February 10-12, 1999, M/DBP Stage 2 FACA: Health Effects Workshop
MEETING SUMMARY

Stage 2 Microbial/Disinfection Byproducts
Health Effects Workshop

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Park Hyatt
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Prepared by

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Welcome and Introduction

On February 10-12, 1999 the U.S. EPA held the Stage 2 Microbial/Disinfectant Byproducts (M/DBP) Health Effects Workshop. Cynthia Dougherty, Director, Office of Ground Water and Drinking Water, opened the meeting by welcoming the guests and reviewing the purpose of the workshop. [See Attachment I.A for meeting agenda, Attachment I.B for list of meeting participants and Attachment I.C for list of speakers and bios.] The purposes of this Workshop are to provide participants with:

- A summary of the major questions associated with the health risks from the disinfection byproducts (DBPs) and microbial contaminants that need to be addressed for the Stage 2 M/DBP rules.
- A summary of the current literature on the health effects from DBPs and microbial pathogens (this would include both cancer and noncancer endpoints for DBPs).
- A summary of ongoing and planned health effects research in support of the Stage 2 M/DBP rules and when the information will be available.
- Perspectives on characterizing the risk from DBPs and microbial pathogens.

The objective of this meeting is to focus on research, not to decide its meaning in the regulatory context, but to share the state of the science. This is the second in a series of three public workshops to review scientific issues surrounding the Stage 2 Disinfection Byproduct (DBP2) and Long Term 2 Enhanced Surface Water Treatment (LT2) rules. The first was the Statistics Workshop held November 19, 1998.(1) A

third public meeting is the March 10-12, 1999 - ICR, Treatment, and Methods Research Workshop to discuss occurrence and treatment.[2]

[1] The Final Meeting Summary and Attachments for the November 19, 1998 Statistics Workshop are available by contacting Detra Stoddard at RESOLVE at [dstoddard@resolv.org] or (202) 965-6218.

[2] The ICR Workshop Meeting summary, when complete, will be available by contacting Detra Stoddard.

Dorothy Patton, EPA/ORD, presented the three key aspects of the Health Effects Meeting: (1) the health effects discussion and the many aspects that allow a comprehensive look at the spectrum of available research and information, (2) the collaboration of many organizations, and (3) discussions for how to proceed in the future.

This meeting summary presents a short summary of the presentations and discussions that occurred at the workshop. Attachments include presentation materials and other more detailed background and supporting information. RESOLVE would like to acknowledge the technical support provided by ISSI, Inc., funded by EPA OST/OW, to produce the February and March M/DBP Stakeholder meeting summaries.

Background

History of Stage 1 Rules and Regulation

Stig Regli, EPA, presented the history and overview of the Stage 1 Disinfection Byproducts and Interim Enhanced Surface Water Treatment rules, baseline risk for the final Stage 1 rules and the timeline and major regulatory questions that need to be addressed for the Stage 2 rules [Attachment II.A].

In 1994, participants in the Stage 1 FACA discussions agreed to wait for more health effects and risk information to become available before developing the Stage 2 regulations. A commitment was made by the drinking water industry and EPA through the Information Collection Rule (ICR) to collect more information on occurrence and treatment for pathogens and by-products. There was also a recognized need for more research, and as a result, research funding was increased.

The Stage 1 rule was promulgated in 1998. A broad range of possible cancer risk reduction was assumed for the Stage 1 rule. While quantification of actual risk reduction was highly controversial, EPA assumed a risk reduction of 25% considering reductions in exposure from TTHMs as a surrogate for DBPs in general. For noncancer risk, there was not enough information and the Regulatory Impact Assessment prepared by EPA did not quantify this risk.

The following questions are important to be answered during Stage 2 Rule development:

1. What is the magnitude of the risk from exposure to chlorinated byproducts in drinking water?

- What is the risk from brominated DBPs versus non-bromide DBPs?

11. What is the relative risk of developing diseases when exposed to waters disinfected with chlorine compared to waters disinfected with non-chlorine (alternative) disinfectants?
12. Which individual DBPs pose the highest risk?
13. How much does exposure to DBPs vary within the distribution system?

The time line for the Stage 2 rules was proposed by EPA: 1) Rule development - Spring 1999-Spring 2000 and 2) Rule proposal - early Spring 2001. In response to a question on how Stage 2 FACA can begin discussions before relevant data may become available, Regli explained that the rule could be

proposed with two or more alternatives which are followed depending on the findings of the new information (e.g., if outcome A, then rule A; If outcome B, then rule B). A Notice of Data Availability (NODA) in summer 2000 could describe such new information and regulatory consequences.

Overview of DBP Formation and Occurrence

Stuart W. Krasner, Metropolitan Water District of Southern California, presented an overview of DBP formation and occurrence including; current industry disinfection practices, DBP chemistry and the major factors that effect DBP formation, and the occurrence of DBPs from the use of different disinfectants [Attachment II.B].

Disinfectant is added at the beginning of the plant for multiple reasons; primarily for microbial inactivation, but also to prevent algal growth, for taste-and-odor control, and for inorganic oxidation. As a result of the Stage 1 DBP rules, chlorine dioxide, and ozone are being used more frequently for primary disinfection and chloramines for secondary disinfection and to maintain residual.

DBPs are formed when disinfectants react with precursors such as natural organic matter (NOM) and bromide in water. NOM comes from decaying vegetation, etc. and bromide comes from salt water intrusion into source waters, etc. All disinfectants form DBPs. DBPs are formed in one of two reactions: 1) Halogen substitution reactions resulting in halogenated by-products and 2) oxidation reactions. Secondary by-products are also formed when multiple disinfectants are used.

Temperature, time and pH, along with the disinfection process and other source water characteristics, determine what DBPs will be formed. Most reactions that form DBPs occur in the first 24 hours. The pH determines, in part, which DBP will be formed, resulting in risk/risk tradeoffs. For example, lowering pH to control for trihalomethane (THM) formation can result in the increased formation of trihaloacetic acids. Reaction time is also an important variable - for example, chloral hydrate is unstable at high pH levels, and over time, it degrades to chloroform, which results in an increase of THMs over time.

Bromide also presents risk/risk tradeoff questions: an increase in bromide in source water results in less formation of chloroform and a greater formation of bromodichloromethane. Moderate-to-high bromide levels increase the formation of bromoform. Increased bromide levels result in higher levels of brominated DBP species. Regulations are currently based on total THM instead of individual THMs.

The following points were discussed by participants:

- If the temperature difference is great, then there is less THM formation in the winter. Seasonal effects flip flop in some regions because, even if temperature difference is not drastic, some areas may be more effected by increases in total organic carbon during runoff events. It is very site specific.
- Very few systems have high bromide levels, but with respect to population exposure, it is very large. For example, Texas and California have high bromide levels.
- If two drinking water systems are connected, then secondary by-products can potentially form if different disinfection methods are used.
- While THMs and haloacids increase over time, some DBPs can decrease over time - especially with alkaline pH. If little chlorine remains, then some DBPs (i.e. haloacids) are degraded by nonpathogenic microorganisms in the water supply.

Overview of Types of Health Effects Data and How Data are Used in Decision Making

Jeanette Wiltse, EPA, presented an overview of the role of health effects data in EPA's decision making process [Attachment II.C]. Wiltse described two distinct domains of risk assessment and risk management, and presented a summary of the risk assessment paradigm and information EPA uses in

assessing risks from DBPs. The risk assessment paradigm consist of: 1) Hazard Index, 2) Dose Response, 3) Exposure Assessment, and 4) Risk Characterization.

Health endpoints considered in a Risk Assessment are cancer (natural disease common to differentiated organisms) and non cancer (acute and chronic). Non-cancer effects include organ effects, reproductive effects, developmental effects, neurotoxic effects and other systemic effects (does the animal lose weight, etc.). It has been difficult to look at mixtures of DBPs, and research is now being developed to do animal testing with mixtures.

The advantages of human data over animal data were discussed. Human data are clear, but there are the issues of cost and ethics. Animal data are clear empirically, but extrapolating the data to humans is difficult.

In response to a question, Wiltse stated that the terms "linearity" and "threshold" should not be interchanged. Threshold is intercept, whereas linearity is based upon the shape of the curve.

Improving Exposure Assessments in Epidemiology

Jay Nuckols, Colorado State University, presented an overview of exposure assessment in the context of epidemiological studies, the state of the science with respect to DBPs, and issues in context with the Stage 2 rulemaking process [Attachment II.D].

Exposure means that a dose is reaching a target. Surrogates for exposure are used because they can be used quantitatively to predict exposure to a population or individual. Nuckols reviewed DBP studies and surrogates for exposure and emphasized that THMs have been used as a surrogate for exposure for all DBPs.

Two main types of epidemiology studies are: 1) Case-Control (contact participants to gather information) and 2) Ecological studies (those that get information indirectly).

The problems encountered in exposure assessment are:

1. Survey methods are limited since respondent to survey may not be knowledgeable and may have a problem with recall;
2. Reconstruction is difficult -- there is a possibility that what is being measured is not the true exposure; and
3. Population size/geographical extent is a problem in obtaining sufficient statistical power.

Nuckols made the following points regarding epidemiological studies:

- Exposure gradient must be defined over space and time.
- There is a wide variation of exposure among different utilities.
- Need to consider all routes of exposure-- historically epidemiological studies only concentrated on ingestion; new studies will now look at exposure from showering, inhalation, and absorption through skin.
- Established that significant exposure is from inhalation and dermal contact.
- All confounders must be considered.
- Statistical power must be established. Look at distribution of exposure to answer questions.

The following points were emphasized by participants concerning approaches for epidemiological studies:

1. Utilities should be characterized by data, distribution, and modeling (water quality and demand).

2. The population should be classified (water quality and water use/consumption by individuals--wash, cook, bathe, drink, work, etc.).

DBP Health Effects: Cancer Risk

Epidemiology: Overview of Current Science

Michael McGeehin, CDC, discussed environmental epidemiology of DBPs and cancer [Attachment IIIA]. McGeehin defined epidemiology as the study of disease in a population. Environmental epidemiology is defined as the study of disease in population with environmental data.

Different types of epidemiological studies include: 1) Ecological studies, 2) Case-Control studies, and 3) Cohort studies. Ecological studies are studies that are put together on previously collected data, and are used to compare groups rather than individuals. Ecological studies are usually considered a good basis for a hypothesis. Case-Control studies are the most common type of study. Case-control studies are most useful for studying rare diseases. A problem with case control studies is recall bias. For example, persons with disease (cases) will look at exposure differently than people who have not had the disease (controls). Cohort studies are studies in which people with exposure are followed in time to see if they develop disease. There are also retrospective cohorts. A problem with cohort studies is that they are very expensive and time consuming.

Criteria for determining causation are: 1) strength of association (relative risk, odds ratio); 2) consistency of association; 3) biologic gradient; 4) biologic plausibility; and 5) temporally correct.

Results of epidemiological studies have shown evidence of moderate association between DBP exposure and bladder cancer, but no causality for bladder cancer. Population Attributable Risk (PAR) studies for colon cancer and for rectal cancer were inconclusive, however, for bladder cancer it was established that 2 to 17% of the disease would not occur if exposure were eliminated. Studies have shown a moderate association between DBP exposure and bladder cancer.

In response to a question, Nuckols explained that the exposure for the EPA PAR included the surrogate, chlorinated waters, and THMs. Exposure was underestimated in studies that did not detail the exposures.

Epidemiological Research and Approaches on DBP Cancer Endpoints

Terry Harvey, EPA, presented a brief overview of ten EPA human epidemiology research studies that will be carried out in FY 99 and 00) [Attachment III.B]. EPA Office of Research and Development's strategy for cancer epidemiology research is to:

Improve cancer risk estimates through a retrospective evaluation of exposure information in completed studies. Attempt will be made to connect animal toxicities to human responses. This will include DBP mixture issues. Harvey indicted that ORDs program for the use of public health approaches (like epidemiology for DBPs) is at a re-evaluation point and he encouraged collaborations on this issue.

In response to questions Harvey made the following points:

- EPA will look at all available studies and data to try to tease out the needed exposure and effects data - regardless of the data collection method.
- Biomarkers of exposure and effects, such as enzyme effects in the body, may be a longer term scientific approach to the issue of human doses/responses.
- Exposures/effects may be affected by the ranges in human effect genetic pools showing genetic polymorphisms that may exist and account for cancer incidences.
- ORD has not given up on trying to understand the cancer-based endpoints for DBPs.

Case-Control Study in Ontario, Canada: Examining the Association Between Cancer and Exposure to Chlorinated Byproducts

Will King, Health Canada, reviewed the relationship between environmental factors and disease occurrence using a case control study to study DBPs and Bladder Cancer [Attachment III.C].

The study area was Ontario, which has 11.6 million residents, that live mostly along the Great Lakes:

- 80 percent of the study population get their drinking water from a Public Water Supply (PWS).
- 70 percent of the population is served by surface water.

The cases had bladder, colon and rectal cancer, with the cancer information coming from the cancer registry. The controls came from telephone marketing. Exposure assessment was determined using a questionnaire and a survey to water treatment facilities. This study focused on THMs. Different approaches to estimate levels of THM over time was done. A model was used to predict THM concentration from 30 years ago, since they were not measured.

Increased DBP exposure was correlated with bladder cancer. For colon cancer, an increased risk was not seen until there were higher levels of DBPs. Unlike bladder cancer, there is a difference in risk based on gender - greater in males for 35 - 40 years of exposure. There was no increase of risk for rectal cancer in men or women.

The probability of developing cancer by the age of 75 was discussed. For most individuals, developing bladder cancer is not a "huge" risk. Therefore, the extrapolation of risk to an individual as a probability of bladder cancer is not very high. However, studies have shown that about 38% of the population is exposed to increased levels of DBPs, and

suggests that 15% of bladder cancer in Ontario is attributable to DBPs-- 165 cases and 75 deaths.

King made the following points in response to questions from participants:

- The exposure model was validated by comparing DBP occurrence model predictions with actual occurrence data.
- Latency and duration from exposure to effect were not studied.
- The model was adjusted for significant confounders. Gender and smoking were examined as sub-analysis of data - interactions were considered. Non-smokers were defined as those who smoked less than one cigarette per day per year.
- The study looked at both invasive and noninvasive tumors as subgroups and saw no difference.
- The model considered only maximum THM values, not averages.

Case-Control Studies in Iowa and Maryland: Examining the Association Between Cancer and Exposure to Chlorination Byproducts

Kenneth Cantor, National Cancer Institute, presented the Iowa case-control study. The Maryland study is much more limited so was not included as originally planned [Attachment IIID]. Iowa was chosen as a location for this case control study for the following reasons: 1) a high quality cancer registry; 2) an adequately sized and cooperative population; 3) one-third of the population is on chlorinated surface, one-third of the population is on chlorinated ground water, one-third of the population is on non chlorinated water; 4) treated surface waters in Iowa historically had high byproduct levels.

Results of the study indicate: there was an association between the duration of exposure to chlorination byproducts and risk of rectal cancer among both sexes and risk of bladder and brain cancer among males. The association for bladder cancer with duration of byproduct exposure was also observed among

smokers. There was an attempt to differentiate between effects among men and among smokers, but this was not possible in the context of this study. There was no excess risk for colon cancer with duration of chlorinated surface water use. Conclusions about brain cancer should not be drawn from this one study. Overall results of the study indicate that there is an association with bladder, rectum, and brain cancer in males, and rectum cancer in females, for exposure to THMs, used as a surrogate of exposure to the chlorination byproduct mixture.

Nitrate in water and a possible association with bladder cancer was not considered in this study. If nitrate is a risk factor for bladder cancer (not known at this time), it would likely act to depress the observed levels of association with chlorination byproducts. This is because nitrate is a larger problem in non-chlorinated private wells than in chlorinated surface waters, and people who consumed water from private wells were considered to be "unexposed" in this study.

Cantor made the following points in response to questions from participants:

- Pesticides were not included as a confounder in exposure assessment. Pesticide levels are in most cases too low to influence cancer.
- As a followup to results from Iowa, he is conducting a brain cancer study in another geographic area. Results from this followup study will not be available for at least another year.
- The study did not look at nitrate in surface or ground water.
- In the Ontario (King) study, elevated risks were found for colon, and not rectal cancer, whereas in Iowa, this result was reversed. This might reflect differences in the composition of the byproduct mixture to which populations in the two areas are exposed, or other factors.

Toxicology of Individual DBPs: Overview of Current Science and Current and Future Research and Schedules for Completion

Fred Hauchman, EPA, presented an overview of current and future research on toxicology for individual DBPs and schedules for completion [Attachment IIIE]. The two types of toxicology studies are: 1) chronic and subchronic bioassays in rodents; and 2) short and intermediate term studies, including those being done on transgenic mice, fish species (i.e. medaka), mammalian and non mammalian cells, and structure activity relationships.

Hauchman made the following points on the "state of science" on DBP toxicity:

- Carcinogenicity of a number of DBPs has been evaluated.
- Many studies (from NTP) have been conducted with corn oil as the vehicle for administering DBP. (Different results occur when the DBP is administered via corn oil gavage compared to drinking water ingestion.)
- Types of tumors observed include liver cancer (not concordant with epidemiology), colon cancer and other types of tumors (brominated DBPs).
- Variability within and between DBP classes: mutagenicity (brominated versus chlorinated), potency, and modes of action.

Additional data needs include: modes of action data for risk assessment and carcinogenicity studies of selected brominated and chlorinated DBPs administered in drinking water. New studies are underway or planned to provide screening level data on the carcinogenicity, neurotoxicity and immunotoxicity of several DBPs (reproductive toxicity research is discussed elsewhere). There is not much information on the potential neurotoxicology and immunotoxicology of DBPs to determine if these effects are likely to be of public health concern. Data from the new long-term cancer studies will not be available for Stage 2, but pre-chronic data from these long-term studies on cancer hazard and mode of action data will be available.

EPA is devoting significant resources on DBP research in the coming years. The focus of the research will be developing hazard identification and dose-response information to support DBP risk assessments for the toxic endpoints described above.

Hauchman made the following points in response to questions from participants:

- Medaka fish studies can be completed in less than a year from start to finish, whereas chronic rodent cancer bioassays typically require several years to be completed. Some data suggest that the results of Medaka and rodent studies for some contaminants may be comparable qualitatively, and it is believed that Medaka may be a useful model for studying modes of action. However, additional research is necessary to further evaluate the utility of this approach for DBP risk assessments.
- The proceedings of a 1995 workshop in Chapel Hill, NC, entitled "Disinfection By-Products in Drinking Water: Critical Issues in Health Research", may be obtained by contacting the International Life Sciences Institute (ILSI). Information on the second international conference on the Safety of Water Disinfection, which will be held in Miami, FL in November 1999, can also be obtained by contacting ILSI.

Research Evaluating the Toxicity of Bromate, Chlorate, and Haloacetic Acids

Doug Wolf, EPA, presented an overview of the toxicity and associated carcinogenicity of haloacetic acids, bromate, and chlorate and reviewed the collaborative work being conducted between the EPA and National Toxicology Program at NIEHS [Attachment IIIF].

The goals of this research are to identify key events, determine dose-response relationships to key events, and provide data that would indicate which risk characterization approach is most relevant (Linearity versus Nonlinearity).

Wolf emphasized the following points concerning Bromate: 1) Bromate results from the oxidation of bromide; 2) It occurs most prevalently with ozonation; 3) Exposure data for humans exists including data from intentional and accidental poisonings; 4) It can be fatal in humans; and 5) It causes loss of hearing, kidney toxicity. The focus of current research is to determine if the production of oxidant damage in the nucleus is the key event that is driving the cancer response and if this key event is common to all three tumor sites in the rat.

There is little toxicology data available for chlorate (some toxicology data reveal that it causes oxidant damage to the red blood cells), and there is no cancer data available. Bioassays have started, so the in-life portion will be completed in 2 years, and final data will be available in 4 years. Additionally, there is some evidence that chlorate causes thyroid damage, but it is unknown whether it is a primary effect or secondary to hormone effects (altered iodine uptake).

Wolf reviewed data and information on the toxicity of Haloacetic Acids:

- Dichloroacetic Acid: Liver tumors in mice and rats
- Monochloroacetic Acid: Not carcinogenic
- Trichloroacetic Acid: Liver tumors in mice
- Brominated acetic acids: Little information

Future work on Haloacetic Acid data include:

- NTP Studies: Dibromoacetic acid, Bromodichloroacetic acid, Bromochloroacetic acid, Dichloroacetic acid
- Short-term dose-response studies

- Cancer bioassays by drinking water exposure EPA Studies
- Comparison of brominated and chlorinated acetic acids
- Define the Key Events in cancer development

The following points were made during the discussion that followed Wolf's presentation:

- Bromate and chlorate are strong oxidants, the relationship between response and extrapolation is an SAR (Structure Activity Relationship) issue. They are both strong oxidants, but in the animals they react differently. Chlorate and bromate both affect the thyroid but their modes of action may be different. Chlorate may be similar to perchlorate which has been shown to block iodine uptake and thereby cause thyroid lesions through a hormonal mediated mechanism. Bromate may be different, there is no evidence that it blocks iodine uptake and there is evidence that it causes oxidant damage in the kidneys.
- Interpreting transgenic rodent studies with bromate is difficult because mice do not respond to bromate. The rat study will permit examination of mutations associated with tumor development.
- Chlorate effluent in raw water from paper mills exists, so the treatment plant is only one source of chlorate.

Research Evaluating the Toxicity of Bromodichloromethane

Rex Pegram, EPA, presented an overview of the methodology and findings from past research on brominated trihalomethanes [Attachment IIIG]. Pegram made the following points: 1) bromine is a better leaving group than chlorine, so compounds containing bromine are generally more reactive; 2) bromine-substituted compounds are more lipophilic, so they can enter more readily into target tissues; and 3) brominated THMs are among the most prevalent DBPs in drinking water, especially when there are higher bromide levels in the source water. Bromodichloromethane is the most potent rodent carcinogen among the THMs and it causes cancer at multiple sites. Cancers of the lower GI tract were induced by two of the brominated THMs in rats and were also correlated with greater exposure to THMs in epidemiological studies. The THMs are volatile, so inhalation is an important exposure route that has not been studied very much thus far. The parent compounds are not believed to be toxic unless metabolized to reactive metabolites that interact with proteins, lipids and DNA to cause toxicity. The brominated THMs are more reactive than chloroform, and are especially more likely to react with DNA and cause mutations.

Human and rodent pharmacokinetic data is expected to be ready for Stage 2. Please see Attachment IIIG for a more detailed discussion of the implications and conclusions of this research, future studies, and a list of references.

Mixture Studies for DBPs

Linda Teuschler, EPA, presented an overview of past, current and future DBP mixtures research [Attachment IIIH]. Mixtures research is important because individuals are exposed to complex mixtures of DBPs, not to single chemicals alone, in the drinking water. Currently, little data on mixtures of DBPs exist, but some preliminary data are available. High dose studies for individual DBPs provide little insight to the health effects of mixtures (even of low dose mixtures). Research on defined mixtures of DBPs have found evidence of increased responses above that expected for single chemicals for liver toxicity in mice and rats exposed to mixtures of THMs, and for liver cancer in mice exposed to dichloroacetic acid and trichloroacetic acid mixtures.

The ILSI Expert Panel Recommendations for Toxicological Testing (March 1998 Report) recommended that DBP risk cannot be assessed by single chemical testing approaches alone. The report suggested the use of modern approaches (e.g., studies relating chemical structure to toxicity, molecular biology and toxicology, knowledge of mechanism of action), the use of a 3-tiered testing approach (i.e., *in vitro* tests; short term screening tests such as medaka fish or 90 day animal studies; long term chronic bioassays),

and a focus on three scenarios: 1) defined (simple) mixtures of less than 10 DBPs; 2) whole mixtures produced by simulating disinfection scenarios; and 3) real drinking water samples or their extracts.

The risk assessment questions that mixtures research should address include:

- Is there a real risk to human health? How can we better understand the real human health effects, exposures and risks?
- How and when do we use animal data to estimate human risk? What biologic processes occur to cause toxicity? Are there models other than animal studies that can be used for mixtures?
- To calculate the mixtures risks, is it correct to: add up the risks for individual chemicals at certain doses? Add up the doses of those chemicals and then estimate risk? Use whole mixture data from animals or humans to estimate risk?
- What is the health risk for various drinking water treatment options?

Teuschler made the following additional points:

- Toxicology and statistical research must go hand in hand to maximize the amount of information that can be obtained for the given resources.
- EPA is currently collaborating with other groups to perform research on mixtures that includes both cancer and noncancer studies and biologic modeling on defined mixtures, new models such as the medaka, FETAX frog embryo assays and new *in vitro* systems, and statistical research on the risk characterization of DBP mixtures across drinking water treatment scenarios. Preliminary results are available for many of these efforts. However, final results will not be available for approximately 1 to 3 years for many of this mixtures research.
- Mixtures Risk Assessment is not new. EPA has published several documents that provide risk assessment guidance on chemical mixtures (U.S. EPA 1986, 1989,1990). Cumulative risk (i.e., multi-chemical/multi-route risk assessments) is also being investigated, as required by the Food Quality Protection Act (FQPA) for pesticides.

Teuschler made the following points in response to questions from participants:

- Only studies done by Beth Jarger show mixtures changing in the digestive tract with metabolic changes in bacteria and alteration by DBP.
- Current EPA guidelines suggest handling mixtures by reviewing all available data for the risk assessment. If available health effects and exposure data are on the complex mixture itself in humans, then use that for the risk assessment. If available data are on a similar mixture that is sufficiently similar to the exposure of interest, then the risk assessment can be made using the similar mixture data as a surrogate. If the available data are only on the components of the mixture, then use a component based approach. Because of the complexity of assessing mixtures and the lack of adequate data in many cases, the guidance allows for flexibility in the evaluation of mixtures.

Summary of WHO Environmental Health Criteria Monograph for Disinfectants and DBPs

Hend Galal-Gorchev, World Health Organization (WHO) *retired*, presented a summary of findings from WHO Environmental Health Criteria Monograph for Disinfectants and Disinfectant By-Products, Summary and Conclusions of International Programme on Chemical Safety (IPCS) Task Group, 17-21 August 1998 [Attachment III.I]. The monograph was authored by four experts and various Disinfectants and DBPs (chlorine, MX, bromate, chlorite, etc.) were evaluated. WHO health-based Guidelines are non-enforceable recommendations that are similar to EPA's MCLG.

Galal-Gorchev made the following points concerning the Characterization of Hazard and Dose-Response: 1) for some chemicals, there were no adequate data to do risk assessment; and 2) for some chemicals,

the task group did not have enough information to determine if they were threshold or non-threshold chemicals.

Epidemiological evidence is insufficient to support a causal relationship between bladder cancer or colon cancer and long-term exposure to chlorinated drinking water or DBPs. The results of currently published studies do not provide convincing evidence that chlorinated water or THMs cause adverse pregnancy outcomes.

DBP health effects conclusions were reached independent of concern about balancing risks from microbial contaminants. The main risk-balance recommendation is that disinfection should not be compromised to control for DBPs because the risk from waterborne diseases, such as cholera and typhoid fever is greater than the potential for risk from DBPs.

Panel Discussion on Characterization of Cancer Risks

Bob Morris, Tufts University; Kenneth Cantor, NCI; Bob Tardiff, Sapphire Group; Betty Meek, Health Canada; Jeanette Wiltse, EPA; Phillipe Daniel, Camp Dresser & McKee, Inc.

Phillippe Daniel framed the discussion of the panel by presenting an overview of the major points of concern in DBP risk management [Attachment III.J]:

1. Treatment alternatives select for DBP mixtures that are qualitatively and quantitatively different (each alternative creates its own unique DBP mixture.)
2. Trade-offs exist not only between microbial and chemical risk, but also between types of DBP mixtures.
3. Evaluating the toxicological trade-offs is hampered by significant data gaps.

The following is a summary of points made by panelists regarding the panel discussion questions, and other comments that arose in the course of open discussion:

Question 1: Based on the current database, what are your observations regarding the cancer effects from exposure to DBPs?

- It is very difficult to reduce risk to a single number. It is difficult to know what the risk management implications are of the estimated relative risks for cancer from DBPs.
- There is evidence of colon and bladder risk.
- Among epidemiological data for bladder cancer, based on incidence data [mortality rate is 1/3 of incidence rate], the criteria that would point to causality are consistency and duration of response. The available data fulfil a number of the traditional criteria for causality for epidemiological data.
- For colon and rectal cancer, the jury is out.
- For pancreas, brain, and renal cancer, mixed results.
- Genetic polymorphisms in animal studies suggest that the effects of chlorination byproducts may be enhanced in individuals with certain genetic characteristics. This notion is being incorporated into epidemiologic studies to identify sensitive or insensitive subpopulations. This type of study should provide insight into mechanism and a bridge between the experimental and human findings.

Question 2: What are the consistencies and inconsistencies in the epidemiological and toxicological data?

- Advantages of the epidemiological studies for DBPs is that they investigate effects of the mixtures to which humans (i.e., the species of interest) are exposed. Disadvantages are that the results

cannot be generalized to populations exposed to varying mixtures. Toxicological data are generally limited to effects of single chemicals for which extrapolation to humans is required.

- Need bridge between human observations and animal toxicological studies - one approach would be the use of human tissues in toxicological studies.
- There is a need to coordinate epidemiological and toxicological study design and interpretation. The bridge between epidemiological and toxicological study data are mechanistic data. Better coordination of epidemiological and toxicological research may resolve some of the apparent discrepancies.
- Focus on compounds of interest in epidemiology based on toxicology.
- Design toxicological studies with plausibility in mind.

Question 3: What are the largest remaining data gaps and uncertainties that need to be addressed to better characterize the cancer effects from exposure to DBPs?

- More dose/response information would be very helpful in understanding the cancer risk from DBPs.
- One future approach would be a pooled analysis of raw data from existing studies. There are now 30 studies of DBP and cancer association. Meta analysis can combine the results of the studies, but the data has to be reduced to a single number. For bladder cancer, relative risk is often greater than 1, but the Confidence Interval (CI) crosses 1. So there is an elevated risk, but it is non significant (trend only). For rectal cancer, different exposures cause cancer at different points. One recommendation is to use only raw data from individual studies (dose response relationship, supply characteristics that may influence associations, influence of non-drinking water exposures on DBP risk.) A limitation is the confounding effect of pooling data from populations with a varying mixtures.
- More mode of action research is needed to gain a better understanding of mechanisms.
- There are major obstacles to studying carcinogeneses of DBPs such as complex mixtures, latency of cancer formation, and ethical considerations with respect to human experimentation.
- Dose/response data is needed. Current studies tell us very little about dose/response relationship of DBPs and health effects. Information about biological occurrence is needed, as well as a gradient of dose response information down to low exposures. Research is needed on the link between biological activity and dose:
    - King and Finnish studies have additional exposure information and there may be other studies which have dose data.
    - Existing epidemiological studies could be used to evaluate exposure data.
    - Refine newer better epidemiological studies with better exposure analysis to tease out dose response.
    - Conduct mechanistic studies to bridge animals-humans.
- In the next year it may be feasible to develop and carry out a plan to evaluate the epidemiological studies with respect to exposure data. The toxic equivalents of various DPBs is still unknown. One approach would be to concentrate efforts on an identified set of chemicals.
- Quick and dirty studies could be planned to collect needed data.
    - Currently planned and ongoing studies could be revamped to collect useful information fairly quickly.
    - Short term treatment based mixture evaluations (screening).
    - Include brominated waters in ongoing studies to compare levels of risk.
- Considerations for future work. We must consider that exposure to DBPs has changed greatly in the past few years due to changes in treatment, changes in pH in water, enhanced coagulation, compliance with first TTHM rule, Long-Term 1 Enhanced Surface Water Treatment rule and other recent activities.
    - It will be important to consider chemical to chemical tradeoff (think about range, or given set, rather than individuals).

Additional observations and discussion points

- Risk assessment will require multidisciplinary teams to work together. The data may not be as inconsistent as we currently think.
- Causation is an interpretation of criteria or guidelines that are not standardizable and not prescriptive. Professional judgement must be applied as to whether sufficient weight of evidence exists -you cannot definitively prove causality. For public health decisions, however, the choice of criteria against which weight of evidence for causality in epidemiology studies is assessed is a very important issue to discuss. Criteria may include; consistency of association, dose/response, temporal association and plausibility.

DBP Health Effects: Reproductive and Developmental Risks

Reproductive and Developmental Effects of Exposure to DBPs: An Overview of Epidemiological Data

John Reif, Colorado State University, presented a summary of the epidemiological methods used and outcomes of studies conducted to date, with a focus on TTHMs and discussed the limitations of existing studies of DBPs, including exposure assessment [Attachment IV.A.].

Key points surrounding Adverse Reproductive Outcomes: 1) women experience adverse reproductive outcomes at low doses; 2) there is an accurate recall of exposure since the latency period for developmental effects is short (9-12 months) compared to the latency period for cancer (up to 20 years), which permits an estimation of exposure according to critical periods in fetal development; 3) there have been no human reproductive studies of males endpoints published to date; 4) male infertility has not been studied in relation to exposure to DBPs.

Spontaneous abortion can result from abnormal development or congenital abnormalities, or there maybe problems with the process of pregnancy and uterine growth. Many spontaneous abortions also occur in first month, and they are unrecognized as such since many women may not know that they are pregnant. This accounts for at least 5% or more of spontaneous abortions.

Bias occurs when risk is underestimated if both the normal and diseased group are equally misclassified. Additionally, a confounder must be a risk factor and be associated with the outcome of interest in order to bias the results.

There have been 10 studies published on DBP reproductive effects; 4 are relevant only to water treatment; 6 addressed THM levels. These six studies were discussed in further detail by Reif, see Attachment VI.A. for a more detailed description of these studies.

Reif made the following points in response to questions from the audience:

- The Tuthill (1982) and Italian studies are both reasonably good papers. There is some individual data, findings were suggestive, but not clear. Total THM concentration was low (20-30 µg/L), which is very different than 80-100 µg/L. Also, comparisons of large city vs. small town is not the best. Good aspect of the study is that it provides growth data (head circumference.)
- We cannot answer questions regarding the temporal trends and spatial trends in variation in rate of spontaneous abortion that occurs across the US because of ascertainment from health care provider. One option is to match your control and study groups temporally and spatially. Inferring between high and low may be complicated by these factors.
- Infertility in men has not been assessed. Infertility, however, could be assessed by time to conception.

DBP Health Effects: Reproductive and Developmental Epidemiology: Current and Future Research

Rebecca Calderon, EPA, presented an overview of expert panels discussions from 1993 and 1997, and the schedule for current and planned DBP Health Effects research [Attachment IV.B]. EPA released two reports on reproductive and developmental epidemiology, one in 1993 and the other in 1997. In 1996, John Reif released an Environmental Health Perspective review of the studies - the take home message was that exposure and health end points need to be more carefully assessed. The studies need to be refined with existing data.

Calderon made the following points during her presentation:

- A need for biomarkers is clear.
- Future studies will begin to focus on variability in the distribution system.
- Epidemiologists can no longer do research design for DBPs by themselves. A multi-disciplinary team, consisting of epidemiologist, toxicologists, chemists, engineers, etc., needs to be used to improve the quality of the studies.
- Several publications resulted from the Pilot Study to Evaluate GIS for Exposure in DBP Epidemiology Studies - including M. Gallagher in the *Epidemiology* Journal last year and a publication in 1994.
- The Replication of the Spontaneous Abortion Study will be a repeat of the Waller study. The RFP is now on the Internet, and proposals are due on 4/1/99. The study is large and will require a budget of 3 to 4 million dollars.
- The development methods of the Male Reproductive Effects study contain methodological problems regarding male reproductive studies. Most of the information received are from infertility clinics and not from the general population. EPA is working on a new container to collect semen samples, by mail, from the general population.
- The pharmacokinetics of bromodichloromethane (BDCM) in humans study will be completed in time for the regulations. It will focus on the bromodichloromethane pharmacokinetic in humans and development of a pharmacokinetic model.

In response to questions from the audience Calderon made the following points:

- Dibromoacetic acid is a key male reproductive toxin, but many communities do not have this contaminant, the male reproductive study will target high level communities.
- Semen will not be collected in the next version of the NHANES. Maybe semen will be collected in a future study.
- An idea is to collect a water sample simultaneously with a semen sample.
- Refrigeration is not important for the sperm. The goal is to get the semen to lab in 48 hours. After 48 hours the sample begins to degrade.
- The population base for the sperm study has not yet been determined.

California Studies: THMs in drinking water and Spontaneous Abortion

Kirsten Waller, Sequoia Foundation, reviewed study design and study results for exposure assessment for TTHMS in drinking water and spontaneous abortion and presented an overview of other California current and planned research and schedule for completion [Attachment IV.C.].

The Trihalomethanes in Drinking Water and Spontaneous Abortion study was published in the March issue of the journal *Epidemiology*. The three communities in California used in this study were chosen because of their water source.

Exposure assessment for the study was performed by identifying drinking water utilities by using the address of the study participants. Quarterly reports from the utilities were obtained to receive THM information. Individual utility data was used, and data were obtained from 78 of the 85 utilities identified in the study. There was no interpolating or extrapolating for unknown data - data that was available for the

first trimester was averaged. If THM measurements, for the first trimester, were not available (60% of the cohort had this data) then a 90 day window was used. For 9 percent of the women, an annual THM average had to be used as the level of exposure. An average of how much water the women drank on a daily basis, and bottled water usage was obtained during telephone interviews.

The odds ratio for SAB (spontaneous abortion) of women that drank less than five glasses of water per day with total THM levels greater or equal to 75 µg/L was 1.2. This number is non-significant. The odds ratio of women that drank greater than five glasses of water per day with total THM levels greater or equal to 75 ug/L is 2.0, which is significant. The odds ratios were adjusted for all confounding factors (95% confidence interval).

The strongest association for SAB were seen in women that drank more than 5 glasses of water per day with bromodichloromethane levels greater than 18 ppb. The Odds Ratio was 3, but the number of cases was small, so this outcome is unstable.

A number of studies are currently in progress in California including: (1) reanalysis using a Geographical Information System (GIS) for exposure assessment keyed to specific sampling sites, (2) reanalysis of risks for individual THMs using more complete data, (3) THM modeling in two distribution systems, (4) THMs and menstrual function/sperm parameters, and (4) THMs and adverse pregnancy outcome in a new cohort with high TTHM but low BDCM.

In response to questions from the audience Calderon made the following points:

- There is some dose-response data on THM level, but it is modest. Tried calculating dose (as David Savitz) and saw a lower but still elevated risk, but they may have diluted the effect. Indication of dose-response and with intake and with showering, too. Showering may be more important than ingestion.
- Threshold appeared to be seen in the study. However, much weight cannot be put on it. There is not enough information to make that determination. There was evidence of biological plausibility.
- There were potential sources of mis-classification in the study. If mis-classification is taken out, we expect that the odds ratio will go up.
- The odds ratio for smoking and age were in the 2 range. Odds ratio for other risk factors were from 1.8 - 2.5.
- Only routine data was looked at in the study, periods of non-compliance such as earthquakes where high chlorination may have taken place, were not taken into consideration.
- Data from the utility companies were used, and only information that are required by the regulations was available.
- Seasonality did not appear to be a confounder.
- THMs are higher in the summer and spontaneous abortion may be related to THMs, however, it is not known if there is any data that show that during the summer there are spikes in spontaneous abortion.
- Spontaneous abortion rates were looked at for water source, whether the water was surface, mixed or ground. Spontaneous abortion rates were not looked at for individual utility companies.
- The study did not look at where the women lived before their pregnancy.

CDC/EPA Birth Defect Studies

Michele Lynberg, CDC-NCEH, presented a summary of the CDC studies (currently underway or planned) examining the relationships between exposure to DBP and birth defects [Attachment IV.D]. Evaluating the causes of birth defects is difficult and requires a large study population and periconceptional exposure information. Currently, several related birth defects studies are being conducted by CDC and its collaborators (EPA, UNC, CSU) using available birth defect information from: 1) the Metropolitan Atlanta Congenital Defects Program (MACDP), an established surveillance system in existence since 1968; 2) the Atlanta Birth Defects Risk Factor Surveillance Project (BDRFS), a case-control study based on the

MACDP, conducted during 1993 - 1996; and 3) the National Birth Defects Prevention Study (NBDPS) which began in 1997 and is based on the surveillance systems in metropolitan Atlanta and 7 other geographic areas (Alabama, Arkansas, California, Massachusetts, New Jersey, New York, and Texas). These birth defects databases are being linked to historical water utility data and reported water use characteristics.

Information was collected from seven water utilities (ten treatment plants) in metropolitan Atlanta. To coincide with the BDRFS case-control study, all existing THM (monthly and quarterly) and chlorine residual (almost daily) data were abstracted for the years 1992-1996. Data collection is complete and analysis is under way. Three exposure matrices are being used: measured THM, predicted THM (based on correlations with chlorine residual), and calculated chlorine demand values. Spatial and temporal boundaries were assigned around sampling locations and subjects date of conception, respectively. Simultaneously, correlation equations between THM and calculated chlorine demand have been developed so that THM values can be predicted in areas where no THM monitoring data are available.

A multi-site study, based on the National Birth Defects Prevention Study, is being undertaken through an interagency agreement between CDC and EPA. The time line for the currently funded birth defects research, which continues through 2001, was discussed.

In addition, there is potential for expansion of the multi-site study to other participating Centers for Birth Defects Research and Prevention. The investigators also plan to conduct an exposure assessment study in Cobb County, Georgia (where the highest level of chlorinated THM in Atlanta occur) and in a selected county in Texas where the prevalence of brominated compounds is high.

Lynberg made the following points in response to questions from participants:

- Quality and amount of prenatal care has not been shown to influence the rate of birth defects. In addition, because exposure to DBPs occurs prior to prenatal care, this should not influence any observed birth defects risk.
- We are evaluating seasonality as an exposure sample. Some seasonality in certain birth defects is seen, but it is not a large contributor.
- Nutritional status is being evaluated in the main CATI interview with a 15 minute questionnaire.
- Nuckols and Singer are looking at bromide levels in the source water and variety of THM species formed at various sites across the US to assist in the planning of future studies.

New Jersey Studies

Judith Klotz, NJDPH, presented an overview, and the results of the New Jersey studies done for reproductive outcomes and drinking water contaminants [Attachment IV.E]. The first study included a wide variety of reproductive outcomes, while the second concerned only neural tube defects. The original study was published in the *American Journal of Epidemiology* in 1995. Neural tube defects were the most significant outcome related to increased THM (dose-response effect).

In the follow-up study there were 112 cases and 248 controls. However, addresses at time of conception could not be determined for 25 percent of subjects, but addresses at birth were known. Data on water source were obtained from the NJ Department of Environmental Protection and the local water companies, and water monitoring data were retrieved from the NJ DEP. Timing in the study was very important, because of the time of susceptibility for neural tube defects. Tap sampling for THMs, Haloacetics, etc. was done 1 year after the study period.

Haloacetics had insufficient data to draw conclusions. Haloacetonitiles showed no clear relationships.

The studies had different designs, and covered a different geographic area. However, they had showed similar associations for NTDs and THMs.

Toxicological Aspects of DBPs - Introduction

Fred Hauchman, EPA, presented a brief history of research on DBP toxicological data. Funding in these areas have been major thrusts for EPA's research program. The sciences of environmental epidemiology and toxicology should go together systematically in the future to look at biomarkers and mechanism of action.

Reproductive Toxicology: Overview of Current Science and Current and Future Research and Schedules for Completion

Gary Klinefelter, EPA, presented overviews of what we know about reproductive toxicology of DBPs and what is planned for the future, current testing strategies and pertinent results, and the importance of predictive mechanisms and biomarkers [Attachment IV.G]. In 1993 a panel of ILSI and EPA experts was formed to review epidemiology and animal data. The panel recommended that data gaps are filled by using screening strategies especially with fertility data, mode of action, and biomarkers. Data gaps still exist, but progress is being made.

Klinefelter covered the history of reproductive toxicology, the work of the Research Triangle Park (RTP) Drinking Water DBP research team, DBP testing strategy, NTP's 35 day reproductive and developmental screen, the test in males and results of other DBP toxicological research - see Attachment IV.G for more detail.

Klinefelter also made the following point in discussing the slides:

- Haloacetic acids perturb the normal events of sperm maturing. They also cause changes in sperm's motility and morphology[3] (3)

.

[3]Ability to move and the structure of the sperm.

- There are major differences in sperm production between humans and test animals. In humans at least 50% of the sperm can be abnormal before considering any effects from THMs.
- SP22 is a sperm protein that is predictive of fertility and may be a good biomarker. As the level of SP22 decreases in the sperm, the sperm loses motility.
- The RTD Drinking Water DBP Research Team Colorado Study will be the first study where chemicals will be studied to determine their effects on reproductive development in men.
- For the bromate in RACB study, NTP will be doing rigorous research to see if a 10% decrease in Bromate means anything. The bromochloroacetic acid in the two generations study will be the first EPA sponsored multi generational chemical study. It is hoped that an epidemiological study is applied to the Reproductive Epidemiology - SP22 Biomarker.

Haloacetic Acids effect male reproduction (fertility). Some effect at low doses, may be mediated through specific proteins. When we look at gestation through puberty these effects will be maintained. Brominated acids are more potent than the chlorinated species.

Developmental Toxicology: Overview of Current Science and Current and Future Research and Schedules for Completion

Sid Hunter, EPA, presented an overview of developmental toxicology literature and an overview of the current research program being conducted through the National Toxicology Program and EPA, National Health and Environmental Effects Research Laboratory including bromodichloromethane. Hunter also included an overview of Screening Studies (What questions can we ask?) and Mechanistic Studies (What are the questions?) [Attachment IV.H].

Hunter made the following points during his presentation:

- Critical effects differ for the THM in different animal strains. Bromodichloromethane administration results in full litter loss in the Fischer 344-rat but not in the Sprague-Dawley rat strain at an equivalent dose.
- Several of the haloacetic acids produced effects in rats, especially in the heart and the eye.
- For bromochloroacetic acid, there was a decrease in implantation and live births.
- For the NTP studies, a maximally tolerated dose was achieved. Therefore, the amounts of chemicals that are given to animals during these studies cannot be increased because an increase in chemical results in a decrease in water consumption.
- Bromodichloromethane (BDCM) research: at days 6-10 of gestation, full litter loss was seen in 75% of litters in the Fisher -344 rat. Susceptible period for structural malformations in rodent developmental toxicity studies is day 6-15 for teratogenicity. Major organ formation in humans occurs during 3-8 weeks gestation. The exposure period for BDCM that produces full litter loss in the rat corresponds to week 3 of human gestation.
- Whole embryo culture system was discussed. In vitro tests (including whole embryo culture) assess the effects of chemicals directly on the cell, tissue or embryo. For Hunter's whole embryo culture studies of the effects of haloacetates, he used a benchmark dose (equals dose to produce 5 percent increase in neural tube) to compare the potency of the chemicals.
- In vitro tests permit examination of parent chemical vs. various metabolites. Metabolites may be less toxic or equipotent to the parent and for several chemicals the metabolite is the active toxicant. For most environmental chemicals including DBPs, there is limited information about the temporal exposure to parent or metabolites in vivo. This is a drawback of the in vitro systems because the length of exposure may be a critical piece of information in understanding the toxic potential. Within the embryo, there are multiple target tissues that can result in a single malformation. Pathogenesis must be used to tease this out.

In response to questions from participants, Hunter made the following points:

- In developing the assay, we are not sure rats are more sensitive than rabbits to THM teratogens.
- Most of the current mechanistic studies will be complete for review by next March.
- Metabolites will be discussed in the studies.
- Some data on luteinizing hormone are available. DBPs will be tested as endocrine disrupters by OPPT.
- One way to translate between dose applied and occurrence is to translate the data into gallons per day to achieve the NOAEL dose, but understanding internal dose is a much more critical piece of information than administered dose. These are risk assessment and risk communication issues.

Panel Discussion on Characterization of Reproductive and Developmental Risk

John Reif (Colorado State University); Gary Kimmel, (EPA); Phillipe Daniel (Camp Dresser & McKee, Inc.); Fred Hauchman (EPA); Kirstin Waller (Sequoia Foundation); Judith Klotz (NJDPH); Gary Klinefelter (EPA)

Panelists were asked to answer the questions below in opening statements. Panelists then followed with an open discussion, including questions from the audience, of the DBP reproductive and developmental

risks. *The following is a summary of points made by panelists regarding the panel discussion questions, and other comments that arose in the course of open discussion:*

Question 1: Based on the current database, what observations can be drawn on the reproductive and developmental effects from exposure to DBPs? Is there biological plausibility that exposure to DBPs can attribute to the types of adverse reproductive and developmental effects seen in the laboratory and human studies?

- Epidemiological studies show an association between some DBPs and reproductive and developmental health effects.
- As we look further, we are continuing to see association between fetal growth parameters; same magnitude, weak association, fetal growth, birth weight.
- Confounding (from a strict epidemiological standpoint) is not a large problem.
- Misclassification is still not fully understood.
- Determinants of exposure and assessment are:
  - Tap water samples
  - Biomarkers of exposure, if not outcome
- Bromodichloromethane emerges as an important DBP from epidemiological studies.
- Brominated haloacetic acids are key from toxicological standpoint, and co-occur with other brominated DBPs.
- Spontaneous abortion and growth parameters (birth weight) are important from an epidemiological standpoint.
- Dose response is unknown; scientific results may not be sufficient for regulation development at this point-- dose response is not equal depending upon use of results. There is however, some evidence of the existence of dose response relationship.
- There is a plausible congruence of toxicological and epidemiological data; follow-up is required for causality, but there is an association. Evidence that is sufficient for scientific standpoint may not be sufficient for a regulatory or health protective standpoint.
- Different designs that are yielding consistent results strengthens our confidence in the study designs, and the data. More so than if all studies use a similar design.
- There is a better concordance between epidemiology and toxicology for development than there is for cancer.
- Biological plausibility is good because similar events occur between rats and humans (spermatogenesis).

Question 2: What are the largest remaining data gaps and uncertainties that need to be filled to better characterize the reproductive and developmental health effects from exposure to DBPs?

- Epidemiology: we currently have little to no data on alternative disinfectants.
- Toxicology: there are many unidentified compounds and mixed matches of HAAs
- Environmental epidemiology is an "impossible exercise" because of the complexity: Good tools are available, however, more are needed. The field is still in its infancy.
- Improved methods of exposure assessment are needed.
- More human information is required on all endpoints. Some endpoints have not yet been considered.
- Information on other DBPs and mixtures should be collected. Little data on alternative disinfectants (ozone and chlorine dioxide).
- Types of studies that might address data gaps:
  - Whole animal studies for mixtures pair with animals in field (on streams) epi zoology
  - Animal embryo studies occurring invitro, modify to investigate gene environments (folates) see what might modify effect of mixture of DBPs
  - Nested case control study: Once you find individuals affected by endpoints of interest detail work could be done on sample (biomarkers, residential history).

- We still lack information on DBP effects on reproductive development, mixtures, mechanisms, epizoology, and biomarkers.
- Studies have become more consistent in recent years; both data evaluation and results.
- Laboratory toxicological studies are being performed to identify specific endpoints.
- Protein endpoints are being developed as biomarkers.
- Dose response relationships are coming for risk assessments of individual compounds.
- Be careful about committing resources to wrong method, before the method is adequately validated.
- We should conduct a meta-analysis of the ten studies that have been completed.
- All exposure pathways, not just ingestion, should be considered -showering and other uses may result in exposure.
- Multi-generational study would address how you bring in RfD or effective dose for acute exposure endpoints and pregnancy sequella versus lifetime cancer risk outcomes.
- We can take data from generational studies to derive an RfD as long as we have dose response data. Cancer was of greatest importance in past, today this is changing. We are working on developing a complete integrated framework for risk assessment for all types of data to develop a hazard characterization for key endpoint(s). This is tied closely to mode of action, which requires a richer data set than was previously available.
- The data base is too small to address dose response relationships or causality.
- Unidentified compounds still exist in the mixtures (only THMs, haloacids and a few other classes have been characterized). Mixture dynamics are still unknown.
- Whole animal studies for mixtures should be paired with animals in field (epizoology).
- Whole animal embryo studies to investigate gene - environment interactions, e.g. folate.
- Nested case control studies should be performed-- detailed analysis of cases with endpoints).
- Associations for Haloacetic Acid data and reproductive results.
- Dose-response data for individual compounds versus structure activity relationship evaluations (resource issue).
- Alternate test methods for classes of compounds.
- SES issues and susceptible populations with respect to exposure.
- Randomized clinical trial to provide bottled water versus tap water for others. Testing ingestion only.
- Some data gaps could be filled through cross sectional design studies that may provide information on different water types and different DBP species.
- Comparison of two treatment processes from same source water or changes in treatment in a given community and do epidemiological study.

Other observations and discussion points (but no agreement)

- Physician awareness issue - there is an outreach effort to make health professionals aware of issues. Bottled water does not have standards that are superior to municipal water, so can we say with any certainty that DBP levels in bottled water are lower than in tap water? Waller found that 75% had non detectable THM, and 25% had a mean of 10 µg/L total THM. One sample had total THMs of 40 µg/L. Because events occur early in pregnancy, you cannot suggest that women drink bottled water, since they may not know they are pregnant when the fetus is most susceptible.
- Some OBGYNs do not think that there is currently sufficient information to offer patients guidance on drinking water risks (i.e., reproductive and developmental health effects). Presently, some counsel patients to drink bottled water if they perceive there is fear, since maintaining adequate fluid intake is of great importance. Physicians and other health care providers do not have a lot of information at their disposal. Pregnant women are a risk averse community - uncertainty is considered to be a risk.
- Are there consistencies between spontaneous abortions, birth defects, and cancer as they all have genetic component? They do not all have genetic basis. There are similarities between

cancer and fetal development - many controlling processes are similar. This is being examined by the EPA.

- Other causes of spontaneous abortions that may be related to infectious agents should be assessed to determine whether this is occurring through coordination with state health department. There are accepted epidemiological standards regarding how big odds ratios should be and about their confidence intervals. For example, there are certain risks that have high odds ratio - smoking is 10. With other events that are environmental you have an odds ratio of 1.5 - 3; this is a relatively weak association. Other confounders are not known. Many are statistically significant. Risks are low, but for public health the problems are important from a public health perspective - do THMs have a public health impact?
- We are moving away from using the concept of threshold versus non-threshold approaches toward a better understanding of mechanistic action. Recent cancer guidelines do not assume low dose extrapolation.
- In systems with free chlorine as a disinfectant, DBP formation depends on precursors that are present - some increase and some decrease. In systems that chloraminate, DBPs vary less (i.e., have more consistency - less spatial variability).

Summary of Reproductive and Developmental DBP Health Effects Panel Discussion

Phillippe Daniel, Camp Dresser & McKee, Inc., presented the following points in a summary of the key points from the Panel's discussion:

- Bromodichloromethane is a key compound from epidemiological studies and toxicological studies. Toxicological studies also indicate that other DBPs are important.
- Alternative treatment methods produce different DBPs, and there is a large cost associated with these treatments. Source water factors, such as Bromide, impact mixture species. The type of treatment also gives different mixtures, but data gaps hamper understanding mixtures since 50% of DBPs are uncharacterized.
- Precursor removal can increase risk by changing the bromine/total organic carbon ratio. If reproductive effects are real, then try for a little variation in the treatment technique. For instance, chloramine can be used.
- In the next year, and given the current data, priorities should be to: (1) Accelerate reconstruction of exposure which is needed to tease out the role of bromate and (2) show data and wrestle with uncertainty and values that must be considered.

Microbial: Background for Long Term 2 Enhanced Surface Water Treatment Rule

Pathogen Occurrence in Tap Water

Mark LeChevallier, American Water Works Service Company, gave an overview of what is known about occurrence of protozoa and viruses in drinking water and reviewed the current understanding of pathogen occurrence as it relates to distribution system maintenance strategies [Attachment V.A.1].

Data on protozoa in tap water are mixed with respect to protection for *Cryptosporidium* [Attachment V.A.2]. Monitoring data shows low levels of pathogens in tap water. Most systems rely on chlorine for disinfecting, but it is not effective against *Cryptosporidium*. Past outbreaks have been related to treatment barrier breakdown, except for Las Vegas. When barriers were replaced in these systems the disease was no longer evident. A broad survey was done in 1995 to determine protozoa existence in drinking water: 55 percent of plants were positive for *Crypto* oocysts. The meaning of this high rate of occurrence of oocysts in systems that had no apparent water associated cryptosporidiosis disease is unclear. The analytical method used in this summary (immunofluorescenct staining) does not indicate viability or infectivity so does not answer public health significance.

Epidemiological studies are difficult to interpret and associate to a particular pathogen or etiological event. Best evidence of pathogen occurrence would be a *C. parvum*[4] cell culture viability/infectivity assay which can be applied to drinking water monitoring which is (viable) in drinking water.

[4]C. parvum is a strain of *Cryptosporidium* which is known to cause disease.

LeChevallier described a project being conducted by the AWWSC to measure live, infectious *C. parvum* in the filter effluents of 80 surface water treatment plants. The two-year study will begin in the Spring of 1999 and be completed in 2002. Turbidity and particle count data will be collected to correlate breakthrough of live oocysts with treatment optimization.

Since 1995 about 4.5% of waterborne disease outbreaks were due to viruses. There are no methods available to culture all viruses (e.g. Norwalk) that are hazardous to public health. The data do not indicate that viruses survive when other pathogens are removed and inactivated. Phage was found in most surface water supplies. ICR data showed that 13% of raw water samples taken were positive for viruses (1 - 28 Most Probable Number/100 L). All of the finished water samples (30) were negative.

Additional treatment barriers to protect drinking water are:

- Watershed protection: It may only reduce, not eliminate *Cryptosporidium*.
- Improvement of filtration and filter variation by using two stage filters or membranes.
- Improving the disinfection barrier: It may reduce *Cryptosporidium* by 1 or 2 logs, although recent research indicated that a 4 log reduction can be achieved by UV light disinfection.

LeChevallier reviewed a list of newly emerging organisms [Attachment V.A.3]. *Mycobacterium avium* is high priority because it is chlorine resistant (it is acid fast, waxy, and not water permeable) and it can regrow in biofilms after treatment. It normally infects immunocompromised persons (e.g. AIDS patients) when it is ingested. The relative contribution disease of *M. avium* from drinking water is unknown.

Preventing contamination should be a priority. Pressure fluctuations in the distribution system can cause contaminated water to leak into the system. Many factors influence water pressure and can cause fluctuations. Water leakage out of the distribution system of 10 and 40 percent is not uncommon; water leakage in to the distribution system can not occur if positive pressures are maintained, but this is not always the case. Hydraulic modeling can be used to understand pressure surges. High speed pressure gauges can detect these pressure transients.

LeChevallier also discussed the effect of monochloramine disinfection on the risk of nosocomial Legionnair's disease (see Attachment V.A.4).

The following points were discussed by LeChevallier in response to questions from participants:

- What is the relationship between turbidity and particle counting?
- Particle counts are more sensitive than turbidity measurement. Particle counting can be a tool, but this measurement is unreliable.
- The AWWSC project is being conducted to determine how current treatment standards are for protection against *Cryptosporidium.* In essence, can the organism pass through a filter in a system that was in compliance with a turbidity level at 0.3 NTU, 0.1 NTU?
- The viability of oocysts is not determined in Method 1622. DAPI staining is used to show whether DNA is inside but this is not an indication of viability. Others have used PI exclusion, but this technique does not correlate to animal infectivity and is difficult to read.

- AWWSC believes that primary treatment is effective at removing *M. avium* given our limit of detection. Coagulation and filtration should impact *M. avium* because they are hydrophobic (waxy)
- AWWSC is currently 5 - 20 years away from using sensors on a real time basis in distribution system, the sensors that are available today may not be reliable. This monitoring technology could be added to an automatic meter reading system.
- The size of *Mycobacterium* is 1 by 5 um.

Overview of Types of Health Effects Data (Microbes) and How Data is Used in Decision Making

Stig Regli, EPA, reviewed the major regulatory questions for Stage 2 microbial rule and discussed information used to assess risks from pathogens and how this influences regulatory criteria. Regli also covered the relevance of waterborne disease outbreak information to development of the regulations, reviewed how various types of epidemiology studies can contribute to regulatory decision making, and presented an overview on how dose response data influences regulatory focus, and how is it used in regulatory impact analysis [Attachment V.B].

In 1994 stakeholders involved in the regulatory negotiation agreed to:

- Propose multiple Interim Enhanced Surface Water Treatment Rule (IESWTR) options for systems serving greater than 10,000 people.
- Propose LT2ESWTR for systems over 10,000 when more data became available.
- EPA committed to augment research.
- Utilities committed to collecting ICR data.

In 1997 EPA convened another FACA to develop recommendations on how to best move forward with the IESWTR in the absence of ICR data. As a result of these deliberations, EPA issued a NODA in November 1997 and promulgated the IESWTR in December 1998. EPA is also developing a LT1ESWTR that will pertain to systems serving less than 10,000 people. The major focus of the IESWTR and LT1ESWTR is to improve performance and reliability of physical removal of pathogens through tighter turbidity standards.

Major regulatory questions for LT2ESWTR include:

- What risks remain subsequent to implementation of the IESWTR, LT1ESWTR, & Filter Backwash Rule?
- What uncertainties can we accept in estimating risks from treatment deficiencies and distribution systems problems following implementation of IESWTR, LT1ESWTR and Filter Backwash Rule?
- What level of risk from drinking water are we willing to accept?

Regli made the following points in response to questions from participants:

- EPA is using infection as endpoint, which is a conservative indicator for illness. Also, the incidence of secondary spread is added depending on the fraction of people believed to be affected.
- The main issue with cross referencing actual waterborne disease with the prediction of 300,000 cryptosporidiosis cases (sick people) is not knowing the rate of unreported disease. The basis for 300,000 cases was derived from estimates of source water occurrence and treatment efficiency and applying a dose response curve to the estimated finished water *Cryptosporidium* occurrence.
- What is lacking from the emerging pathogens program is a formal risk assessment. The National Center for Environmental Assessment (NCEA) is currently developing a formal risk assessment, including costs and a microbial risk assessment.

- To be conservative, EPA considered protecting at the genus level in developing the IESWTR - we do not yet know the extent to which species other than *C. parvum* may be infectious, so we are on the side of protection by specifying a maximum contaminant level goal for the genus - *Cryptosporidium*. EPA believes that this approach is consistent with the SDWA. Guidance manuals will be released with the IESWTR.

Epidemiology: Overview of Current Science Including Sensitive Subpopulations

Dennis Juranek & Deborah Levy, CDC, discussed the use of epidemiology in understanding microbial risk [Attachment V.C]. Levy began by presenting an overview of the nation's waterborne disease outbreak surveillance system. For passive surveillance, unit of analysis is outbreak not single cases and there are many factors that affect whether waterborne disease outbreaks are recognized and investigated by state or federal health agencies.

In many outbreaks, it is common for the etiological agent to be unidentified. Fifty (50) percent of outbreaks come from a well water source; 31.8 percent of outbreaks come from surface water. Actual likelihood of outbreaks being detected and reported is effected by the size of the outbreak, severity, public awareness that an outbreak is occurring, investigator interest, and resources of local health department. Surveillance system sensitivity is probably low. Many factors contribute to not reporting, such as: a person not going to a physician when ill, lack of physician awareness, lack of lab testing and confirmation, lack of reporting by the physician, lack of timely review by local health dept, etc. Hence, there is probably more waterborne disease occurring than is reported.

The Foodborne disease active surveillance network (Foodnet) included questions about water. It was done through random phone calling of the general population, physicians, and labs. The survey revealed that 11 percent of the population had diarrheal episodes (1.4 episodes/year); of these 8 percent visited a health care provider and 2 percent provided a stool specimen. The probability of the general population being tested for *Cryptosporidium* is related to the specialty of the practitioner. An infectious disease practitioner is more likely to test for *Cryptosporidium*. Doctors generally assumed that the lab would automatically test for *Cryptosporidium*. Only 5 percent of labs routinely test for *Cryptosporidium* or cydospora; 50 percent routinely test for *E. coli* oocysts; none routinely test for viruses.

Payment waterborne disease studies estimated that 14 percent of diarrhea was attributable to waterborne illness. The results suggested that the distribution system was a potentially significant source. Extrapolation of this data to the US population suggests that there may be as many as 50 million cases of diarrhea per year.

The risk of *Cryptosporidium* the drinking water is unknown; it depends on the quality of source water and the efficacy of treatment facility. The risk of infection depends upon immunity and behavior. The risk of developing severe disease depends on immune status. The populations at risk of severe disease from *Cryptosporidium* are: AIDS patients, persons with congenital defects in the immune system, and those taking immunosuppressive medications. Age alone is not an indication of increased risk. In Milwaukee, the elderly were less likely to be infected, but they were more likely to be hospitalized. Secondary spread of *Cryptosporidium* is most common from 0 - 2 year old children.

Juranek made the following points in response to questions asked by meeting participants:

- In the Payment study, the disinfectant was ozone followed by low levels of chlorination to provide a disinfectant residual in the distribution system. However, there was often no detectable chlorine at many points in the distribution system.
- Passive surveillance does not pick up outbreaks very well. We are trying to determine which tracking methods work for what conditions (e.g., antidiarrheal medication sales, nursing home reporting).

Microbial Pathogens and Epidemiology: Current and Future Research and Schedules for Completion, EPA/CDC/AWWARF Household Intervention Study

Rebecca Calderon, EPA, presented an overview of current and future microbial pathogen and epidemiology studies [Attachment V.D]. The goal of these studies is to look at surveillance to determine what human disease is occurring in the population, and the relationship with pathogen occurrence. Two questions need to be answered about endemic illness regardless of etiology and individual pathogens.

Calderon discussed the following studies:

- The Community Intervention studies enteric disease rate will be determined before and after changing treatment. Relative source, contribution of water vs. food, and human contact will be assessed to determine the extent to which these factors may be relevant to contributing to disease.
- The Two-City Serological study looking at filtered surface water versus high quality ground water in a serological study.
- The Estimating Incidence of Waterborne *Cryptosporidium* study using HMO nurse hotline as a reporting mechanism.
- The susceptible population projects focusing on susceptible subpopulations including children and HIV positive persons.
- The infectious dose studies focusing on patient exposure and monitoring. Animal models are also being used.

There were 22 identified waterborne outbreaks in 1995-1996 in the US. In 34 percent of the cases, the etiological agent was not identified. International projects do not depend as much on monitoring and reporting as in the US. Studies are planned or ongoing in Great Britain, Italy, New Zealand and France.

Epidemiology: The Use of Serology to Study Prevalence of Cryptosporidiosis Due to Drinking Water

Jeff Griffiths, Tufts University, presented an overview of serology studies to estimate occurrence of cryptosporidiosis due to drinking water, and the Pediatric Serologic epidemiology study comparing populations served by unfiltered water from protected watershed and filtered water from unprotected watershed [Attachment V.E].

There is no one method that is superior or complete for assessing *Cryptosporidium* exposure. All tests seem to underestimate exposure and, hence, are conservative. Serological methods are important because they permit comparisons among studies. *Cryptosporidium* is a complex parasite B immunity is short-lived. Once infection occurs, T cells and gamma interferon are crucial to recovery. Antibodies are helpful, but are not crucial to recovery. The Western blot and ELISA serological testing methods are used to detect infection. The Western blot may be the superior testing method, but it is costly and technically complex. No one method gives you the complete picture.

In the Providence Rhode Island data the serological testing revealed a correlation between oocyst antibodies and age. Over 50 percent of children aged 1-2 had oocyst antibodies and/or tissue changes. In Massachusetts, serological testing in children was able to occur because the department of public health had obtained blood samples. The zip codes of the children were known, so that the water source and supplier could be identified. The tests revealed that those in protected water supplies were less likely to have anti-oocyst antibodies (IgM, IgA, & IgG). It was predicted that only 1 in 10,000 infections are reported (range is 3,000 - 40,000). The results of this study supports the idea that we should have a protected water supply that is free of animal runoff or human sewage.

Other important unanswered questions/issues for *Cryptosporidium* exposure include:

- There is very little data on children, or other susceptible populations
- Is there one life-long marker of past *Cryptosporidium* exposure in past?
- We need prospective comparisons of the different serological techniques.
- Need to better understand the relationship between seropositivity and clinical disease.
- We need to link serological data with water data, e.g. what is the linkage between actual waterborne *Crypto* and a concrete marker of exposure.

Jeff Oxenford, AWWARF, presented the results of a two-city study that compared the rates for *Cryptosporidium* infection between populations being served surface water vs. ground water. Approximately 500 sera samples were collected from blood banks in each city and were analyzed by the Western Blot technique. Detail surveys were also obtained to identify other possible exposures. The project included extensive QA/QC procedures and received extensive review.

Studies results did show a significant different between the serological response in two cities with the surface water city having a higher response than the ground water city. Weak correlations were found with age, travel, gender, working or school located in another city, and using an alternative water source. No correlations were found between serological response and episodes of diarrhea, handling livestock, drinking untreated water or swimming.

What are the implications of the study? While a difference was observed between the two-cities studied, caution must be maintained in interpreting the results. Questions that remain include: 1) What is the endemic level of *Cryptosporidium* infection in the U.S.? Were infection rated in these two cities within ranges typically found? 2) Will this relationship hold for other cities? 3) Were there other sources of *Cryptosporidium* that could account for the differences? 4) What is the relevance of serological response to disease?

In response to questions from participants Griffiths and Oxenford discussed the following points:

- No level of antibodies is completely protective - only partial protection is conferred.
- The Two city study used blood banks for sources of sera (500 samples/city) and a questionnaire to assess risk factors and demographics (e.g., children in day care, diarrhea, pets/livestock, bottled water, travel outside US, swimming in lake, stream or pool?)
- The Two-city study used Western blot serology method measuring infection, NOT disease.

Time Series Studies on GI Illnesses Related to Turbidity

Bob Morris, Tufts University, presented time series studies and their use [Attachment V.F]. Waterborne disease outbreaks are, by nature, time series events. An outbreak is characterized by a period of contamination of the water supply followed at some time lag by an increase in disease in the community served by that water supply. In order to understand how time series studies fit in with other methods for studying waterborne disease it is useful to look at the mechanisms by which pathogens spread in the community and how available methods assess the role of water in that spread.

The pathways by which fecal-oral pathogens (i.e., GI pathogens spread by ingestion of fecal material) spread are depicted in Figure 1, Attachment V.F.. Waterborne pathogens reach host populations from the distribution system. Once hosts are exposed, they may become infected and develop symptomatic disease, and may, in turn, spread the disease through direct contact, or through contamination of surfaces, food, or swimming pools. Thus, cases of infection and disease caused directly by exposure to contaminated water will tend to be amplified through secondary spread of the pathogen. This has important implications for studies that focus only on disease caused directly by the spread of pathogens in drinking water since they will not include the effects of amplification outside of the home.

Figure 1 also depicts the host transitions that occur in a person following exposure to a pathogen. The exposed individual may or may not develop an infection and, if infected, may or may not develop symptomatic disease. The relationship between exposure and disease depends upon the characteristics of the host (e.g., immune status, nutritional status, age). A person with symptomatic disease may or may not seek medical care and, in turn, may or may not be tested, diagnosed or reported. Consequently, studies of waterborne disease that rely on passive surveillance data will only characterize a small portion of the symptomatic disease.

Recently introduced time series methods consider the incidence of disease in the entire community as a function of changes in drinking water quality over time. These studies can utilize any sort of data describing exposure and a relevant health outcome as time series. The ability to use routinely collected data such as HMO or emergency department billing data to describe the occurrence of gastroenteritis allows these studies to be conducted relatively inexpensively without requiring the use of passive surveillance data which has the limitations described above.

The time series method was first used in 2 studies (Morris et al.) of the 1993 waterborne outbreak in Milwaukee which looked at emergency room visits, gastrointestinal events, and water treatment plant's turbidity levels before the outbreak to determine if gastrointestinal events and turbidity levels were correlated. A non linear model - temporal exposure response model (TER) was used for data analysis. A time lag between increased turbidity levels and the onset of a gastrointestinal event was factored in (a time lag between increased turbidity and onset of gastrointestinal event was noted at 6-7 days with a second peak occurring about 1 week later).

Results show that there may have been a low level contamination prior to the 1993 outbreak - this was particularly evident for children. A subsequent study conducted in Philadelphia by Schwartz *et al* had similar results.

The advantages and disadvantages of time series studies mentioned by Morris are summarized below:

Advantages:

- Low cost (can look at many cities).
- Short time to completion (could use historical data).
- Can directly relate to indicators used in standard setting (could use changes in turbidity or other indicators that are regularly measured).
- May provide tool for long term surveillance (not expensive to maintain information collection system once it is established).
- Could extrapolate findings to National Estimate if method were validated by detailed study in a few cities and then employed in a larger number of cities.
- Confounders would have to be correlated in time with water quality (temporal changes).
- Can be used to study factors related to water source and water quality that influence the association between monitoring data and disease.
- Lots of things will cause GI illness but these studies should pick up those that are correlated specifically with drinking water quality.
- Analysis of the specific time lag between changes in water quality and changes in a health indicator may be related to the incubation period for the infectious agent and may be useful in identifying the responsible pathogen.

Disadvantages:

- Does not relate individual exposure to outcome.

- May be difficult to isolate effects of the distribution system. Stratification of the population by distance from treatment plant might give some indication of this effect. (Note that this is not the only study design that has this problem).
- Rare events difficult to analyze.
- Stage I IESWTR may reduce efficacy of current surrogates for water quality such as turbidity.

This tool can be used retrospectively and prospectively. You can look at historical data to establish a signature for a given pathogen, and once you understand the relationship, you may be able to predict the impact of a change in water quality. Improvements in exposure assessment and measurement of health outcomes can improve the sensitivity of this technique. A recently funded CDC study will use serological data as the health outcome with the data analyzed as time series to give a pathogen specific time series study.

Dose-Response: Overview of Current Science

Charles Haas, Drexel University, presented an overview of how dose response studies are conducted, their limitations in study design and what general conclusions that can be drawn. Haas also discussed what is involved with extrapolating dose response data from healthy populations to sensitive subpopulations, and from high dose to low dose [Attachment V.G].

A "typical" dose-response study does not consider minimal infectious dose, but median infectious dose. Multiple endpoints, multiple outcomes, number of infections, and number of subjects that develop illness, are measured for each dose group. Models should be biologically plausible. Threshold approaches to model dose response should not be used. No human data substantiate models that allow thresholds and there is no biological plausibility for these models.

Morbidity data is more scarce than infectivity data. The fraction of people that are ill once infected is simple to calculate, since there is no dose dependency. This is true for most pathogens except for *Vibrio cholera* - which has dose dependent relationship with morbidity.

Remaining questions include:

- How should we measure multiple pathogen exposures (consider each day as single statistical period separately) and immunity?
- How should we measure sensitive populations - sensitivity or severity issue?
- What is the acceptable risk level?
- Is there variability between strains (issue with *Cryptosporidium* but not *salmonella*)?
- What is the suitability of animal models (analogous to chemical risk assessment studies; animals may explain pathobiology and generate dose response data)?

Cryptosporidium parvum Volunteer Study

Cynthia Chappell, University of Texas-Houston, presented an overview of the *Cryptosporidium parvum* (dose-response) Volunteer Study. The objective of the study was to:

a. determine the natural history of the *C*. *parvum* infection in healthy adults;
b. determine the infectious dose of *C*. *parvum*; and
c. delineate the immune response to *C*. *parvum* infection in infected hosts.

Volunteers in the study had to be negative for specific anti-*Cryptosporidium* antibodies by ELISA, healthy (not IgA deficient and they had to have a certain number of T cells), and they could not have elderly persons or children under 2 years of age living in the household. Additionally, they had to pass a written examination about the organism and the study. The study population was mostly Caucasian, but had

representation in Hispanic, black and Asian ethnicities; consisted of males and females, and the age range was 18 - 50 years (mostly 25 - 35). To date there are about 110 study volunteers.

The challenge dose was prepared from calves - genotype 2 isolates. The route of administration was via gelatin capsule. Stool was collected for a total of 6 weeks: on a daily basis for the first 14 days and three times a week for the next 4 weeks. A physical exam was done daily for 2 weeks and twice weekly for the next 4 weeks. Each volunteer kept a personal health diary to record any and all symptoms experienced during the study, including gastro-intestinal symptoms.

The duration of the infection was 1 - 11 days (median 6 days). Mean fluid loss was 1180 grams with a maximum fluid loss of 2846 grams over 5 days. All cases were self-limited and cleared the fecal oocysts (detected by direct fluorescence). 88 percent of the volunteers cleared their infections within 14 days; one excreted organisms for 38 days. The incubation period was approximately 5-7 days and did not vary significantly among isolates.

Susceptibility to re-infection was tested: 19 individuals were re-challenged with a single dose of 500 oocysts. Total oocyst shedding from re-infection was decreased dramatically. The prepatent period or duration of oocyst shedding were not significantly different from primary challenge, and the illness attack rate remained the same (58%) when compared to the original infection. However, there were fewer unformed stools after re-challenge compared to the original infection.

Serologically positive volunteers were selected on the basis of IgG reactivity. These individuals developed infection and illness, but required much higher oocyst doses to do so. ID50 in these individuals was approximately 20-fold higher than in seronegative subjects. Also, the number of oocysts shed and the number of infected individuals shedding oocysts were significantly decreased, indicating a much reduced potential for secondary transmission. Antibody positive volunteers had longer diarrhea duration compared to antibody negative volunteers, indicating that seropositive people exposed to high levels of oocysts may have a more severe illness than those who were antibody negative. The reason for this observation is unclear, but may indicate an enhanced mucosal response to the infection. In these individuals, diarrhea may be considered part of the protective response- that is, an enhanced shedding of infected cells and removal of parasites via the stool. However, no increase in total stool weight was noted.

These studies have provided many tissue and oocyst samples for continued studies. Next research steps will be the examination of genotype 1 isolates, as well as non-*parvum* species studies (important for immunosuppressed patients).

In response to questions from participants Chappell made the following points:

- Volunteers remained serum IgG negative after receiving a primary challenge, but about 70% converted for IgM, Prior to rechallenge, none had IgM remaining, but about 33% developed IgG following rechallenge. Thus, seroconversion for IgG may require multiple exposures."
- We have verified antibody (+ or -) by multiple methods and find that the immunoblot confirmed this, but the immunoblot differs from the ELISA results. There is no absolute method for confirmation of infection, so the interpretation and validation of the serological tests are hampered. In the future, PCR methods may help to identify persons with very light infections.
- However, serological assays may always suffer from some limitations. We have studied secretory antibodies from stool samples of our volunteers and saw strong correlation between oocyst excretion and *Cryptosporidium*-specific fecal IgA. Thus, secretory antibodies may provide a more useful marker of active or recent infection in outbreak situations.

Studies on the Infectivity of Norwalk and Norwalk-Like Viruses

Christine Moe, University of North Carolina, discussed the infectivity of Norwalk and Norwalk-like viruses [Attachment V.I]. Norwalk virus (NV) and related small round structured viruses (SRSVs) are the leading

cause of epidemic viral gastroenteritis and are important waterborne and foodborne pathogens. Risk assessment for these viruses in water and food requires data on dose-response.

A double-blinded human challenge study was conducted to determine the dose-infectivity relationship for NV in healthy human volunteers with various levels of pre-existing anti-NV IgG. The study was conducted in 3 rounds. Each round had 15 subjects randomized to one of 3 doses of safety-tested NV inoculum which was tittered by electron microscopy and endpoint titration RT-PCR. Subjects were monitored for gastrointestinal (GI) symptoms for 5 days post-dosing and returned for Day 8, 14 and 21 follow-up visits. Viral excretion was determined by RT-PCR, and seroconversion was measured by enzyme immunoassay. Six doses, ranging from approximately ten (10) PCR detectable units (PDU) to 1 x 10^7 PDU, were examined with 5 or 10 subjects per dose. Overall, 20 subjects became infected: 19 excreted virus and seroconverted and 1, at the lowest dose, seroconverted but had no GI symptoms or detectable NV RNA in stools.

The predominant symptoms were nausea and vomiting, and the duration of illness was less than 48 hours. Although the illness associated with infection was mild, about 50% of infected subjects reported that they could not have gone to work during their illness. Viral shedding was typically for 6-8 days post-dosing (maximum 20 days). The highest infection rate (70%) was observed among ten people who received a mid-range dose, suggesting that response to NV ingestion may be related to host factors as well as dose. Pre-existing IgG antibodies to NV did not protect against infection. The data did not show a simple dose-response relationship. The best fit to the data was with a two-population beta-Poisson model that modeled the dose-infectivity relationship for subjects with pre-existing IgG separately from those without pre-existing IgG. Subjects with pre-existing IgG were infected at lower doses and infection was related to dose. The results of this study will be valuable for estimating the risk of NV and related SRSV infection associated with exposure to contaminated water and food and to establish exposure limits for SRSVs to reduce waterborne and foodborne disease. Further studies of low NV doses with a larger sample size are needed.

Panel Discussion on Characterization of Microbial Risk

Mark LeChevallier, AWWSCO; Dennis Juraneck, CDC; Charles Haas, Drexel Univ.; Jeff Griffiths, Tufts Univ.; Stig Regli, EPA; Stephen Edberg, Yale Univ.

Panelists were asked to answer the questions below in opening statements. Panelists then followed with an open discussion of the risks related to microbial contamination in drinking water, including questions from the audience.

Question 1: What is the public health risk associated with microbial contamination?

- Hydration is critical to public health and access to high quality water is important.
- Data from epidemiological studies suggests that microorganisms are capable of reaching the population and responsible for causing a substantial component of the disease we see.
- We need to understand the relative risk of waterborne pathogens (relative to food, other sources/variables)?
- Microbes are predominant risks in water - it is easy to see the benefits of risk reduction for the consumer. For example, if there are 400 million cases of gastrointestinal illness, if only 1% are attributable to drinking water (which does not appear unreasonable) then there are 4 million cases per year would be attributed to drinking water - a significant public health risk. Seropositive data from epidemiology studies evaluating exposure from *Cryptosporidium* suggests that the attributable risk of infection from drinking water in systems using surface water may be significant.
- Balancing microbial risks and chemical risks is very important yet difficult. We must try to draw a relationship between the microbes and the DBPs.
- Exposure does not necessarily confer long-term protection. Re-exposure can cause subsequent illness. Also different strains have different degrees of infectivity. There is no evidence of a

threshold existing for any isolate; i.e. some probability of infection can be assumed for any exposure level.

Question 2: Source water related? Distribution system related?

- Both source and distribution system related risks are important.
- Microbial contamination is likely related to source water and distribution system sources. We will probably see the contribution of the distribution system component increase in the future.
- Risk is immediate and high if treatment barriers are compromised, due to high volume of contaminated water and the large number of people exposed.
- Communities with highly contaminated source waters will have to be addressed.
- Imperfect methods of removal may result in both source and distribution related endemic disease, whereas outbreaks are likely due to treatment deficiency. About 50% of HIV positive people do not know they are HIV positive, so immunostatus of population is not always known.
- Patchy data exists on distribution system related outbreaks. Risks from distribution system may be more important than risks from inadequately treated water - e.g.; sewer lines adjacent to distribution system lines may be a much bigger issue than poor quality source water, depending upon how well the distribution system is managed. The distribution system is a contributor, possibly greater on the East coast, because the systems are in order.
- Source water maybe more immediate, higher volume source of risk. Risk in distribution systems may be less obvious, more chronic, less prone to trigger a violation, and may overtime be a bigger source of cumulative risk.
- We need to understand chronic endemic exposure, including distribution system problems.

Question 3: What are the key pieces of information needed to estimate risks?

- The odds ratios in existing studies are very low; studies need to be better funded or results are muddied.
- Gastro-intestinal immunity differs from systemic immunity-- age is not a clear indicator of immune status.
- Infection is related to: the number of microbes, virulence characteristics, and virulence by modifying the immune status of the host.
- How safe is safe? Can you make water treatment cost effective for most? What about for immunocompromised population? It may be much more cost effective for these populations to take additional steps to protect themselves rather than through centralized treatment.
- Long-term sequellae (i.e., chronic health effects) are important - pancreatic disorders and Type I diabetes mellitus may be associated with gastro-intestinal illnesses. 1.4 cases per person per year of diarrhea per person per year are not bad - temporary immunity may be significant, but long term sequella are unknown. We do not know if there is there any association between sequella and exposure frequency. Sequella are likely related to a small percentage of the infected population with specific immune system characteristics.
- How does endemic waterborne disease compare to the overall realm of endemic disease?
  - 1.4 cases of diarrhea per person per year is reported of unknown etiology.
  - Need to reproduce waterborne disease epidemiology studies in different parts of the country so that we have an accurate national picture.
- Develop inexpensive, simple panels of tests: bio, physical, chemical test to determine if microbes are in the water.

Question 4: To what extent may there be risks to sensitive Subpopulations?

- Outbreak data and endemic data (time series studies) suggest that there is a low level background cryptospordiosis infection rate.
- We need to take into account the population served, not just water supply characteristics (immunocompromised populations, those who don't know they are immunocompromised.)

- Sensitive Subpopulations
  - Define them, levels of immunity, deficits, what do they react to?
  - There may be particular risks to sensitive subpopulations from pathogens in the water, however can be limited due to behavioral adaptation.
  - We need to define immunologic status for each organism, the risk for individuals acquiring infection, or for being susceptible to disease.
  - Yes, need to target, but think about how to address (question develop standard for all populations?)
- There can be risks, but they are limited. Risks to children and hospital populations are real. Who do we regulate for? You must define immunological status for each organism (*Cryptosporidium* vs. *Giardia*). Subpopulation sensitivity has 2 facets: risk of infection and disease, and risk of recovery from illness: dose susceptibility versus severity susceptibility.

Question 5: To what extent is research available to address this question? (What further research is needed?)

- Pierre Payment data suggests the need for follow up with other household intervention and monitoring data. Incidence of gastro-intestinal illness is likely on the order of millions per year.
- The impacts of immune status on infectivity needs to be explored. It is possible that we will find pockets of communities with much incidence.
- There remains an open question of how incidence of disease attributable to source water compares to that attributable to distribution system sources.
- The risk assessment and infectious dose are critical pieces, but they tell us about how we respond to the organisms. More important to identify the presence/absence of the organisms and how they exist in the water.
- The behavior of the organism in the water will indicate what to do about the organism.
- Invest in methods to look for live *Cryptosporidium* in drinking water.
- Increased monitoring and research in distribution systems and treatment plants.
- Technology transfer is needed from specialized research labs to utilities.
- ICR will give EPA public health data, but not organism specific data, and will not tell utilities what to treat for.
- Organism specific studies are expensive and difficult to execute.
- What data do we have now, and what will be available in the near future:
  - Fraction of *Cryptosporidium* that are *parvum* and that are viable (% of total counts).
  - Estimation of inactivation efficiencies.
  - Estimation of filter performance.
  - AWWSCo will test all their plants next year.
  - Explore benefits of other disinfection practices.

Question 6: To what extent do you believe it is appropriate to use Cryptosporidium as a target organism for controlling pathogens in general?

- Cryptosporidium strain differences may result in several orders of magnitude differences in infectivity.
- Investment monies should be focused on identifying live *Cryptosporidium* in drinking water.
- Culture change is needed for utilities regarding the use of technology to detect *Cryptosporidium.*
- Indicators for microbes in the water and indicators for success of treatment are needed. Not for all water sources because with certain waters (groundwater for example) *Cryptosporidium* may not be relevant.
- There is a need to determine the level and potency of disease causing organisms.
- *Cryptosporidium* will not work for distribution system. There is a need to focus on other target pathogens (e.g. regrowth of coliform).
- Viruses will need to be addressed (proximity of water and sewage lines). *Cryptosporidium* appears to be a good target organism for defining the adequacy of treatment on surface water

supplies. However, adequacy of treatment also must consider viruses because if systems provide adequate removal for *Cryptosporidium* by physical removal, this may not be adequate for viruses. Adequate treatment criteria might include some total removal, inactivation requirements for *Cryptosporidium* and some minimum inactivation requirements for viruses.
- *Cryptosporidium* is the best pathogen to determine disinfection effectiveness, but watch out for other organisms that may arise.
- Risk assessment approach that involves indirect measurement are not applicable-- you need to measure infectious organisms.

Other Observations

- UV might be effective on *Cryptosporidium*, but higher doses may be needed for *Giardia.*
- Get the methodology and technology transfer from specialized labs to people in the field. This may not be a market driven investment.
- ICR is problematic and questionable because of a lack of viability assay. For example the Sidney, Australian scare-- 3 boil water alerts. No detectable increase in number of observed cases. Using risk assessment down to 5th decimal place may be questionable. At best one order of magnitude.
- To achieve the safest drinking water possible, you better develop simple panels of testing to determine whether treatment processes are working. WHO stated that simple inexpensive test done frequently are superior to infrequent expensive tests.
- Reliability is a key issue, but in the context of level of loading-- degrees of contamination of source water and reliability of the treatment (e.g., turbidity-- similar tools for disinfection are needed). More studies are needed on risk attribution.
- Ability to quantify endpoints to determine level of comfort. Organism specific information is important to permit targeted treatment by the facility, but such data are expensive and difficult to interpret.
- Benefit of other disinfection practices should be evaluated, such as UV data.
- CDC and EPA will continue working together. The coordination has been solidified by *Cryptosporidium* studies. Further interaction with researchers and state health departments will also continue.
- Relative importance of Norwalk virus should not be overlooked. Very infectious and reinfection occurs.
- Research opportunities are available for next 6 months to be completed within a year - input is needed; these should be sent to Abby by E-mail: **(AARNOLD@RESOLV.ORG**). What is research need and time line. These proposals will go to M/DBP council.

Closing Remarks

Cynthia Dougherty, EPA, thanked all of the participants for taking part in the workshop. Dougherty reminded participants that their present efforts need to be focused on short term needs to support decision making, and policy conclusions from this workshop. Beginning with the March 30, 1999 M/DBP Stage 2 Federal Advisory Committee organization meeting, participants will begin to discuss policy conclusions the EPA should draw from the data presented. Dougherty thanked all participants for their contribution to the success of the Workshop and their continued efforts and adjourned the meeting.