches were used. The results were then compared with the monitoring data available. Uncertainty and variability of these exposure estimates were derived with modeling using available measured data as parameter inputs. Our assessment indicates that human exposures to chloroform in drinking water via inhalation and dermal absorption are comparable to the exposure level from ingestion.

Several animal studies have shown that chloroform is carcinogenic (NCI, 1976; Jorgensen et al., 1985; DeAngelo, 1995; Matsushima, 1994). Studies of chloroform-induced genotoxicity yielded mixed results (Rosenthal, 1987). A number of researchers concluded that the weight of evidence supports the view that chloroform is not genotoxic. Some investigated the association between chloroform-induced cell proliferation and increased cancer incidence.

This assessment researched the correlation between certain parameters of cytotoxicity (short-term induced proliferation; hyperplasia and necrosis at necropsy) and tumorigenicity in target tissues (i.e., liver and kidney) reported by the available studies. Various variables such as species, strain, sex, target tissue, dose level, dose vehicle, and exposure duration were specified. The evaluation attempted to focus on both qualitative and quantitative aspects.

There is a good correlation between short-term induced cell proliferation of chloroform and the occurrence of liver tumors in female $B6C3F_1$ mice (-/- drinking water, and +/+ corn oil gavage) (NCI, 1976; Jorgensen et al., 1985; Larson et al., 1994a). It appears that there is no correlation between short-term induced cell proliferation and tumorigenicity in [1] liver of male Fischer-344 rats (-/+, drinking water) (DeAngelo, 1995; Larson et al., 1995), [2] liver and kidney of Fischer-344 male rats (+/-, inhalation) (Matsushima, 1994; Larson et al., 1994b), and [3] kidney of male $B6C3F_1$ mice (+/-, corn oil gavage) (NCI, 1976; Larson et al., 1994c). Data also suggest that there is no correlation of long-term hyperplasia and necrosis at necropsy with kidney tumorigenesis in male Osborne-Mendel rats in bioassays using either drinking water or corn oil gavage as vehicle (NCI, 1976; Jorgensen et al., 1985; Stanford Research Institute, 1985).

Cancer risks were derived with the linearized multistage model (LMS) using the kidney tumor data of Osborne-Mendel male rats administered with chloroform by corn oil gavage (NCI, 1976) or by drinking water (Jorgensen et al., 1985). Because of the concern about possible effects of unequal survival rates in rats in different dose groups, the data for these studies were therefore also modeled using the time-to-

tumor method. There are no significant differences between the estimated kidney cancer risks derived with the two modeling approaches (i.e., LMS or time-to-tumor) using data of rats exposed to chloroform in corn oil (gavage) or in drinking water.

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Session Summary

Linda S. Birnbaum
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Dr. Nesnow presented the mechanistic data that the U.S. EPA's National Health and Environmental Effects Research Laboratory has been developing for construction of a dose-response model for dichloroacetic acid. He also reviewed the different stages of hepatic cancer and talked about how the development of different types of altered foci can be linked to the tumors. Some of these foci appear to progress directly to carcinomas, while others have to go through multiple stages before reaching that stage.

Dr. Nesnow and his group have made a great deal of progress in developing this biologically based dose-response model. In contrast to the now classical two-stage Moolgavkar model, their model has at least five different stages and is much more complex. That, of course, means it has many more parameters, which may mean that it has many more uncertainties associated with it. Dr. Nesnow's presentation underscored the extensive data-gathering efforts needed to develop these kinds of models.

Dr. Nesnow showed a great deal of mechanistic data, including genetic toxicology, standard histopathology, and immunohistochemical data. He presented information on one or two of the different markers for which they have information, as well as some of the metabolic information for dichloroacetic acid and some of the data on cell proliferation.

Dr. Pegram talked about bromodichloromethane as a case study and presented both pharmacokinetic and mechanistic evidence. He reviewed the information on trihalomethanes and cancer and provided fairly strong evidence that brominated compounds may present a greater risk than chloroform. He very briefly presented a physiologically based pharmacokinetic model for bromodichloromethane and showed how this model did a good job of describing some of the data that have been generated. Dr. Pegram also talked about the different mechanisms that may be involved, including the different kinds of metabolic pathways as well as the different kinds of toxicity that can come from these chemicals. He stressed that different metabolic pathways may be associated with the different kinds of toxic endpoints and that the brominated compounds may, in fact, have a previously unknown pathway for generating genotoxic products. He also presented fairly convincing data that bromodichloromethane has the potential to be a genotoxic carcinogen.

Dr. Conolly demonstrated some modeling using chloroform as a case study. This was the first time we actually saw a prediction of human response based upon animal data. He built his work upon the Chemical Industry Institute of Toxicology (CIIT) data set that links cytotoxicity and carcinogenicity. The CIIT representatives at this workshop have been very careful to state that this is not a one-to-one relationship, but it appears that the cytotoxicity may be necessary but certainly not sufficient to induce the regenerative hyperplasia.

Dr. Conolly gave a relatively unusual definition of pharmacokinetics and pharmacodynamics, but he definitely linked the physiologically based pharmacokinetic model to the pharmacodynamic responses. He used short-term proliferation as a surrogate for the tumor response, and he really brought up the issues of model uncertainty. He did not talk at all about a benchmark dose approach, but I really hope that CIIT is going to pursue that approach with the very rich data set they have.

Dr. Chiu presented data to show that there is significant inhalation and dermal exposure to drinking water, which suggested that perhaps even people who drink bottled water that has essentially no trihalomethanes will have significant exposure to these compounds from showering and bathing. This exposure can be substantial, particularly for subpopulations such as teenagers who tend to take very long showers.

Dr. Chiu also talked about the qualitative and quantitative evaluations and the multiple studies of chloroform cytotoxicity and carcinogenesis. We heard quite a bit of discussion after her presentation about the validity of this approach. At some point we will have to reconcile some of the apparent discrepancies. I knowingly used the word "apparent" because we may be looking at apples and oranges in some of these comparisons.

Session Discussion

Chaired by Linda S. Birnbaum
National Health and Environmental Effects Research Laboratory,
U.S. Environmental Protection Agency

DR. BIRNBAUM: I will mention a few areas we might want to cover in our discussion. One area is how physiologically based pharmacokinetic models or biologically based dose-response models can be used in risk assessment, and what specific research is needed to make these models more useful. Clearly, these models are data-intensive. Earlier, I stressed the need for an iterative approach—in other words, starting with a simple model, conducting research to generate new data that can be used to enrich the model, and then conducting additional research to further enhance the model. But we also need to know the minimum data sets needed to develop these models appropriately and we need to say when enough is enough.

The uncertainty issue needs to be addressed. We can develop beautiful biologically based dose-response models, but will they help us reduce uncertainty in risk assessment compared to the default models currently in use? Will these new models allow us to conduct dose or response extrapolations. Another key issue is linking the epidemiology and toxicology data.

How generic are these models? Can we go from one scenario involving a single chemical and exposure route to, for example, a different chemical? Can we develop models for complex mixtures, mixtures not only of trihalomethanes, for example, but also mixtures of trihalomethanes and haloacids or mixture of haloacids, trihalomethanes and inorganic by-products? Let's first address the issue of model uncertainty.

DR. CONOLLY: I see these data-rich, mechanism-based models as valuable tools for characterizing uncertainty. For years, physiologically based pharmacokinetics has been regarded as an attempt to lower risk numbers. I have never agreed with that. This attitude misses the point about modelling the biology that gives rise to the toxicity. The point of the exercise is to try and understand the mechanisms—to try to understand what is going on physiologically and biochemically that links exposure with the development of a toxic response. Models are just one part of our attempt to understand these linkages, but to the extent that they can provide that type of understanding, they can help illuminate that black box.

That kind of illumination can help to reduce the uncertainty associated with the prediction of risk at the end of the risk assessment process. The more we understand the mechanisms, the more confidence and less uncertainty we will have in our risk predictions. So, I think the point of collecting these admittedly

expensive data sets is so that we can plausibly argue that our risk assessments are associated with less uncertainty.

DR. PEGRAM: We can reduce the uncertainty in these models by looking carefully as best we can at what is going on in humans. This has been done with some models but not others. There are plenty of readily available tools now to look at metabolism, tissue samples, and microsomes. Some of these tools are even commercially available. At the very least, we need to look at the *in vitro* metabolism of these chemicals in human samples to see whether it produces the same type of metabolites as rodents produce. This will greatly enhance our ability to extrapolate to humans. It is an important component of reducing uncertainty.

DR. NESNOW: This is probably quite controversial, but I really think that categorizing chemicals as genotoxic carcinogens, nongenotoxic carcinogens, and cytotoxic carcinogens has gotten us into the box we are in now. Most chemicals have multiple activities. For example, they can be genotoxic, they can affect cell replication, they can affect cell death, and they can affect many other processes. Biologically based dose-response models allow us to incorporate all this biology and biochemistry into the model and then examine what is driving the model. They also get us away from these artificial categorizations that tend to limit our thinking about the types of research we might do on a chemical. I see this integrative function as the major new direction for use of these models.

DR. CHIU: I echo that view. Chemicals can do many different things. It is important to realize them all.

DR. CONOLLY: This comment concerns chloroform risk assessment. If we generally agree that developing biologically based dose-response and physiologically based pharmacokinetic models is a good idea for the reasons that Dr. Nesnow mentioned, that also commits us to working with the data we collect. If we do not agree, then why bother to collect the data? Why bother to do experimental toxicology at all?

I make this comment because the CIIT has collected an unparalleled data set on chloroform over the past 6 to 7 years. I do not think that any other laboratory in the world has collected an equivalent data set for any other chemical. If that data set is not taken seriously in risk assessment, then one has to question the value of the whole process.

DR. CHIU: The U.S. EPA has been taking the CIIT data very seriously. We have spent a lot of time evaluating the CIIT studies, as well as all other chloroform studies. However, the CIIT studies are only looking at one corner and a very narrow time frame. The correlation is really mixed for the data concerning cytotoxic cancer. We are continually evaluating the data and we hope we can draw some conclusions in the future.

DR. BIRNBAUM: In developing these extensive data sets, we are measuring many different kinds of parameters. The question is, how can we best use the resources? Which of the parameters is most important to measure? Is there any kind of formal approach to sensitivity analysis?

DR. NESNOW: These are complex questions. The approach that EPA and the National Toxicology Program are using is to try to design the chronic bioassay to collect enough biological specimens throughout the course of the assay so that you can potentially answer a wide variety of questions over time. Initially, we might look for the typical things associated with chemical carcinogenesis and tumor

progression. But if something new comes up—new antibodies for example, or if a new test becomes available, we have the option of going back and examining the specimens we collected. The key is setting up the protocol for the chronic or subchronic bioassay, and collecting, storing, and cataloguing all the tissues, so that you have the specimens you need to conduct a variety of tests without having to redo the bioassay.

The major cost of bioassays is the animal studies and the pathology. Other tests such as immunohistocytochemical tests are expensive, but nowhere near as expensive as repeating the entire bioassay. So, you can develop a very rich data set if you plan well and collaborate with other people who are knowledgeable about different types of tests. For example, we are at the cutting edge of what is known about mechanisms of action of cancer. As we learn more and more about these mechanisms and which tumor oncogenes and tumor suppressor genes or enzyme processes are important, we will be able to go back and look for these things in the bioassay specimens.

DR. CONOLLY: Over the past 15 or 20 years, we have learned that physiologically based pharmacokinetic modeling gets easier as you accumulate experience. A well-equipped laboratory could put together a fairly reasonable physiologically based pharmacokinetic model for a volatile organic pretty quickly now, because you know what data you need to collect to do that.

It is not quite as easy for some other classes of chemicals, such as metals, because the modeling is inherently more complex. But models are beginning to appear for some metals. Once a few models are in place, then you know what you need to do for those classes of chemicals. So, things get easier with experience.

If you intend to study a particular chemical and you think it would be a good idea to get a modeling effort going up front, you have to see what has been done in the field for that class of chemical and whether the available literature provides guidance on how to go about developing a model. Generally speaking, physiologically based pharmacokinetic modeling has advanced sufficiently to provide a lot of guidance. We seldom are starting from scratch in any modeling exercise. A lot of attention has been paid to the kinds of physiological parameters needed to describe pharmacokinetic models both for rodents, for humans, and even recently for some other species. So, I am reasonably optimistic about our ability to develop these kinds of models.

DR. PEGRAM: I agree with Dr. Conolly. We can employ sensitivity analysis to help determine which model parameters we really need to focus on and to give us some direction on where to invest our money. For example, some chemicals are really sensitive to things like inhalation rate in the model. Similarly, some of those chemicals may affect inhalation rates when the animals are exposed. In these cases you need to determine what the effect is and spend some time with that. Likewise, with some chemicals the partition coefficients may have a big impact on outcome, and we may need to spend more time there.

DR. BIRNBAUM: Are both of you saying that these kinds of models could begin to be used generically?

DR. CONOLLY: There is always a tradeoff here. There are somewhere around 70,000 or 75,000 chemicals in commerce now. No one seriously thinks there will ever be 75,000 physiologically based pharmacokinetic models. Clearly, society generally supports large expenditures to evaluate toxicity for a limited number of chemicals, such as dioxins, formaldehyde, and chloroform. There is another set of chemicals that are sufficiently important economically or in terms of exposure that some resources are

available to study them in some detail. But we are going to be limited to the default methodologies for most of those 70,000 chemicals.

It is important to keep this in mind. We do not think enough about how research on mechanism-based modeling can pay us back, in terms of refining the default paradigms over time. Our defaults can get more sophisticated as we learn more.

The current movement to elaborate the default model for cancer risk assessment with a series of mode-specific models under the new cancer risk assessment guidelines is an example of this. We still have some default models in cancer risk assessment, but instead of just having one rigid model, we will have different models for different categories of carcinogens.

DR. BIRNBAUM: But Dr. Nesnow just argued that we should move away from rigid categorization of carcinogens.

DR. CONOLLY: Sure, but you have to keep in mind the tradeoff between the 74,500 chemicals for which we probably will never collect rich data sets, and the relatively small number of chemicals for which we can afford to do this kind of research.

DR. BIRNBAUM: Dr. Nesnow, do you think we will be able to have more generic response models as we gather more mechanistic information?

DR. NESNOW: Response is organ-specific and chemical-specific, so I really do not think we will have generic models. However, we may be able to develop a model for a homologous chemical class for which we have successfully applied various techniques (biochemical, structure-activity relationships, etc.) to understand enough about the similarities and differences in response of the chemicals within the class. If we understand the stages and the various interactions, we can probably apply those concepts just by altering parameters as you do in physiologically based pharmacokinetic models. However, Dr. Conolly is absolutely correct. We simply do not have enough money to take on everything. We have to put our money where it will make a difference.

DR. BULL: Concerning Dr. Nesnow's comments, I want to say that categorization and modeling are not exclusive. With categorization, you are asking what aspect of the chemical is driving the process. The modeling allows you to decide what is driving it.

I also want to respond to Dr. Conolly's comments. Certainly, one-carbon compounds are fairly easy to model when you know which reactive intermediates are responsible. However, other compounds with stable nonpolar metabolites, such as dichloroacetic acid or trichloroacetic acid, that are not reactive intermediates are more difficult.

I have to take issue with some of Dr. Nesnow's interpretation of our data, because the results do not support his conclusion. When administered *in vivo*, roughly 80% of the ^{14}C from dichloroacetate that was found "covalently bound" to protein could be accounted for by metabolic incorporation as glycine and serine. That label was also to be seen in alanine and any other amino acid carbon skeleton that could be derived from glyoxylate, an established metabolite of dichloroacetate. To measure this accurately would have taken many more resources than were available. Essentially, these data show there is very little evidence that dichloroacetate is metabolized to a reactive compound that is capable of binding covalently

to anything. This is just one example of how negative data become positive data in the review literature and in criteria documents.

DR. BIRNBAUM: We have to be careful about generalizing for one-carbon compounds, because both chloroform and bromodichloromethane are one-carbon compounds, yet they behave very differently. We may be able to use a structure-activity approach to give us some insight into whether compounds behave similarly.

DR. BREACH: I would like to broaden the discussion. As a water quality manager, one problem I have with this discussion is the time factor. Back home in my company, I have eight million customers who pay me to keep their water safe. They ask whether their water is safe to drink and I try to give them an honest answer. They understand the risk from pathogens and, if you talk to them, they can understand that the risk from disinfection by-products is pretty small.

My company tries to follow the 80-20 rule. In any business, you want to get 80% of the benefit for 20% of the effort. In this discussion, we have been talking about things that will bear fruit around 5 to 20 years from now. I would like to give my customers a reasonable answer now to a relatively simple question: For disinfection by-products, how low is low enough? What we are all grappling with here is how and where to focus. The answers may be more complicated.

This morning we heard that there are a few compounds that people generally seem to worry about. I would much rather get all the expertise at this workshop focused on those four or five compounds to give me an 80% probability of risk assessment in a short period of time than waiting for 100% certainty in 20 years' time.

DR. RABINOWITZ: A lot of the issue is extrapolation. I am particularly concerned about the high- to low-dose extrapolation. The problem I have with the model that CIIT is proposing for trichloromethanes is that it is based on something that happens at high dose but that apparently has a threshold and therefore does not happen at low dose. Can we be sure that this threshold process is not masking some other process that might occur with some other chemical at low doses. Can we confidently use the CIIT model for extrapolation to low doses?

DR. CONOLLY: One way to try to answer your question is to consider when we have a level of comfort with our understanding of the toxicity for a particular chemical. We have to live with the data that we have. My sense is that there is a fair level of comfort these days with the idea that cytolethality and induced replication is the precursor for the tumors we see in mice and rats. Whether the U.S. EPA is ready to accept that story to the point of using it as the paradigm for risk assessment is evidently up in the air at present.

We could do chloroform research for the next 100 years, spend a trillion dollars on it, and still ask the question that Dr. Rabinowitz posed. His question is, in a sense, unanswerable, because we can always ask it. I would prefer questions that we can hope to address by doing more research.

DR. RABINOWITZ: I am saying that we need to be able to specify the mechanisms of action, and not just specify one mechanism of action. A model needs to be inclusive.

DR. CONOLLY: We would argue that is exactly what we have done. We have surveyed the literature. Dr. Butterworth tried to make the argument yesterday that the weight of the evidence on genotoxicity is that chloroform does not exert genotoxic effects. Dr. Pegram showed some confirmatory data on that point today.

We have developed extensive dose-response information, showing the threshold nature of the cytotoxicity in the target organs and the correlation with carcinogenesis. So we would argue that we have addressed the major plausible mechanisms of action, and that our conclusions are based on the data. What else can you do?

DR. RABINOWITZ: Maybe I should not have been as specific about chloroform as I was. But I just want to put forward the idea that high-dose effects will mask low-dose effects in any chemical. So, how do you get at the low-dose effects when you apparently have only high-dose effects? As you said, you do this through understanding the mechanism of action.

DR. BIRNBAUM: Dr. Pegram's data support this point. Dr. Pegram (and Dr. DeAngelo with bromodichloromethane) demonstrated that you have a lower tumorigenic response at high doses than at lower doses. This may be a function of inhibited metabolism at high doses, so that you may not see things at very high doses that are going on at lower doses.

Maybe we need to use a little common sense in risk assessment regarding high- to low-dose extrapolation for responses at concentrations that are a billion or more times higher than any potential exposure.

DR. WEISEL: I would like to come back to the earlier point about integrating the models and the experiments. Dr. Pegram indicated a couple of things in humans that we should start looking at. When you do these experimental integrations, it is very important not just do it in the animals at the high levels, but also to incorporate the human part at the lower levels, so that you go to the whole extreme for the pharmacokinetics. We cannot do this for the pharmacodynamics now, but we can for the pharmacokinetics. We are at the point where we can set up a whole study to look at animal responses to high doses and also to incorporate the low doses and see if they match. We can also get the metabolites through the whole system.

The other part of this is understanding some of the population dynamics and the statistics. Earlier, it was indicated that ventilation is an important factor in the sensitivity analysis. We know now the human ventilation range. When we extrapolate, we can consider whether this is an important factor, or whether other differences about the partitioning between animals and humans may be more important. We have to bring the two groups of extremes together more frequently than we do now.

DR. BARBEE: I think that studies such as those presented yesterday and today that are conducted at very high doses are of limited value for risk assessment. These studies have to be done, but we need to get down to levels that are more reflective of the physiological and biochemical processes that occur in humans exposed to disinfection by-products in the real world. I do not necessarily know how we go about that, but we do need to dose animals at levels that reflect the biochemical and physiological processes in humans. If we do not do this, we will have a very difficult time reaching closure on the issue of uncertainty. As Dr. Conolly said, we have to believe the data. We have to believe that animals are a proper surrogate for human risk assessment.

DR. BIRNBAUM: Dr. Pegram, do you know whether the glutathione theta in humans is a polymorphic form like the mu?

DR. PEGRAM: It is. The theta class is polymorphic and appears to be expressed in about 30% of caucasians and 50% of asians. Dr. Cantor was asking for a polymorphic metabolic activity that we might use in epidemiological studies. This might be a good one to try out.

MR. FAWELL: Dr. Pegram, dibromochloromethane appears to be negative on carcinogenicity and really throws the structure activity-relationship for the brominated trihalomethanes out. Have you given any thought to why this happens?

DR. PEGRAM: We have not studied dibromochloromethane at all. However, we also know that bromoform is even more reactive in the mutagenicity assay than bromodichloromethane. So, we could also ask why we do not see a greater tumor response with bromoform than with bromodichloromethane. I think those answers are only going to come from some good mechanistic studies, especially pharmacokinetic studies.

My guess is that, even though they may be more reactive, they never reach the important targets such as DNA or the macromolecules that must be reached to elicit these responses. It could also have to do with absorption rates. For example, if these chemicals are administered in corn oil, as in the National Toxicology Program studies, absorption could be greatly affected. There really needs to be a drinking water study for bromoform. Dr. DeAngelo mentioned yesterday that EPA will start such a study soon.

DR. BULL: The data you presented on bromodichloromethane almost imply that the liver tumor response is killed in the high-dose or maximum-tolerated-dose type of assay. If that is a real result, it could drive the risk assessment down for bromodichloromethane by a long way.

DR. PEGRAM: A long way, which is one reason that we intend to repeat that study, and especially look at the low-dose range in drinking water. As Dr. DeAngelo said yesterday, he has already planned that study. After we do some more mechanistic work to give us guidance in dose selection, we are going to do the study.

MR. REGLI: For most water supplies, there is fairly good proportionality between trihalomethane or chloroform reduction versus bromodichloromethane. In other words, if you get a 20% reduction of chloroform, you are likely to see the same range of reduction for bromodichloromethane. The relationship between specific by-products versus total trihalomethanes or chloroform tends to break down when you have a higher concentration of bromide species that is bromoform or dibromochloromethane. So, proportionally, we are in the same ballpark in terms of risk reduction.

At EPA, when we assess the national impact and benefits of the disinfection by-products rule, the risk that we use as the starting point for assessing the benefits of risk reduction ranges over four orders of magnitude. If, for example, the risk assessment for bromodichloromethane, which is much more risky than chloroform, changes the national perspective on the risk level, the risk reduction will still have the same proportions that we estimated. We started out with potential risks of about 10,000 cases of cancer and went down to 1,000, 100, or 10. If bromodichloromethane makes a big contribution to risk, that may put us in the ballpark of 100 to 1,000. In terms of risk reduction and benefit, we would still be in the same ballpark as our analysis already indicates.

DR. CONOLLY: Dr. Pegram, given the mechanistic differences that are becoming evident between chloroform and its brominated analogs, do you think the case is being made for not having a trihalomethane standard but rather separate standards for chloroform and the brominated analogs?

DR. PEGRAM: Yes. I think the data at least support taking a look at that. If the trihalomethanes have such different biological activities, especially differences in cancer mechanism, as the data I presented suggest, it seems really difficult to justify the continued use of a total trihalomethane regulation. I think we need to really look at the individual compounds.

DR. BIRNBAUM: Dr. Conolly, in the model you presented, how are you modeling human drinking water exposure?

DR. CONOLLY: We have built alternative absorption descriptions into the model. One alternative is an assumption of 12 hours a day of active drinking involving one sip per hour, so the total drinking water consumption is broken into twelve sips instead of one big bolus as has been used in earlier models. The one big bolus is really analogous to a gavage experiment. The idea is that if we break that up and fractionate the dose over time, we will decrease the instantaneous rate. So, we can model exposure any way we want, but we need to figure out what the most realistic description of human exposure to drinking water would be and incorporate that into the model.

DR. BIRNBAUM: Dr. Boorman, do you think it would be worth going back and revisiting some of the pathology data from earlier studies in light of the changes in pathology definitions, such as the revised classifications of liver tumors, that we now have? The slides are in the archives. Would it be worthwhile to reread some of the liver and kidney slides to see whether the numbers would change and whether that might impact analyses such as Dr. Chiu presented?

DR. BOORMAN: Dr. Wolf at CIIT has gone back and looked at many of the kidney slides. There have been a lot of changes and a lot of improvement. The quality of the slides is not ideal, for either the National Cancer Institute study or the Jorgensen study. Some slides are missing, for example, and the number of sections per kidney is different. We should not be too critical because we simply are able to do better studies now.

I commend Dr. Chiu and others for going back and looking at the data because that is certainly the least expensive way to try and get more data, but we have to be cautious. If Dr. Wolf publishes the data, it may be worth taking a look at them.

DR. WOLF: One example of the types of concerns with those earlier studies is the issue of nephropathy with the Jorgensen study. While it is true that 90% of the animals did show nephropathy over the course of the entire study, the water restriction and subsequent diet restriction greatly impacted the severity of the nephropathy. The control animals had terrifically severe nephropathy and the high-dose animals and the vehicle control had very mild nephropathy.

When both National Cancer Institute and Jorgensen studies were done, there was no mechanism to record the severity of a lesion. They could only record whether a lesion was present or absent. Therefore, the data were reported as "+" nephropathy or no nephropathy. Thus, the written record lacks information about the severity of the nephropathy. In fact, the control animals, as well as the 200 ppm- and lower-dosed animals had markedly severe nephropathy, which would probably be given a score of 3 or 4 by

today's standards. In contrast, the nephropathy exhibited by high-dose groups (i.e, the 900 and 1,800 ppm groups) as well as the vehicle control would have received a very low score. These considerations have to be part of any retrospective analysis of these studies.

DR. WEISEL: We need to better integrate exposure and toxicology in our risk assessment, especially when the environmental exposures are at low concentrations. In our risk assessments, we lump exposure from various routes together to get total dose.

At higher exposure concentrations, the chemical will tend to get distributed throughout the body regardless of the route of exposure. But, when we are exposed to low concentrations, the route of exposure may determine where the chemical goes in the body. If you give a chemical orally at low concentrations, for example, it may end up at the liver and nowhere else. There are some nice pharmacokinetic models now that show this. Chemicals that enter the body in low concentrations via other routes of exposure end up elsewhere in the body.

With this in mind, we do not necessarily need to worry about kidney cancer from ingestion of chloroform, bromodichloro-, or any of these species because they will be completely taken care of by the liver. Are there any suggestions how to bring this all together in a risk assessment?

DR. CHIU: With the risk assessment of radon in drinking water, we separated the routes of ingestion and inhalation and eventually combined them together. With chloroform, we still need a lot more data.

DR. WEISEL: After what we heard today, I suggestion that we do not limit this approach to chloroform. Chloroform is probably not the active player. Maybe the pharmacokinetic modeling efforts should be focused on some of the other players.

DR. CHIU: The U.S. EPA has a risk forum technical panel trying to address the exposure of volatiles in drinking water. We hope to have some interim guidance coming out on this.

EPIDEMIOLOGY PANEL

Critical Issues in Epidemiologic Research on Disinfection By-products

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Dr. Kenneth P. Cantor
Division of Cancer Epidemiology and Genetics,
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Dr. Michele C. Lynberg
National Center for Environmental Health,
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Dr. Philip C. Singer
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This presentation provides a brief summary of findings from epidemiologic studies of cancer and adverse reproductive outcomes and makes recommendations for further research. Epidemiologic studies of bladder and colorectal cancer have generally found an increase in risk associated with the consumption of chlorinated surface water, although a causal association has not been conclusively established. The findings from interview-based case control studies are considered more reliable than those from ecologic and death certificate-based studies. Future studies of drinking water and cancer should attempt to reduce exposure misclassification by collecting lifetime residence history and water source/quality data and by estimating water consumption from various sources, and they should control for confounding by other exposures associated with site-specific cancer risk.

Only a few epidemiologic studies of reproductive effects and chlorinated water consumption have been conducted. Associations with low birth weight and congenital malformations (including neural tube and cardiac defects) were suggested, but the interpretation of these findings is problematic due to such problems as poor control of important risk factors or small numbers of exposed cases. Further research in these areas is urgently needed. In addition, measures of fertility, fecundity, and male reproductive function should be included in future studies.

In studies of both cancer and reproductive effects, biologic markers of exposure, effect, and susceptibility should be included to the fullest extent possible. These measures will help us improve estimates of exposure and our understanding of mechanisms of action, including gene-environment interactions. A better understanding of genetic susceptibility will have the added benefit of identifying populations at especially high risk for these adverse health outcomes.

Further research is also required to refine exposure assessment in order to reduce misclassification. Epidemiologic studies of cancer and reproductive outcome should examine risk for classes of disinfection

by-products other than the trihalomethanes. Numerous collaborative opportunities currently exist. Such collaborative efforts, which build on ongoing cohort and case-controls by integrating water source, water quality, water distribution, and water consumption data, provide the best and most cost-effective approach to furthering our scientific understanding of this area. Data from experimental studies in animals should be used to identify appropriate endpoints and exposures for further epidemiologic research.

The ultimate goal of research related to the health effects of disinfection by-products is to protect public health. Epidemiologic studies of the effects of these exposures are critical to reaching this goal.

Panel Discussion

Chaired by Dr. John S. Reif
Department of Environmental Health,
Colorado State University

DR. REIF: My colleagues and I on this panel will briefly point out a few features of epidemiologic research related to disinfection by-products. We will also discuss the potential for incorporating some of the findings into future studies involving populations.

First, although the number of studies has been limited, there is evidence that disinfection by-products may pose a risk for human populations, both for cancer and reproductive and developmental endpoints. These findings serve to stimulate further research, particularly toxicological research, but the evidence is far from adequate to infer a causal relationship.

Nonetheless, there are some intriguing and consistent features, in both the cancer and reproductive areas, that suffice to drive additional work in this area. For example, in the area of bladder cancer, Dr. Pegram yesterday dismissed the endpoint of bladder cancer as a parallel model for animal toxicology studies. That may be premature because four interview-based studies of bladder cancer in humans, including those conducted by Dr. Cantor and by our group, all showed associations between bladder cancer and consumption of chlorinated surface waters—especially long-term consumption. We consider these studies to be much more reliable than studies based on death-certificate analyses which used an ecological approach. Thus, the epidemiological data certainly can be used to drive the risk assessment approach and the toxicological approach, as Dr. Pegram attempted to do yesterday. We support those efforts.

In the reproductive area, Dr. Klotz and her colleagues in New Jersey are using a case-control approach to study neural tube defects. They are measuring disinfection by-products at the tap in the homes of both cases and controls, and relating these data to diagnosis of neural tube defects in infants. Refinements in exposure assessment will enable us to improve on earlier epidemiology study designs, which often were rather crude, and add to the epidemiological information base that currently exists.

We have found rather consistently in epidemiological studies that the risks tend to be associated with the consumption of chlorinated surface water, rather than with concentrations of trihalomethanes per se. In my opinion, this supports some of the suggestions made at this workshop that perhaps it is not trihalomethanes, but rather other disinfection by-products, such as haloacids, that drive the risk.

An additional point has to do with misclassification. When misclassification occurs nondifferentially in epidemiologic studies (in other words, errors are similar in defining exposure to by-products in persons with and without the disease), risks are driven towards the null value (that is, towards 1.0 or towards no difference). This suggests that use of appropriate exposure classification in human studies would yield somewhat higher estimates than the modest risks described to date. That is another rationale for improving the quality of epidemiologic studies and conducting additional studies in selected populations.

Further, laboratory studies can evaluate only a limited number of mixtures containing a limited number of components. Epidemiologic studies are, of necessity, mixture studies. Clearly, the mixtures vary over time and space, and one needs to be aware of the mixture components. But mixtures are the exposure of interest in epidemiologic studies.

Finally, several pieces of evidence suggest that interactions with DBPs may occur in humans. For example, in two case-control studies of bladder cancer, Dr. Cantor finds important evidence of interaction with both male gender and cigarette smoking. That suggests something about the possible mechanism of action for initiation of carcinogenesis in the bladder epithelium. I would urge toxicologists who are designing animal studies to think about using co-carcinogens or to model in other appropriate ways the kind of interactions demonstrated in epidemiologic studies.

In closing, I want to encourage interaction between toxicologists and epidemiologists. I think there is fruitful potential for further epidemiological research done in concert with good laboratory studies. Dr. Singer will now talk about issues concerning exposure assessment.

DR. SINGER: To follow up on Dr. Reif's points, many of the previous epidemiological studies have suffered from misclassification because of our inability to properly assess the exposure of subjects to chlorinated water and to various constituents of that chlorinated water. This misclassification has led to relatively low risk estimates, values that suggest only a modest association at best.

On the first day of the workshop, I talked about some new simulation models that have been developed for predicting exposure to trihalomethanes, overall exposure to chlorinated water, and possibly exposure to haloacetic acids. These new models, coupled with residence time information to the customer's tap, provide epidemiologists with a much better way of assessing exposure at the subject's tap than in previous studies. This approach is particularly attractive for studies of outcomes with relatively short latency periods, such as reproductive health effects, which can often be done prospectively in addition to being done retrospectively. There are excellent opportunities to improve the epidemiology by more accurately assessing subject exposure. We can use these predictive models not only to predict exposure at the subject's tap, but also to design epidemiologic studies with a broad range of exposures to different types of disinfection by-products. If you have information about the water quality of a particular system, the kind of treatment practices provided, and the range of residence times within the distribution system, you can get a much better idea of the anticipated range of exposures among the subjects.

Many previous epidemiological studies have suffered because they have not involved water utility operations personnel. Many practitioners are willing to participate in such studies and have a plethora of monitoring information that is not readily available from compliance monitoring reports. We are on the verge of being able to do some good epidemiological studies by making better use of the exposure assessment capabilities that are now available.

DR. CANTOR: I will discuss some epidemiological approaches that are being used in other drinking water situations that might be valuable additions to studies of disinfection by-products. First, I would like to mention two studies of intermediate measures of effects of biological damage. One of these studies concerns exposure to nitrate. Some 10 to 15 years ago, it was found that people who eat proline and nitrate at the same time will excrete n-nitrosoproline in their urine, which is a good indicator that an n-nitroso reaction is taking place endogenously. This finding has now been applied to an environmental situation. Sidney Mirvish at the Eppley Institute has studied two populations of 20 to 25 men in Nebraska. One group lives in areas with high nitrate levels and the other in areas with low nitrate levels. Mirvish demonstrated a statistically significant difference between these groups in the amount of n-nitrosoproline excreted after proline administration.

The second study used a marker of biological damage: micronuclei in exfoliated bladder cells. This marker is especially relevant to bladder cancer. Marcella Warner and colleagues successfully applied this marker in an epidemiological study in Nevada. She detected an excess of micronuclei in a population highly exposed to arsenic as compared to a population with low arsenic exposures. We are now designing a study to examine exposure to chlorinated by-products in a similar way.

Another very promising area not yet used in drinking water studies is biological susceptibility factors. This is particularly appropriate for case-control studies of cancer. One classic example of this approach is a study of bladder cancer among men occupationally exposed to aromatic amines. The study clearly showed that men who are slow acetylators are at much higher risk for bladder cancer than men who are fast acetylators.

Several presenters at this workshop have spoken about promising developments. Dr. Pegram yesterday talked about his finding of glutathione transferase theta related to bromodichloromethane metabolism. In fact, this is a polymorphism in the human population. If we can use this as a tool, we would expect to detect a stronger association - one that is not diluted because only a small portion of the study population is affected by the exposure. This would also strengthen the biological plausibility of some of the effects we are seeing. There undoubtedly are several other polymorphisms in the human population for either activating or detoxifying enzymes relevant to these exposures.

These methodologies are starting to be used in epidemiologic studies of cancer and in intermediate studies of activation and deactivation. They offer promising leads for work in the near future.

DR. LYNBERG: I will talk briefly about reproductive outcomes associated with drinking water contaminants. A number of recent studies have suggested an association between several types of birth defects (most commonly neural tube and cardiac defects) with various water contaminants, including disinfection by-products. Other studies have shown adverse reproductive outcomes (mostly low birth weights) with these same drinking water contaminants. In general, the associations reported are modest in magnitude and based on small numbers, making them difficult to interpret. However, assuming they represent a true association and do not result from misclassification, even relatively weak risks associated with drinking water would potentially be of great public health significance because of the ubiquitous nature of drinking water exposure.

In 1993, a select panel of experts was convened by ILSI and prepared a report for the U.S. Environmental Protection Agency (U.S. EPA) entitled "A Review of Evidence on Reproductive and Developmental Effects of Disinfection By-products in Drinking Water." The panel recommended adding a water quality

component onto already existing population-based studies of adverse reproductive and developmental outcomes. The purpose of this brief presentation is to discuss ways that epidemiologists can collaborate with scientists in other fields. Since 1968, the Centers for Disease Control (CDC) has been doing surveillance on birth defects under the Metropolitan Atlanta Congenital Defects Program—one of several birth defects surveillance systems across the country. Under the Atlanta program, we ascertain all structural malformations in the five-county metropolitan Atlanta area.

The Birth Defects Risk Factors Surveillance Project is intended to further our knowledge about risk factors for birth defects. Each year we interview about 300 case mothers and 100 controls. The hour-long interview covers occupational and environmental exposures, mother's illness, drug use in pregnancy, family history, and many other subjects. Also, as part of this study, we sample blood from the mother-infant pairs, so we have the capacity for biomonitoring both exposure and susceptibility using existing biomarkers.

We have funded two other studies using the same interview instrument in Iowa and the Bay area of California. The capabilities of these studies include rapid case ascertainment, collection of interview information, the application of biomarkers, and the capacity to study isolated and multiple defects separately. Other advantages to using this surveillance system approach include the opportunity to cover diverse water supplies with naturally occurring variation, large sample size, and extreme cost efficiency. For example, the large costs of finding and interviewing cases and collecting biologic samples are already covered. This is an excellent opportunity to study birth defects, since it can provide fairly high quality data in return for relatively minimum investment of resources.

We must not forget that the ultimate reason we collect data on drinking water contaminants is to protect human health. We should take advantage of existing epidemiological studies for health outcomes by piggybacking drinking water studies onto them so that we can better understand the human health effects of these exposures.

MR. KRASNER: To my mind, improving exposure assessment is most important. I echo what Dr. Singer said, but I would take it a step further. In my experience, it is sometimes very disappointing to find out how many large and seemingly sophisticated utilities can produce very good water and know how to do that empirically, but do not always understand exactly how they got to that point.

Based on my experience with many utilities, I recommend asking for information on coagulant doses and turbidities when checking a utility's historical database. I believe there are formulas (such as the one that Malcolm Pirnie developed on the relationship of coagulant dose, total organic carbon, and turbidity for the EPA's regulatory negotiation) that you can use to back out the total organic carbon levels in the water.

DR. SINGER: The water distribution system is an important part of exposure assessment, but is very complex. For example, people furthest from a treatment plant are not necessarily exposed to the highest levels of disinfection by-products, because sometimes large pipes distribute water to those parts of town faster than to the closer parts of the town. The people who know most about the distribution system are the people who supply the water. They are the best people to help in any spatial distribution analysis of subjects' exposure to disinfection by-products.

DR. WEISEL: I also want to address exposure, but from a slightly different perspective. Human exposure to compounds in drinking water is affected not only by the concentrations at the tap but also by

how people use the water. One of Dr. Chiu's slides showed exposure through inhalation, dermal, and ingestion routes for trihalomethanes. While we think we understand trihalomethane exposure to some extent, we do not understand exposure to any other disinfection by-products right now. For example, we have no idea whether nonvolatile species are carried on particles and ingested. We do not understand the dermal absorption of these compounds.

Another complication is that people's activities vary greatly. For example, someone mentioned that teenagers tend to take 30-minute showers. That is real. Different people will have different exposure durations.

Another issue is that the controls currently are annual averages. This is fine if you are looking at cancer endpoints, but does not work for reproductive endpoints. What matters are the peak exposures, not the average annual exposures. We need to consider this differently than we have done so far. I would appreciate comments on how to do that.

DR. SINGER: I will comment on that. With available models we can make a reasonably good quantitative assessment of the levels of trihalomethanes that customers are getting exposed to on any given day. Chlorine doses and chlorine residuals are measured on a routine basis. So, in fact, we do have much more effective ways of mapping out what the distribution system looks like and assessing what individuals are exposed to on a daily basis rather than relying on annual averages or even quarterly averages. The issue of exposure once the water leaves the tap and is consumed clearly is an important question that should be addressed.

SESSION 5
PREDICTIVE TOXICOLOGY, MIXTURES, AND EVOLVING RISK ASSESSMENT APPROACHES

Introduction

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The first two days of this workshop focussed largely on the toxicity of individual disinfectant by-products. Unfortunately, we still have not yet identified or have sparse information on a number of individual disinfection by-products in drinking water. We have also learned that drinking water is a complex mixture and that its composition varies from one location to another and from season to season. Both the quantitative and qualitative composition of drinking water mixtures is influenced by several factors, including the source water characteristics, the conditions of treatment, and the distribution network.

In this session, we will hear about various approaches to assessing the risks from drinking water mixtures. Dr. Jennifer Seed will review what we know about drinking water as a complex mixture and what we have learned from the available studies. Mr. Phillippe Daniel will consider the issue of risk assessment from both an engineering and a practical perspective.

We must also address situations when we do not know enough and what type of approaches we can take given the available data. Using a case study, Dr. Denise E. Robinson will present a parallelogram approach for hazard assessment for certain drinking water by-products. Following her presentation, Dr. Ann Richard will address the issue of structure-activity relationships and how we could apply this type of technique to disinfection by-products.

This will be followed by two talks on evolving risk assessment approaches. Dr. Robert Kavlock will give a presentation on embryologically based dose-response models. Dr. Robert Tardiff will discuss the application of new risk assessment approaches to disinfection by-products using cancer as an endpoint.

Drinking Water as a Complex Mixture: What Have We Learned?

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Regulatory agencies have traditionally set drinking water standards for individual chemicals. However, it is well known that chlorine reacts with naturally occurring humic substances in water to form trihalomethanes and other halogenated compounds. The nature and extent of disinfection by-products associated with chlorine disinfection, as well as other disinfection processes, is strongly influenced by many factors including pH, temperature, and concentration of precursors, which in turn may vary with the season. The potential toxicity of many of the disinfection by-products has been studied, however, there is

concern that interactions among the many by-products in drinking water may alter their toxicity. If such interactions occur, the adverse effects from drinking water exposure may be greater than, less than, or equal to the sum of the effects of the individual components.

Three basic approaches have been used to examine the toxicity of drinking water as a complex mixture: assessments of the toxicity of (1) concentrates of drinking water, (2) humic and/or fulvic acid solutions treated with various disinfectants, and (3) defined mixtures of chemicals. The available toxicological database for each approach, the inherent uncertainties associated with each approach and the applicability of the studies for risk assessment was discussed.

These studies, as well as the results of studies recently conducted by the TNO Nutrition and Food Research Institute in the Netherlands and the studies of defined mixtures conducted by the National Toxicology Program, have important implications for future steps in the risk assessment of disinfection by-products. All of the studies are consistent with the hypothesis that adverse systemic effects are unlikely to occur as long as the dose levels of the individual components are well below their respective thresholds. One option for future efforts would be to further examine this hypothesis with respect to by-products of particular concern.

Considerations in Assessing the Risks of Mixtures

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While there are many problems in comparing water treatment technologies, the core problem we currently encounter is that of selecting an optimal strategy when the quantitative and qualitative yields of disinfection by-products (DBPs) are different. The U.S. Environmental Protection Agency (U.S. EPA) currently regulates individual contaminants using maximum contaminant levels. This approach does not recognize the dynamics and complexity of DBP chemistry. For engineering decision-making, a meaningful comparison of qualitatively and quantitatively different mixtures of disinfection by-products is essential.

Factors Influencing Mixture Composition

A variety of factors govern the qualitative and quantitative yield of disinfection by-products associated with different technologies: source water factors (e.g., natural organic matter concentration, bromide ion level, pH, temperature) and treatment process variables (e.g., pH during treatment and distribution, location of disinfectant application, and chemical doses). Of particular importance are pH and bromide ion concentration.

pH: Different pH levels favor formation of different disinfection by-products. For example, formation of trihalomethanes is favored at higher pH, as is bromate production. Haloacid formation, on the other hand, is apparently favored at lower pH. Depending on the relative significance of different compounds, the pH could be adjusted to select for certain disinfection by-products and not others.

Bromide: Bromide occurs in source waters throughout the United States in concentrations varying from less than 5 μg/L to greater than 1,000 μg/L. The presence of bromide causes a shift

in DBP speciation from chlorinated to brominated compounds. Depending on the bromide concentration, varying proportions of chloro-, chlorobromo-, and bromo-substituted compounds will be formed. The overall DBP concentration also increases when bromide is present because bromine is twice as heavy as chlorine. Bromate, an oxidation by-product, can be produced even at very low bromide concentrations (Amy et al., 1994). Table 1 presents a typical DBP profile for a plant using free chlorine to treat water containing a moderate concentration of bromide ion.

Three observations can be made from Table 1. First, bromine substitution increases DBP mass concentrations, making compliance with the current trihalomethane regulatory level of 100 μg/L difficult. Second, there are few health effects data for many of the compounds listed, especially the bromochloro-DBPs. Third, many bromochloro- compounds go undetected due to limitations of current analytical methods.

Approaches

Assuming simple additivity (i.e., no synergisms or antagonisms), the most simple (and the most naive) approach to assessing carcinogenic risk is to multiply the disinfection by-product concentration by the unit risk number for those disinfection by-products for which cancer potency factors are available. Totaling the numbers for a given disinfection by-product profile provides an overall number.

While this method has significant limitations (e.g., lack of health effects information on all compounds of interest, uncertainty associated with the unit risk numbers, the question of whether risk is additive, multiplicative, etc.), it provides valuable insight. For example, due to the greater potency of haloacids, increasing the chlorination pH results in a decrease in calculated carcinogenic risk since trihalomethanes increase and haloacids decrease. Or, consider ozonation of bromide-containing water: the calculated cancer risk is mostly attributable to bromate, highlighting the importance of its control.

A refinement of this approach would be to group the compounds according to mechanism or target organ. The dilemma, however, is lack of information. How can the lack of health effects information be approached? Will current toxicological research provide insights into how to make these comparisons? Much work is needed to assess the uncertainties concerning mechanisms, mixtures, and risk assessment.

Table 1: Typical Disinfection By-product Profile in Bromide-containing Water

Disinfection By-product	Concentrations[1,2] µg/L
Trihalomethanes	
Chloroform	9.1
Bromodichloromethane	26.3
Dibromochloromethane	42.8
Bromoform	15.6
Haloacetonitriles	
Trichloroacetonitrile	0.1
Dichloroacetonitrile	2.1
Bromochloroacetonitrile	3.8
Dibromoacetonitrile	6.5
Haloketones	
1,1-Dichloropropanone	0.5
1,1,1-Trichloropropanone	0.7
Haloacetic Acids	
Monochloroacetic acid	1.0
Dichloroacetic acid	7.6
Trichloroacetic acid	6.0
Monobromoacetic acid	2.3
Dibromoacetic acid	14.1
Miscellaneous	
Chloropicrin	0.1
Choral hydrate	2.8
Cyanogen chloride	2.2
2,4,6-Trichlorophenol	0.4
Aldehydes	
Formaldehyde	7.6
Acetaldehyde	4.5
Total organic halogens (TOX)[3]	230.0

[1] 3rd quarter Santa Clara Valley Water District results, Rinconada (McGuire et al., 1989).

[2] Raw water conditions:
Bromide - 0.28 mg/L
Chloride - 93 mg/L
Total organic carbon (TOC) - 2.86 mg/L

[3] Disinfection by-product total = 0.33 TOX (molar)

The crude approach discussed above, aided by a decided lack of toxicological sophistication, can be successively refined (based on sensitivity analysis) in the following sequence:

1) Use the maximum tolerated dose/LD_{50} approach to calculate a cancer potency factor for those compounds that have not been studied in a long-term rodent bioassay.

2) Compare the disinfection by-product profiles using short-term *in vitro* tests (if available) and by comparing compounds by ratio for common endpoints.

3) Use computational chemistry and structure-activity relationships to compare disinfection by-products and estimate relative potency factors.

4) Use an expert committee to evaluate the results and develop an evaluation. A probabilistic approach could be used to estimate the uncertainty. Compounds could be segregated according to postulated mechanisms.

Case Study

Consideration of the bromide problem in California is illuminating. Extensive pilot work has been done on these sources, providing a good database for the DBP concentrations associated with alternative disinfection schemes. In 1989, the process deemed most feasible for plants in this area was to use ozone followed by chloramine as an alternative to chlorine followed by chloramine. This reduced the trihalomethanes to well below 10 μg/L and decreased the haloacid concentrations from approximately 14 μg/L to less than 5 μg/L. The TOX was reduced from 230 μg/L to 50 μg/L. Taste- and odor-causing compounds were effectively oxidized.

A concern emerging later, however, was bromate formation. Bromate may be produced at levels ranging from 5 to 30 μg/L. Assuming a bromate level of 10 μg/L and using simple additivity, bromate formation offsets the trihalomethane, haloacetic acid, and TOX reductions because bromate has a much higher carcinogenic potency factor. Consequently, the estimated carcinogenic risk index from ozonation can actually increase to 110 as compared to 40 from chlorination. If bromate is reduced to below detection limits (currently 5 μg/L), as has been shown feasible by recent studies, the carcinogenic risk index from ozonation drops to 10 (i.e., or one-fourth the current risk associated with a chlorine-chloramine disinfection strategy).

In this case, no health effects data are available for brominated haloacetic acids (even though significant concentrations of monobromoacetic acid and dibromoacetic acid are produced). Formation of bromochloroacetic acids is almost certain in this water, and is likely to be higher with chlorine-chloramine disinfection than with the ozone-chloramine. The brominated haloacetic acids are difficult to analyze, but some progress has recently been made. Even when they are detected, however, insufficient data are available to assess the human health significance of these compounds. Lack of health effects data hinders meaningful comparisons of treatment processes.

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Use of a Parallelogram Approach for Hazard Assessment

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A paradigm for predicting chemical risks for a compound for which limited data are available has been proposed by Robinson et al. (1995). The approach involves a graphical comparison of the toxicity of two compounds that are similar in structure, metabolism, and mechanism of toxicity. All available information is utilized, including acute and chronic toxicity data as well as mechanistic data obtained from several routes of exposure. The graphical comparisons are utilized to make predictions about the potential toxicity of the chemical for which incomplete data are available.

The comparisons are visualized as a parallelogram, with each of the corners representing available toxicity data for a particular route of exposure for one of the two compounds. Such data might include tumor incidences from chronic bioassays, measures of acute toxicity, cytotoxicity, and mechanism or mode of action data such as DNA binding, DNA-protein crosslink formation, or cell proliferation. The slopes between the corners of the parallelogram represent the relative toxicity of the two compounds or the routes of exposure.

A comprehensive comparison of two aldehydes, acetaldehyde and formaldehyde, will be presented as a case study for the application of the parallelogram approach. These aldehydes are of interest because of their ubiquitous presence in the environment, both from naturally occurring sources as well as by-products of industrial and combustion processes. Concern about human exposure to aldehydes has primarily focused on the inhalation route of exposure due to significant air concentrations present in urban environments as a result of the combustion of fossil fuels. Both formaldehyde and acetaldehyde have been shown to be carcinogenic in several rodent species via the inhalation route of exposure.

Human exposure to aldehydes through the oral route is also widespread. Aldehydes are found in many fruits, vegetables, fish, and meats and as by-products of fermentation processes. In addition, both acetaldehyde and formaldehyde are by-products of several methods of drinking water disinfection, including chlorination and ozonation. Chronic exposure to formaldehyde in drinking water failed to elicit a tumorigenic response in rodents. Data are not available for oral exposure to acetaldehyde. Given that drinking water and dietary exposures to acetaldehyde occur, and that acetaldehyde is a carcinogen

following inhalation exposure, it was of interest to evaluate the potential for carcinogenicity by the oral route of exposure.

In rodent models, the toxic effects of both aldehydes exhibit a contact site pattern of toxicity, with lesions occurring only in tissues associated with the site of exposure and subsequent absorption. The postulated mechanism of toxic action involves the formation of DNA-protein crosslinks at doses that overwhelm the normal aldehyde dehydrogenase metabolic pathways in those tissues. Quantitative data on tumor incidences, no-observed-adverse-effect levels for subchronic exposures, and measures of DNA-protein crosslink formation in the target tissues form the basis for the comparisons between the two aldehydes and the inhalation and drinking water routes of exposure.

It should be emphasized that this approach is predictive in nature and is not strictly quantitative. However, the parallelogram approach can provide an estimate of relative potency and can play a useful role in identifying important toxicologic and mechanistic data gaps as well as in evaluating priorities for future research. Additional evaluations are necessary to further validate the utility of this approach. Future work will focus on identifying appropriate data sets for other chemical pairs. Potential candidates include ethyl acrylate and its congeners, methyl and butyl acrylate. Also under consideration are propylene and ethylene oxide.

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Structure-Activity Relationships: Predictive Toxicology as Applied to Disinfection By-products

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Most research and testing to investigate the adverse health effects of disinfection by-products has focused on relatively few chemicals that are prominent members, in terms of known health effects or exposure, of larger classes of disinfection by-products—e.g., haloacetic acids and halomethanes. Since data are often lacking for structurally related disinfection by-products, studied chemicals are often treated as representatives of the class for regulatory purposes. Risk extrapolations based solely on relative exposure levels, however, may be inappropriate if alternate mechanisms of action or large differences in biological potencies exist within the class. For example, while a great deal of research has focused on di- and trichloroacetic acid (DCAA and TCAA), much less in known about the health effects of other bromo-, iodo- and mixed haloacetic acids that result from disinfection of waters containing natural sources of bromine and iodine.

Structure-activity relationship (SAR) studies of disinfection by-products are directed toward probing relationships between structure and biological activity within chemical classes for two purposes: (1) to provide a rational framework for predicting relative potencies for untested chemicals within the class; and

(2) to generate insight into the underlying mechanism for the observed biological activity. The ability to derive useful SAR models depends foremost on the quality and nature of the available data. Secondly, it depends on the ability to frame the problem in terms of a common mechanism of action, or a key rate-limiting or discriminating step in the biological interaction leading to divergent activities or mechanisms. SAR capabilities exist for modeling physical properties (e.g., acid dissociation constants and vapor pressures), as well as extrinsic properties such as biodegradation and bioavailability. In contrast, very few SAR models have been specifically developed for predicting the relative toxicities of disinfection by-products. Three SAR investigations on haloacetic acid DBPs and related compounds will be described to illustrate the varied approaches and potential utility of SAR for modeling the toxicity of disinfection by-products.

The first project, conducted with Dr. Sidney Hunter of the U.S. EPA's Developmental Toxicology Division, involved successful quantitative structure-activity relationship (QSAR) modeling of the developmental toxicity of haloacetic acids in mouse whole embryo culture. This represents one of very few published QSAR analyses for a developmental toxicity endpoint. Whole embryo assay results were initially generated for acetic acid and a series of eight haloacetic acids; two additional acids were subsequently included for model refinement and validation. The best overall regression related log[1/(benchmark concentrations for induction of neural tube defects)] to the energy of the lowest unoccupied molecular orbital (E_{lumo}) and the acid dissociation constant (pKa) with $r=0.97$.

The ability to derive a single, statically robust QSAR for the class of haloacetic acids (HAAs) strongly supports the following conclusions: (1) that a common mechanism applies to the developmental toxicity of the HAAs, and (2) that additivity of effects of HAAs would be observed in mixtures. In additional, the QSAR parameters, pKa and E_{lumo}, provide support of postulated mechanisms implicating changes in intercellular pH and/or redox metabolism in developmental toxicity, suggesting new lines of experimentation.

This QSAR model was also used to prospectively predict the whole embryo toxicity of a number of mixed HAAs (e.g., monobromodichloroacetic acid, etc.), most of which were commercially unavailable for testing. One mixed HAA, monobromomonochloroacetic acid (MBMCA), recently was obtained through custom synthesis and tested in the assay. The QSAR prediction and experiment were in excellent agreement, both finding the activity of MBMCA to be very similar to dibromoacetic acid (DBAA), rather than to DCAA.

From a risk assessment view, *in vivo* whole animal results are considered more informative than whole embryo assay results, since the latter neglect the important influence of the maternal compartment on toxicity. From a structure-activity point of view, however, the distinct advantage of the whole embryo culture assay over *in vivo* animal studies is that measured toxicity can be directly related to a known chemical exposure of the embryo without the confounding influence of maternal factors. Such simplification enabled the present QSAR development, and the resulting QSAR offers valuable insight into the mechanism of embryo toxicity that can potentially contribute to understanding of the larger, more complex, *in vivo* animal teratogenicity problem.

A second project (conducted with Dr. Carl Blackman of the U.S. EPA's Environmental Carcinogenesis Division) involves QSAR modeling of halogenated disinfection by-products and proposed metabolites that inhibit gap juncture-intercellular communication (GJ-IC), which is an endpoint of interest due to its possible relationship to tumorigenicity. SAR was used during the phase 1 stage of the investigation (after

five chemicals had been tested: DCAA, TCAA, trichloroethanol, perchloroethanol, and chloral hydrate) to generate a tentative model based on molecular size and partitioning characteristics for predicting relative potencies among these chemicals. This QSAR model was subsequently used, in phase 2, to direct testing to four related disinfection by-products (chloroacetic acid, bromoacetic acid, dibromoacetic acid, and tribromoacetic acid). The assay results for these by-products would challenge and refine the initial model, and the QSAR-predicted potencies for these chemicals would be used to narrow the experimental search for the effective dose. A third phase of testing for model validation is underway. The current model suggests molecular features relevant to the mechanisms for GJ-IC. Subject to further validation, QSAR could be used to probe possible relationships between the GJ-IC effect and other *in vitro* or *in vivo* measures of toxicity.

The third project is attempting to consider the carcinogenicity of HAAs, and illustrates the problems and challenges of applying SAR concepts to sparse data and complex biological endpoints. Experimental studies indicate that TCAA and DCAA induce carcinogenicity in different rodent species by different mechanisms, which implies early discrimination of these chemicals in biological systems based on their molecular structure. Since little is known of the mechanism of carcinogenicity for either of these compounds, and few carcinogenicity data exist for other HAAs, our approach has been to calculate general reactivity properties of the HAAs with respect to putative mechanisms for metabolic activation. Computational studies are underway to characterize relative reactivities of HAAs with respect to dehalogenation through reductive, free radical mechanisms. Such computations ultimately may help to support or refute proposed elements of a mechanism of carcinogenicity, and could provide a reactivity ranking of the HAAs relevant to other forms of toxicity.

The author acknowledges productive collaborations with Dr. S. Hunter and Dr. C. Blackman of the U.S. EPA's National Health and Environmental Effects Research Laboratory.

The Road to Embryologically Based Dose-Response Models

Robert J. Kavlock
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The goal of research in developmental toxicology is the prevention of adverse reproductive outcomes (early pregnancy loss, birth defects, reduced birth weight, and altered functional development) in humans due to exposures to environmental contaminants, therapeutic drugs, and other factors. To best achieve that goal, it is important that relevant information be gathered and assimilated into the risk assessment process. One of the major challenges of improved risk assessment is to better utilize all pertinent biological and mechanistic information. This may be done in three fashions: qualitative (e.g., demonstrating that the experimental model is not appropriate for extrapolation purposes); semi-quantitative (using information to reduce the degree of uncertainty present under default extrapolation procedures), or quantitative (formally describing the relationships between exposure and adverse outcome in mathematical forms, including components that directly reflect individual steps in the overall progression of toxicity).

This presentation reviewed the recent advances in the risk assessment process for developmental toxicants and hypothesize on future directions that may revolutionize our thinking in this area. The road to these changes sometimes appears to be a well-mapped course on a relatively smooth surface. At other times the

path is bumpy and obscure, while at still other times it is only a wish in the eye of the engineer to cross an uncharted and rugged environment.

During the last five years, significant changes in the risk assessment process for noncancer health effects of environmental contaminants have begun to appear. The first of these changes is the development and beginning use of statistically based dose-response models (the benchmark dose approach) that better utilize data derived from existing testing approaches. Accompanying this change is the greater emphasis on understanding and using mechanistic information to yield more accurate, more reliable, and less uncertain risk assessments.

The next stage in the evolution of risk assessment will be the use of biologically based dose-response (BBDR) models that begin to build into the statistically based models factors related to the underlying kinetic, biochemical, or physiological processes perturbed by a toxicant. Such models are now emerging from several research laboratories. The introduction of quantitative models and the incorporation of biological information into them has pointed to the need for even more sophisticated modifications. The term embryologically based dose-response (EBDR) models is offered to describe these modifications, which would be based on the understanding of normal morphogenesis. Such models would represent a quantum leap in our thinking, however, their complexity presents daunting challenges to both developmental biologists and developmental toxicologists. Nevertheless, the remarkable progress in understanding mammalian embryonic development at the molecular level that has occurred over the last decade should eventually enable these hypothetical models to be brought into use.

The development of BBDR and EBDR models for developmental toxicants is a costly, labor-intensive endeavor. We do not yet know the extent to which such efforts will reduce uncertainties in the risk assessment process, as the models have not yet advanced to that stage of use. Because of the expense and effort, these efforts should only be directed at chemicals (1) that have what appears to be appreciable risk, (2) for which there is widespread human exposure, and (3) for which the regulatory costs would be high. Some disinfectant by-products meet several of these criteria and might be likely candidates for model construction. One of the major limitations for disinfection by-products is the current lack of mechanistic hypothesis with which to begin the research effort.

Application of New Risk Assessment Approaches to Disinfection By-products

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Estimating the risks to human health from ingestion, inhalation, or skin contact with disinfection by-products (e.g., trihalomethanes) has been controversial, and has stimulated wide-ranging experimentation for over two decades. Early attempts to estimate risks have mirrored scientific techniques used to judge the safety of chemical substances present not only in tap water, but also in food, consumer products, air, and soils. Risk assessment approaches and techniques have been evolving at a relatively rapid pace over the past several years. The changes are mostly scientific and occasionally cultural.

Scientific changes deemed noteworthy in risk assessment relate to five areas: (1) weight of evidence for carcinogenicity, (2) estimation of toxic potency via mode(s) of action, (3) estimation of toxic potency for non-cancer toxicity via the "benchmark dose," (4) characterization of exposures as distributions rather

than as point estimates, and (5) the application of mode-of-action information to estimate the risks from exposures to mixtures. The changes in approaches to defining and describing the weight of evidence for carcinogenicity also have a cultural origin.

Considerable effort has been expended over the past two decades to find ways to succinctly and consistently summarize the collection of data that characterizes the carcinogenic properties of chemicals. Best known are the classification schemes of the International Agency for Research on Cancer (IARC) and the U.S. EPA, both of which rely on alpha-numeric designations to distinguish among various degrees of persuasiveness that a substance might or might not cause cancer in humans. The designations are buttressed by extensive data summaries and evaluations; however, in practice, the designations are often presented without benefit of supporting documentation that conveys important nuances for estimating risks. Many have criticized this approach as being overly simplistic because it loses sight of the wealth of toxicologic and epidemiologic information that often makes up the comprehensive understanding about the cancer-causing properties of substances.

In the latest revision of the U.S. EPA's cancer guidelines, the alpha-numeric designations are replaced with explanatory text. While the text is partially standardized to facilitate both differentiation of classification and comparability among reviewed substances, it offers latitude in describing confidence in conclusions and alternative explanations that relate to hazards for humans. This change represents an important advance since it fosters a fuller understanding of the scientific underpinnings of the conclusions, while averting the limitations of the simpler approach.

Historically, the estimation of toxic potency for carcinogens and non-carcinogens has been based largely on quantitative descriptions of toxicologic dose-response relationships, often with little consideration of complex biological factors that could alter the dose-response relationship. Often, a single dose-response function has been used to estimate risk to large population groups. This practice has resulted in risks appearing to be over- or underestimated, in some cases by several orders of magnitude. Over the past few years, mechanistic information (i.e., information describing the behavior of chemicals at the subcellular level as well as the ways in which tissues respond to toxic challenge) has been applied to modulate quantitative dose-response functions. Studies of the modes of action (e.g., disruptions of endocrine homeostasis, alterations of cell cycles, changes in rates of damage and repair, alterations in the ratio of activation and detoxification of toxicants, the presence of genetic polymorphisms, and the range of toxicokinetic behaviors under assorted conditions of exposure) in laboratory animals and humans have provided insights into the likely existence of tolerance levels of exposures and into the variability of those tolerances among members of the exposed population. The outcome has been prominent for chloroform and dichloroacetic acid, both chlorination by-products. As a result, the risks estimated based on mode-of-action information have yielded higher tolerable levels than the risks obtained using the default assumption of linearity at low doses. This has promoted greater confidence in the conclusions about risks.

For many years, a no-observed-adverse-effect level/uncertainty factor (NOAEL/UF) approach has been used to estimate toxic potency for non-cancer chronic toxicity. This process relies on identifying and selecting a NOAEL that best represents the highest dose below which the injurious effects of a chemical are considered unlikely. Since the NOAEL is generally obtained under conditions different from those most likely to occur in the exposed population, a variety of uncertainty factors (UF) are applied to the NOAEL to obtain a NOAEL that relates specifically to the exposed population.

The "benchmark dose" approach has been developed as a refinement to the NOAEL/UF methodology. This approach, proffered by the U.S. EPA, uses an entire dose-response data set, rather than a single point estimate, models the dose-response function as the most likely estimate and the upper 95% confidence interval, and selects either the ED10 (10% effective dose-response) or the ED5 from the upper 95% confidence interval as the point from which to consider applying uncertainty factors. If multiple dose-response sets are available, each can be modeled, and mode-of-action information can be used to select one data set deemed most relevant to the human situation. A major value of the benchmark dose over the NOAEL is that the benchmark dose incorporates the slope of the toxicological dose-response relationship. This slope is often an important determinant of toxic potency, and is generally a more precise determinant than the default slope obtained from statistical modeling.

Another recent enhancement to risk assessment is the use of probability distributions in characterizing exposures of populations to substances of interest. The traditional approach, by contrast, uses point estimates. Distributional analysis, implemented via techniques such Monte Carlo simulations, can incorporate several independent variables (e.g., body characteristics, concentrations of compounds in several media, rates of intake, absorption, and distribution in the body) that influence the frequency and magnitude of exposure and determine the distributions of these characteristics—alone and in combination. When combining the relevant parameters, the distributional analyses provide a more precise description (and graphical representation) of the numbers of individuals at risk of possible injury. Thus, this tool is capable of better rendering a sense of public health impacts from defined types of exposure regimens. This approach offers the advantage of replacing numerous default assumptions about exposures to compounds such as the by-products of chlorination. One illustration of an assumption that can be replaced is: all individuals are exposed continually throughout their lifetime to the maximum reported concentration. Using distributional analyses, the actual exposure from such a scenario is apt to be actually only a small fraction of the more simplified inference.

The distributional analyses are also useful in describing the excess risk of disease from exposure to a chemical as compared to background incidence rates in the general population of the same disease resulting from other causes. The incidence of compound-related toxicity that can be justifiably attributed to the compound(s) of interest can be readily identified by superimposing the distribution of disease incidence (such as neurological impairment) caused by the agent being investigated on the distribution for the overall disease incidence. In this way, the entire incidence is not improperly attributed solely to the compound.

The toxicity of complex mixtures whose composition varies over time has generally been characterized by testing mixtures whose composition has been arbitrarily fixed in time. The result is information with limited application to actual situations of human exposure. Correspondingly, it is impractical to test every mixture to determine its toxic potential. The practical solution lies in the reasoned use of either modes-of-action information or, in the absence of mechanistic data, the application of the concept of functional additivity. For a mixture of, say, chlorination by-products, mode of action information is capable of inferring that the simultaneous exposures to chloroform and MX in tap water are unlikely to produce a carcinogenic potential that is either additive or synergistic. By examining the toxicity data for individual substances in a mixture, it may be possible to render judgments about the likelihood of toxic interactions. Equally important, such an approach provides a basis to design research using biologically plausible hypotheses rather than repetitive testing of mixtures that relies on traditional endpoints included solely because of their availability of historical use.

The concept of functional additivity also has a role in characterizing the risks from exposures to complex mixtures. Functional additivity relies on descriptive pathologic findings from traditional toxicity studies of individual compounds to group compounds according to their target organs for ranges of effective doses. For instance, all renal toxicants in the range of the ED5 and ED20 in a 20-compound mixture are treated as one entity for purposes of judging the risks of renal impairment from exposures to the mixture at the same and lower doses. Their dose-response slopes can be compared, and either a NOAEL or benchmark dose can be defined for the study populations, and later extrapolated to humans. If sufficient information is available, a ranking of toxicity equivalency can be constructed; however, such situations are presently relatively rare.

The changes in the practice of risk assessment contribute not only to increased confidence in the estimated risks faced by those exposed but also to significant insights about how to design and conduct subsequent research and testing.

Session Discussion

Päivi Julkunen, Chair
The Coca-Cola Company

DR. BREACH: This session has been one of the most important of the whole workshop, because it is actually beginning to address the real issues of risk and tradeoff. There are thousands of utilities out there who share the same goal we all have of producing safe water for their customers. They also have to consider value for money, because they cannot just throw money at a problem, particularly if the problem is not real.

We are all trying to grapple with the need for decision-making to set standards. I see two dimensions to the problem. One is the magnitude of overall risk from disinfection by-products. How low a concentration do we generally want to achieve for disinfection by-products? Do we really want to make surface water into ground water? You could probably do it, but the costs would be astronomical. I am not sure the health benefits are justifiable. The other element is the tradeoff between different disinfection by-products. We have heard a lot about which disinfection by-products are more or less important.

I would like to know how can we combine those two elements into some forced decision-making process because we have to make decisions. There are some elegant statistical techniques that force people to make decisions on available evidence. Could we not use those statistical techniques with the expertise available in this room and elsewhere to make some recommendations that allow utilities to make decisions with real value and benefits to their customers now, because this is what the utilities have to do?

It is instructive that the World Health Organization quite recently was brave enough to try to put some markers in that quicksand—to put numbers against parameters. They may not be perfect numbers, but they at least allow us to begin the decision-making process.

Should we not take those two elements and put them with these numbers that have already been stuck in the quicksand, so that we can at least develop a ranked priority and some idea of magnitude of risk that utilities can use now to make decisions that really protect public health now, and not just in 20 years' time?

DR. DANIEL: I think there is a third dimension. The first two dimensions, as Dr. Breach mentioned, are the overall magnitude of disinfection by-product risk and the risk tradeoffs between different compounds. For decision-making, however, we are pushed into a third dimension of framing the risks of disinfection by-products with other risks, such as microbial risks.

The dilemma is not so much scientific as it is social science in nature. It concerns what tradeoffs people are willing to make between low probability chronic risk, delayed effects, long latency, and high uncertainty versus the short-term immediate morbidity effects associated with microbial disease. We need some good tools for eliciting what tradeoffs people are willing to make and their willingness to pay for various improvements.

DR. TARDIFF: I entirely agree. I would add that there is an important perspective in doing that, which is that the decision is not really up to a regulatory agency but rather to the people. The decision relies on people being sufficiently informed about the nature of the risks or concern to determine how much they are willing to pay for certain degrees of safety. I think that many people in the United States believe that absolute safety is feasible for potable water. That is largely a function of ignorance, because they do not understand the world as it currently exists.

DR. DANIEL: What we as scientists have is a perception of public perception. Often, we gather public opinion by interrupting people for a few minutes while they are shopping in the mall or eating dinner. We analyze their answers numerically, perform some statistics, and dignify the results by calling them public opinion. Actually, it is public ignorance.

We need a systematic way of eliciting public opinion. A political reformist named James Fishkin wrote a book called Democracy and Deliberation. He recommends a grand jury type of approach for eliciting public perception. This process involves convening a focus group and bringing in experts from different sides to answer the group's questions.

DR. WOLF: The mixtures issue is difficult. Generally, we tend to assume that the effects of mixtures will be either additive or synergistic compared to the effects of the compounds acting individually. At the Chemical Industry Institute of Toxicology, we have been dealing with unleaded gasoline, which is a complex mixture, for many years. Unleaded gasoline is a pretty noxious solution of many known rodent carcinogens and some known and probable human carcinogens, including such compounds as benzene, formaldehyde, and aromatic hydrocarbons.

One would predict that, when given to rodents, such a mixture should produce tumors everywhere in the body. However, wholly vaporized gasoline administered to rats and mice at greater than 2,000 parts per million produces tumors only in male rat kidneys and female mouse livers. The male rat kidney tumors are due to alpha-2μ-globulin accumulation, so those data are no longer used for the risk assessments. That leaves only the female mouse liver tumors. I mention this to illustrate that mixtures of different disinfection by-products might not be as risky as our predictions might suggest.

DR. SEED: I would like to comment on that. There are two magic words we have been hearing throughout this workshop. One is "low-dose" and the other is "mixtures." In the past, we have assumed additivity when doing risk assessment on mixtures. Because we are toxicologists, we have believed there is good biological reason for assuming some synergism or antagonism, depending on the chemical.

The issue now coming to light is how to combine low-dose with mixtures. If we assume there is additivity (assuming a common mechanism) or synergism in cases, at what point does this result in what we would characterize as an adverse effect? A lot of data are coming out right now from the TNO Nutrition and Food Research Institute in the Netherlands and from Japan that address this issue and that are highly pertinent to the mixtures issue we are dealing with here. These investigators have challenged or tested the additivity/synergism assumption in a variety of ways. I cannot remember the exact compounds, but they tested different kinds of mixtures—one where the compounds are presumed to act via a common mechanism and others where they are not. In both cases, they are basically not seeing any noncancer threshold effects at environmentally low levels of exposure. If something is going on at the molecular level, it does not translate into anything discernable at the organism level.

However, for many cancer endpoints, there is some evidence for synergism and antagonism at low levels, so this needs to be worked out more. Gasoline is another example. Many of the examples I presented today are bearing this whole issue out. We have got to keep this in mind when we talk about mixtures. Instead of feeling that we are not doing an adequate job of assessing the disinfection by-products mixtures, maybe we should ask whether we have a mixtures problem.

DR. KAVLOCK: I see a real dichotomy between the epidemiologists and the toxicologists on these issues. Earlier this morning, the epidemiologists said that better exposure assessments would only serve to increase the relative risks. They said that identifying susceptible subpopulations probably will magnify the risks.

So, the presumption is that better epidemiology will make things look more hazardous and more risky. While we do not want to have chemicals in the environment that are causing low birth weight in humans, the animal data that Dr. Seed showed this morning and the review of synergisms in binary and tertiary mixtures suggests there is not a lot of evidence, except in very few circumstances, for marked synergism in animal toxicology studies. Dioxin and glucocorticoids is one example where there probably is a two-order-of-magnitude interaction. But that is a real outlier. With most of the data, you do not see interactions of that magnitude.

How can we go to the public and ask them to trade off risks if we cannot tell better characterize the risks they are going to trade off. We can tell people that 132 spores of *Cryptosporidium* will make them sick, but we cannot tell them they will have a reproductive risk from exposure to 8 parts per billion of dibromoacetic acid.

DR. BIRNBAUM We need to be careful when we talk about low dose. A low dose for one chemical is not a low dose for another chemical. We have to look at the ratio of exposure to dose and understand what that is. For explicitly toxic compounds, the level we are exposed to may not be all that low, while for less toxic compounds, it may effectively be several orders of magnitude lower. So, we need to define our terms very carefully.

DR. TARDIFF: I agree. When I was talking about low dose, I meant lower than the maximum tolerated dose. Many of the studies that have been done serve as good screening experiments, but the doses used appear to be phenomenally high. We need to study lower doses than that, although I am not suggesting doing toxicological research at parts per trillion.

DR. SEED: I agree that we have got to define "low" doses. For the studies I was talking about, the investigators defined low in terms of the no-observed-adverse-effect level for that effect as a single compound.

MR. REGLI: A major, but not necessarily new, hypothesis seems to have emerged from the discussions at this workshop that the brominated fraction of disinfection by-products poses significantly greater risks than the chlorinated fraction. Much of the mixtures work to date has not focused on this issue. I would like to get feedback on the feasibility of comparing the magnitude of risk associated with mixtures that meet certain boundary parameters—for example, comparing a mixture with a certain folic acid makeup, bromide concentration, chloride levels, and perhaps pH to a mixture that meets those same conditions except has no bromide.

I suggest this approach because a major problem with regard to mixtures is the stability of the fraction. Another problem is the levels at which animals may react. For example, if the salinity is too high, you cannot conduct the experiment. But if the magnitude of the risk is near that suggested by the epidemiology, we could see effects at much lower concentrations than people have presumed.

I am imagining an experiment where you would create a reproducible black box. In other words, you define what endpoints of response you are interested in, and then you impose the black box upon the population and see what the response is.

DR. TARDIFF: Around 40 years ago, the National Cancer Institute conducted the first such studies, in which the product concentrates, as crudely defined as they were, were administered to the animals by subcutaneous injections. In the early 1970s, the U.S. EPA conducted a whole series of attempts to assemble various kinds of mixtures and then separate them out into at least three chemical fractions. There was also a series of experiments Dr. Bull was connected with that involved introducing a specific disinfectant. The results were generally disappointing. For one thing, it is very hard to get representativeness and to have something sufficiently definable that you can understand what it means in a dose-response function.

DR. BULL: I think you could theoretically do some experiments, but the mixture would have to be reproducibly produced to be useful. Going after wild-type mixtures is a mistake in my opinion, not only because it is cost prohibitive, but also because it is dangerous to generalize from samples that are not representative of the water from which they are derived or do not represent the variability of that source in time. It is a situation begging for both false positives and false negatives to occur. I do not think we can get reasonable results for the reasons that Dr. Seed outlined earlier. Probably our best experiment of this type was the one in which we added chlorine to commercially obtained humic acids in the laboratory to avoid isolation artifacts that occur in taking real samples. However, even in this case we ran into the limitation I identified earlier. The hydrochloric acid generated as a reduction product of chlorination needed to be neutralized. At some point around one thousand times the concentrations seen in drinking water the animal is drinking salt water and cannot survive. This limited what we could administer, and we saw no effects.

I could see that experiment being done again with bromide. You might see a response, but again you would probably be limited in what you could administer. For example, if you assume that dichloroacetic acid, trichloroacetic acid, and brominated compounds are the biologically active components of your mixture, you could use the individual potencies of these compounds to roughly calculate the level at which

you might expect to see an effect. You would not reach an effective concentration in that mixture for any of them, so you would be spending money on a potentially negative result. That is my concern.

DR. TARDIFF: I do not think those mixture studies can work either. A mechanism-of-action approach might be more successful. For example, after a while you might see that there are three cell proliferators in the mixture. You could test those three. You now have a rationale for doing that and looking at various alternative molar concentrations that might mimic what is going on in the water supply. I think that would be a far better use of our resources than the black box approach.

MR. REGLI: Perhaps one way of getting a handle on what that black box contains would be to see how the chemical distribution spectra of the synthetic black box mixture compares to the spectra of a field sample for key parameters such as bromide, total organic carbon, and ultraviolet absorbents.

DR. SIMMONS: There have been advances in experimental designs and statistical analysis that can help us with the mixtures problem. Someone alluded earlier to the 5^3 study. That was very courageous and took a lot of animals. If you want to do a 5^4 study with just trihalomethanes, you would need 625 dose groups, and that is simply not feasible.

There are, however, methods that allow prediction of the additive response based on the characteristics of the dose-response curves for the individual chemicals. So, you do not need to test 625 groups, but you do need to know the dose-response curves of the individual chemicals. We toxicologists should be talking more with our statistical counterparts. We can move forward without the use of so many animals.

SESSION 6
PRIORITIES FOR RESEARCH: SUMMARY OF THE FINAL PANEL DISCUSSION

Final Panel

Dr. Curtis Klaassen, Chair	University of Kansas Medical Center
Dr. Linda Birnbaum	U.S. EPA
Dr. Gary Boorman	National Institute of Environmental Health Sciences
Dr. Richard Bull	Battelle Pacific Northwest Laboratory
Mr. Phillippe Daniel	Camp, Dresser, and McKee
Mr. John Fawell	Water Research Centre
Dr. Hend Galal-Gorchev	World Health Organization
Dr. John Reif	Colorado State University
Dr. Robert Tardiff	EA Engineering, Science, and Technology

Key Conclusions and Recommendations[1]

- Microbial contamination of water supplies poses a clear public health hazard when treatment is inadequate. Any efforts to reduce potential health risks associated with disinfection by-products (DBPs) must not compromise the microbiological quality of drinking water currently achieved through disinfection and physical removal.

- While available data suggest that the risks from DBPs appear low at the current levels of exposure, the ubiquitous human exposure to DBPs in drinking water raises the concern that even small risks could have public health significance. Therefore, additional research is needed to further investigate whether DBPs pose a public health risk.

- Drinking water research involves a variety of cross-disciplinary issues. Therefore, researchers are encouraged to seek input from and, as appropriate, to collaborate with scientists in other fields when designing and conducting DBP research. Such communication will help ensure that the limited resources available for research are wisely targeted.

- Many DBPs from the various disinfection processes have yet to be identified. Further analytical research to identify DBPs should focus on low-molecular-weight compounds (e.g., below 5,000 daltons) that occur in relatively high concentrations, with a particular emphasis on polar compounds, which can now be identified using emerging analytical techniques.

- Research efforts should focus on those compounds that appear, based on the available concentration and/or toxicity data, most likely to be of public health concern. Screening processes should be devised to prioritize DBPs for further research based on comparison of specific toxicity and exposure parameters.

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[1] These recommendations and conclusions represent areas of general agreement among panelists and other workshop participants who contributed to the discussion; however, they do not necessarily indicate complete consensus among all discussants.

- In addition, a minimum concentration should be established that could serve as a cutoff for research decisions. DBPs occurring in drinking water at concentrations below this minimum would not be considered a likely public health concern and would not have priority for research.

- When prioritizing compounds for research, information on a compound's stability or potential for degradation should also be considered to provide perspective on the degree of exposure and, thus, the potential public health concern associated with specific DBPs.

- Insufficient data are available for many classes of DBPs to support a predictive structure-activity relationship (SAR) model for use in setting research priorities; however, in a few cases, sufficient data may be available to develop preliminary SAR models for use in hypothesis generation and testing prioritization.

- Research to characterize the toxicity of the highest priority DBPs should not be limited to high-dose hazard identification, but rather should focus on determining whether these compounds cause effects at low doses.

- For some DBPs, mechanistic research will be important to provide a meaningful basis for extrapolating the data below the detection limits of cancer and other toxicological bioassays. Mechanistic research should also be an emphasis in the future to provide a basis for interpreting potential differences among species and designing mixtures and interactions studies.

- To provide a meaningful basis for low-dose extrapolation, studies of interactions and mixtures should be predicated on the development and testing of specific hypotheses about how the chemicals in question may be anticipated to interact.

- Epidemiology should be hypothesis-driven and supported by improved exposure assessment to enable epidemiological assessment of the total possible risks associated with DBPs from multiple sources and testing of hypotheses for specific DBP classes.

- In general, sufficient screening-level toxicity data are available for the known by-products of chlorination. More detailed mechanistic research would be valuable for a few chlorination by-products.

- No further chronic bioassay research on chloroform is necessary, because extensive research indicates it is unlikely that chloroform, at the average concentrations found in drinking water, poses a significant human health risk.

- Communication between the scientific community, decisionmakers, and the public about the benefits and risks associated with drinking water disinfection should be improved and should form part of a comprehensive risk management strategy.

- Information on how the risk of microbiological contaminants compares to the risk posed by DBPs would be useful in a number of developing countries where there is a high level of public concern about DBPs, particularly potentially carcinogenic DBPs, in drinking water.

Importance of Disinfection

Panelists emphasized that microbial contamination of water supplies is a clear public health hazard when treatment is inadequate. Disinfection of drinking water, therefore, is essential to protecting public health, and any efforts to reduce potential health risks associated with disinfection by-products (DBPs) must not compromise the microbiological quality of drinking water currently achieved through disinfection and physical removal (e.g., filtration).

Identifying Disinfection By-products

Approximately half the halogenated compounds in chlorinated drinking water have still not been identified. Likewise, over half the disinfection by-products produced by alternative disinfectants (e.g., ozone, chloramines) have not been identified. Panelists agreed that efforts to identify additional disinfection by-products should focus on those chemicals most likely to be of public health concern. There was general agreement that, as a first cut, further analysis should focus on low-molecular-weight compounds (e.g., below 5,000 daltons) that occur in relatively high concentrations. Chemicals of higher molecular weight are less likely to be toxicologically significant. Research on disinfection by-products of molecular weight above this cutoff would be deemed of low priority in the absence of information to the contrary.

It was suggested that the pool of compounds of interest could be reduced even further to polar organic compounds of low molecular weight, because polar organic compounds have typically not been detectable with existing analytical methods. A variety of analytical techniques are becoming available to identify polar organic chemicals, but they have not been well tested on drinking water. It was suggested that partnerships should be formed between government, industry, and academic laboratories that are knowledgeable about the techniques and have the necessary instrumentation to identify unknown DBPs in drinking water.

Prioritizing Disinfection By-products

Evidence to date suggests that the human health risks from disinfection by-products are low at the current levels of exposure. However, the ubiquitous exposure to these compounds raises concern that even relatively small risks (expressed as an odds ratio, for example), if they are real, could affect a significant number of people. This possibility argues for additional research to further investigate the potential of these compounds to pose a significant public health risk.

The limited resources available and the high cost of chronic animal and epidemiology studies, as well as sophisticated mechanistic research, give additional weight to the need for a prioritization process. Panelists agreed that, as disinfection by-products are identified, research efforts to elucidate potential health effects should focus on those chemicals most likely to be of public health concern. Panelists discussed a number of potential schemes to screen chemicals and set research priorities.

One such scheme, illustrated in Table 1, involves comparing some basic toxicological and exposure parameters to screen and rank DBPs according to their apparent potential to cause health effects at the concentrations found in drinking water. Table 1 lists a limited number of representative chemicals produced during drinking water disinfection using chlorine, ozone, and/or chlorine dioxide. The table was

constructed for illustrative purposes only, thus the list of DBPs is not exhaustive and may not include all by-products of greatest importance. The values given in Table 1 are approximations.

Table 1 allows two types of comparisons that may be useful in providing perspective regarding research priorities for DBPs. The first involves comparing the maximum tolerated dose (MTD—given in column 2) to the Lowest Observed Adverse Effect Level (LOAEL—given in column 3). For purposes of Table 1, the MTD is defined as the dose at which overt toxicity is observed and the animals have a difficult time surviving or thriving. The LOAEL is the minimum dose that has been shown to produce some type of toxic effect. Because of the limited data available for many of the chemicals listed in Table 1, the LOAELs given should be considered approximate, at best.

Comparison of the LOAEL to the MTD provides some idea of whether a compound may cause effects at low doses. When the LOAEL approaches the MTD (i.e., when the LOAEL/MTD ratio [given in column 4] approaches 1/1), the specific toxicities (e.g., cancer, developmental effects) observed at these doses are likely to be expressions of the abnormal physiology produced at such high doses. Chloroform, which has a LOAEL/MTD ratio of ½, is perhaps the best example of this. Chloroform is carcinogenic only at high doses in rodents—doses that approach the MTD and that produce overt toxicity. Because of chloroform's LOAEL/MTD ratio, which approaches 1/1, combined with recent understanding about its possible nongenotoxic mechanism of action, the panel concluded that it is unlikely that chloroform would present a significant carcinogenic hazard at the low concentrations found in drinking water.

The LOAEL approaches the MTD for several other DBPs on Table 1, suggesting that the effects observed may result from nonspecific pathology associated with such high doses and therefore would not manifest at low doses. However, less certainty can be attached to this conclusion than in the case of chloroform, because none of the other chemicals has as rich a database as chloroform.

The second type of comparison facilitated by Table 1 is a comparison of the LOAEL with the estimated daily human dose to the DBP from drinking water. This comparison provides a rough idea of a DBP's potential to pose a human health risk at typical levels of human exposure. Column 5 shows the upper estimate of the daily dose that people are likely to receive from U.S. drinking water. Column 6 shows the human dose/LOAEL ratio. The more this ratio approaches 1/1, the greater the probability that a DBP may be of human health concern at the levels found in drinking water. The human dose/LOAEL ratio provides an important perspective on compounds such as the chlorinated furanone, MX. This DBP initially appears to be of potential concern because of its LOAEL/MTD ratio of 1/50; however, human exposure to MX via drinking water is so low relative to the LOAEL for this compound that it seems highly unlikely that MX in drinking water poses a human health hazard.

Table 1. Suggested Information Matrix for Use in Prioritizing Health Effects Research on Disinfectant By-products

DBP	MTD_{rodent} mg/kg/day	$LOAEL_{rodent}$ mg/kg/day	LOAEL / MTD	Human Dose[a] μg/kg/day	Human Dose / $LOAEL_{rodent}$
Chloroform	180	81[b]	½	1.7[c]	1/50,000
Bromodichloro-methane	100	9[b]	1/10	0.7[c]	1/10,000
Dichloroacetic acid	500	1.2[b]	1/400	0.9[c]	1/1,000
Dibromoacetic acid	250	2[d]	1/125	0.2[c]	1/10,000
Dichloroacetonitrile	25	8[e]	1/3	0.3[f]	1/30,000
MX	50	1[g]	1/50	0.001[h]	1/1,000,000
Bromate	45	0.7[b]	1/60	0.6[i]	1/1,000
Formaldehyde	150	32[b]	1/5	0.7[j]	1/50,000
Chlorite	80	6[k]	1/13	23[l]	1/300

[a] Based on 70-kg adult consuming 2 L/day of drinking water containing the indicated DBP with a concentration at the 90th percentile for U.S. drinking waters.

[b] Based on a lower 95% confidence level estimate of the dose required to produce a 10% increase in tumor incidence in experimental animals using the linearized multistage model (Bull and Kopfler, 1991).

[c] Based on American Water Works Association disinfection/disinfection by-product negotiated regulation database (Gramith et al., 1994).

[d] Based on a lower 95% confidence level estimate of the dose required to produce a 10% increase in tumor incidence in experimental animals using the linearized multistage model. From preliminary data of Bull et al. presented at the workshop.

[e] Based on decreased body weight and evidence of reproductive effects at a slightly higher dose (Bull and Kopfler, 1991).

[f] Based on U.S. EPA survey (Reding et al., 1989).

[g] From preliminary data presented by Tuomisto et al. at the workshop.

[h] Based on limited U.S. study (Munch et al., 1988) and augmented by more complete surveys in Finland (Kronberg et al. [1988]) and in Canada (Andrews et al. [1989]).

[I] Projected bromate occurrence for systems that may use ozonation without practicing some form of control to keep bromate below the proposed U.S. maximum contaminant level of 10 μg/L (Krasner et al., 1993).

[j] Formaldehyde occurrence for systems that may use ozonation without biological filtration (Weinberg et al., 1993).

[k] Based on mild oxidative damage to rat and cat erythrocytes (Heffernan et al., 1979).

[l] Chlorite occurrence for systems that use chlorine dioxide (Bubnis, 1993).

As discussed at the workshop, compounds that have both a LOAEL/MTD ratio in which the denominator is high (when the numerator is set at 1) (suggesting that the compounds may have effects at low doses) and a ratio of human dose/LOAEL that approaches 1/1 (suggesting that human exposure is approaching the doses at which the compound causes effects) would receive highest priority for research. Thus, among the DBPs listed in Table 1, research should be prioritized for dichloroacetic acid and bromate, which are primarily produced by chlorination and ozonation, respectively. Similarly, based on Table 1, secondary priority would be directed toward bromodichloromethane and dibromoacetic acid.

Another proposal for setting research priorities among identified chemicals was to establish a threshold exposure concentration to serve as a cutoff for research decisions. Specifically, for example, the daily allowable dose of aflatoxin, the most potent known human liver carcinogen and one of the best understood carcinogens from a mechanistic standpoint, was suggested as a suitably conservative cutoff. Under this scheme, disinfection by-products for which human exposure is less than this threshold would not be considered a public health concern and would not be candidates for DBP research. One participant expressed some concern that this scheme would fail to flag for research any extremely potent DBPs that were present in very low concentrations. Panelists responded that it was possible but unlikely that a disinfection by-product existed that would exceed aflatoxin in potency.

Another approach proposed for prioritizing identified compounds for study makes use of a correlation that has been found between the MTD and the TD_{50} (the dose that produces tumors in 50 percent of the test animals), which is a measure of cancer potency. In all compounds examined, there was a positive correlation between the MTD and the TD_{50}, and a negative correlation between the MTD and the slope of the dose-response curve in the low-dose region (another measure of cancer potency). The estimated cancer potency values for all known chemical carcinogens (based on rodent bioassays) vary over seven orders of magnitude. The distribution of these estimated cancer potencies can be plotted. It can be assumed that any unidentified carcinogen most likely falls within this potency distribution and its potential potency can be estimated from the MTD determined during a 90-day toxicity study.

Participants discussed the use of structure-activity relationships (SAR) as a tool for setting priorities. There was general agreement that SAR, in the absence of biological activity data for related members of a chemical class, has limited application for priority setting. The predictive value of SAR for any particular compound depends on the degree to which the database for the related group or class of compounds has been developed, and it was agreed that insufficient data are available for many classes of DBPs to support a predictive model. However, it had been noted earlier that even limited activity information within a class can sometimes provide sufficient data for developing preliminary SAR models for use in hypothesis generation and testing prioritization. One panelist mentioned that SAR can frequently indicate whether something is going to be mutagenic, which could indicate a high priority for research.

The use of octanol/water partition coefficients (i.e., logP values) was also proposed as one way to scale concentrations from the standpoint of bioavailability (similar to the current use of logP by SAR practitioners to estimate the percentage of a chemical that reaches the internal site of action). It was suggested that logP, in the absence of other data for use in ranking, could be used to provide an initial, default estimate of relative pharmacokinetic transport within a chemical class.

There was general agreement that information on fate and transport (e.g., information on a compound's stability or potential for degradation) within the treatment and distribution system should be considered to provide perspective on the degree of exposure and, thus, the potential public health concern associated

with specific DBPs. Most disinfection by-products are stable in distribution systems and the concentrations of the chlorination by-products will typically increase in the presence of a free chlorine residual. However, a number of DBPs (e.g., dichloroacetonitrile) can by hydrolyzed (degraded) in distribution systems operated at a high pH. Biodegradation of DBPs is typically a treatment plant issue and not a distribution system issue. If the treatment plant filters are operated biologically, some DBPs (e.g., formaldehyde) can be efficiently biodegraded in the plant and, thus, consumers will not be exposed to such chemicals. Dichloroacetic acid can possibly be degraded in distribution systems, but that appears to happen only in regions of the distribution system that have little to no chlorine residual. With new coliform standards, however, this situation should rarely occur in U.S. distribution systems.

General Research Needs

Many expressed the view that most DBPs are encountered in such low amounts that they likely present only a trivial, if any, health risk (see Table 1). In times of limited resources, these commentors suggested, it is important to target research dollars on specific areas of concern rather than on less focussed approaches. Panelists stressed the importance of a multidisciplinary approach to DBP research to provide cross-fertilization and help ensure the most productive use of research resources in this complex field of public health concern.

As stated earlier, participants emphasized the need to focus research efforts on those DBPS that may actually pose a health risk. There was some concern that more was known about the by-products of chlorination than those of other disinfection processes, such as ozonation. Since regulatory agencies may suggest or require changing municipal drinking water disinfection processes based on perceived health risks, it was felt that overall comparisons should be conducted of the toxicity profiles of by-products produced by different disinfection processes.

It was felt that mechanistic research had proven valuable in putting relative risks into perspective and should be an emphasis in the future, particularly in helping to interpret dose-response relationships, potential differences among species, and as the basis for design of mixtures and interactions studies. For example, it was pointed out that mechanistic research with chloroform has helped to characterize its lack of genotoxicity and provided evidence that, at least in some target organs (e.g., mouse liver), carcinogenesis may be secondary to organ-specific toxicity and regenerative cell proliferation. Similar mechanistic information would be valuable for other key DBPs.

Any new cancer studies should be hypothesis-driven, rather than simply focussing on high-dose hazard identification, which does not provide adequate information for public health decisions. More complete hazard characterization studies should cover wide dose ranges, include mechanistic information, and be planned such that the data can be meaningfully used in health risk assessments. These second generation studies can provide some of the mechanistic data that will allow comparisons between the different by-products or the different disinfection processes.

A strong opinion was expressed that studies of interactions and mixtures should be hypothesis-driven. The risks posed by exposures to complex mixtures at low doses cannot be evaluated cost-effectively from experiments utilizing random combinations of the chemicals with dose ranges several orders of magnitude higher than found in drinking water or by concentrating chemicals from water. Such data provide no basis for low-dose extrapolation. The panel expressed the strong opinion that the impact of mixtures of chemicals can only be meaningfully evaluated by very focused studies of chemicals for which the mode or

mechanism of action has been established. To effectively utilize resources in this area, these studies should be predicated on the development and testing of specific hypotheses about how the chemicals may be anticipated to interact. Mechanistic research would be an important prelude to interactions and mixtures research to develop the basis for the hypotheses to be studied.

Different modes of action are represented within and across the major classes of DBPs. It was suggested that studies of interactions between members of these classes be contemplated, with an emphasis on interactions likely to occur at low doses. One panelist expressed skepticism that, at their relatively low concentrations in drinking water, any two DBPs would have significant synergism. He suggested that society should be more concerned about potential interactions between a disinfection by-product and a substance such as alcohol, to which there is substantial non-drinking water exposure and which is known to increase the toxicity of many other chemicals.

Similarly, another panelist expressed the opinion that hypothesis-driven epidemiology was needed as an alternative to epidemiological studies that lumped all exposure to chlorinated water together as a common exposure. For example, an epidemiological study could compare three types of populations—people with high bromide levels in their chlorinated water, people with low bromide levels in their chlorinated water, and people who drink ground water—or some other perturbation of that scheme. Other participants supported the general idea of using epidemiology to study broad brush differences in water supplies. One panelist pointed out that the approach of comparing populations exposed to two surface water supplies would be particularly advantageous over studies that compared surface water to ground water because it would eliminate potential confounding from non-DBP-related water quality differences between surface water and ground water. He also pointed out that, almost invariably, relatively common diseases involve multifactorial risk factors, so caution is needed when attributing causality to drinking water.

Expanding on the general consensus about the value of research to compare the risks of chlorinated versus other disinfected waters, one participant suggested that research on the by-products of chlorine-chloramine disinfection and ozone-chloramine disinfection might be especially important because these combinations appear to be effective for inactivating *Cryptosporidium*, whereas chlorine alone is not. If additional research bears this out, there may be a shift to alternative disinfectants to better control microbial risk. Another participant questioned whether separate research on the by-products of combined technologies would be needed. He thought that research that focussed on the prioritized by-products produced by the individual disinfectants might well provide sufficient data on mixed chlorine-chloramine or ozone-chloramine processes.

One participant pointed out that data presented at the workshop by Dr. Reckhow indicated that many ozonation by-products are significantly altered by biological activity and therefore never make it to the customer's tap. This alteration may occur both in the filters and the distribution system, and by the subsequent chlorination-chloramination step. For this reason, focussing exclusively on ozonation by-products could be misleading. He also mentioned that some compounds produced by ozonation followed by chlorination-chloramination do need to be addressed specifically because ozonation enhances their subsequent formation. For example, if biological filtration is not used, ozonation followed by chloramination can produce larger concentrations of cyanogen chloride, and ozonation followed by chlorination can produce larger concentrations of chloral hydrate.

Exposure assessment was discussed as another important research area. Several panelists and participants indicated it was important to know total exposure from all potential routes (air, food, water) and not just

assume that all DBP exposure comes from ingestion of drinking water. Showering and bathing, for example, may be additional exposure sources, although one panelist expressed doubt that these sources would prove significant. A number of commentors suggested that research include studies of human activities to examine the type and range of exposure from other sources.

The need to improve exposure assessment for epidemiologic studies was emphasized. Improved exposure assessment would allow epidemiologists to explore the total risks associated with disinfection by-products from multiple sources and to test hypotheses for specific groups of DBPs such as brominated by-products and halogenated acetic acids. Reduction of misclassification that occurs when trihalomethanes are used as a surrogate for other DBPs will improve the accuracy of risk estimates for human populations.

Panelists briefly discussed engineering research to investigate technological options for risk reduction, e.g., technologies that minimize the formation of by-products or remove by-products. One panelist emphasized that many developing countries need practical, inexpensive technological options for reducing the risk from chlorination by-products since switching to alternative disinfectants may be difficult due to cost and technology barriers. Another panelist suggested that biological filtration has shown some potential for removing chemicals that are of toxicological concern, either by themselves or after chlorination or chloramination, and should be examined further.

It was pointed out that many developing countries need a better understanding of the risks of microbial contamination compared to disinfection by-products. In many of these countries, there is a public chemophobia about potential carcinogens in drinking water. Research to quantify and compare the risk of microbiological contaminants and disinfection by-products is needed so that scientists, communication professionals, and decisionmakers can provide a clear message about this issue, in as quantitative terms as possible.

Chemical-Specific Research Needs

Panelists and participants discussed research needs for a few specific disinfection by-products. The limited timeframe did not allow discussion of all potential compounds that might be candidates for research.

In general, sufficient screening-level toxicity data are available for chlorination by-products. More detailed mechanistic research would be valuable for a few chlorination by-products. In particular, there was general agreement that no further chronic bioassay research on chloroform was necessary, because extensive research indicates it is unlikely that chloroform, at the average concentrations found in drinking water, poses a significant human health risk. However, further mechanistic research may be valuable from a scientific standpoint. Discussants noted that chloroform is still a useful marker for trihalomethanes; however, they suggested that the individual trihalomethanes be considered separately because of their different target organs and potentially different mechanisms of action.

One participant recommended that further research be done on cyanogen chloride, a fairly major by-product of chloramination. At present, there are only acute inhalation health effects data, but no ingestion data, and the Science Advisory Board has recommended not using hydrogen cyanide as a surrogate because its metabolism is quite different.

As reported by Dr. Romano during the workshop, significant research has recently been conducted on chlorite. In light of this, it was suggested that additional chlorite research was probably not a priority.

A panelist suggested that further research is needed on chlorate, a compound that is not currently captured by any of the regulatory activity yet is potentially present at significant concentrations. There are very few data on chlorate, so basic research is needed to indicate whether or not it might be a concern. The U.S. EPA is planning to begin a two-year bioassay in 1996 to assess the potential carcinogenicity of chlorate in rodents. Studies may also be conducted to evaluate selected noncancer endpoints.

Another participant pointed out that some haloacetic acids that are not being studied are likely to occur at or above concentrations of other haloacetic acids currently under investigation. He recommended studying additional haloacetic acids that are likely to be present in distribution systems at significant levels. Currently there are health effects data on all four trihalomethanes, whereas such information is only available for a few of the haloacetic acids. Because both classes of DBPs are subject to regulation in the proposed Disinfection By-products Rule, health effects information on other haloacetic acids—especially those that can be present at significant levels in distribution systems—needs to be developed.

Risk Communication

Some public concern has been expressed about the safety of drinking water; however, it is not clear to what extent these concerns reflect the views of most consumers. Current information tends to represent an "opinion of public opinion," rather than a reliable assessment of public opinion. It was suggested that additional surveys would be helpful to more accurately understand public concerns.

Participants expressed concern that scientific knowledge is often misinterpreted or misused by the media and emphasized the importance of enhanced communication and education as part of a comprehensive risk management strategy. Effective communication strategies are needed for three communication channels: scientists communicating to risk managers; risk managers communicating to the media; and risk managers/media communicating to the public. All three target audiences—risk managers, the media, and the public—need a better context for understanding risk information. In particular, participants stressed, any description of risk should clearly and impartially describe both certainties, uncertainties, and limitations of scientific knowledge and methods. Too often, numerical point estimates are interpreted to convey a much greater sense of certainty than is warranted by the methods and data.

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POSTER PRESENTATIONS

POSTER #1

Genotoxicity Evaluations of Drinking Water Chlorine Disinfection By-products

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Major disinfection by-products from the chlorination of drinking water include dichloroacetic acid (DCA) and potassium bromate ($KBrO_3$). These chemicals are known to be carcinogenic in rodents, yet little genotoxicity data are available to assess the possible role of chromosome damage in this process. We have used the micronucleus assay to investigate the genotoxic effects of DCA and $KBrO_3$. This assay can detect chromosome breakage and/or malsegregation caused by exposure to the test agents. Mice were exposed to these compounds in drinking water, available *ad libitum* for up to 78 weeks. Micronucleus induction in blood erythrocytes was evaluated following staining with propidium iodide and an anti-kinetochore antibody to determine centromere status. Our results show that at the highest dose of DCA tested, a small but significant increase in the frequency of micronucleated cells was detected in the normochromatic erythrocyte (NCE) population following exposure for longer than 10 weeks. In addition, a small but statistically significant dose dependent increase in the frequency of micronucleated polychromatic erythrocytes (PCEs) was observed after a 9 day chronic exposure. $KBrO_3$-exposed mice showed a doubling in the frequency of micronucleated cells as compared with the negative control group. Furthermore, our data suggest that both DCA and $KBrO_3$ function as clastogens since the overwhelming majority of the micronuclei observed did not stain with the anti-kinetochore antibody, and thus presumably lack centromeres. This suggests that the micronuclei induced by these chemicals represent chromosome fragments.

POSTER #2

Mutagenic Effects of Chlorine Disinfection By-products

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Four disinfection by-products from the chlorination of drinking water, chloral hydrate (CH), 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX), dichloroacetic acid (DCA), and trichloroacetic acid (TCA) were evaluated for their ability to cause mutagenic damage in mammalian cells *in vitro*. L5178YTK$^{+/-}$ -3.7.2C mouse lymphoma cells were used to detect mutations effecting the thymidine kinase (tk) locus and to analyze for gross chromosome aberration induction. Our test results have demonstrated that all four compounds are mutagenic in mouse lymphoma cells and that MX, DCA, and CH induced gross chromosome aberrations. MX, DCA, and CH are direct acting mutagens; TCA required an exogenous activation system to be mutagenic. The four compounds vary in their mutagenic

potency. MX is the most mutagenic, inducing 1026 mutants/10^6 survivors and 34 aberrations in 24 of 100 metaphases at a dose of 0.75 μg/L. TCA with activation is the least mutagenic, inducing 126 mutants/10^6 survivors at a dose of 3000 μg/L. Cytogenetic analysis was not performed on TCA because of its weak mutagenic response.

These studies demonstrate that the four compounds are mutagenic in mammalian cells *in vitro* and, therefore, have the potential to induce DNA mutation in somatic cells *in vivo*. It is reasonable to postulate this ability to damage DNA as a mechanism for their carcinogenicity in animals.

POSTER #3

Glutathione *S*-Transferase-Mediated Mutagenicity of Trihalomethanes

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Trihalomethanes (THMs) are the most prevalent disinfection by-products generated in chlorinated drinking water. Among the THMs, chloroform ($CHCl_3$) generally occurs at the highest concentration in finished water, but the concentrations of the brominated THMs ($CHBrCl_2$, $CHBr_2Cl$, and $CHBr_3$) can exceed that of $CHCl_3$ when source waters with high bromide levels are chlorinated. Each of these four THMs was carcinogenic in rodents in chronic oral dosing studies. The determination of whether carcinogens produce cancer through a genotoxic mode of action is critical in the selection of appropriate risk assessment strategies. In standard mutagenicity assays, $CHCl_3$ is generally negative while the 3 BrTHMs are weakly positive. The objective of this study was to assess THM mutagenicity in a strain of *S. typhimurium* TA1535 which was transfected with rat GSH *S*-transferase 5-5 (+GST) (Thier et al., PNAS 90:8576). The +GST strain and its nontransfected counterpart strain (-GST) were employed in a plate incorporation assay and exposed for 24 hr to the vapor of individual THMs at concentrations from 0-4800 ppm in sealed Tedlar® bags. Base pair revertants were produced in the +GST strain in a dose-dependent fashion by $CHBrCl_2$ and $CHBr_3$, but not by $CHCl_3$. At 3200 ppm, revertants per plate induced by $CHBrCl_2$ and $CHBr_3$ averaged 373 ± 86 and 1935 ± 255, respectively. These results indicate that bromination of THMs confers the capability for GST-mediated transformation to mutagenic intermediates, suggesting the possibility of a similar activation route in humans. Further, the lack of participation by $CHCl_3$ in the GSH-dependent pathway demonstrates that different THMs can induce adverse effects via different mechanisms.

POSTER #4

Concentrations of Ames Mutagenic Chlorohydroxyfuranones and Related Compounds in Finnish Drinking Waters

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In extracts of Finnish drinking waters, the concentrations of a total of thirteen Ames mutagenic compounds were determined. The compounds were mainly chlorinated 5-hydroxy-2-furanones, i.e., the extremely potent mutagen MX [3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone], and compounds with structural similarities to MX. The objectives of the study were to make a comprehensive survey of the occurrence of such compounds in drinking waters, and to estimate the significance of the compounds as contributors to the overall Ames mutagenicity in the waters.

Drinking water was collected from 35 localities in Finland. The acidified water samples were passed through XAD-8 resin and eluted with ethyl acetate. The mutagenic activity was measured in the extracts using the Ames Salmonella typhimurium strain TA100 without metabolic activation. The compounds were analyzed in methylated extracts using GC-MS in the selected ion monitoring mode.

The results showed that the compounds were present in mutagenic extracts in concentrations ranging from 0.5 to 78 ng/L. The contribution of MX to the overall mutagenicity ranged from 16 to 90%, and the contribution of the other compounds was usually below 10%.

The study confirms that MX is the most important individual compound active in Ames tester strain TA100 occurring in disinfected drinking water derived from surface water.

POSTER #5

NOAEL and LOAEL Estimation for Acute Hepatotoxicity of $CHCL_3$ and BDCM Administered in an Aqueous Vehicle to F344 Rats

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Chlorination is one of the most effective and least costly disinfection processes used for our finished drinking water supply. However, humic material in the water supply reacts with the residual chlorine to form trihalomethanes (THMs) as well as other halogenated compounds which have been shown to be carcinogenic in rodents. Chloroform ($CHCl_3$) and bromodichloromethane (BDCM) are the two most prevalent THMs formed during chlorination of drinking water but their toxicity has most often been studied using corn oil as the vehicle of administration. The objectives of this study were to assess hepatotoxicity after exposure to single, low dosages of $CHCl_3$ and BDCM given orally in an aqueous

vehicle to estimate a lowest observable adverse effect level (LOAEL) and/or no observable adverse effect level (NOAEL) and to compare toxic potency. Ninety-day-old male Fischer-344 rats (n=6) were gavaged with either 0, 0.5, 0.75, 1.0 or 1.5 mmoles of $CHCl_3$ or BDCM/kg body wt in 10% Emulphor® (5 ml/kg body wt). At 24 hr post-gavage, serum was collected for determination of clinical chemistry indicators of liver damage. Data were analyzed by analysis of variance; when $p \leq 0.05$ the data were analyzed further by the Ryan-Einot-Gabriel-Welsh Multiple F test. Both $CHCl_3$ and BDCM induced dose-dependent hepatotoxicity; serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and sorbitol dehydrogenase (SDH) were elevated significantly over control ($p \leq 0.001$) at 1.5 and 1.0 mM/kg. At 0.75 mM/kg, SDH was elevated significantly ($p \leq 0.001$) by both $CHCl_3$ and BDCM (other hepatotoxic markers were not significantly affected at this dose level). At 0.5 mM/kg, significant increases over control were not detected ($p \leq 0.05$) for any of the end points measured. In conclusion, these acute oral hepatotoxicity data suggest apparent NOAELs and LOAELs of 0.5 mM/kg and 0.75 mM/kg, respectively, for both $CHCl_3$ and BDCM. Further, at the dose levels tested, the two THMs appeared to be equipotent hepatotoxicants.

POSTER #6

Analysis of DNA Strand Breaks Induced *In Vivo* and *In Vitro* by Trihalomethanes (THMs)

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The chlorinated and brominated trihalomethanes are found in finished drinking water as by-products (DBPs) of the disinfection process. The exposure of large populations over long time periods to low concentrations of THMs has raised concern about the safety of chlorinated drinking water. Studies conducted by the National Toxicology Program demonstrated the carcinogenicity of THMs when gavaged in corn oil. The focus of NHEERL's Water Carcinogenesis Program has been to identify cancer hazards using laboratory rodents exposed to DBPs in the drinking water, define a dose response, and examine carcinogenic mechanisms which might underlie the carcinogenic process.

Chloroform (CHCl3) has been uniformly negative in genetic toxicity assays; the data for the genotoxicity of the brominated THMs, bromoform (CHBr3), chlorodibromomethane (CHBr2Cl), and bromodichloromethane (CHBrCl2) have been conflicting. We studied the ability of the THMs to induce DNA strand breaks (SB) [1] after administration to rats and mice by gavage (aqueous) and in drinking water and [2] in rat hepatocytes and CCRF-CEM cells exposed *in vitro*. SB were measured by the DNA alkaline unwinding assay (DAUA). Methyl methane-sulfonate (MMS), N-nitrosodimethyl-amine (DMNA), and N-nitrosodiethylamine (DENA) were used as positive controls.

Incubating CCRF-CEM cells with 1, 5, and 10 mM brominated THMs for 2 hr produced DNA SB. The order of activity was CHBr > CHBr2Cl > CHBrCl2; CHCl3 was inactive. After a 22 hr recovery period, cells which had been exposed to > 5 mM CHBr3 or 10 mM CHBr2Cl did not recover as evaluated by the trypan blue exclusion assay. Treating rat hepatocytes in primary culture with 1 - 10

mM CHBr3 and CHBr2Cl for 4 hr failed to induce DNA SB without concomitant cell toxicity (release of LDH into the culture media). CHBrCl2 and CHCl3 produced no toxicity or SB.

Brominated THMs (0.3 and 0.6 mmol/kg; 4 hrs) administered by aqueous gavage to male B6C3F1 mice and F344 rats failed to increase the number SB above background in the DNA of liver, kidney, and duodenum. CHBr3 (1.25 g/l) and CHBr2Cl (1 g/l) in the drinking water of mice for 2 weeks increased DNA SB in the duodenum. CHBrCl2 failed to increase DNA SB in any of the three tissues from the mouse (0.75 g/l) after 2 weeks of exposure or the rat (0.6, 1.2, and 2.4 g/l) following a 5 week exposure period.

These data demonstrate that the brominated THMs administered in an aqueous vehicle possess a specific type of genotoxicity (DNA SB) in cells with low metabolic capability (CCRF-CEM, duodenal epithelial cells), but not in cells with an active P450 enzyme system (hepatocyte, kidney tubule). However, metabolism by gut microbes cannot be ruled out in the case of CHBr3 and CHBr2Cl genotoxicity in the duodenal cells.

POSTER #7

The Evaluation of the Carcinogenicity of Chloral Hydrate in the Male B6C3F1 Mouse and F344 Rat

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Chloral hydrate is used as a pediatric anesthetic and is found in finished drinking water as a by-product of chlorine disinfection. Chloral hydrate at 1 g/l in the drinking water increased hepatocellular carcinoma in male B6C3F1 mice (Daniel et al., *Fund. Appl. Toxicol.*, 19:159, 1992). Male B6C3F1 mice were exposed to 0.05, 0.8, and 1.4 g/l chloral hydrate in the drinking water for 104 weeks. The calculated mean daily doses for CH were 5, 87, and 165 mg/kg/day respectively. Male F-344 rats exposed to 0.05, 0.8, and 2.7 g/l CH in the drinking water over the same time period had a mean daily dose of 3, 50, and 174 mg/kg/day respectively. Chloral hydrate increased hepatocellular carcinoma in the mouse at doses greater than 0.05 g/l (0.69 carcinoma/animal), 0.5 g/l (1.03 carcinoma/animal; $P<0.05$), and 1.4 g/l (1.44 carcinoma/animal; $p<0.05$) vs. control (0.76 carcinoma/animal). In the rat, chloral hydrate treatment did not increase hepatocellular carcinogenesis above that of the control group (0.03 carcinoma/animal): 0.05 g/l (0.05 carcinoma/animal), 0.8 g/l (0 carcinoma/animal), or 2.7 g/l (0.03 carcinoma/animal). There were no significant chloral hydrate dose effects on the body weights of either mice or rats. Chloral hydrate did not have any effect on the activity of palmitoly Co A oxidase, a marker of peroxisome proliferation in either species, but increased the BRDU labeling index of hepatocytes at 26 weeks (0.5 and 1 g/l) and 52 weeks (1 g/l) in the mouse. No effects on the hepatocyte labeling index were measured in the rat. This study demonstrates that CH is a hepatocarcinogen in the mouse, but not the rat when administered in the drinking water.

POSTER #8

Chloroform-Induced Toxicity and Cancer in Male Osborne-Mendel and F344 Rats

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Chloroform induced kidney tumors in male Osborne-Mendel (Os-M) rats when administered for 2 years by gavage at 180 mg/kg/d or at 1800 ppm in the drinking water. F344 rats exposed via inhalation to 90-ppm chloroform vapors 5 d/wk for 2 years developed no chloroform-induced tumors. The relative sensitivity of the two strains to the cytolethal and regenerative cell proliferation effects of chloroform has not been established. Male Os-M and F344 rats were administered a single dose of 0, 10, 34, 90, 180, or 477 mg/kg chloroform in corn oil by gavage. Bromodeoxyuridine (BrdU) was given i.p. 2 h prior to necropsy, which occurred 48 h postdosing. The labeling index (LI), percentage of cells in S-phase, was quantified with BrdU immunohistochemistry. The epithelial cells of the proximal tubules of the cortex were the primary targets for cell proliferation in the kidney of Os-M and F344 rats. A dose-dependent increase in the LI was present in Os-M rats given doses of 10 mg/kg and above and in F344 rats given 90 mg/kg and above. The highest LI response in Os-M rats was 4.5 fold over control (FOC) and in F344 rats was 3.7-FOC. There was no increase in the hepatocyte LI in the Os-M rats. A significant increase in the hepatocyte LI was present only in the F344 rats administered 477 mg/kg. A dose-dependent increase in the unit length labeling index was present in the nasal ethmoid turbinates of Os-M and F344 rats administered 90, 180, or 477 mg/kg. Previous studies indicated that events secondary to chloroform-induced toxicity and regenerative cell proliferation play a critical rate-limiting role in chloroform carcinogenesis. Inhalation studies in F344 rats have shown a concordant lack of induced proliferation and a lack of induced liver and kidney tumors. Regenerative cell proliferation in the kidneys of the Os-M and F344 rats indicates that the strains are approximately equally susceptible to chloroform-induced nephrotoxicity and supports the hypothesis that chloroform-induced cytolethality and regenerative cell proliferation played a role in the induction of renal tumors in the Os-M rat.

POSTER #9

Promotion of Dichloroacetic Acid (DCA) Initiated Hepatocarcinogenesis by Phenobarbital

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DCA is a complete hepatocarcinogen and tumor promoter in the male B6C3F1 mouse. The role of DCA as an initiator is uncertain since chloracetic acids are non-genotoxic in a variety of test systems. To investigate the role of DCA as an initiator, 3 groups of male B6C3F1 mice were given drinking water containing DCA (3.5 g/l) beginning at 28 days of age. Two groups received distilled water.

After 30 weeks, DCA was removed from the drinking water of 2 groups of animals; one group was given phenobarbital (PB, 0.06%) while the second group was given distilled water until sacrifice at 100 weeks. The third group continued to receive DCA for 100 weeks and a fourth group was given PB instead of distilled water beginning at 30 weeks. After sacrifice, livers were weighed and blocks of liver as well as all visible lesions were fixed in 10% phosphate-buffered formalin and processed routinely. Histologic sections were cut at 5μ and stained with H & E. On average, 6 histologic sections per animal were evaluated for pathologic changes. Hepatic lesions were classified as follows: altered foci (AF) were small lesions with altered H & E staining characteristics: hyperplastic nodules (HN) had alterations in liver architecture and staining characteristics; adenomas (AD) had alterations in liver architecture and staining characteristics and also showed displacement of triads and compression of adjacent tissue; carcinomas (Ca) were comprised of cells with a high nuclear/cytoplasm ratio and with nuclear pleomorphism and atypia, that showed evidence of invasion.

DCA exposure for 100 weeks promoted the development of putative pre-neoplastic, neoplastic and malignant legions. While both DCA exposure for 30 weeks and PB exposure for 74 weeks promoted development of hepatic lesions, given together, the effect of these compounds was synergistic. Based upon the H & E staining characteristics, the data suggest that DCA initiates *eosinophilic* pre-neoplastic lesions which are promoted to progress to neoplastic and malignant lesions by PB.

POSTER #10

Immunohistochemical Detection of P4502E1 Induction in Livers from Female and Male Chloroform-Treated B6C3F1 Mice and F344 Rats

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Cytochrome P4502E1 is thought to be the principal hepatic enzyme that metabolizes chloroform. The present study relates the site and amount of P4502E1 immunoreactivity (IR) to chloroform-induced hepatic degeneration and necrosis. Female and male B6C3F1 mice and F344 rats were treated with chloroform by corn oil gavage with doses ranging from 3 to 477 mg chloroform/kg bwt/d (mkd) for 4 d or 3 wk. Livers were examined microscopically after HE staining and P4502E1 immunohistochemistry. The extent of P4502E1 IR was determined from 10 randomly chosen lobules on each of 3 sections of liver from each animal. The number of layers of IR cells around each central vein and the number of layers of cells from the central vein to the adjacent portal triad were counted and used to calculate the percentage of labeled cells and percentage difference from control. Female mice given 90 mkd or greater for 4 d or 34 mkd or greater for 3 wk had dose-dependent centrilobular hepatocyte degeneration and/or necrosis. Male mice had dose-dependent hepatic lesions at all time points. Female rats had mild centrilobular degeneration or necrosis after 100 mkd or greater for 4 d, and 200 mkd or greater after 3 wk. Male rats had hepatic centrilobular degeneration after 4 d of 90 mkd or greater and only the highest dose (180 mkd) after 3 wk of treatment. P4502E1 IR in control animals was typically a single layer of hepatocytes around the central vein. Female mice treated with 90 mkd or greater had 1.5- to 2-fold increase in P4502E1 IR after 4 d and 3 wk of treatment. Male mice and female rats had 2- to 3-fold time- and dose-dependent increased P4502E1 IR. Male rats had 1.5- to 2-fold dose-dependent increased IR of P4502E1 at 4 d but not at 3 wk. The population of

hepatocytes with P4502E1 IR correlated well with hepatocytes that were degenerate. Chloroform induces its own metabolizing enzyme in mouse and rat livers in hepatocytes in which there is an hepatotoxic effect.

POSTER #11

Renal Toxicity of Bromodichloromethane (BDCM) and Bromoform (TBM) Administered Chronically to Rats and Mice in Drinking Water

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BDCM and TBM are drinking water chlorination byproducts which are carcinogenic in rats after chronic gavage in corn oil. Epidemiological evidence has linked consumption of chlorinated water containing trihalomethanes to urinary tract and lower gastrointestinal cancer in humans. In this study, male F344 rats were provided drinking water containing 0.25% Emulphor and either 0, 0.12, 0.6 and 1.2 g TBM/L or 0, 0.08, 0.4, 0.8 g BDCM/L for 52 wk. Male B6C3F$_1$ mice received water containing either 0, 0.08, 0.4, and 0.8 g TBM/L or 0, 0.06, 0.3 and 0.6 g BDCM/L. Urine was collected over 12 hr intervals and blood samples taken at sacrifice to measure indicators of renal toxicity. In rats, urinary protein concentrations were elevated (≈75%) above controls at all three TBM dose levels and at 0.08 and 0.4 g BDCM/L. Urinary AST was increased by 52 and 80% in the 0.6 and 1.2 g TBM/L groups. A slight elevation of urinary N-acetyl-ß-glucosaminidase (NAG) was noted in rats only at the highest TBM water concentration, but BDCM caused increases in urinary NAG (30-45%) in each rat dose group. In mice, urinary protein and alkaline phosphatase were increased at all three TBM water levels and at 0.6 g BDCM/L. Urinary NAG was elevated (≈75%) at 0.4 and 0.8 g TBM/L, and both NAG and ALT were increased (66 and 92%) at 0.6 g BDCM/L. Significant elevations of BUN were observed in all TBM mouse groups, but no BDCM groups. Mice therefore appeared more susceptible to the nephrotoxic effects of TBM than BDCM. These clinical data provide evidence indicating that chronic consumption of both BDCM and TBM in drinking water causes glomerular and proximal tubule damage in rats and mice.

POSTER #12

Differential Effects of Dichloracetate (DCA) and Trichloracetate (TCA) on Cell Replication in Initiated Cells, *In Vivo*

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DCA and TCA are major by-products of the chlorination of drinking water. Both compounds are capable of inducing hepatic tumors, but neither one appear to induce point mutations. Therefore, alternative mechanisms need to be explored for risk assessment purposes. TCA falls into the

peroxisome proliferator (PP) class of carcinogens. However, the carcinogenicity effects of DCA appear divorced from PP and more closely associated with changes in carbohydrate metabolism.

Male B6C3F1 mice were treated with 2.0 g/L their drinking water for 38 weeks (DCA) or 50 weeks (TCA). The animals were then randomly assigned to groups of 12 and provided 0, 0.02, 0.1, 0.5, and 2.0 g/L of the same haloacetate for two additional weeks. Livers were evaluated for apparent hyperplastic nodules (HN) or tumors which were sectioned into a minimum of six slices for histological examination. Normal livers were stained for PAS and BrdU, HNs were stained in serial sections for *c-Jun, c-Fos*, and BrdU. The normal tissue (NH) was examined for glycogen (-) poor areas that corresponded to altered hepatic foci (AHF) that had not been apparent upon gross examination. DCA was found to depress cell replication rates in NH at concentrations up to 0.5 gm/L, whereas TCA required higher doses. The replication rates of cells within AHF did not appear to be affected by DCA, but high doses selectively stimulated replication within c-Jun staining areas of larger nodules and tumors. There was no selective staining of *c-Jun* within TCA-induced lesions, although *c-Fos* levels were elevated. Replication rates within lesions induced by TCA were quite high compared to DCA-induced lesions and were apparently unaffected by continued treatment with TCA.

Thus, the net effect of DCA treatment was to provide selective advantage for c-Jun expressing cells within HN and tumors. While some selective advantage accrues to initiated cells within the liver of TCA-induced tumors by virtue of inhibited replication of normal cells, once well established the nodules and tumors produced by TCA do not appear to have a marked dependence upon continued treatment. We suggest that this reflects very different mechanisms in the genesis of the lesions induced by these two haloacetates. It seems clear that DCA is acting primarily, if not exclusively as a tumor promoter, whereas TCA appears to be affecting some earlier event, perhaps mediated through suppressed apoptosis, an effect associated with other peroxisome proliferators. (Supported by AWWARF/NWRI Contract 701-92/699-516-92)

POSTER #13

Melatonin Modulates Intercellular Communication Changes Caused by Perchloroethylene and Metabolites

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When chlorine is used to purify drinking water, it reacts with organic matter in raw water forming chloracetic acids and their corresponding chloroaldehydes. These by-products have been shown to affect intercellular communication (IC) with different efficiencies in cultured cells. Melatonin, a prominent naturally occurring hormone, is known to have oncostatic properties. We have recently reported that physiological concentrations of melatonin can enhance IC in 10T1/2 cells. We now ask if melatonin can modulate the inhibition of IC caused by these water purification by-products. Here we examined that potential using Clone 9 cell cultures (normal rat liver cells). In this study we selected concentrations of chemicals that had been previously shown to reduce IC in 24 hours by approximately

one third compared to controls. The chemicals and the concentrations chosen are: dichloroacetic acid (DCA) at 10 mM and 20 mM, trichloroacetic acid (TCA) at 2.5 mM, chloral hydrate (CH) at 5 mM, trichloroethanol (TCEth) at 5 mM, and perchloroethylene (PERC) at 0.1mM. Lucifer Yellow scrape-load dye transfer was used to assay for IC. The doses of melatonin used are in the physiological range: 1, 2.5, 5, 10, 20, 40 and 80 $x10^{-10}$ M. Melatonin had no effect on IC in Clone 9 cells not exposed to a chemical, nor when the cells were exposed to TCEth or PERC. Exposure to DCA at 10 mM and CH at 5 mM, caused an increase in IC at a melatonin concentration of 40 $x10^{-10}$ M, with a maximum increase at 20 $x10^{-10}$ M. Cells exposed to DCA at 20 mM demonstrated increased IC at melatonin concentrations of 10 and 20 $x10^{-10}$ M. IC in cells treated with TCA showed increased IC at melatonin concentrations of 2.5 $x10^{-10}$ and at 10 $x10^{-10}$ M with a maximum increase at 5 $x10^{-10}$ M. These results demonstrate for the first time that physiological concentrations of melatonin can modulate the inhibition of IC caused by some of the water purification by-products or metabolites. This modulation may be the basis for some of the oncostatic properties attributed to melatonin. Additional work is necessary to establish the mechanism for this process and to determine if it operates *in vivo*.

POSTER #14

Evaluation of the Neurotoxicity Produced by Dichloroacetic Acid in Rats

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Dichloroacetic acid (DCA) is a known neurotoxicant in rats and humans. We have characterized DCA neurotoxicity using a neurobehavioral screening battery under varying exposure durations (acute, subchronic, and chronic) and routes of administration (oral gavage and drinking water). Studies were conducted in both weanling and adult rats. Limited neuropathological evaluations, and comparisons between Long-Evans and Fischer-344 rats, were included in some studies. DCA produced neuromuscular toxicity comprised of limb weakness and deficits in gait and righting reflex. Other effects included chest clasping and ocular abnormalities. Neurotoxicity was permanent at high dose levels, whereas intermediate dose levels had slowly reversible effects. The severity, specificity, and recovery of the neurological changes were route-, age-, and strain-dependent. Neurotoxicity was evident at exposure levels as low as 16 mg/kg/day for three months. Preliminary neuropathological assessments indicated damage to the spinal cord but not peripheral nerves. The data from these studies reveal DCA effects to be more pronounced and long-lasting than has been previously assumed. More research is needed to fully assess the neurotoxic effects of DCA including the cellular and molecular sites of action.

POSTER #15

Absorption, Excretion, and Disposition of ^{14}C-MX in Mouse

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Although 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX) has been shown to induce point mutations in bacteria and chromosome damage in mammalian cells *in vitro*, it has given negative or weakly positive results *in vivo*. The fate of MX in mice was investigated by oral administration of ^{14}C-labelled MX. Plasma levels, urinary and fecal excretion and tissue distribution were studied over a series of time intervals from 0.25 hours (h) to 120 h. Absorption and excretion, primarily in urine was rapid and extensive. At 120 h, less than 1% was retained in the carcass. Peaks of activity were seen in kidney, bladder wall, and stomach wall after 0.25 and 1 h and in stomach wall after 24 h but no peaks were seen at 120 h. These findings were confirmed by whole body autoradiography. At 120 h, levels in plasma were lower than that in whole blood. The form of plasma binding *in vivo* was different to that *in vitro*.

POSTER #16

The Effect of Gavage Vehicle and Volume on Bromodichloromethane Toxicity*

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Bromodichloromethane (BDCM) ranks second in concentration only to chloroform (CHCl3) among known chlorination disinfection by-products in finished drinking water. CHCl3 toxicity is modulated greatly by gavage vehicle with administration of high dosages in corn oil producing greater hepatic and renal toxicity than aqueous administration. The volume of oil vehicle also modulates CHCl3 toxicity with substantially greater toxicity seen with the higher volume. Following oral exposure to BDCM in oil, greater hepatic damage resulted relative to administration in an aqueous vehicle (Lilly et al., 1994). However, the effect of vehicle on BDCM-mediated nephrotoxicity was dependent on the dosage of BDCM.

To study the effects of gavage vehicle and volume on BDCM toxicity, adult male Fisher-344 rats (n=6, ~70 days old) were gavaged with 0, 150, or 300 mg BDCM/kg in corn oil or 10% Alkamuls solution at a total gavage volume of either 2.5 or 10 ml/kg. Body weight was decreased significantly when 300 mg BDCM/kg was administered in oil in both low and high gavage volumes. At 48 hr, there were no significant elevations in serum hepatotoxicity indicators (aspartate aminotransferase, alanine aminotransferase, and sorbitol dehydrogenase) from administration of BDCM in the aqueous vehicle at

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either gavage volume. In oil, serum hepatotoxicity indicators were elevated significantly at 300 mg BDCM/kg in the high gavage volume. At 48 hr, there were no significant elevations of serum indicators of nephrotoxicity, blood urea nitrogen and creatinine. In summary, at these BDCM dose levels, the only evidence of hepatotoxicity was in the high volume oil administration. In conclusion, both gavage vehicle and volume affect BDCM hepatotoxicity.

POSTER #17

The Effect of Corn Oil on Hepatic Cytochrome P-450 2E1

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Health risk assessments of drinking water contaminants have typically been based on the results of toxicity studies in which the chemical is administered to the animal by oral gavage in a digestible oil vehicle such as corn oil (CO). There is increasing concern regarding the relevance of studies where CO was the gavage vehicle for risk assessments of chemicals found in drinking water. It has been demonstrated that both gavage vehicle and gavage volume affect the hepatotoxicity of chloroform and bromodichloromethane (a drinking water contaminant, second in concentration to chloroform among known trihalomethane disinfection by-products in finished drinking water).

To examine the potential influence of CO on metabolism of xenobiotics, adult male Fischer-344 rats (n=6, ~70 days old) were gavaged with 0, 2.5, 5 or 10 ml CO/kg. Hepatic CYP2E1 activity, measured as p-nitrophenol hydroxylase activity (nmol/mg microsomal protein/min), was determined 18 hr after the CO gavage. CYP2E1 activity was increased significantly at 5 (154% of control) and 10 (156% of control) ml CO/kg. At 10 ml CO/kg, relative liver weight, but not body weight or absolute liver weight, was decreased significantly. In an effort to separate the effect of a bolus gavage from the effect of CO itself, rats were gavaged either with 0, 2.5, 5 or 10 ml CO/kg or with 2.5 or 10 ml water (H_20)/kg. Statistically significant increases in CYP2E1 activity occurred again at 5 (150% of control) and 10 (261% of control) ml CO/kg whereas no significant increase was detected in rats gavaged with water. Feed consumption was decreased in rats receiving 10 ml CO/kg compared to the groups receiving either 0 ml CO/kg or 2.5 or 10 ml H_20/kg. In conclusion, CO gavage increased hepatic CYP2E1 activity, indicating that gavage with CO may affect study outcome for those chemicals whose toxicity is dependent on CYP2E1-mediated metabolic activation. (Funded by EPA/UNC-CH TA 901915.)

POSTER #18

Chlorinated Drinking Water and Risk of Bladder, Colon, and Rectal Cancers: A Case-control Study in Iowa

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A case-control study of incident cancers of the colon (N=685), rectum (N=655), and bladder (N=1452), with 2,434 population-based controls, was conducted in Iowa (USA) in 1986-89. Past exposure to chlorination by-products was estimated by combining residential history (including water source information) and fluid consumption data from a postal questionnaire, with historical water utility data and measures of drinking water contaminants. Logistic regression models were used to calculate odds ratios, adjusting for potential confounding due to other risk factors. **Rectal cancer** risk was associated with measures of exposure to chlorination by-products, with ORs of 1.1, 1.6, 1.6, and 2.6 (p (trend) <0.01) with duration of exposure to chlorinated surface water ($<=19$, 20-39, 40-59, and 60+ years), relative to never-exposed; and ORs of 1.3, 1.3, 1.5, 1.9, and 1.6 (p (trend) <0.005) for increasing milligram measures of lifetime trihalomethane intake. Associations were stronger among subjects with low fiber diets, or limited physical activity, with ORs of approximately 3 among highly exposed subjects, relative to those with the lowest exposures. In contrast, **colon cancer** was not associated with estimates of past chlorination by-product exposure. Risk of **bladder cancer** showed little overall association with exposure to chlorination by-products. However, among males and among smokers, bladder cancer risk increased with exposure duration (after careful control for cigarette smoking), with odds ratios among male smokers of 1.0, 1.2, 1.6, and 2.1 (p (trend) <0.01). This suggests that the effects of chlorination by-products upon bladder cancer risk may be enhanced by other endogenous or exogenous factors.

POSTER #19

Trihalomethane Development in Drinking Water in Function of Disinfectants

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Trihalomethanes pose a problem in Croatia only in the two water supply systems where gaseous chlorine is a disinfectant and which provide water to a population of 200,000. In these, trihalomethane levels of about 10 μg/L have been recorded. Currently, there are no reports of epidemiological or toxicological effects on Croatian water consumers due to trihalomethanes. To safeguard human health and to establish the most suitable disinfectant, a study looked at one of the two water supply systems.

Artificial lake water is used by the Butoniga water supply system, the largest World Bank water management investment in Croatia. Although its peak capacity is 2,000 l/s, being at the testing stage the system uses only 50 l/s water, retaining water in the test line for as long as 166 hours.

Both the presence of trihalomethanes and their development from chlorination with chlorine dioxide and chlorine gas preparations were meticulously examined. The dynamics of sample collection (in 3 turns at 7 points) took account of the water supply system's water flow rate. Trihalomethane levels were assessed using two gas chromatography methods, the extraction procedure and direct space head technique, in parallel.

Water was disinfected with 280 μg/L ClO_2. Whereas chlorite maximums recorded were 100μg/L, the monitoring did not include chlorates.

The maximum values found were: chloroform 8.902 μ/L , bromodichloromethane 12.962 μg/L, dibromochloromethane 4.271 μg/L, and bromoform 1.115 μg/L.

For showing trihalomethane levels above MAC (10 μg/L in each parameter), some samples present a human health hazard as defined in the 1994 Drinking Water Safety Regulation.

While no trihalomethanes were evolved by the chlorine dioxide-treated water, the chlorine gas-treated water evolved them. Their development is assumed to be due to a reaction of chlorine with the organic matter (mainly algal decomposition by-products) and, to a smaller extent, humin acides present in water.

The rise in trihalomethane levels was proportional to the duration of its retention in the water supply system, i.e., to the length of contact.

Based on all the above, and to minimize human health risks, we recommend that chlorine dioxide be used in water disinfection.

POSTER #20

Mechanism of Genotoxicity of Bromate

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Long-term oral administration of bromate has been shown to induce kidney tumours in F344 rats, by what is considered to be a genotoxic mechanism. However, it has been postulated that bromate causes DNA damage by an indirect mechanism and that risk may be significantly overestimated by using the linearised multistage model. We are currently studying the mechanism of carcinogenicity of bromate for the UK Water Industry (UK WIR). The results of work carried out to date provide evidence for the general lack of direct oxidation of DNA by potassium bromate ($KBrO_3$) both in isolated DNA and in cellular systems *in vitro*. Only minimal effects on 8-hydroxydeoxyguanosine (8-OHdG) formation were seen using isolated DNA (in the absence of added lipid) and only at the relatively high concentration of 15mM $KBrO_3$. These effects were exacerbated by the addition of iron. The preliminary data also indicated a lack of ability of $KBrO_3$ to elevate 8-OHdG in both human lymphocytes and in S. typhimurium strain TA102. Results from a Comet assay showed measurable DNA strand breakage (associated with 8-OHdG formation) in both mouse and rat kidney but not in the liver. These results

support the view that the genotoxic activity of potassium bromate is indirect and may be the result of DNA damage caused by active oxygen radicals generated during lipid peroxidation, a process which is likely to have a threshold.

POSTER #21

Mutation Spectra In Salmonella of MX and Organic Extracts of Chlorinated, Chloraminated, or Ozonated Drinking Water

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Chlorinated drinking water contains a complex mixture of organic mutagens, and it is of interest to determine the types of mutations induced by the whole mixture as well as by a representative mutagen within that mixture. Thus, drinking water samples were prepared by chlorination (Cl_2), chloramination (NH_2Cl), ozonation (O_3), or O_3 followed by Cl_2 or NH_2Cl; and the organics were extracted by XAD/ethyl acetate and evaluated for mutagenicity in Salmonella. The extracts were 2-8X more mutagenic in TA100 than in TA98, and the mutagenic potencies of the water extracts ranked similarly in both strains: $Cl_2 > O_3 + Cl_2 > NH_2Cl > O_3 + NH_2Cl > O_3 >$ raw. 3-Chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX) accounted for ~20% of the mutagenic activity of the chlorinated extracts. The mutations in ~2,000 revertants of TA98 and TA100 were analyzed by colony probe hybridization and PCR/DNA sequence analysis. The water extracts and MX produced similar mutation spectra, which consisted in TA100 of predominantly GC to TA transversions. This spectrum resembles that produced by large, aromatic compounds and is distinct from that produced by alkylating agents and the semi-volatile drinking water mutagen dichloroacetic acid. In TA98, MX and those water extracts resulting from the introduction of the chlorine atom produced 50-70% hotspot 2-base deletions and 30-50% complex frameshifts (frameshifts with an adjacent base substitution--mostly GC to TA transversions as found in TA100). No other compound or mixture is known to induce such high frequencies of complex frameshifts. These results suggest that MX and "MX-like" compounds (possibly halogenated aromatics) account for much of the mutagenic activity of the nonvolatile organics in drinking water and that these halogenated organics are particularly capable of promoting misincorporation by the DNA polymerase. This study provides further evidence that the mutation spectrum of a complex mixture reflects the dominance of one or a few classes of chemical mutagens with the mixture.

POSTER #22

Carcinogenicity and Mutagenicity of Four Trihalomethanes

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Expert committees advising the UK Department of Health on carcinogenicity and mutagenicity are considering the data on chloroform, bromodichloromethane (BDCM), dibromochloromethane (DBCM) and bromoform, in the light of the current UK regulatory limit for trihalomethanes in drinking-water, recent WHO guideline values, and a European Commission proposal for a revised Drinking Water Directive.

The committees concluded that there is no convincing evidence that chloroform is genotoxic. The carcinogenic effects in animals are associated with chronic tissue damage; therefore, a threshold would be expected at the no effect level (NEL) for sustained cell toxicity and repair in the target organs (kidneys and liver). The NEL could be used with a safety factor approach to set a regulatory standard.

In vitro data on the other three compounds indicate mutagenic potential, but well-conducted *in vivo* assays are essentially negative. The committees agreed that, provided negative results were to be obtained in an *in vivo* liver UDS assay for each compound, and a bone marrow micronucleus assay for bromoform, there would be adequate reassurance that these compounds did not have significant genotoxic activity *in vivo*. These studies have been performed at Huntingdon Research Centre for the Drinking Water Inspectorate and are negative (rat liver UDS assay, gavage doses up to: 450mg BDCM/kg, 2000mg DBCM/kg, 1080mg bromoform/kg; mouse bone marrow micronucleus assay, gavage doses up to 1000mg bromoform/kg).

POSTER #23

Developmental/Neurotoxicological Study of the Chlorite Ion

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When chlorine dioxide (ClO_2) is used to disinfect drinking water, it degrades primarily into chloride (Cl^-) and chlorite ions (ClO_2^-). Although the toxicity of chlorite has been known for many years, developmental/neurological studies conducted during the 1980s brought new potential concerns into focus. These studies do not meet current standards, however. In the absence of better data, the U.S. EPA proposed a maximum contaminant level goal (MCLG) for ClO_2^- of 0.08 mg/L based on this data. At this concentration, it would effectively eliminate the use of ClO_2 as a drinking water disinfectant. To resolved this concern, a comprehensive 2-generation reproduction/neurodevelopmental study was proposed and is currently underway. This state-of-the-art study will provide the U.S. EPA with developmental toxicity data in order to conduct a risk assessment and reevaluate the current MCLG.

In this study, rats will be exposed to either 0, 35, 75, or 300 ppm $NaClO_2$ in their drinking water for two generations. The parental animals (F_0) will be treated for 10 weeks prior to mating. They will be dosed through gestation, lactation, and weaning. The F_0 animals will then be sacrificed and subjected to a gross necropsy. Selected tissues will be retained for histopathology. The F_0 males will be evaluated for sperm motility. Reproductive parameters that will be measured/calculated include litter size, sex ratio, pup weights, and pup survival.

Pups (F_1) will undergo either developmental or neurological evaluations. Pups selected for developmental evaluations will be observed for day of eye and ear opening, righting reflex, startle response, sexual development, etc. They will be exposed to test material at the same concentration as their F_0 parents after weaning, and will then be bred to produce the F_2 generation. The other pups will undergo neurotoxicological evaluations. Half of this group will be tested for auditory function, or learning and memory, followed by a function observational battery (FOB). The FOB includes home cage observations, reactivity to stimulus, open field observations, righting and tactile responses, etc. The other half of this group will be sacrificed for neuropathological evaluation of assessed for motor activity in specially designed chambers followed by sacrifice for neuropathological evaluation.

POSTER #24

The Association Between Volatile Organic Chemicals and Birth Defects in Metropolitan Atlanta - A Proposed Study

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A number of recent epidemiologic studies have suggested an association between several types of birth defects and various water contaminants, including disinfection by-products and solvents. Scientists within the Centers for Disease Control and Prevention's National Center for Environmental Health propose to evaluate the impact of volatile organic chemicals (VOCs) (including disinfection by-products and solvents) in drinking water on the occurrence of birth defects in metropolitan Atlanta using the Birth Defects Risk Factor Surveillance Project (BDRFS). BDRFS, which began in 1993, is an additional component to the Metropolitan Atlanta Congenital Defects Program, a population-based surveillance registry. BDRFS is an ongoing case-control study, with interviews of mothers of 300 infants with structural malformations and 100 controls per year. In addition to maternal interviews, BDRFS includes biologic sampling of mother-infant pairs with the capacity for laboratory evaluation of biologic markers of exposure and susceptibility. BDRFS provides an excellent, cost efficient opportunity to further the scientific understanding of this relationship between birth defects and VOCs. We propose to obtain drinking water samples from case and control homes over the next two years. Blood samples (which are already being obtained) would also be analyzed for VOCs.

POSTER #25

Occurrence and Comparison of Disinfection By-products in Population-based Tapwater Samples

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As part of a statewide population-based epidemiologic study of certain birth defects, disinfection by-products have been sampled in homes of New Jersey residents during 1994 and 1995. Over 160 homes utilizing surface water sources have been sampled and analyzed for haloacetic acids (HAAs) and haloacetonitriles in addition to trihalomethanes (THMs) using Methods 551 and 552 of the United States Environmental Protection Agency. Public drinking water monitoring data for THMs pertaining to the specific locations and tap water sampling dates have also been retrieved.

The tapwater analyses results indicate that total HAAs frequently exceeded total THMs in our population. THM concentrations in tap water samples collected in homes closely resembled those retrieved from monitoring data submitted by water utilities to state authorities for the same locale and timeframe. Our observations indicate widespread occurrence of high concentrations of haloacetic acids and the importance of specific assays of haloacetic acids in drinking water in order to estimate population exposures to these by-products.

POSTER #26

Biomarkers of Exposure to Chlorination By-products

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Multi-route exposures to chlorination by-products (CBPs) from residential tap water result in increases in their concentrations within the body and subsequently in bodily fluids. In support of a case control study of the relationship between birth defects and water contaminant levels in N.J., trihalomethanes (THMs) were measured in breath during home visits and at the end of showers and haloacetic acids were measured in first morning urine. The biomarker determinations were made along with a questionnaire being administered and tap water concentration measurements in order to compare different approaches to estimating exposure to CBPs. Little elevation in the background breath concentrations of THMs is being identified for the participants using chlorinated water containing THM levels above 25μg/L compared to a control group, while the breath THM concentrations at the end of a shower are much higher for that group. This relationship is consistent with the relatively short biological half life of these compounds in the body following environmental exposures. Urinary excretion rates of trichloroacetic acid, and to a smaller extent dichloracetic acid, have a strong dependence on the water concentration of the haloacetic acids, indicating water use is a major exposure route for these compounds. Water concentration and differences in water uses, as reported by the

subjects, are being compared to the levels of CBPs measured in breath and urine to establish the uncertainty in the exposure estimate using only water concentration.

POSTER #27

In Vitro Dermal Absorption of Bromodichloromethane (BDCM) and Chloroform ($ChCl_3$) in the Rat

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Exposure to disinfection by-products (DBPs), such as BDCM and $CHCl_3$, can occur during bathing, showering and swimming. Thus one potential route of entry of DBPs is through the skin. The objective of this study was to examine the *in vitro* dermal absorption of BDCM and $CHCl_3$. Female F344 rats (ca. 90 days) were euthanized and their pre-clipped dorsal skin was removed. Split-thickness skin (250 μm thick) was prepared with a dermatome. The skin was then mounted onto teflon cells of a flow-through diffusion system. Media (containing 10% fetal bovine serum) was pumped below the skin at a rate of 3 ml/hr. Skin integrity was tested and the skin was not used if >0.045% of a dose of [3H]-H2O penetrated it in 1 hr. The cells were then capped and [14C]-BDCM (95, 190 and 380 μg) or [14C]-$CHCl_3$ (231, 462 and 921 μg) in 250 μl water were applied onto the skin. Fractions of media were collected over a 4 hr period. Recovery of non-volatilized radioactivity ranged from 30-50% of the dose for both compounds. Cumulative BDCM absorption into the media for the 3 doses, based on % of the applied dose, ranged from 8-20%; skin wash (methanol) accounted for 27-28%; digested skin was 1-2%. Cumulative $CHCl_3$ absorption ranged from 9-14% of the dose; skin wash was 19-22%; digested skin was 2%. Maximal penetration rates for both compounds ranged from 4-9% of the dose/hr, and occurred by 1 hr post-exposure. Thus dermal exposure, at least *in vitro* in the rat, represents an absorption pathway for BDCM and $CHCl_3$.

POSTER #28

Alteration of Metabolism of Dichloroacetate (DCA) with Prolonged Treatment with DCA in Drinking Water

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DCA is an important by-product of the chlorination of drinking water that induces hepatic tumors in both rats and mice and has been shown to possess neurotoxic, developmental, and reproductive effects. Clinical investigation of DCA's clearance from blood of humans has shown that the half-life of DCA was progressively increased with sequential doses (Curry et al., *Clin Pharmacol. Ther.* 37:89-93,

1995). Such dramatic changes are most frequently associated with alterations in metabolism and can have important implications for chronic toxicity.

The present study has sought to determine whether pretreatment of male F344 rats and B6C3F1 mice with either 0.2 or 2 g DCA/L of drinking water for 14 days would significantly modify the metabolism of doses of ^{14}C-DCA administered on the 15th day (i.v. in rats, p.o. in mice). Pretreatment of rats at both 0.2 and 2 g/L sharply inhibits the conversion of (UL-^{14}C)-DCA to $^{14}CO_2$. For example, the initial rate of conversion was reduced from approximately 11.6 ± 3.7 mg. eq. DCA kg^{-1} h^{-1} to 0.86 ± 0.18 using a 100 mg/kg dose following the 2 g/L pretreatment schedule. At this same dose, the terminal half-life of DCA in blood was increased from 2.5 to 11 h and the urinary excretion of unchanged DCA was increased 12-fold. Monobasic acid metabolites of DCA in the urine were also increased; MCA (3-fold), glyoxylate (2.5-fold), glycolate (8.4-fold), and acetate (2.2-fold). However, oxalate excretion was essentially unchanged. The effect on DCA metabolism was observed with all doses utilized (as low as 5 mg/kg). Metabolism of DCA in mice was essentially unaffected by pretreatment with 2 g/L with a dose of 100 mg/kg, but some minor modifications were observed when the dose was increased to 1000 mg/kg.

These data demonstrate that there are very substantial interspecies differences in the metabolism and pharmacokinetics of DCA as treatment periods are increased. Therefore, it will be necessary to determine whether DCA or a metabolite is responsible for a specific toxicological effect before one can determine whether there are intrinsic species differences in the sensitivity to this chemical. Moreover, it is possible that DCA can affect the metabolism of other disinfectant by-products, an important consideration for studying the overall risks associated with disinfectant by-products. (This research was supported by AWWARF/NWRI Contract 701-92/699-516-92)

POSTER #29

Assessment of the *In Vivo* Metabolic Interaction of Chloroform (CHCL3) and Trichloroethylene (TCE) in Rats by Closed Chamber Methods*

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Concurrent oral exposure of rats to CHCl3 and TCE results in reduced hepatic and renal toxicity relative to CHCl3 alone (Lilly et al., 1992). The present study investigated the *in vivo* metabolic interaction of CHCl3 and TCE by closed chamber methods. Male F-344 rats (~70 days old) were exposed individually (n=3) to 4 initial concentrations of CHCl3 or TCE of approximately 100, 500, 1000 or 3000 ppm. The uptake curves were examined with a physiologically-based pharmacokinetic (PBPK) inhalation model. Both TCE and CHCl3 metabolism were modeled successfully by a single saturable pathway. The allometrically scaled maximum velocities (V_{maxc}) for CHCl3 and TCE were

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estimated as 4.1 and 4.4 mg/hr/kg, respectively. Sensitivity analysis indicated that 500 ppm of either TCE or CHCl3 was an optimal concentration for estimation of V_{maxc}. Rats were then exposed to the following initial concentrations of the binary combinations: 500 ppm CHCl3 with 10, 500 or 2000 ppm TCE; and, 500 ppm TCE with 10, 500 or 2000 ppm CHCl3. The uptake curves of TCE and CHCl3 during combined exposures were examined by a PBPK model that included an equation to accommodate 3 different mechanisms of inhibition: competitive, noncompetitive, or uncompetitive. The metabolic interactions of TCE and CHCl3 were best described as competitive. TCE competitively inhibited CHCl3 metabolism; similarly, CHCl3 competitively inhibited TCE metabolism. Inhibition of CHCl3 metabolism by TCE in rats may contribute to the antagonism of CHCl3 toxicity by TCE. (Funded by EPA/UNC-CH TA901915.)

POSTER #30

Development of a PBPK Model for Chloral Hydrate Metabolism in Humans and Rats

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Chloral Hydrate (CH) is a by-product of drinking water chlorination, a short-lived intermediate metabolite of trichloroethylene (TRI), as well as being an approved sedative/hypnotic drug. A study was undertaken to gather metabolism data on the disposition of CH that can be used in the development of PBPK models for several disinfectant by-products that are its metabolites and TRI.

CH and two of its metabolites, trichloroacetate (TCA) and dichloroacetate (DCA), have been shown to be hepatocarcinogenic in mice. However, we choose to study the rat for ease of documenting the influence of enterohepatic circulation and trichloroethanol-glucuronide (TCE-gluc) on the kinetics of trichloroethanol (TCE), TCA, and DCA.

CH was administered to both humans and rats to provide physiological explanation for the prolongation of the half-life of TCA when it appears as a metabolite of CH or TRI in humans as well as other species. Human volunteers were administered either 500 mg or 1500 mg of CH, the blood and urine sampled over a 7 day period, and the specimens analyzed for CH and its metabolites, TCE, TCE-gluc, DCA, and TCA. Rats were infused with CH at dose levels varying from 12 to 200 mg/kg and the bile, blood, urine and feces collected and analyzed. Approximately 30% of TCA in the blood of rats is attributable to enterohepatic circulation (EHC) of TCE-gluc.

Therefore, it appears reasonable to conclude that the EHC is acting as a reservoir for TCE until it can be converted to TCA and removed from the cycle. This explains the extended half-life and persistence of TCA in the blood when it is derived from either CH or TRI in both humans and rats and probably other species. PBPK models are being developed for both humans and rats for CH as well as TRI. (This research supported by EPA # CR 822577).

POSTER #31

A Biologically Based Model of Bromodichloromethane (BDCM) Toxicity: Developing a Pharmacokinetic Model

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BDCM, a by-product of water chlorination, is carcinogenic to rats and mice. We are developing a biologically based dose-response model to support a cancer risk assessment including a pharmacokinetic model of the oral uptake and metabolism of BDCM administered in various dosing vehicles. The pharmacokinetic model discussed in this paper consists of six compartments (liver, kidney, fat, slowly and richly perfused tissues and gut). Liver metabolic rate constants of 0.5 (K_m; mg/l) and 13.0 (*Vmaxc*; mg/hr/kg) were estimated from plasma bromide ion data collected after constant concentration 4-hour inhalation exposures of 100-3200 ppm BDCM. The gut compartment consists of 3 to 5 sequential subcompartments with each subcompartment having an absorption constant (KA; 1/hr), a bioavailability term (A; unitless), and time of compartment emptying (T; hrs). Blood and exhaled breath BDCM concentrations from 90d-old male F-344 rats gavaged with 50 or 100 mg BDCM/kg in corn oil or 10% Emulphor® were obtained to calibrate the model. Simulations of the complex, multi-peak blood concentration-time profiles after corn oil gavage resulted in KA, A, and T values for the subcompartments ranging, respectively, from 0.4-1.4, 0.3-1.0, and 0.45-6.0. Aqueous BDCM gavage was described with a single GI tract subcompartment and KA values ranging from 0.45-1.4 with A=1.0. Levels of plasma bromide ion were also described using this model, providing greater confidence in the accuracy of previously determined values for BDCM metabolic rate constants. This pharmacokinetic model, together with tissue activities of activating enzymes, will permit estimation of tissue exposure to BDCM and its metabolites for various oral dosing scenarios used in the bioassay studies and for anticipated human exposures.

POSTER #32

Risk Assessments for Airborne Chloroform Based on Inhalation Studies

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Chloroform-induced cytotoxicity and cell regeneration were characterized in male and female $B6C3F_1$ mice and F-344 rats exposed 6 hr/d for 7 or 5 d/wk to chloroform atmospheres of 0, 0.3, 2, 10, 30, or 90 ppm (mice) or 0, 2, 10, 30, 90, or 300 ppm (rats) for up to 13 wk. The labeling index (LI), percentage of cells in S-phase, was quantified with bromodeoxyuridine immunohistochemistry. Results for *MICE* - *Liver*: concentration-dependent histologic changes and increased LI of 14-20 fold over control (FOC) in both sexes for the 7 d/wk groups and 6-FOC for the 5/wk groups. *Kidney*: histologic

changes in male mice with an increased LI in the cortex of 2-FOC with 7 d/wk and 3.5-FOC with 5 d/wk 90 ppm 13-wk exposures. Results for *RATS* - *Kidney*: a concentration-dependent histopathology and increased LI in both sexes with the LI in the cortex 5- to 6-FOC in 90 ppm and 10-FOC in 300 ppm 7 d/wk 13-wk exposures. In the 5 d/wk exposed rats, the LI was lower in males and unchanged in females. *Liver*: hepatocyte toxicity and regeneration observed only with 300 ppm exposures. Body weight changes indicate 90 ppm as a MTD for long-term inhalation studies in both species. To estimate human risk to airborne chloroform, the EPA uses the linearized multistage model applied to mouse liver data from a gavage bioassay to estimate a 10^{-6} risk level or virtually safe dose (VSD) of 0.000008 ppm. Because chloroform appears to act through a nongenotoxic-cytotoxic mode of carcinogenesis, an alternative risk assessment can be based on noncytotoxic doses. In the present study, the NOEL for cell proliferation in the female mouse liver and male rat kidney was 10 ppm. Applying an uncertainty factor of 1000 yields a VSD of 0.01 ppm. This estimate relies on inhalation data and is more consistent with the mode of action of chloroform-induced carcinogenesis.

POSTER #33

A Computer Support System for Policy Decisions on Disinfection By-products

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Decisions on the selection of treatment strategies consistent with regulatory goals require information on input water quality, alternative treatment options, organization of options into treatment trains, concentrations of DBPs after treatment, concentrations of micro-organisms after treatment, numbers of health effects produced by DBPs with and without treatment, numbers of health effects from micro-organisms with and without treatment, weighting of cancer and non-cancer health endpoints, cost of treatment trains, demographic characteristics of exposures, and uncertainty in all of these factors. Based on this information, it is possible to rank treatment trains with respect to cost, health effects caused by DBPs, health effects averted by disinfection, and the confidence with which the goal of protecting public health at reasonable cost is met. The Institute for Environmental Studies is developing a computer-based Decision Support System in which the factors listed above are visualized easily using advanced graphical packages. The system draws on formal tools of uncertainty and sensitivity analysis to determine the confidence with which specific policy goals are reached by different treatment trains and to determine where major sources of uncertainty lie. This analysis may be used to identify the impact of improved knowledge from different areas of research (e.g. treatment chemistry, sampling of supplies, interspecies extrapolation, microbial inactivation kinetics) on the uncertainty for selecting treatment strategies, allowing the rational allocation of research resources. It also may be used in environmental negotiations between parties affected by regulations, providing visually-enhanced access to complex scientific, engineering, and economic information.

POSTER #34

The Performance of Disinfection and By-products of Selected Disinfectants

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Introduction

Recently in Japan, there have been three impacts on drinking water quality management: The introduction of risk assessment and risk management, revision of drinking water quality standard, and the application of advanced water treatment technologies. Under these conditions, a research committee on alternative disinfection technologies was set up in 1989, and a wide range of studies has been carried out. A pilot plant composed from five disinfection processes for a comparative study of alternate disinfectants was manufactured. This study paper summarized the results of the comparative experiments.

Experiments

Five kinds of disinfectants were examined, including chloramine and free chlorine which are already authorized to apply in drinking water production in Japan; chlorine dioxide (ClO_2), ozone, and ultraviolet rays (UV) that may be applicable as alternative disinfectants. The inactivation effects, bacterial regrowths, mutagenicities, disinfection by-products (DBPs), and deodorizing effects of 2-methylisoborneol (2-MIB) were compared with these five disinfectants.

Mutagenicities (Ames Assay) were examined in two kinds of water, i.e., the finished water from conventional treatment system (FWC) and the finished water from advanced treatment system (FWA).

The Ames Assay was implemented using Salmonella typhimurium TA98 with or without S9.

Results and discussions

1) Bacterial regrowths

Chloramine showed the lowest inactivation rate on both general bacteria and heterotrophic bacteria, the regrowths easily occurred. Under the conditions of pH7.5, ClO_2 revealed higher inactivation effect than of free chlorine.

For ozonation, under the dose rate of 1.0mg/l or more, inactivation was completely done in about 10 minutes for both the FWC and the FWA. Conversely, for the BAC treated water (including turbidity particles), it was not completely inactivated even if ozone was applied at the rate of 1.5mg/l. After the disappearance of residual ozone, bacterial regrowth would likely occur.

For UV radiation, even though the inactivation effect is high, the regrowth occurred in 24 to 48 hours afterwards.

In characteristic comparison of UV and ozone disinfection, the lower regrowth rate was observed in the ozonated sample probably due to the difference in the inactivation mechanisms.

Higher inactivation rate and lower regrowth potential of each disinfectant were proved in the FWA, as compared with the FWC.

For chloramine and free chlorine, higher inactivation effect was confirmed at lower pH value.

For ozone and UV, the inactivation rates were slightly higher at pH 6.0. Conversely, for ClO_2, there is slight difference in disinfecting effects in the range of pH 6.0 to 8.0.

2) DBPs

The production of trihalomethanes (THMs) was almost zero except for the free chlorine disinfection. Although a concentration of chloroform was low in the FWA, a shift to brominated THMs was observed during chlorination.

Chloral hydrate (CH), dichloroacetonitrile (DCAN), dichloroacetic acid (DCAA), and trichloroacetic acid (TCAA) were formed slightly in the case that FWC was disinfected with free chlorine. With the FWA, however, none of them were formed during any disinfection.

Formaldehyde (FALD) was formed during ozonation of the FWC. For the FWA, no FALD was detected even in the case of ozone application because FALD precursor had been removed in advance of disinfection.

3) Combined disinfection

For the disinfection by combination of ozone and chloramine, almost an hour was needed for inactivation of heterotrophic bacteria; though, complete inactivation (more than 99.99% inactivation) in a short time after the injection of post-disinfectants except chloramine was observed. Moreover, no regrowths were proved even if disinfectants had been dissipated for all post-disinfectants.

4) Deodorization

It was found that ozone had the most effective to degrade the odorous compound such as 2-MIB produced by algae. In case of ClO_2 and UV, it was confirmed that deodorizing effects increased in accordance with an increase of their doses.

5) Mutagenicities

Among the five disinfection methods examined with the conventional water, chloramine, free chlorine, and ClO2 were on the trend to develop the direct mutagenicity (will present Table of results at the workshop.) All methods including ozone and UV, however, did not show any

increase of the indirect mutagenicities. The free chlorine disinfection proved the most conspicuous tendency to develop the direct mutagenicity and to reduce the indirect mutagenicity. The ozone disinfection decreased the both mutagenicities. The UV radiation hardly influenced the mutagenicities.

Any mutagenicities were not observed in the FWA before and after disinfection. Therefore, it was considered that development of mutagenicities was also related with the organic properties of the waters which would be disinfected.

Conclusions

A plenty of information was obtained from the field study for five kinds of disinfectants using the pilot plant connected with the full-scale drinking water treatment plant. The results show that single application of disinfectant has a limitation in the total quality management of finished drinking water at the tap.

Therefore, the combined methods (hybrid disinfection) of ozone or UV for inactivating microorganisms in the raw water (primary disinfection) followed by free chlorine, chloramine, or ClO_2 for maintaining disinfecting effects in a water distribution system (post-disinfection), is considered to be an excellent technology from various viewpoints.

POSTER #35

An Evaluation of Methods for Predicting Total Trihalomethane Concentration in Finished Drinking Water

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The objective of this study was to examine the use of two different models for the prediction of trihalomethane (THM) concentration in chlorinated drinking water. The goal of this research was to improve the information used in exposure assessment for estimating health effects. An intensive sampling program involved the collection of THM samples for eight months in Chapel Hill, N.C. In addition, utilities throughout North Carolina were surveyed to obtain historical data on THM compliance monitoring for the period from 1990 to 1994.

The first method of predicting THM concentrations is based on the consumption of chlorine between the treatment facility and the consumer's tap. Utilizing information collected for the treatment plant's chlorine dose as well as the residual at the point of sampling, THM formation can be correlated to chlorine consumption. For the Orange Water And Sewer Authority (OWASA), a regression of the data yielded an r^2 value of 0.72. Currently, this model is being used to examine similar information collected from other N.C. utilities.

The second model utilizes the EPA's Water Treatment Plant Simulation Program (WTP) as well as the EPANET hydraulic simulation model to predict THM concentration in the OWASA distribution system. These results were compared with measured THM levels from the monthly samples. The WTP model was found to consistently underpredict THM concentration, but with an empirical correction to account for this variation, the model gave reasonably good predictions.

POSTER #36

Predicting Trihalomethane Exposure in Drinking Water Distribution Systems

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This paper reports on an ongoing study related to drinking water epidemiology and disinfection by-products. The goal of this study is to develop an algorithm for predicting trihalomethane (THM) concentrations in drinking water using the census block group (average population 1000) as the unit of analysis.

A computer simulation model (EPANET) has been applied to the Northglenn, Colorado water district (service population 28,000). The district uses conventional treatment processes, including disinfection by chlorination, and has finished water total trihalomethane concentrations (TTHM) ranging from 20 to 70 micrograms per liter (ug/l). EPANET is a dynamic model, which predicts hydraulic performance and chlorine decay at about 150 locations (model nodes) within the water distribution system. The district is composed of all or part of 24 census block groups that were used in the US Census Bureau 1990 decennial census. At least one model node is located in each of these units. Water quality and pressure monitoring was conducted in the distribution system in order to calibrate and validate the model, and to determine the correlation between chlorine residuals and other parameters, and THM concentrations in the system. A high degree of association (r^2 up to 0.83) between chlorine residual and concentration of TTHM, with strong seasonal trends, has been found thus far. A resultant algorithm is being developed to predict risk to THM exposure for each census block group. The results have been incorporated into a geographic information system (GIS) for use in a reproductive epidemiology study.

POSTER #37

Analysis of Polar Carbonyls Using Capillary Electrophoresis/Electrospray Mass Spectrometry

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The discovery of toxicologically worrisome chemicals in water used for consumption has relied heavily on advances in analytical chemistry. We must thus recognize the interplay between the emergence of significant environmental health issues and the development of technologies to cope with these issues. A modern issue is the identification of polar and potentially hazardous multi-functional carbonyls that may account for the unidentified mutagenicity in drinking waters.

We have conducted a pilot study that establishes the promise of Girard's Reagent P (1-(carboxymethyl) pyridinium chloride hydrazide) derivatization in combination with capillary electrophoresis/electrospray mass spectrometry (CE/ES/MS) for the identification of polar carbonyls. This approach is more suitable for the analysis of such carbonyls than 2,4-dinitrophenylhydrazine (DNPH) or O-(2,3,4,5,6-pentafluorobenzyl) hydroxylamine (PFBHA). DNPH has a propensity to form osazones from α carbonyls and hydroxy- and carboxyl- carbonyls are difficult to analyze by using PFBHA. The charged nature of the derivatives enables sensitive detection of Girard's Reagent P derivatives and acquisition of molecular weight information by using ES/MS. Optimum derivatization conditions for representative multi-functional carbonyls were established, an affinity column clean-up procedure was developed to remove the excess reagent, and we have established capillary electrophoretic separations of a complex mixture of carbonyls.

POSTER #38

Bromate Ion Formation Resulting from Bromine Treatment

Bernard Bubnis[1], Gilbert Gordon[2], Al Yeoman[3], and Bill Beaumann[4]
[1]Novatek, Oxford, OH, [2]Miami University, Oxford, OH, [3]BioLab, Decatur, GA, [4]Everpure, Inc., Westmont, IL

The U.S. EPA has raised concerns about the potential for a bromine product currently used by the US Navy to form bromate ion. The agency has required that the potential for bromate ion formation and exposure during shipboard use be evaluated for the polybrominated trimethylbenzylammonium anion exchange resins currently used to disinfect potable water aboard ship.

The objective of this study was to measure bromate ion formation resulting from the use of free available bromine (FAB) under a set of conditions that would encompass the conditions expected aboard ship while in service.

The conditions chosen were conservative and provide not only the normal situation but also extreme situations that are not likely to be encountered.

Temperature	20°C
pH	4, 5, 6, 7, and 7.5 (buffered)
Concentration	0.5, 2, and 5 mg/L FAB
BrO_3^- Measurement	Ion chromatography (Method 300)
Water Type	Doubly distilled/deionized and reverse osmosis (RO)

Studies were performed in doubly distilled and RO waters at pH 4, 5, 6, and 7.5 in 0.001 M acetate or phosphate buffer with 0.5, 2, and 5 mg/L FAB as bromine added. BrO_3^- was not detected under any pH or mg/L FAB conditions.

The results of holding studies when free available bromine (FAB) was added to doubly distilled deionized and RO waters in concentrations ranging from 0.5 to 5 mg/L FAB at 20°C at pH 4, 5, 6, 7, and 7.5 indicate that **bromate ion is not formed under conditions expected aboard naval ships using bromine resin for water disinfection.**

POSTER #39

Bromodichloromethane Inhalation Selectively Inhibits Cytochrome P450 Metabolism in Rat Liver

John W. Allis, Barbara L. Brown[1], and Rex A. Pegram[1]
National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, NC

During the development of a physiologically-based pharmacokinetic model for bromodichloromethane (BDCM), a by-product of water chlorination, it became evident that the inclusion of multiple metabolic pathways resulted in better fits to the data from either gas-uptake experiments or measurement of bromide ion concentration in the blood. The data also suggested that BDCM might be inhibiting one of these pathways. Two isoenzyme of the cytochrome P450 (CYP) family have been implicated as important metabolizers of trihalomethanes in rat liver, CYP 2E1 and CYP 2B1, with the former thought to be the more important. To address the possibility that BDCM is an *in vivo* characteristic of three isozymes after exposing rats to BDCM exposures in humans.

Naive male 90-day old F344 rats were exposed to constant concentrations of 0, 100, 200, 400, 800, 1600 or 3200 ppm BDCM for four hours and were sacrificed immediately afterwards. Liver S-9 fractions were prepared and frozen in small aliquots. Microsomes were subsequently prepared for measurement of total cytochrome P450 and the catalytic activities of p-nitrophenol hydroxlase (PNP), ethoxyresorufin-O-deethylase (EROD) and pentoxyresorufin-O-dealkylase (PROD).

CYP 2E1 metabolizes many small organic chemicals and is important in the metabolism of BDCM. PNP activity, a marker for this isozyme, was not affected by BDCM exposure at any level measured. However, PROD, a marker for CYP 2B1, was decreased by about 40% at the highest exposure. This

isozyme also often contributes to metabolism of low molecular weight compounds. In addition, we found a decrease of up to 30% in EROD activity, a marker for CYP 1A1, which was surprising in that CYP 1A1 is not believed to metabolize BDCM. EROD activity has been found previously to decrease in rats gavaged with BDCM (Thornton-Manning et al.,) Toxicology 94:3, 1994), but the basis for this sensitivity is not understood. No change in total hepatic cytochrome P450 content was measurable. These results may provide insight into the mechanism of BDCM metabolism, suggesting that CYP 2B1 may be responsible for creating a highly reactive species.

PROGRAM
AND
PARTICIPANT LIST

Workshop on

Disinfection By-products in Drinking Water: Critical Issues in Health Effects Research

October 23-25, 1995
The Carolina Inn
Chapel Hill, North Carolina

PROGRAM

MONDAY, OCTOBER 23

8:00 - 8:30 **Registration and Coffee**

8:30 - 8:45 **Welcome and Introductions**
Dr. Denise Robinson, ILSI Health and Environmental Sciences Institute (HESI)
Dr. Fred Hauchman, U.S. EPA National Health and Environmental Effects Research Laboratory
Mr. Stuart Krasner, Metropolitan Water District of Southern California

SESSION 1: DISINFECTION BY-PRODUCTS: CRITICAL ISSUES
Chair: Dr. Denise Robinson, ILSI Health and Environmental Sciences Institute (HESI)

8:45 - 9:00 **Research Needs: Regulatory Perspective**
Mr. Stig Regli, Office of Ground Water and Drinking Water, U.S. EPA

9:00 - 9:30 **Disinfection By-products: From Source to Tap**
Dr. Philip Singer, University of North Carolina

9:30 - 10:00 **An Integrated View of Toxicology and Epidemiology**
Dr. David Hoel, Medical University of South Carolina

10:00 - 10:20 **Break**

10:20 - 10:50 **Assessing the Health Risks of Drinking Water**
Dr. Daniel Krewski, Environmental Health Directorate, Health Canada

SESSION 2: CHLORINATION
Chair: Ms. Jennifer Orme-Zavaleta/Mr. Michael Cox, Office of Water, U.S. EPA

10:50 - 11:05 **Introduction**
Mr. Michael Cox

11:05 - 11:35 **Influence of Water Quality on Formation of Chlorination By-products**
Ms. Patricia Synder Fair, Office of Water, U.S. EPA

11:35 - 12:05 **Recent Reproductive Effects Associated with Disinfection By-products**
Dr. Gary Klinefelter, National Health and Environmental Effects Research Laboratory, U.S. EPA

12:05 - 1:35 **Lunch** (Posters available for viewing from 1:05 to 1:35)

1:35 - 2:05 **Effects of Haloacetic Acid Water Disinfection By-products on Embryonic Development**
Dr. Sid Hunter, National Health and Environmental Effects Research Laboratory, U.S. EPA

2:05 - 2:35 **Assessing the Cancer Risk of Chloroform**
Dr. Byron Butterworth, Chemical Industry Institute of Toxicology

2:35 - 3:05 **The Carcinogenicity of Disinfection By-products in Drinking Water: Results from the Water Carcinogenesis Research Program at the U.S. Environmental Protection Agency's National Health and Environmental Effects Research Laboratory**
Dr. Anthony DeAngelo, National Health and Environmental Effects Research Laboratory, U.S. EPA

3:05 - 3:35 **Carcinogenic Properties of Brominated Haloacetates**
Dr. Richard Bull, Battelle Pacific Northwest Laboratory

3:35 - 4:00 **Break**

4:00 - 4:30 **Genotoxicity and Carcinogenicity of MX and Other Chlorinated Furanones**
Dr. Jouko Tuomisto, National Public Health Institute, Finland

4:30 - 5:30 **Panel Discussion**

4:30 - 4:45 **Session Summary** - *Mr. Michael Cox*
4:45 - 5:30 **Discussion**

6:30 - 8:00 **Poster Session and Reception**
Presenters with even numbered posters will be available at their poster to answer questions from 6:30 to 7:15 and presenters with odd numbered posters will be available from 7:15 to 8:00. This arrangement will provide optimal conditions for both the participants and presenters to view and discuss the posters and enjoy the reception.

TUESDAY, OCTOBER 24

8:00 - 8:30 **Registration and Coffee**

SESSION 3: ALTERNATIVE DISINFECTANTS
Chair: Dr. Fred Hauchman, National Health and Environmental Effects Research Laboratory, U.S. EPA

8:30 - 8:45 **Introduction**
Dr. Fred Hauchman

8:45 - 9:15 **Overview of Alternative Disinfectants**
Dr. David A. Reckhow, University of Massachusetts at Amherst

9:15 - 9:45 **Toxicity and Risk of Bromate**
Mr. John Fawell, Water Research Centre

9:45 - 10:10 **Long-term Carcinogenicity Bioassays on Formaldehyde and Acetaldehyde Administered in Drinking Water: Results and Implications**
Dr. Morando Soffritti, Institute of Oncology, Italy

10:15 - 10:45 **Update on Chlorite Toxicology Studies**
Dr. Robert Romano, Chemical Manufacturers Association

10:45 - 11:15 **Break**

11:15 - 11:45 **The Need for Understanding Mechanisms When Using Data from Rodent Nasal Toxicology Studies to Develop Drinking Water Standards**
Dr. Kevin Morgan, Chemical Industry Institute of Toxicology

11:45 - 12:30 **Panel Discussion**

11:45 - 12:00 **Session Summary** - *Dr. Fred Hauchman*
12:00 - 12:30 **Discussion**

12:30 - 2:00 **Lunch** (Posters available for viewing from 1:30 to 2:00)

SESSION 4: BIOLOGICALLY BASED RISK ASSESSMENT
Session Chair: Dr. Linda Birnbaum, National Health and Environmental Effects Research Laboratory, U.S. EPA

2:00 - 2:15 **Introduction and Overview of Approaches**
Dr. Linda Birnbaum

TUESDAY, OCTOBER 24 ***continued***

2:15 - 2:45 **Overview of the Mechanisms of Carcinogenic Action of Dichloroacetic Acid in the B6C3F$_1$ Mouse: Application to the Construction of a Biologically Based Dose-response Model**
Dr. Stephen Nesnow, National Health and Environmental Effects Research Laboratory, U.S. EPA

2:45 - 3:15 **Use of Mechanistic and Pharmacokinetic Data: Bromodichloromethane as a Case Study**
Dr. Rex Pegram, National Health and Environmental Effects Research Laboratory, U.S. EPA

3:15 - 3:45 **Break**

3:45 - 4:15 **Use of Mechanistic Data: Chloroform as a Case Study**
Dr. Rory Conolly, Chemical Industry Institute of Toxicology

4:15 - 4:45 **Risk Characterization for Chloroform in Drinking Water: Issues and Estimates on Exposure and Cancer Risk**
Dr. Nancy Chiu, Office of Science and Technology, U.S. EPA

4:45 - 5:30 **Panel Discussion**

4:45 - 5:00 **Session Summary** - *Dr. Linda Birnbaum*
5:00 - 5:30 **Discussion**

5:30 - 6:00 **Posters available for viewing**

6:00 - 7:30 **Dinner**

WEDNESDAY, OCTOBER 25

8:00 - 8:30 **Registration and Coffee**

<u>GENERAL SESSION</u>

EPIDEMIOLOGY PANEL: CRITICAL ISSUES IN EPIDEMIOLOGY RESEARCH ON DISINFECTION BY-PRODUCTS

8:35 - 9:00 **Epidemiology Panel Discussion**
Dr. John Reif Chair, Colorado State University
Dr. Kenneth Cantor, National Cancer Institute
Dr. Michele Lynberg, Centers for Disease Control
Dr. Philip Singer, University of North Carolina

SESSION 5: PREDICTIVE TOXICOLOGY, MIXTURES, AND EVOLVING RISK ASSESSMENT APPROACHES
Session Chair: Ms. Päivi Julkunen, The Coca-Cola Company

9:00 - 9:05 **Introduction**
Ms. Päivi Julkunen

9:05 - 9:20 **Drinking Water as a Complex Mixture: What Have We Learned?**
Dr. Jennifer Seed, ILSI Risk Sciences Institute

9:20 - 9:45 **Considerations in Assessing the Risks of Mixtures**
Mr. Phillippe Daniel; Camp, Dresser, and McKee

9:45 - 10:00 **Use of a Parallelogram Approach for Hazard Assessment**
Dr. Denise Robinson, ILSI Health and Environmental Sciences Institute

10:00 - 10:15 **Break**

10:15 - 10:40 **Structure-Activity Relationships; Predictive Toxicology as Applied to Disinfection By-products**
Dr. Ann Richard, National Health and Environmental Effects Research Laboratory, U.S. EPA

10:40 - 11:05 **The Road to Embryologically Based Dose-Response Models**
Dr. Robert Kavlock, National Health and Environmental Effects Research Laboratory, U.S. EPA

11:05 - 11:30 **Application of New Risk Assessment Approaches to Disinfection By-products**
Dr. Robert Tardiff, EA Engineering, Science, and Technology Inc.

11:30 - 12:00 **Discussion**

12:00 - 1:00 **Lunch**

SESSION 6: PRIORITIES FOR RESEARCH
Chair: Dr. Curtis Klaassen, University of Kansas Medical Center

1:00 - 1:30 **Synopsis: What Have We Learned?**
Dr. Curtis Klaassen

WEDNESDAY, OCTOBER 25 *(continued)*

1:30 - 2:35 **Panel Discussion: Research Priorities**
Moderator Dr. Curtis Klaassen

Dr. Linda Birnbaum, National Health and Environmental Effects Research Laboratory, U.S. EPA
D. Gary Boorman, National Institute of Environmental Health Sciences
Dr. Richard Bull, Battelle Pacific Northwest Laboratory
Mr. Phillippe Daniel; Camp, Dresser, and McKee
Mr. John Fawell, Water Research Centre
Dr. Hend Galal-Gorchev, International Program on Chemical Safety (IPCS) World Health Organization,
Dr. John Reif, Colorado State University
Dr. Robert Tardiff, EA Engineering, Science, and Technology Inc.

2:30 - 3:15 **General Discussion**

3:15 - 3:30 **Wrap-up**
Dr. Curtis Klaassen

3:30 **Adjournment**

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