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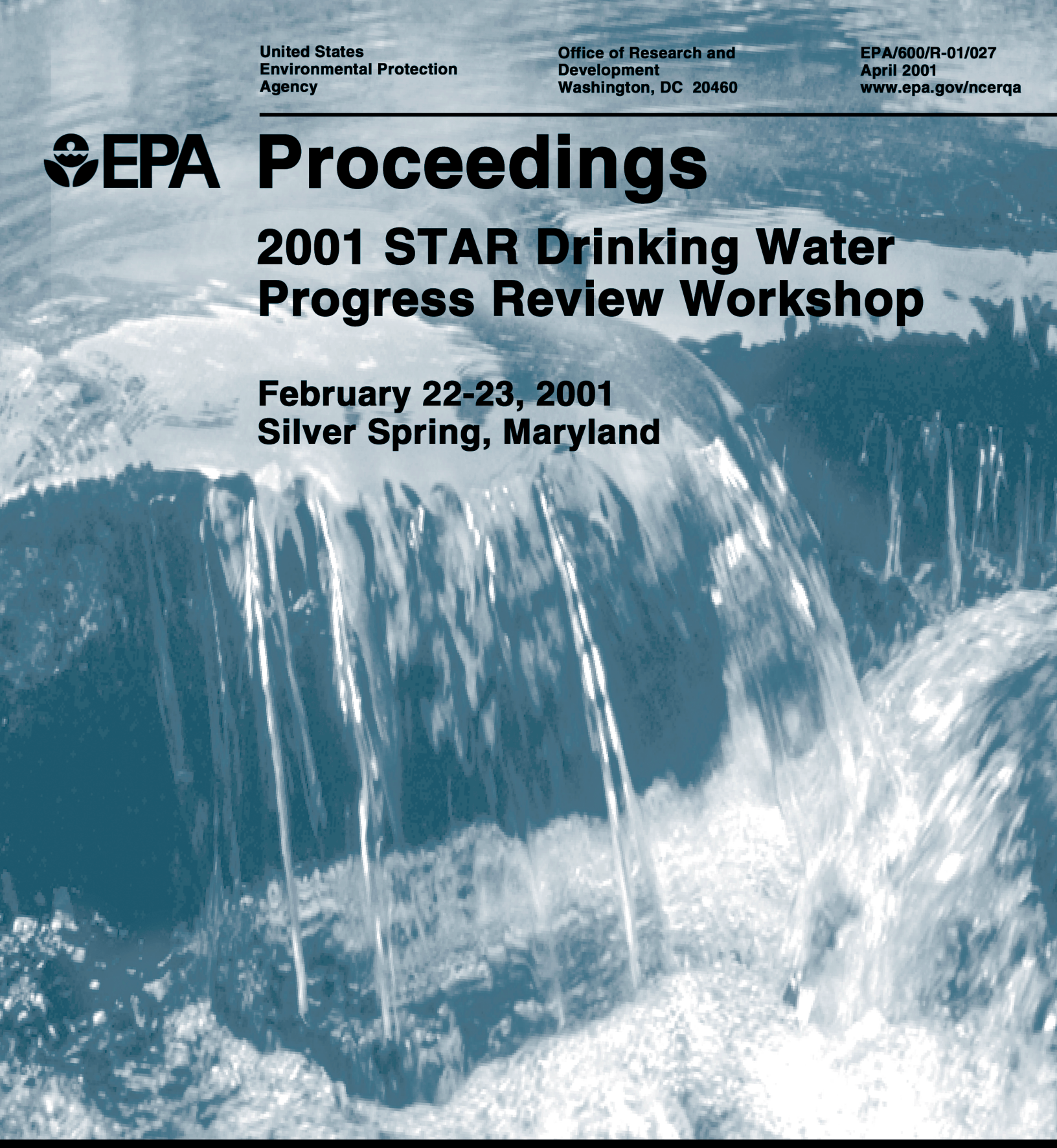
Proceedings

2001 STAR Drinking Water Progress Review Workshop

February 22-23, 2001
Silver Spring, Maryland



NATIONAL CENTER FOR ENVIRONMENTAL RESEARCH

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Introduction

The mission of the U.S. Environmental Protection Agency (EPA) is to protect public health and to safeguard and improve the natural environmentCair, water, and land. Achieving this mission requires applying sound science to assessing environmental problems and evaluating possible solutions. The National Center for Environmental Research (NCER) at EPA is committed to providing the best products in high-priority areas of scientific research through significant support for long-term research.

One high-priority research program identified by EPA's Office of Research and Development (ORD) is Drinking Water. The Safe Drinking Water Act (SDWA) was originally passed in 1974 to protect public health by regulating the Nation's public drinking water supply. The law was amended in 1986 and 1996. The 1996 amendments greatly enhanced the existing law by recognizing source water protection, operator training, funding for water system improvements, and public information as important components of safe drinking water. This approach ensures the quality of drinking water by protecting it from source to tap. The SDWA applies to every public water system in the United States, and the responsibility for making sure these public water systems provide safe drinking water is divided among EPA, states, tribes, water systems, and the public.

Research described in this progress review was funded through EPA's Science to Achieve Results (STAR) Program, which is managed by NCER. Grants were awarded through an open, peer-reviewed competition of proposals submitted in response to Requests for Application (RFA) for each of the years from 1997 to 1999. The research being supported by these grants, along with work conducted in the EPA laboratories and other outside research institutions, is addressing key research questions identified in the Research Plan for Arsenic in Drinking Water, the Research Plan for Microbial Pathogens and Disinfection By-Products in Drinking Water (both available at http://www.epa.gov/ORD/WebPubs/final/), and research on drinking water contaminants identified as priorities for additional research (the Contaminant Candidate List, CCL).

This progress review provided an opportunity for investigators to interact with one another and to discuss the progress and findings of their research with EPA and other interested parties. If you have any questions regarding the program, please contact the program manager, **Cynthia Nolt-Helms, at 202-564-6763 or nolt-helms.cynthia@epamail.epa.gov.**

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Section 1.

Chemical Contaminants: Exposure, Effects, & Assessment

Development of Biomarkers for Haloacetonitriles-Induced Cell Injury in Peripheral Blood

Ahmed Elsayed Ahmed
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Drinking waters are contaminated with a mixture of halogenated hydrocarbons that are disinfection by-products. Among these are a number of toxic and carcinogenic halogenated acetonitriles (HANs) that are known to induce a variety of acute and chronic adverse effects in humans and laboratory animals. The goal of this project is to develop unique biomarkers, in a readily accessible compartment such as blood, for HAN exposure and HAN-induced cell injury. This injury may result from HAN-induced alkylative or oxidative damage to cellular macromolecules. To evaluate the mechanism of HAN-induced DNA damage, the effect of HANs on the conversion of native supercoiled plasmid DNA to circular or linear forms was quantified electrophoretically. The results indicated HAN-induced oxidative DNA damage *in vitro*. H_2O_2 mediation of dibromoacetonitrile (DBAN) induced DNA damage is a possible mechanism of HAN genotoxicity.

In a dose-response and time course study, fibroblasts were exposed to various concentrations of the water disinfection byproduct, DBAN. The results indicate that there was a concentration and time-dependent depletion of cellular antioxidants, while markers for oxidative stress and cellular damage were significantly increased (see Figure 1). The effect progressed and was followed by an increase in membrane damage and cellular death. Treated cells exhibited features of apoptosis at low exposure levels and severe membrane damages and necrosis at higher concentrations of the chemical.

The ability of fibroblasts to withstand DNA damage induced by DBAN was evaluated by determining the magnitude of DNA repair processes in the cell using a base excision repair (BER) assay. BER was upregulated in cells exposed to low concentrations of DBAN, and was downregulated at higher concentrations and longer exposure periods (see Figure 2). These studies indicate that DBAN activates oxidative stress. Depending on its magnitude, oxidative stress signals induce up- or downregulation of gene expression of BER enzymes. The existence of human diseases associated with impaired DNA repair graphically illustrates the importance of these processes of quality control. Chemically induced disruption of BER might be found in humans, and may lead to a decrease in defense mechanisms against various types of carcinogenic DNA damage.

Currently, the goal is to closely investigate the sequence of the signals leading to the regulation of BER induced by the drinking water disinfectant by-products and how to intervene in their progression. These signals will constitute an array of mechanism-based biomarkers for HAN exposure and effects. Future studies will focus on the characterization and quantitative determination of oxidative and alkylative damages to cellular macromolecules following inhalation exposure of animals to HANs. These studies should provide a basis for the development of regulatory guidelines and policies governing the tolerance levels for chronic human exposure to HANs.

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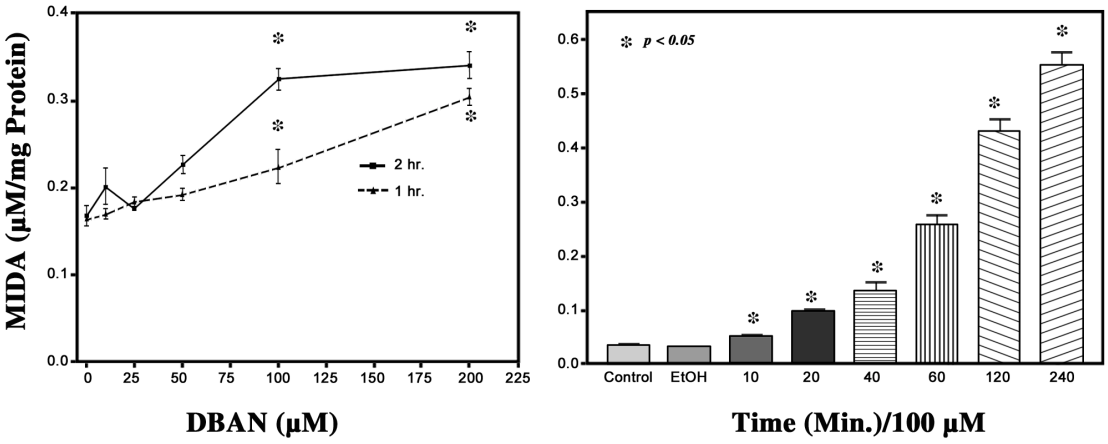

Figure 1. Effect of dose-response and time on DBAN-induced lipid peroxidation.

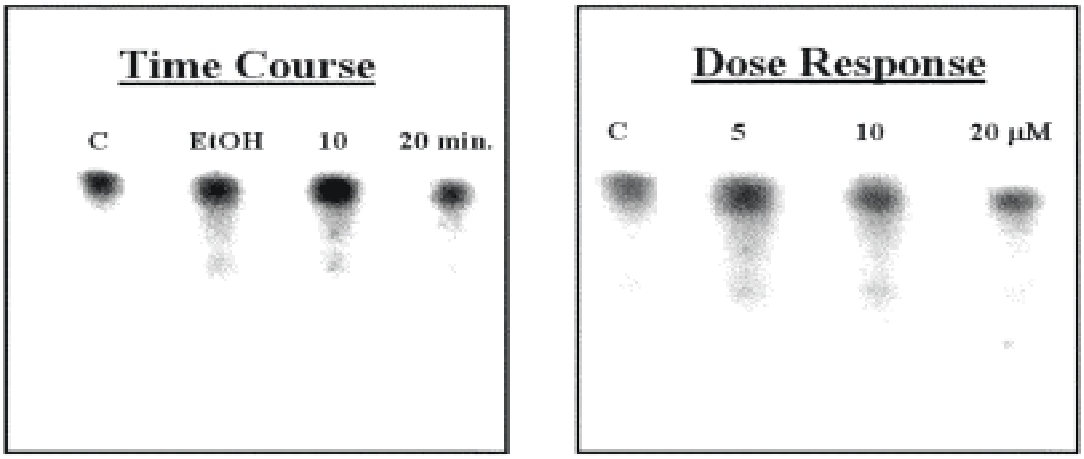

Figure 2. Effect of DBAN on DNA base excision repair.

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Metabolic Fate of Halogenated Disinfection Byproducts *In Vivo* and Relation to Biological Activity

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Halogenated byproducts of drinking water disinfection are of concern because of their widespread human consumption and the current uncertainty over their health effects. Haloacids as a class are the most abundant of the halogenated disinfection byproducts. Many of these compounds are suspect carcinogens, and in addition, may form macromolecular adducts that could be useful as biomarkers of exposure to disinfectants. This project attempts to elucidate mechanisms of biological activity and disposition of haloacids.

The initial hypothesis was that dihaloacetic acids could be metabolically activated to genotoxic intermediates by a glutathione-dependent enzyme pathway, and that tricyclic adducts would be formed on the DNA bases. The goals were to: (1) use the Ames plate incorporation assay to measure the production of genotoxic species in the form of mutations, and manipulate the metabolic activation conditions to favor glutathione conjugation, cytochrome P450-linked oxidation, or NAD^+-dependent oxidation, by addition of the appropriate cofactors, alone or in combination, to define the metabolic pathway(s) that produce genotoxic products, with the intent of using that information to prioritize the synthesis of potential DNA adducts towards those molecules that were plausibly produced by pathways linked to mutagenicity; (2) synthesize such potential DNA adducts; and (3) investigate the formation of DNA adducts following treatment with haloacids *in vivo*.

Evidence was obtained from mutagenicity assays that glutathione-mediated pathways did not lead to mutagenic products from dichloroacetic acid—in fact, they were more likely to enhance the toxicity of this compound. However, NAD^+ enhanced the mutagenicity of dichloroacetic acid, hypothetically by acting as cofactor for oxidation of an intermediate alcohol to a carboxyl group. This conclusion was investigated to see whether it held true for the brominated analogues. The purpose at this stage was to prioritize the different haloacetic acids for *in vivo* metabolic studies; preference was given to the most mutagenic compounds for investigation of potential *in vivo* DNA adduct formation.

The brominated haloacids each exhibited some interesting features. Monobromoacetic acid clearly was toxic to the bacteria, because few or no colonies (either revertant or background) were able to grow at the two highest doses, and this toxicity was mitigated when glutathione was included in the assay mix. NAD^+ did not appear to have any effect on plate counts with or without glutathione. With dibromoacetic acid, plate counts increased at low doses, just barely reached a doubling of the background rate, and fell off at the highest dose. A similar pattern was seen when glutathione was added alone. In the presence of NAD^+, either alone or in combination with glutathione, no increase in plate counts was seen, or was toxicity especially marked. The only compound of those tested to exhibit a clear mutagenic response was the mixed haloacid, bromochloroacetic acid (BCA). BCA more than doubled the background rate, consistently, either alone or in the presence of NAD^+. This increase in plate counts was not observed when glutathione was added, either alone or in combination with NAD^+. Synthesis of putative DNA adducts has been unproductive to date (results not shown).

The original hypothesis developed was that a glutathione-dependent pathway could contribute to formation of genotoxic metabolites from dihaloacetic acids. The results presented here confirm earlier findings that the presence of glutathione does not enhance the mutagenicity of haloacetic acids in the Ames plate incorporation assay. However, while glutathione appeared to increase the toxicity of dichloroacetic acid, for the brominated species bromoacetic acid and bromochloroacetic acid, the effects of glutathione amounted to detoxication, in the form of a decrease in toxicity for MBA and of mutagenicity for BCA. Thus, interaction with glutathione is biologically important for this class of compounds; the outcome of that interaction would appear to differ depending on the nature of the halogen substituent.

Earlier conclusions that thioether conjugates derived from haloacetic acids are unlikely to contribute greatly to the genotoxicity of these compounds, and that synthesis of DNA adducts inferred to be derived from this route can be assigned a lower priority, remain unchanged. Nevertheless, sufficient evidence has been developed to show that glutathione-dependent pathways play important, and perhaps different, roles in the overall biological activity of the haloacetic acids. BCA, although not the most abundant dihaloacetic acid produced from drinking water disinfection, would appear to be the most mutagenic haloacetic acid; hence, it is expected to offer the highest chance of observing DNA adducts in any quantity, in addition to the high likelihood that it will form adducts similar to those arising from dichloroacetic acid.

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Genotoxicity and Occurrence Assessment of Disinfection Byproducts in Drinking Water

Roger A. Minear and Michael J. Plewa
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The objectives of this project were to: (1) calibrate short-term genotoxicity assays based on mutation in *Salmonella typhimurium* and direct DNA damage in cultured mammalian cells using regulated disinfection byproducts (DBPs); (2) compare the relative genotoxicities of chlorinated versus brominated DBPs; (3) compare the relative genotoxicities of chlorination byproducts versus brominated byproducts; (4) compare the relative genotoxicities of DBPs derived from singular versus combination (sequential) use of ozonation and chlorination; and (5) provide a DBP occurrence database for extrapolating genotoxicity results to practice.

This project merged quantitative chemistry and biology components. A new method was developed to evaluate brominated and chlorinated DBPs based on an ion chromatograph system as the detector, instead of a coulometric titration cell. Gas phase HBr and HCl that corresponded to brominated and chlorinated compounds were dissolved in water, and the aqueous sample was applied to ion chromatography for separation and quantification.

Comparison among chlorination, chloramination, and chlorine dioxide treatment of Suwannee River fulvic acid with bromide ions showed that the ratio of the brominated fraction of total organic halogen (TOX) to the chlorinated fraction of TOX during chlorine dioxide treatment was much higher than those during chlorination and chloramination. The cytotoxic and mutagenic properties of known DBPs were quantitatively compared using bacterial and mammalian cell systems. A rapid microplate cytotoxicity assay using *S. typhimurium* was developed and calibrated. The cytotoxicity data were incorporated into the analysis of the genotoxic potency of each DBP. Selected DBPs were assayed for mutagenicity in strains TA98, TA100, and RSJ100 under preincubation test conditions. The rank order of decreasing cytotoxicity in TA100 was 3-chloro-4-(dichloromethyl)-5-hydroxy-2-(5H)-furanone (MX) >> bromoacetic acid (BA) >> bromoform (BF) > dibromoacetic acid (DBA) >> tribromoacetic acid (TBA) > chloroform (CF) >> dimethylsulfoxide. The rank order of the mutagenic potency of the DBPs from the highest to lowest was MX >>> BA >> BF > DBA >> dichloroacetic acid (DCA) > chloroacetic acid (CA) >> TBA ~ trichloroacetic acid (TCA). Rapid, quantitative mammalian cell cytotoxicity and genotoxicity assays using cultured Chinese hamster ovary (CHO) cells also were developed and calibrated. The CHO cell cytotoxicity rank order from highest to lowest was bromonitromethane (BNM) > dibromonitromethane (DBNM) > BA > MX > DBA > CA > potassium bromate > TBA > DCA > TCA. Genotoxicity analyses of the DBPs were conducted using single-cell gel electrophoresis (SCGE), which detects genomic DNA damage at the level of the individual nucleus. Using SCGE genotoxic potency, the rank order was BA > DBNM > tribromonitromethane > trichloronitromethane > BNM > MX > CA > dichloronitromethane > DBA > ethylmethane-sulfonate ([EMS] positive control) > TBA >> TCA ~ DCA (see Figure 1).

Comparative toxicological analyses of DBPs and DBP mixtures isolated from disinfected water using a variety of biological endpoints are important for risk assessment and assist the formulation of public regulatory policies that protect the environment and the public health. Data to date show that there was no significant correlation between the relative cytotoxicity and genotoxicity data of the DBPs when compared between *S. typhimurium* and CHO cell assays. Order ranking and magnitude of responses of some DBPs are quite different between the bacterial and mammalian assays.

Complex DBP mixtures have been isolated from real waters after chlorination, chloramination, or ozonation. Cytoxicity and genotoxicity analysis of these samples currently are underway and will be the focus of the work in the final project year.

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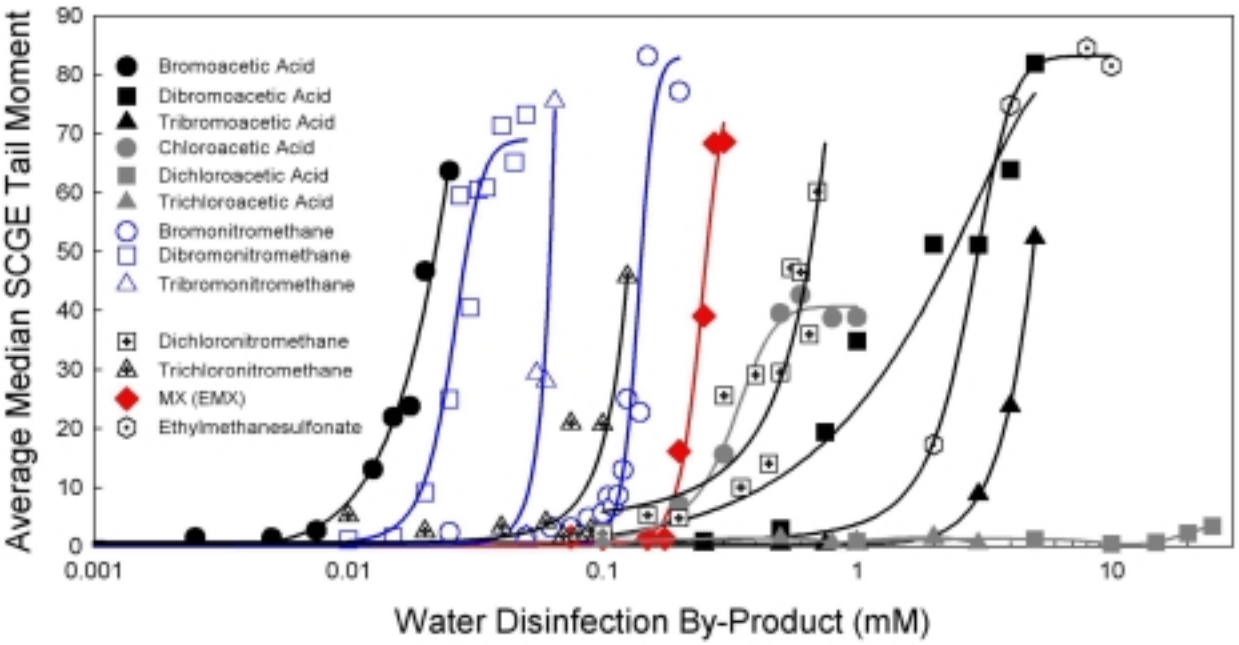

Figure 1. Single-cell gel electrophoresis analysis of genomic DNA damage in CHO cells induced by selected DBPs. For comparison of the relative genotoxic activities of the DBPs, the standard chemical mutagen ethylmethyl-sulfonate was included. The halonitro-methanes were provided by Dr. Susan Richardson, U.S. Environmental Protection Agency.

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Assessment of Human Dietary Ingestion Exposures to Water Disinfection Byproducts via Food

James H. Raymer [1], E.D. Pellizzari [1], Y. Hu [1], B. Childs [1], K. Briggs [1], H. Weinberg [2], and V. Unnam [2]
[1]Research Triangle Institute, Research Triangle Park, NC; [2]School of Public Health, University of North Carolina, Chapel Hill, NC

The overall objective of this research project is to estimate the magnitude of exposure to disinfection byproducts (DBPs) in drinking water via their ingestion after uptake into food during cooking or home processing. Although the potential for contamination has been shown, the magnitude of this contamination has not been studied. This research will specifically address the uptake of compounds known to arise from the process of water disinfection (ozonation in conjunction with a secondary process such as chloramination), including nonhalogenated aldehydes, ketones and acids, trihalomethanes, haloacetic acids, bromate, chloropicrin, and haloacetonitriles. The main hypotheses to be tested are: (1) foods prepared using contaminated water become contaminated; (2) food is a significant source of DBP exposure; (3) DBP concentrations in food can be predicted with knowledge of DBP concentrations in tap water and foods consumed; and (4) dietary exposures of children are higher than for adults living in the same household.

Work to date has focused on the investigation of a method for nonhalogenated aldehydes and ketones in composite foods and beverages, the stability of haloacetonitriles and haloacetic acids (HAAs) to boiling, the partition of HAAs into foods during cooking and rinsing, and on a method for the determination of bromate in food. A method for nonhalogenated aldehydes and ketones based on derivatization of carbonyl compounds with o-(2,3,4,5,6pentafluorobenzyl)-hydroxylamine to form the corresponding oxime was developed and applied to foods and beverages. The sample extracts were very complex and contained many of the target analytes without the addition of any known DBPs. During boiling of spiked water, haloacetonitriles were lost very rapidly, while some HAAs were more persistent. Chloroacetic, dichloroacetic, bromochloroacetic, and dibromoacetic acids were unaffected following boiling for 60 minutes while bromodichloroacetic, chlorodibromoacetic, and tribromoacetic acids were lost quickly (within 10 minutes); trichloroacetic acids were of intermediate stability.

The partition of HAAs into carrots, green beans, pinto beans, chicken, and lettuce following cooking or contact with water spiked to contain HAAs was studied. The foods selected for study were spaghetti, dried beans, carrots, green beans, chicken, and lettuce and were chosen based on different cooking conditions, different chemical compositions (i.e., starch, vegetable, protein), and the fact that they are commonly consumed by children. Foods were cooked according to package directions using either reagent water or water spiked to contain HAAs. Following cooking, foods were homogenized and extracted as previously described (Raymer et al., *J Exposure Anal Environ Epidemiol* 2000;10: 808-15).

In some cases, more than 60 percent of HAAs in the cooking water were taken up by the food during cooking (see Table 1). In general, chlorodibromoacetic and tribromoacetic acids are not detected following cooking, consistent with the rapid loss of these compounds during boiling. The soaking of lettuce also resulted in uptake of HAAs (1.8–7.8% of the total available). Results for spaghetti indicated that as much as 15 percent of the available HAAs are adsorbed/ absorbed during cooking in spiked water. Given the large volumes of water used to cook pasta, the masses absorbed could be much higher than for other types of food.

In addition, the data obtained to measure uptake following rinsing with spiked water indicate that additional HAAs—up to 10 percent—are taken up from the rinsing water. This was true whether the pasta was cooked in reagent water or in spiked water. It also was clear that the HAAs that typically show low recovery following cooking (bromodichloroacetic acid, chlorodibromacetic acid, and tribromoacetic acid) can become available to contribute to exposure following contact of the pasta with fresh, DBP-containing water.

Initial evaluation of a method to extract bromate from food (spaghetti) showed that material extracted from pasta interfered with measurement of the recovery of bromate. A specific and sensitive method for bromate based on ion chromatographic separation/ postcolumn reaction method developed for water was adapted for this work. Analysis of water recovered from the cooking of spaghetti showed that dilution was needed to affect detection of the bromate; whether this was due to adsorption of bromate onto suspended/ dissolved solids or a suppression of the chromatographic detection was not clear. Bromate was detected in reagent water following its use to cook pasta, which indicated its presence in the pasta. Initial extractions of homogenized, cooked pasta indicated fewer interferences than with the recovered water, but that method's precision was not very good. Further method work will be conducted during the next year.

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Table 1. Percent uptake of haloacetic acids into foods during cooking

Compound	Concentration in Water (ppb)	Percent Uptake (%RSD)				
		Carrots	Green Beans	Pinto Beans[g]	Lettuce[h]	Chicken[g]
Chloroacetic acid	500	NR[b]	17[c] (38)	62[d] (3.1)	2.5[d] (42)	15[c] (3.2)
Bromoacetic acid	100	57[c] (3.8)	21 (50)	ND[e]	4.5[d] (9.1)	ND
Dichloroacetic acid	150	64[c] (24)	48[c] (39)	85[d] (3.4)	3.9[d] (19)	11[c] (22)
Trichloroacetic acid	50	24 (294)	33 (92)	INT[f]	3.1[d] (75)	14[c] (121)
Bromochloroacetic acid	100	40[c] (9.7)	35[c] (8)	37[d] (5.0)	4.2[d] (5.4)	7.1[c] (45)
Dibromoacetic acid	50	57[c] (8.0)	60[c] (18)	51[d] (16.6)	7.2[d] (6.7)	11[c] (23)
Bromodichloroacetic acid	100	26[c] (43)	2.2 (182)	5.3[d] (87)	1.8[d] (40)	NR
Chlorodibromoacetic acid	100	ND	ND	ND	6.5[c] (5.5)	ND
Tribromoacetic acid	100	ND	ND	ND	7.8[d] (12)	ND

[a] Uptake = [(HAA mass in food cooked in spiked water – mass in food cooked in reagent water) x 100] ÷ total HAA mass in cooking water; N= 3.
[b] NR = Not reported. A decrease in concentration was measured following cooking in spiked water.
[c] Significant at $p < 0.05$.
[d] p value not calculated because n = 1 for control case; a substantial increase was noted for these compounds relative to the food cooked in reagent water.
[e] ND = Not detected.
[f] INT = Interferent. Very high trichloroacetic acid concentration in the blank made estimation of the uptake difficult.
[g] Pinto beans soaked in reagent water and cooked in water spiked with HAAs; chicken soaked and cooked in spiked water.
[h] Lettuce soaked for 5 minutes in water containing HAAs.

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Bioavailability of Haloacetates in Human Subjects

Irvin R. Schultz [1], Robert Shangraw [2], and Torka Poet [1]
[1]Battelle, Pacific Northwest Division, Richland, WA; [2]Oregon Health Sciences University, Portland, OR

The objective of this research project is to characterize the absorption, disposition, and oral bioavailability of chlorinated and brominated haloacetates in human volunteers after consumption of drinking water containing a natural mixture of these compounds. It is hypothesized that accurate assessment of the oral bioavailability of haloacetates can be achieved by the simultaneous administration of an oral dose of ^{12}C-labeled haloacetate with an intravenous dose of ^{13}C-labeled haloacetate. It is hypothesized that measurable plasma levels of dichloroacetate, bromochloroacetate, and dibromoacetate can be detected from the debromination of bromodichloroacetate, dibromochloroacetate, and tribromoacetate.

This research will directly test the hypothesis that prolonged exposure to low concentrations of dihaloacetates reduces their metabolism and increases their systemic bioavailability in humans. These experimental results will be used to validate a physiologically based pharmacokinetic (PBPK) model for haloacetates in humans that is based on *in vitro* metabolism parameters obtained with human tissue homogenates (see Figures 1 and 2). Dichloroacetate (2 mg haloacetate/Kg) in 1 pint of water will be given to volunteers. After 5 minutes, ^{13}C-labeled dichloroacetate will be given by intravenous injection (via a catheter placed in the arm).

A similar experiment will be performed using mixtures of chlorinated and brominated haloacetates in rhesus monkeys. In a second experiment, volunteers will consume 1 pint of tap water previously verified to contain the seven haloacetates of interest. For all experiments, serial blood samples will be removed using the intravenous catheter, and the blood plasma will be analyzed simultaneously for both the ^{13}C haloacetates and ^{12}C haloacetates (using gas chromatography-mass spectrometry or liquid chromatography-MS/MS techniques). The area-under-the-curve ratio for the oral and intravenous doses will be determined to estimate the oral bioavailability.

This project will provide critical data needed to make accurate and reliable exposure estimates of haloacetates to humans consuming municipal drinking water supplies. In addition, this project will identify the consequences of low-level exposure to haloacetates on their subsequent metabolism and disposition. This information is needed to assess whether individuals who consume water containing high levels of byproducts experience greater than predicted exposure due to decreased elimination of haloacetates. This project also will allow for the direct testing of physiologically based pharmacokinetic model predictions of haloacetate dosimetry in humans.

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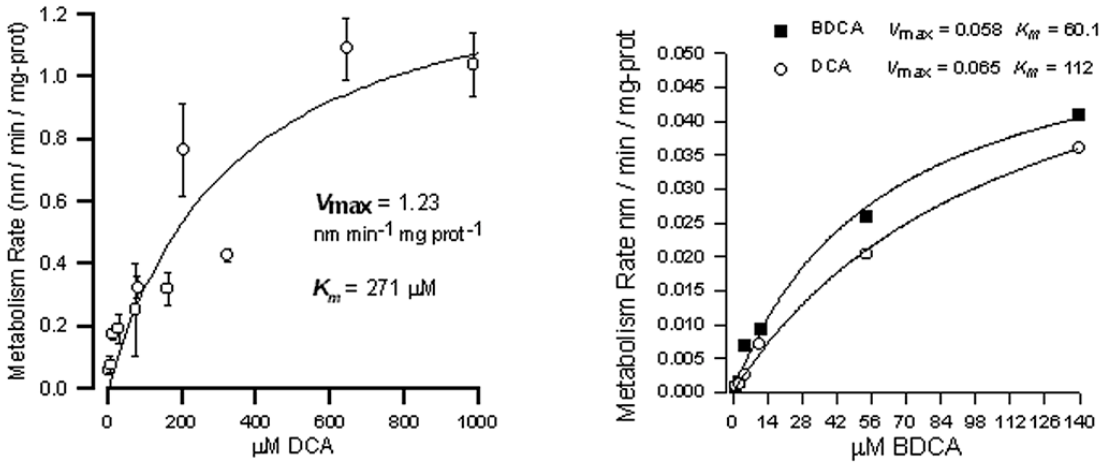

Figure 1. Left graph: DCA metabolism in human liver S9. Right graph: BDCA metabolism (total, solid squares) and DCA formation (open circles) in human liver microsomes. Formation of DCA accounts for most of the consumption of BDCA. Solid lines on both graphs are the nonlinear least squares fit of the observed data to the Michaelis-Menten equation.

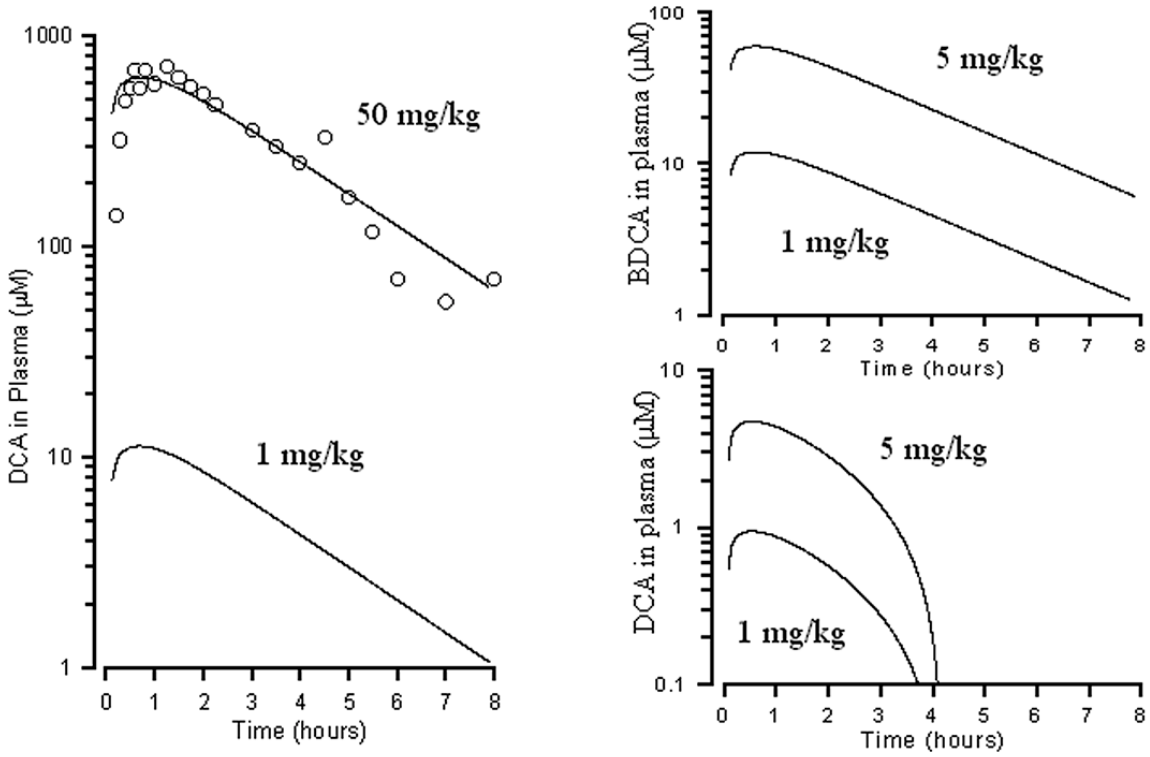

Figure 2. Left graphs: PBPK model predictions of DCA plasma profiles in humans after 1 and 50 mg/kg oral doses. Observed data (circles) is from Curry et al. (1991) *Biopharm Drug Dispos* 12:375-390. Right graphs: PBPK model predictions of BDCA and DCA plasma profiles in humans after 1 and 5 mg/kg oral doses of BDCA.

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Physiologically Based Pharmacokinetic Modeling of Haloacid Mixtures in Rodents and Humans

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The goals of this research project are: (1) characterization of the comparative toxicokinetics and metabolism of chloro, bromo, and mixed chlorobromo haloacids (HAs) in rodents; and (2) development of a physiologically based pharmacokinetic (PBPK) model that can predict the tissue distribution and elimination of HAs during chronic oral exposure in mice, rats, and humans.

Prior experiments established that di- and tri-HAs have distinct pharmacokinetic properties, and that prior exposure to di-HAs can alter their metabolism. Current studies have focused on characterizing the kinetics of di- and tri-HAs at lower doses and as a mixture of HAs. *In vivo* experiments used mice and rats given either intravenous or gavage doses of an HA or HA mixture and the blood plasma concentration-time profile and urinary excretion characterized in individual animals. Both control (naïve) and animals pretreated with a di-HA for 7 days at various drinking water concentrations were used to measure the effects of prolonged HA exposure on the disposition. *In vitro* metabolism experiments of tri-HAs used mouse and rat microsomes. These experiments were performed under varying oxygen tensions ranging from zero (pure N_2 atmosphere), 2 percent O_2, and normoxic (normal atmosphere).

Di-HA elimination by naïve rats was so rapid that only higher doses (1–20 mg/kg for the intravenous and 5–20 mg/kg for the gavage) achieved blood plasma concentrations above the analytical limit of detection. The oral bioavailability was approximately 30 percent in naïve rats at the 5 and 20 mg/kg doses. Oral bioavailability increased to 39 percent and 82 percent at 5 and 20 mg/kg doses, respectively, in HA-pretreated animals. The oral absorption plasma profile of di-HAs was complex and exhibited secondary peaks several hours after dosing. This phenomenon appeared to be enhanced in mixtures of HAs. The metabolism of di-HAs is known to be mediated by the enzyme glutathione-S-transferase Zeta (GSTzeta).

Pretreatment of rats with a di-HA greatly diminished the GSTzeta activity and metabolism of all di-HAs. This effect on metabolism could be observed after pretreatment with drinking water levels as low as 1 mg/Kg (see Figure 1). Microsomal metabolism of tri-HAs proceeded by reductive debromination forming a di-HA free radical intermediate, which was stimulated under a reducing environment. The V_{max} for the loss of parent trihaloacetate was 4–5 times higher under nitrogen headspace than under atmospheric conditions. Intrinsic metabolic clearance was of the order Tri-Bromoacetate > Chlorodibromoacetate>>Bromodichloroacetate.

Eadie-Hofstee plots for the consumption of the parent tri-HA appeared linear, suggesting that a single P450 enzyme is responsible. Carbon monoxide and diphenylene-iodonium (a specific P450 reductase inhibitor) blocked the metabolism. However, inhibitors of specific P450 proteins (CYP 2E1, 2D6, and 3A4) failed to block metabolism significantly.

These results indicate that low-level exposure to di-HAs decreases metabolism, causing an increase in the oral bioavailability. The delayed absorption of di-HAs may allow greater levels to reach the colorectal region than previously thought. These results will be incorporated into the working PBPK model for HAs to predict tissue dosimetry during low exposure rates of mixtures of HAs.

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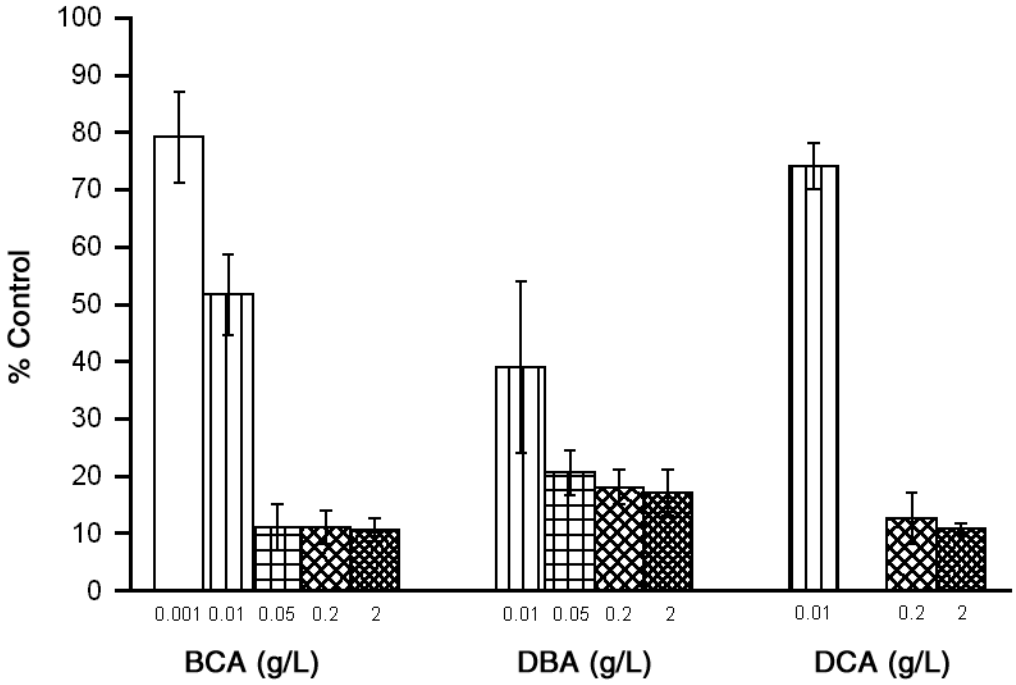

Figure 1. GSTzeta activity in liver cytosol prepared from rats treated for 7 days with a di-HA at various drinking water concentrations (mean ± SD).

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Inhalation and Dermal Exposure to Disinfection Byproducts of Chlorinated Drinking Water

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Inhalation and dermal exposure to haloacetic acids, haloketone, haloacetonitrile, and chlorohydrate from showering and bathing are being determined. The exposures will be determined from: (1) concentration of the DBPs or their metabolites in urine or breath following known exposures; (2) estimation of dermal transport coefficients using *in vitro* measurements across excised skin; and (3) size and number distribution of residential water aerosols generated during showering and disinfection byproduct (DBP) concentration.

The dermal absorption during both *in vivo* and *in vitro* studies was minimal for chloroacetic acid, dichloroacetic acid, bromoacetic acid, trichloroacetic acid, bromochloroacetic acid, and dibromochloroacetic acid. Only trichloroacetic acid had measurable penetration through the skin *in vitro* at pH 7, with a Kp value of 0.00039 cm^2/hr at 37°C. The haloacetic acids were either only slightly above the background levels in the urine (total excess excretion 1–2 μg for dichloroacetic acid) or not detectable in the urine (dibromoacetic acid) following a bath using concentrations of 200 μg/L. Low or nonmeasurable levels of the haloacetic acids in urine after bathing could be due to rapid metabolism of the compound, although a portion of even rapidly metabolized compounds are expected to be excreted prior to being metabolized after being dermally absorbed.

The *in vitro* and *in vivo* results strongly suggest that dermal absorption of haloacetic acids from water at pH of approximately 7 is insignificant. Dichloropropanone and trichloropropanone had Kp values of 0.019 and 0.0081 cm^2/hr, respectively, at 21°C.

The *in vitro* measurements were made under steady-state conditions of high concentrations (g/L), run for 20–50 hours. Chloroacetonitrile rapidly adsorbed across the skin within the *in vitro* studies, made under nonsteady state (μg/L concentrations), with a peak flux of 0.01 μg/hr/cm^2 within minutes of the exposure, followed by a decline after several hours.

For water concentrations of 20 μg/L, a 30-minute bath would provide a dose of 1–3 μg, or 5–15 percent of that obtained from ingestion of 1 L of water. The *in vivo* studies also documented penetration through the skin, although a majority of the compounds are metabolized rapidly, making measurement of the DBP itself difficult. Chlorohydrate was observed to dermally penetrate the skin based on measurement of its metabolite trichloroacetic acid.

Aerosols of 0.5–2.0 microns were produced by showers using different showerheads and temperatures. The aerosol size distribution was characterized by $p = 3.2 \times 10^{-3} d^{-2.4}$, where p is the percentage of aerosols within a certain size range, and d is the diameter of the aerosol.

For water spiked with 25 μg/L of haloketones and 200 μg/L of haloacetic acids, the upper limits of what is expected in a water distribution system, the airborne particulate levels near the shower stream were 10 μg/m^3 for the haloacetic acids and 100 ng/m^3 for the haloketones.

The expected inhalation contribution to the total dose from these aerosols is calculated to be 1 percent or less compared to an ingestion of 1 L of water. It is expected that the haloketones also will have a vapor component that will contribute more to the inhalation dose.

These results provide evidence that dermal and inhalation exposures are not important routes for haloacetic acid, but should be considered for haloacetonitrile, chlorohydrate, and haloketone (see Table 1).

The data necessary for drinking water exposure models, including dermal uptake, particle size, and particle number distributions are being generated. *In vivo* studies need to be completed. Additional work also is needed to develop biomarkers that adequately can measure exposure to these DBPs in field and controlled studies.

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Table 1. Importance of exposure route to total dose relative to ingestion.

Compound Class	Dermal	Inhalation-Aerosol	Inhalation-Vapors
Haloacetic Acid	No	No	No
Haloketone	Yes	No	Probable
Haloacetonitrile	Probable	No	Probable
Chlorohyrate	Yes	No	Probable

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NHEERL Research on Carcinogenic Contaminants in Drinking Water

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Water research in the Environmental Carcinogenesis Division focuses on improved understanding of the mechanisms of mutagenesis and carcinogenesis of water contaminants for incorporation into human cancer risk assessment models. The program uses cellular, animal, and computer models for assessing responses to individual and mixtures of water disinfection by-products (DBPs), arsenic, and chemicals on the U.S. Environmental Protection Agency's (EPA) chemical contaminant list. A significant component of the research effort is directed toward a better understanding of mechanisms underlying tumor development to inform the cancer risk assessment process. As examples, research on potassium bromate ($KbrO_3$), dichloroacetic acid (DCA), arsenicals, and a defined mixture of four water DBPs will be discussed.

Ozone has been proposed for water disinfection because it is more efficient in killing microbes than chlorine and results in much lower levels of trihalomethanes than chlorination. Ozone leads to formation of hypobromous acid in surface waters with high bromine content and forms brominated organic by-products and bromate. The carcinogenicity and chronic toxicity of $KBrO_3$ was studied in mice and rats. $KBrO_3$ is carcinogenic in the rat kidney, thyroid, and mesothelium and is a renal carcinogen in the male mouse. These data were used to predict the human health risk associated with exposure to bromate in drinking water.

DCA is the main component of the haloacetic acids, the second most prevalent group of DBPs after trihalomethanes. DCA is a rodent liver carcinogen that results in tumor development after 2 years, even when mice are exposed to DCA for only 10 weeks. DCA has been shown to depress apoptosis in hepatocytes and is a weak inducer of DNA mutations, as it has been weakly positive in both *in vitro* and *in vivo* mutagenicity assays, including the Big-Blue Mouse®. These and other data are being used to develop a biologically based dose-response model for DCA hepatocarcinogenesis in the mouse. Arsenic is a known human carcinogen and has been shown to promote cancer in the rat urinary bladder, kidney, liver, and thyroid as well as in the mouse lung. It has been generally thought that arsenite is the carcinogenic form, and that methylation is the detoxification and excretion pathway. However, recent evidence shows that methylation of arsenic also may be a toxification pathway. Arsenic may act as both a genotoxic and nongenotoxic carcinogen, thereby acting as an initiator and/or a tumor promoter. Additional research is examining the interaction between arsenic and DNA methylation, as well as the carcinogenic activity of dimethylarsenic acid and the contribution free radicals may have in arsenic-induced cancer. Current default risk assessments for chemical mixtures assume additivity of carcinogenic effects, but this may under- or over-represent the actual biological response.

A rodent model of hereditary renal cancer was used to evaluate the carcinogenicity of a mixture of $KBrO_3$, 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX), chloroform, and bromodichloromethane. Treatment with the mixture did not produce more neoplasms than the individual compounds, suggesting less than an additive response for carcinogenicity. Research within the Environmental Carcinogenesis Division not only attempts to develop methods for detecting environmental carcinogens, but also tries to define the mechanisms of carcinogenesis with the intent of resolving issues, assumptions, and uncertainties in cancer risk assessment for water contaminants. This abstract does not reflect EPA policy.

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Aluminum Toxicokinetics: Oral Absorption From Drinking Water and Brain Retention

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Aluminum (Al) is a neurotoxicant that plays a role in dialysis encephalopathy and similar conditions. Although highly controversial, some epidemiological studies suggest a positive association between drinking water Al concentration and the incidence of Alzheimer's disease. It has been suggested that Al is better absorbed from water than foods. The overall objective of this study is to determine if Al in drinking water significantly contributes to brain Al accumulation.

There were four aims of the study. The first aim was to determine the absolute bioavailability of Al from drinking water.

The second aim was to assess whether water hardness and/or food in the stomach affect oral Al bioavailability. Repeated measurements of both ^{26}Al and ^{27}Al in rat plasma were obtained after concurrent administration of a single stomach feeding of ^{26}Al in water and continuous ^{27}Al intravenous infusion. The stomach feeding was given in the absence ("soft" water) or presence of calcium and magnesium carbonate ("hard" water) and in the absence or presence of stomach contents. Al bioavailability was calculated from the area under the Al concentration × time curve for ^{26}Al compared to the equivalent term for ^{27}Al.

The third aim was to determine the fraction of Al that enters the brain from blood by quantitation of ^{26}Al in the rat brain after its systemic administration.

The fourth aim was to determine the rate of brain Al elimination. Two approaches were utilized. Rat brain ^{26}Al was quantitated at multiple times after systemic ^{26}Al dosing. Additionally, some rats received repeated injections of the Al chelator desferrioxamine to determine whether it affected the brain ^{26}Al half-life. Approximately 0.3 percent of the ^{26}Al was absorbed independent of whether it was given in "soft" or "hard" water and independent of the absence or presence of stomach contents.

After intravenous ^{26}Al administration, approximately 5×10^{-3} percent of the dose appeared in each gram of brain. The half-life of brain Al elimination was approximately 150 and 85 days in the absence and presence of repeated desferrioxamine injections, respectively. Al shows a prolonged residence in the brain. As the human lifespan is about 30 times that of the rat, the 150-day half-life in rat brain translates to a human brain Al half-life of approximately 12 years. Brain Al concentration resulting from continuous Al intake can be predicted from the amount of Al that enters the brain and the half-life of Al in the brain.

For this prediction, 0.3 percent absorption of the 8 mg Al consumed daily in drinking water and food, entry into the brain of 5×10^{-3} percent of the absorbed Al, and a 12-year brain Al half-life were assumed. The results suggest that brain Al in the average 60 year-old human should be approximately tenfold more than is reported. One explanation for this discrepancy is that 0.3 percent is an overestimate of oral Al bioavailability from food. Water provides only about 1 percent of the typical daily Al consumption, whereas food provides about 95 percent.

If Al bioavailability from food is approximately 0.03 percent, the prediction would match observed human brain Al concentrations. Drinking water then would provide approximately 10 percent of the daily absorbed Al that might contribute to brain Al accumulation. The Al concentration in drinking water then might impact human brain Al accumulation. Determination of the oral bioavailability of Al from food is required to clarify the ability of Al in drinking water to significantly contribute to brain Al.

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Section 2.

Chemical Contaminants: Arsenic

Overview of EPA's Arsenic in Drinking Water Regulation

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The goals and objectives of this project are to propose arsenic regulation by January 1, 2000, issue a final rule by June 22, 2001, and reevaluate at least every 6 years. The Safe Drinking Water Act directed the U.S. Environmental Protection Agency (EPA) to revise its 1975 Drinking Water Standard (the Maximum Contaminant Level [MCL]) and list affordable technologies for small systems. EPA may adopt an MCL "that maximizes health risk reduction benefits at a cost that is justified by the benefits."

The Act, as amended in 1996, charged the Agency to develop a research plan by February 2, 1997, to address the uncertainty in assessing health risks from low levels of arsenic to support the rulemaking, and conduct the research in consultation with the National Academy of Sciences (NAS), other federal agencies, and interested public and private entities.

In December 1996, EPA submitted its draft research plan for peer review and issued the final plan in February 1998. The plan identified the short-term research (available before January 2000) that would support regulation development and long-term research to reassess the arsenic MCL, as specified by the statute. In 1996, EPA asked the NAS to review arsenic health effects research and EPA's risk characterization from arsenic exposure. The NAS report, issued in March 1999, concluded that the Taiwanese studies provide the best data available for quantifying risks. The report stated that EPA's MCL of 50 ppb is not adequately protective and should be lowered as soon as possible.

In the preamble to EPA's June 22, 2000, proposed rule, the Agency explained how it used NAS' bladder cancer risk analysis to prepare EPA's initial risk assessment (see Figure 1). In the proposal, EPA identified sources of scientific uncertainty and requested comment on MCL options ranging from 3-20 ppb. Although the possible mechanisms for arsenic-induced cancer are associated with sublinear dose-responses, available data do not meet EPA's criteria for departing from a linear extrapolation.

Studies published in 2000 show that trivalent organic forms of arsenic are more toxic than inorganic arsenic. Current studies do not identify infants and children as being at greater risk.

In the January 22, 2001, final rule, EPA's lower bound risk estimates accounted for the arsenic in Taiwanese food and from food preparation in Taiwan.

More than 2,900 of the 3,000 community water systems projected to require treatment serve fewer than 10,000 people. The MCL of 10 ppb will avoid 37–56 bladder and lung cancers per year, and cost households $38–$327 per year in systems serving under 10,000 people.

Although ecological studies identify health effects, uncertainties about exposure, differences in nutrition, selenium, and linear extrapolation of data affect the risk assessment. Additional mode of action studies, animal studies, population studies, and studies of infants and children could improve future risk assessments.

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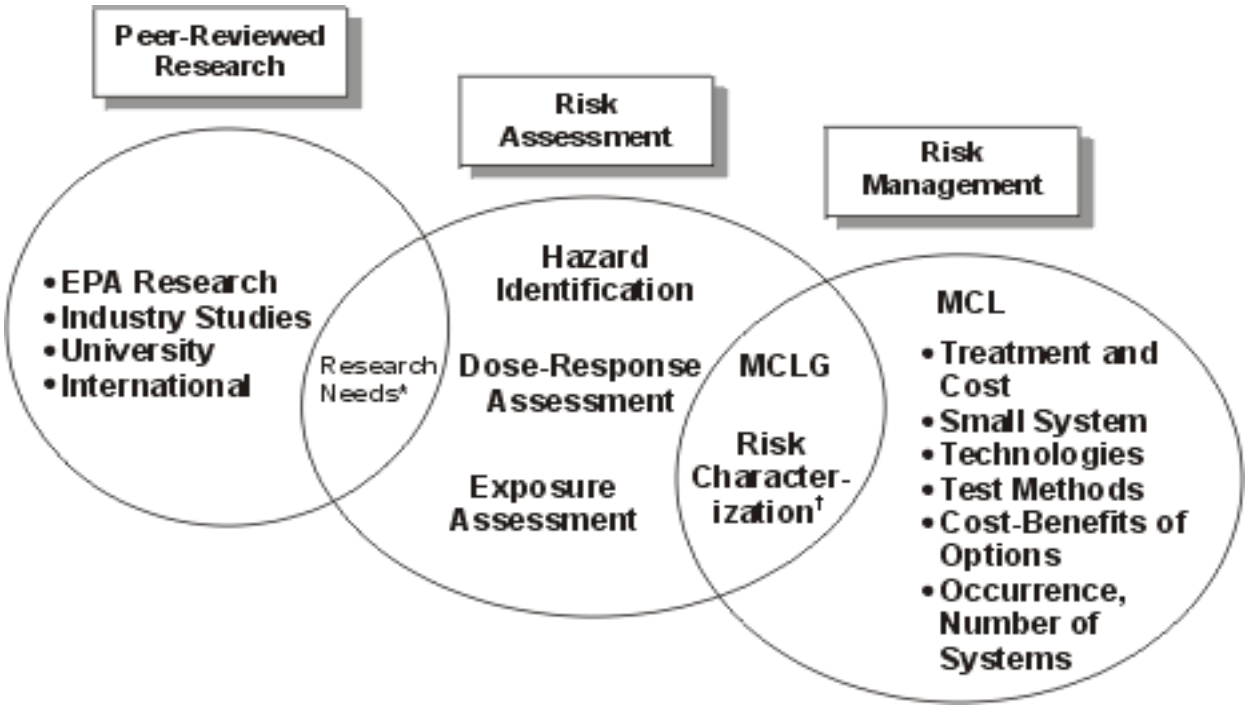

* Arsenic Research Plan (www.epa.gov/ORD/WebPlus/final/arsenic.pdf).
† 1996 Cancer Assessment Guidelines (61 FR 17960).

Figure 1. Role of research and studies in standards development.

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Arsenic-Glutathione Interactions and Skin Cancer

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The goal of this research project is to test the hypothesis that arsenic-induced cancer could result from changes in cellular redox control mediated by glutathione (GSH). Specific aims were designed to: (1) examine the *in vitro* effects of arsenic on the activities of GSH-metabolizing enzymes glutathione S-transferase-pi (GST-π), glutathione reductase (GR), and glutathione peroxidase (GPx); (2) assess GSH-dependent redox status, production of reactive oxygen species (ROS), GSH/GSSG levels, and the activities of GR and GPx in low-dose inorganic arsenite (As^{III}) treated cultured human keratinocytes; and (3) evaluate the role of GSH in arsenic-induced carcinogenesis by examining the effect of varied GSH levels on the rate of skin papilloma induction in normal and GPx-overexpressing mice.

The approach taken by this project was to explore the effects of arsenic on glutathione-regulating enzymes in human skin keratinocytes *in vitro,* and in mice *in vivo*. It was predicted that exposure to physiologically relevant, low doses of As could activate the glutathione-related enzymes due to changes in cellular phosphorylation and/or redox status. If this occurred, it could either potentiate or ameliorate the induction of cellular stress responses, and perhaps skin carcinogenesis. This research has shown that physiologically relevant concentrations of arsenic (less than 100 μM) do not directly inhibit GSH-metabolizing enzymes. Direct enzyme inhibition is only seen at high arsenic concentrations. Low concentrations of As^{III} do, however, cause significant changes in cellular GSH levels and in the relative activity and gene expression of a variety of redox-active enzymes in cultured human keratinocytes or fibroblasts (see Figure 1).

These results show that low, relatively nontoxic concentrations of arsenic can directly modulate cellular redox activity that, in turn, may alter cellular signaling, thereby contributing to the carcinogenic process.

In experiments designed to evaluate the hypothesis that arsenic acts as a progressor or copromoter in the production of skin cancer, this research has confirmed that As does not act as a copromoter with 7,12-dimethylbenz[a]anthracene (DMBA) and 12-O-tetradecanoyl phorbol-13-acetate (TPA) in a standard skin carcinogenesis protocol. This is consistent with the lack of As-induced carcinogenesis in other animal models, and again shows that the biological response of humans to As is quite different from that of rodents. In another mouse experiment using a higher dose of TPA, the significant level of papilloma formation in the DMBA/TPA-treated animals is reduced in the arsenic-exposed animals.

During the last year of this project, additional animal experiments with the GPx-overproducing transgenic mice will be performed. GPx is one of the few identified genes that is downregulated by a low dose of As^{III}, and these animals also are highly sensitive to DMBA/TPA-induced skin cancer. They should provide an interesting additional test of As as a copromoter or progressor.

Frozen samples of skin and other tissues from the first three animal experiments have been stored, and other samples have been preserved in paraffin blocks. These samples will be used to investigate tissue arsenic levels and the levels of GSH-related proteins and mRNA in the skin, bladder, and other tissues from the As-treated and control animals.

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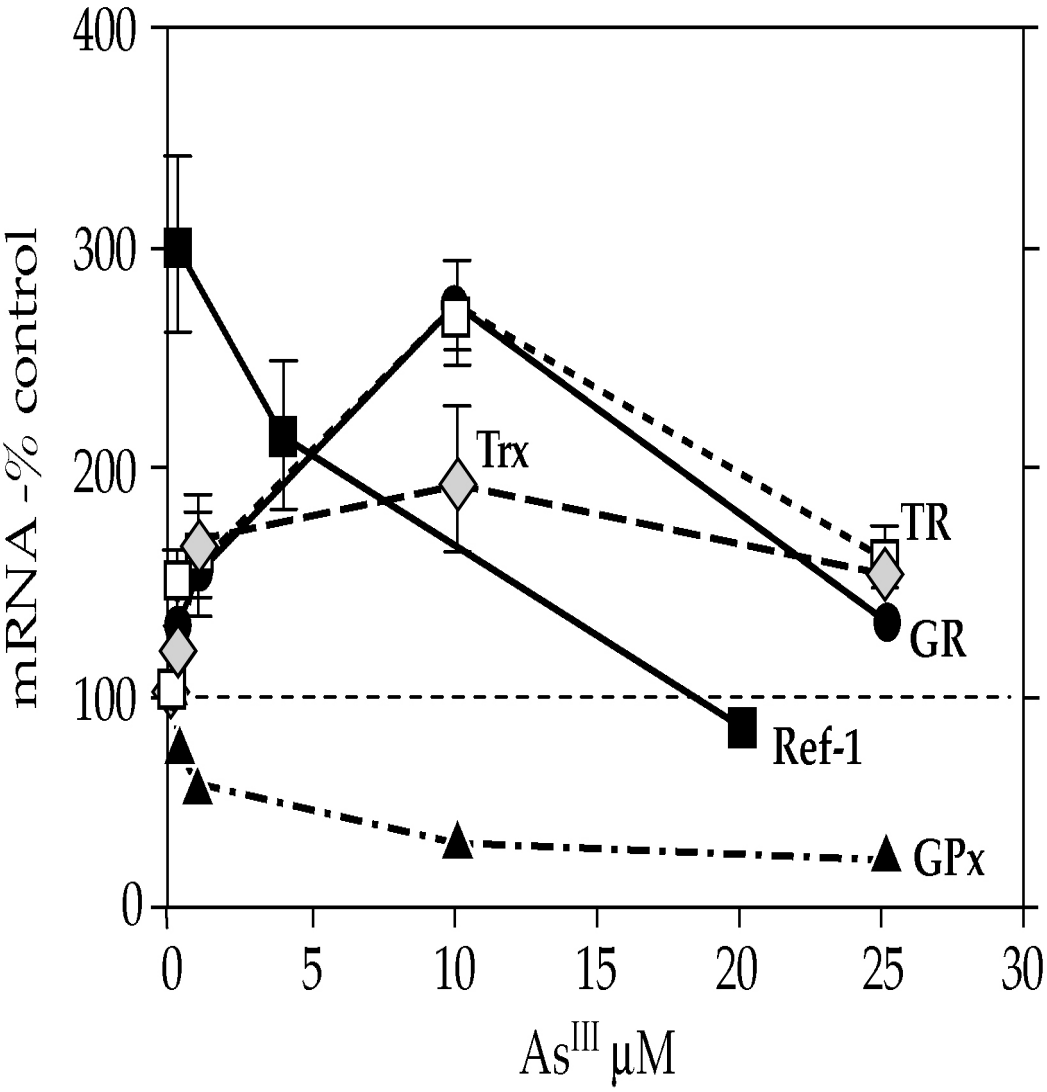

Figure 1. mRNA levels in cells exposed to As for 24 hours.

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A Dose-Response and Susceptibility Investigation of Skin Keratoses and Hyperpigmentation Caused by Arsenic in Drinking Water

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The first detailed assessment of the dose-response relationship of arsenic-induced keratoses and hyperpigmentation at low doses has been completed. Key objectives of the study also included determining whether susceptibility varies by arsenic methylation capability and nutritional factors such as methionine and cysteine. Arsenic methylation was assessed by urinary assays. Nutritional status was determined by blood measurements of key macronutrients and micronutrients as well as by analysis of a dietary questionnaire.

Recently, a case-control study was completed that took advantage of the largest population-based survey conducted of a district in West Bengal, India, with elevated inorganic arsenic levels in its drinking water supplies. The source of the arsenic is geologic.

Potential participants for the case-control study were identified from the 1995 cross-sectional survey for further medical examination and detailed arsenic exposure assessment. The cross-sectional survey included more than 7,000 participants, and measured the residents' current arsenic concentration in their tubewell water supplies. All individuals identified with keratoses and/or hyperpigmentation in the survey, and exposed to $\leq$500 μg/L of arsenic, comprised the case group for the present investigation. The control group consisted of lesion-free individuals randomly selected from the cross-sectional survey database matched on age and sex.

The information used in the earlier survey of arsenic in drinking water was expanded to include information from all current and past water sources used in households and work sites. Data obtained from personal interviews and chemical analyses of drinking water samples were used to assess arsenic exposure. The interviews consisted of questions about lifetime residential history, water sources at home and work, and fluid consumption. The clinical exam involved various dermatologic, neurologic, and respiratory endpoints. A number of participants suspected to have an arsenic-induced skin lesion were photographed. A dietary questionnaire supplemented with results of blood assays will be used to ascertain the participants' nutritional status. Urinary assays will be used to determine arsenic methylation efficiency.

The interviews and sample collection started in June 1998, and ended in December 1999. Participation was excellent (93% in cases and 97% in controls). Ultimately, 192 cases and 213 controls participated. Key preliminary results on the dose-response relation include: (1) strong dose-response trends with peak and average exposures were found based on known years of exposure (test for trend with peak and average exposures, $p<0.0001$); (2) these trends remained after adjusting for sex, age, smoking status, socioeconomic factors, and body mass index; (3) the proportion of cases confirmed as definite or probable by photograph was high (87%)—an implication of this finding is that keratoses and hyperpigmentation are the best biomarkers of long-term effect; and (4) some cases who were initially thought to have consumed very low arsenic levels actually were exposed to higher concentrations in past decades.

For the first time, the dose-response relation of skin lesions and arsenic ingestion at low doses was characterized using a detailed exposure assessment. Also, it was the first study to confirm cases by photograph. Interviewing and sample collection have been completed for 405 total participants. Data entry and editing have been completed, and scientific reports are being prepared for publication regarding the dose-response relation, arsenic methylation capability, and nutritional factors.

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Trivalent Methylated Arsenicals: Novel Biomarkers of Arsenic Toxicity in Humans

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The major goal of this project is to study the effects of inorganic arsenic on a major cellular redox enzyme, glutathione reductase (GR), and on the redox status of cells exposed to arsenic. To better understand the role of metabolism in the toxicity of arsenic, the correlation between effects induced by arsenic and the patterns of its metabolic conversions are systematically examined in live cells. Cells from tissues that metabolize arsenic (liver) and tissues that are targets for carcinogenic effects of arsenic (skin, lung, and bladder) are used in the study. A summary of results obtained during the 3 years (1997-2000) of the study follows.

An inorganic form of trivalent arsenic, arsenite (iAs^{III}), is toxic for most cell types examined only at relatively high concentrations. It is a weak inhibitor of GR and of another major oxidoreductase, thioredoxin reductase (TR), in cultured cells. iAs^{III} is effectively metabolized to methylarsenic (MA) and dimethylarsenic (DMA) species in primary human hepatocytes. However, the capacity of human hepatocytes to methylate iAs^{III} varies significantly among individuals. Human epidermal keratinocytes and human bronchial epithelial cells are much less effective methylators of iAs^{III} than are hepatocytes. Epithelial cells derived from the human bladder do not methylate iAs^{III}. There is no apparent correlation between the capacity of cells to methylate iAs and their susceptibility to acute toxicity of iAs^{III}.

Trivalent methylated metabolites of iAs^{III}, methylarsonous acid (MAs^{III}) and dimethylarsinous acid ($DMAs^{III}$), are significantly more toxic for most cell types than is iAs^{III}. In addition, MAs^{III} is a more potent inhibitor of GR and TR than is iAs^{III} by an order of magnitude. An acute exposure of cells to MAs^{III} results in a significant reduction of intracellular levels of a major cellular antioxidant, glutathione (GSH). Pentavalent methylated metabolites of iAs^{III}, methylarsonic acid (MAs^{V}), and dimethylarsinic acid ($DMAs^{V}$) do not inhibit either GR or TR and are not cytotoxic. In cells exposed to iAs^{III}, inhibition of TR activity strongly correlates with intracellular MAs, suggesting that the trivalent form of MAs^{III} is a product of the metabolism of iAs^{III} in cells.

Treatments with antioxidants (catalase, N-acetylcysteine, or glutathione-ethyl ester) partially protect cultured cells against the toxicity of iAs^{III}, MAs^{III}, or $DMAs^{III}$, indicating that generation of reactive oxygen species may be, in part, responsible for cytotoxicity of trivalent arsenicals. On the other hand, a concurrent exposure to selenite increases cytotoxicity of all three species.

Using an optimized hydride generation atomic absorption spectrometric technique, it was found that trivalent methylated arsenicals, MAs^{III} and $DMAs^{III}$, are indeed products of the methylation of iAs^{III} in human hepatic (HepG2) cells (see Figure 1). Both MAs^{III} and $DMAs^{III}$ also were found in the urine of individuals chronically exposed to iAs from drinking water (see Table 1). The amounts of MAs^{III} and $DMAs^{III}$ positively correlated with total urinary arsenic and thus, with the extent of the exposure to iAs.

In conclusion, trivalent methylated metabolites, MAs^{III} and $DMAs^{III}$, are more potent cytotoxins and enzyme inhibitors than is iAs^{III}. Trivalent methylated arsenicals are natural products of the metabolism of iAs in humans, suggesting that methylation of iAs is not necessarily a detoxification mechanism. In fact, these species may significantly contribute to the adverse effects associated with the exposure to iAs. Because of their pronounced biological effects, MAs^{III} and $DMAs^{III}$ may become sensitive biomarkers of arsenic toxicity in humans.

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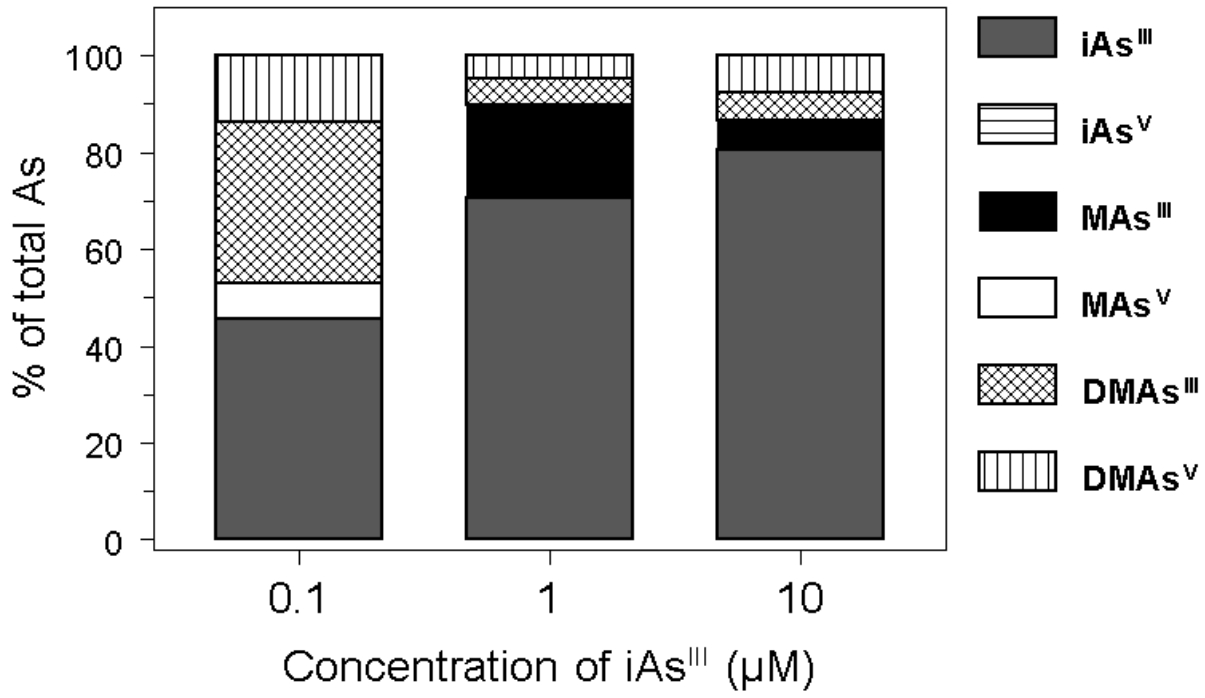

Figure 1. Arsenic metabolites in human hepatocellular carcinoma (HepG2) cells exposed to various concentrations of iAs^{III} for 24 hours. Metabolites in the whole culture (medium + cells) are shown (n=2).

Table 1. Arsenic metabolites in urine (ng/mL) collected from residents of Zimapan region, Mexico.

Age/Sex	iAs^{III}	iAs^{V}	MAs^{III}	MAs^{V}	$DMAs^{III}$	$DMAs^{V}$	Total As
39/f	8.1	4.5	Nd	7.1	Nd	18.6	38.3
19/m	25.2	16.8	2.3	37.7	18.2	40.1	140.3
21/m	59.4	72.1	5.2	119.8	59.8	408.2	724.5
12/m	104.2	122.8	12.3	276.7	114.3	467.2	1097.5
20/f	14.7	10.1	1.2	19.3	5.7	90.5	141.5
11/f	11.2	12.2	1.0	11.2	18.3	52.7	106.6

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Section 3.

Chemical Contaminants: Formation of DBPs

Use of Differential Spectroscopy To Study DBP Formation Reactions

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The major goal of this study is to ascertain the intrinsic mechanism of formation of halogenated disinfection byproducts (DBPs). The novelty of the employed approach is twofold. First, it is designed to explore the nature of intermediates formed prior to the release of individual controlled DBPs (notably, trihalomethanes, haloacetic acids, chloral hydrate). Second, the approach makes use of *in situ* techniques (such as differential absorbance spectroscopy [DAS] and stopped-flow spectrophotometry [SFS]) capable of probing the system with adequate temporary resolution. These techniques were applied to track and quantify the incorporation of halogens into representative organic substrates, which were exemplified by natural organic matter (NOM) and model compounds (e.g., 3,5-dihydrobenzoic acid, [DHBA] and resorcinol). The latter type of species represents halogen attack sites in NOM.

Several major findings have been made. It was determined that the differential spectra of NOM recorded in the conventional mode are dissimilar to those of the model compounds. Nonetheless, a secondary component whose features were comparable to those found for the model aromatic compounds has been detected. The secondary differential spectra appear to be associated with the formation of chlorinated aromatic intermediates that form prior to cleavage of small DBPs from larger NOM precursors. The disappearance of the secondary component of the differential spectra coincided with the onset of release of individual DBPs such as di- and trichloroacetic acids. Time-resolved experiments indicated that the intensity of the features corresponding to the formation of halogenated aromatic units incorporated into NOM molecules is much higher in the SFS mode than it was in the conventional regime. Halogenation of the model compounds in the SFS mode showed that each step of the halogen incorporation consists of two main phases. The first rapid phase corresponds to the formation of a charge-transfer complex, while the slow second phase corresponds to the actual incorporation of the halogen into the aromatic ring. In the case of the model compounds, the charge-transfer complexes exhibit distinct spectroscopic properties, which so far have not been observed for NOM. The reason for this is being explored. It has been suggested that either the rate of halogen interaction with NOM is higher than that with model compounds, or the stereochemistry of NOM does not allow the charge-transfer complexes to form. Thus, the pathway of NOM halogenation is distinct from that of the model compounds.

Despite the need to ascertain the existence of the transient complexes for halogenated NOM, the data of DAS unambiguously indicate that the release of all individual DBPs ranging from trihalomethanes to haloacetonitriles is associated with the breakdown of the halogenated intermediates.

Experiments in which these intermediates were subjected to hydrolysis, oxidation, or thermal treatment demonstrated that the formation of haloacetic acids involves oxidative destruction, while trihalomethanes are released following their hydrolytical breakdown. These data provide important insights into the identity, formation, and breakdown of the halogenated intermediates during chlorination of NOM. The results also support novel technological approaches pertinent to the control and prediction of DBP concentrations in drinking water. Currently, studies of the kinetics and mechanisms of halogenation of model compounds (e.g., flavonoids) are being conducted, as is exploration of the nature of sequential halogen incorporation into the reactive sites. Eventually, it is planned to use the data to create a consistent mechanistic model of DBP formation.

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Brominated DBP Formation and Speciation Based on the Specific UV Absorbance Distribution of Natural Waters

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Understanding the characteristics of natural waters that influence disinfection byproduct formation and treatability is critical for providing safe water and for meeting current drinking water regulations (e.g., the disinfection/disinfection byproducts [D/DBP] rule). Understanding natural organic matter reactivity is of primary importance. Although sophisticated fractionation and characterization of organic mater in natural waters yields important information, one bulk water parameter, the specific ultraviolet absorbance (SUVA), has proved to be a useful and robust predictor of both reactivity with oxidants and treatability. SUVA determination of a water sample yields a single aggregate value that represents the response of a distribution of chromophores within a single natural organic matter (NOM) molecule and among different NOM molecules. Similarly, reactivity of bulk water represents the combined reactivity of many different molecules and molecular moities. The objective of this research is to examine how specific UV absorbance, and more importantly, how the distribution of SUVA in a source water, influences the formation and speciation of brominated DBPs. Such information will be useful for optimizing treatment goals, understanding the effects of treatment processes, and devising strategies to comply with the D/DBP rule.

Two high-SUVA waters (Charleston and Myrtle Beach) and two low-SUVA waters (Troy and Waterford) were fractionated using four physicochemical separation processes (i.e., carbon and XAD resin adsorption, coagulation, and ultrafiltration). For each water, approximately 50–60 fractions were obtained, each having different SUVA values. The fractions were chlorinated according to the uniform formation condition protocol. The formation of trihalomethane (THM), nine species of haloacetic acids (HAA9), haloacetonitriles, chloropicrin, chloral hydrate, and cyanogen chloride were quantified. The relation between the formation and speciation of DBPs and the SUVA of each fraction was examined. Experimental procedures are presented in detail elsewhere (Kitis et al., 2000a,b).

Preliminary results showed that for the three physicochemical separation processes (GAC adsorption, XAD-8 [batch and column] adsorption, and alum coagulation), each fractionated dissolved organic matter (DOM) in natural water samples by preferentially removing higher SUVA components. Similar trends were observed for all water samples tested. The SUVA of DOM remaining in solution was plotted versus adsorbent (GAC or XAD-8) or alum dose applied. The resulting profiles indicated that by increasing the adsorbent or coagulant dose in small increments, it is possible to incrementally fractionate a natural water and to probe the hypothetical SUVA distribution of the sample from high to low values (see Figure 1). However, each process showed a different fractionation endpoint, indicating that low- or non-UV absorbing components of DOM are removed to different extents by each process. As expected, ultrafiltration separated DOM components in solution based primarily on molecular size differences; no preferential removal of higher SUVA components was observed because there was not a strong correlation between SUVA and molecular size.

The results of chlorination experiments and DBP yields obtained thus far clearly indicate that for each water tested, there are strong correlations between the SUVA values of DOM fractions and their THM and HAA9 yields, independent of how the fractions were obtained (see Figure 2). The apparent single trend for each DBP provides evidence that the observed behavior represents the intrinsic reactivity profile of DOMs to DBP formation in a (single) natural water. Also, it was found that low SUVA components of DOM are more efficient at incorporating bromine. The reactivity profile concept (i.e., understanding how reactivity is correlated to SUVA) will allow water utilities to optimize the degree of treatment required to comply with D/DBP regulations. The reactive components that require removal, and the degree of treatment necessary to accomplish this removal, may be directly obtained from the relationship between SUVA removal and the degree of treatment (e.g., alum dose).

Future work will evaluate whether there is any impact of commonly used isolation processes (e.g., reverse osmosis, XAD adsorption) on the DBP reactivity of isolates obtained from natural waters. The observed DBP reactivity profile concept will be further tested for waters having a range of hydrophobicity, measured by the relative proportion of hydrophobic and hydrophilic DOM components. Using physicochemical fractionation, DOM components with different SUVA values will be obtained. The impact of bromine incorporation by these fractions will be examined. Additional physicochemical characterization of the fractions will be attempted to provide insight to the differences in the bromine incorporation by different DOM components. The formation and speciation kinetics of brominated DBPs will be investigated as a function of SUVA, water chemistry, and reaction conditions.

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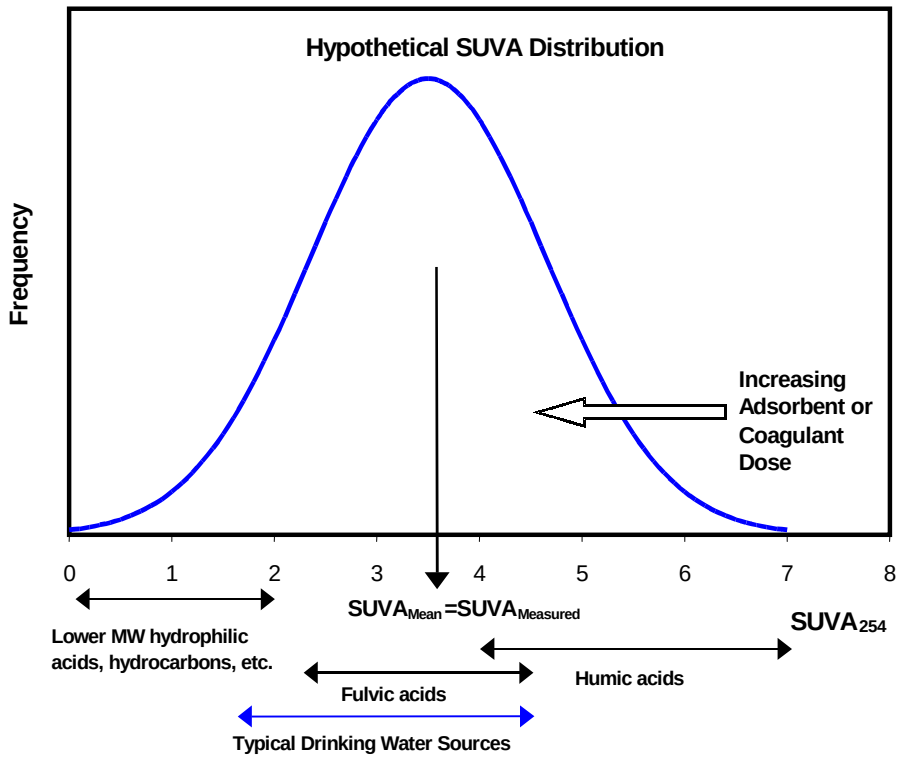

Figure 1. A hypothetical SUVA distribution in a natural water.

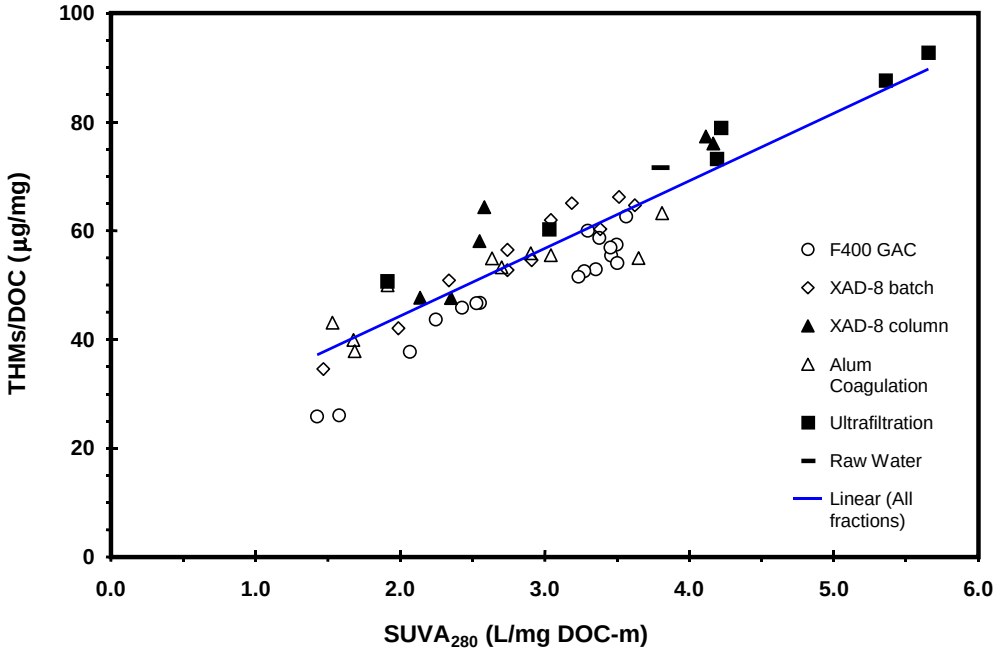

Figure 2. THMs reactivity profile of the natural organic matter fractions obtained from Myrtle Beach water. The solid line represents a linear fit to all experimental data.

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Molecular Weight Separation and HPLC/MS/MS Characterization of Previously Unidentified Drinking Water Disinfection Byproducts

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New approaches are being examined for better characterizing disinfection byproduct (DBP) molecular weight profiles by using tandem mass spectrometry (MS/MS) techniques. The study will include an examination of the differences in DBPs that result from different water disinfection processes. The underlying hypothesis of this work is that new approaches are needed for this assessment and that tandem mass spectrometry, coupled with prior separations, offers promise in that regard.

A prerequisite to making such procedures meaningful is the development of preseparation procedures that will simplify the mass spectral data. The MS/MS system has the potential for assessment of molecular weight in the first stage, followed by generation of specific chemical structural data on selected mass peaks in the distributions via measurement of related fragments from the selected ion. The MS/MS system, hence, has its own separation capabilities.

The work is directed at enhancing these capabilities for complex DBP mixtures with preselection by molecular-size separations using ultra filtration (UF) membranes and size exclusion chromatography (SEC). These preselected molecular-size fractions then would be followed by other high-performance liquid chromatographic (HPLC) techniques. Information has been developed on determining levels of halogenated natural organic matter (NOM), required to obtain measurable mass spectra in both stages of the MS system, using surrogate compounds and their mixtures and chlorinated NOM, represented by Suwannee River Fulvic Acid (SRFA) and a hydrophilic NOM. Studies have been conducted on two instruments, Micromass AutoSpe-OA TOF mass spectrometer at the Metropolitan Water District of Southern California, and a TSQ 7000 ESI quadrapole ion trap mass spectrometer at Kyoto University in Japan. From these studies, information has been found on optimal solvent composition, flow rates, and scan rates for quality spectra. Using individual known compounds, their mixtures, and chlorinated SRFA, variables for the second stage MS were examined as to the balance between obtaining spectral fingerprints and demonstrating the presence of chlorine in the mass selected.

From the complexity of bulk sample spectra, it is apparent that preseparation of the DBP samples will be essential. To that end, chlorination of SRFA and Suwannee River Humic Acid (SRHA) have been conducted using chlorine 36 labeling to obtain molecular size distribution of the chlorinated products on size separation columns (see Figure 1). To date, this has been a 25 x 200 mm BIAX column (Chrom, Germany) packed with Toyopearl HW 50S resin (Japan), with a nominal fractionation range of 100–20,000 g/mol.

It is apparent that chlorine is distributed across the entire molecular size range, SRHA has chlorine at higher molecular size than SRFA (as expected), and radioactivity also shows up at longer retention times than for inorganic chloride. This is not surprising, as work with known compounds has demonstrated that several low molecular weight chlorinated compounds eluted after chloride, including haloacetic acids. Longer chlorine contact times show a slight shift to smaller molecular size distributions (1 hour versus 24 hours versus 5 days).

The levels of sample concentration required to obtain MS data have been defined; however, the preliminary results demonstrate that separations to reduce the complexity of the first stage spectra are going to be essential to reduce the complexity of the second stage MS.

The use of chlorine 36 affords the mechanism for evaluation of the separation process by greatly facilitating data acquisition from SEC and other HPLC columns. Traditional total organic halogen measurements proved to be tedious and impractical.

The following are projected activities for the immediate future: (1) additional HPLC columns will be evaluated for separation of the components prior to MS/MS examination—Protein Pak SEC and C18 columns will be used; (2) solid-phase extraction will be explored as a preconcentration step prior to mass spectrometry analysis; and (3) using a mixture of standards, high resolution electrospray ionization will be investigated, which may provide accurate mass determination at the first stage ($\Delta m = 0.1$ for m/z 500 at 5,000 resolution) and better definition of parent ions for MS/MS.

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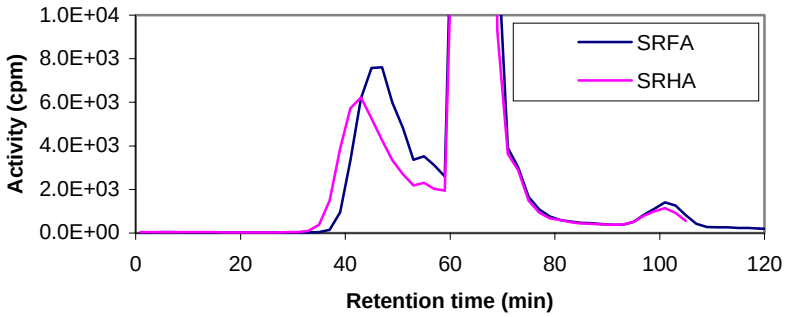

Figure 1. Comparison of SEC-Cl36 chlorinated SRFA and SRHA samples.

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Membrane Introduction Mass Spectrometry Studies of Halogenated Cyano Byproduct Formation in Drinking Water

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Increasing recognition is being given to the importance of nonhumic precursors of disinfection byproducts (DBPs) in drinking water. This especially is an issue in water supplies with low humic matter content and as more effective removal of humic matter is achieved. Proteins, peptides, and amino acids have been implicated as important precursors of halosubstituted nitriles and cyanogen halides under these conditions. Efforts to reduce the use of chlorine disinfectants also have increased the significance of brominated cyano byproducts when bromide concentrations are elevated.

Colorado River Water (CRW), a source of drinking water for more than 20 million people, represents a water supply where these conditions collide; it is both low in humic matter and contains moderately high bromide concentrations. The proposed research seeks to: (1) determine which amino acid precursor compounds represent the most important source of these DBPs and the structural features that contribute to their reactivity, (2) characterize the kinetics and formation mechanism of chlorinated and brominated cyanosubstituted DBPs, and (3) model the formation of halogenated cyano compounds in CRW. Initial phases of the project involve a set of characterization and screening experiments that will provide the basis for selecting a subset of amino acids and peptides for later mechanistic study. The amino acid composition of CRW samples currently is being characterized. Relative reactivities of free and combined amino acids in CRW with chlorine and chloramine will be determined, and the formation potentials of haloacetonitriles (HANs) and cyanogen halides (CNX) due to the chlorination, chloramination, and bromination of model amino acids will be examined.

Upon selecting a short list of amino acid and peptide precursors, the kinetics of amino acid halogenation will be studied by applying a new online technique known as membrane introduction mass spectrometry (MIMS). The method relies on the selective membrane separation of volatile species directly from aqueous solution into the vacuum of a mass spectrometer ion trap (see Figure 1). This "pervaporation" approach offers important advantages for the kinetic study of volatile byproduct formation, such as cyanogen halides and nitriles, as extraction and reaction quenching steps are avoided.

Based on the proposed kinetic studies of cyano byproduct formation, a model for HAN and CNX formation in CRW will be formulated. Simulation results will be compared with actual CRW chlorination and chloramination experiments.

The findings of this research will help water suppliers to assess the precursor sources of HAN and CNX, gain insight into possible control strategies (e.g., controlling algal activity, adjusting solution chemistry), and assess the risk of disinfection strategies. The research also will help to establish optimal approaches for applying MIMS techniques to disinfection byproduct analysis.

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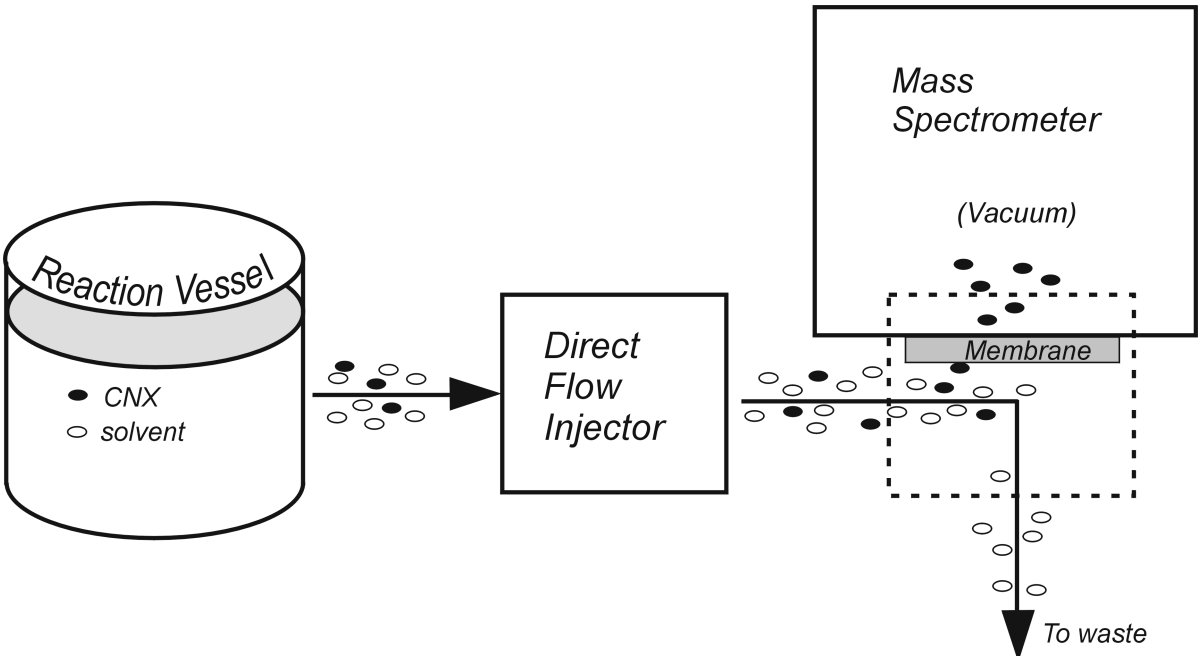

Figure 1. Schematic illustrating the online MIMS measurement of cyanogen halide formation rates.

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Mechanisms and Kinetics of Chloramine Loss and Byproduct Formation in the Presence of Reactive Drinking Water Distribution System Constituents

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This project seeks to enhance understanding of the influence of reactive substances in drinking water on: (1) the fate of monochloramine and the nature of inorganic reaction products, (2) the kinetics of monochloramine loss, and (3) the formation of selected organic disinfection byproducts (DBPs). Results also will be used to develop and extend mechanistic chloramine reaction models to include the effects of these reactive substances.

The approach has been to investigate reactions in batch reactors containing laboratory prepared model waters. These waters contained a variety of reactive organic and inorganic components. Natural organic matter (NOM) includes both extracted humic material (HM) and nitrogenous compounds (NCs) such as dimethylamine. Monochloramine reacts slowly with humic-type organic matter. Most of this loss can be attributed to relatively simple reduction reactions, not substitution to form organic DBPs. The loss is characterized by a biphasic reaction. Modeling results indicate that a one-site reaction model is not adequate to describe monochloramine loss in the presence of HM. The reactivity of monochloramine correlates with the UV absorbance. After resolving several monochloramine loss pathways occurring in the presence of humic NOM, the decrease in UV absorbance with time was observed to correlate with the amount of monochloramine reacting with HM. The stability of both nitrite and monochloramine in their mixtures successfully was modeled by consideration of its oxidation to nitrate by monochloramine via a direct reaction.

Haloacetic acid (HAA) formation was correlated with monochloramine reactivity with HM. Several HAAs also were reactive with iron oxides. The fastest and most extensive decay was observed for monobromoacetic acid. Dichloroacetic acid was the most stable. Several brominated trihalomethanes were reactive in the presence of ferrous hydroxide. Monochloramine reacts with dimethylamine to form dimethylnitrosamine (NDMA), a potent carcinogen. A mechanism is proposed in which monochloramine reacts with dimethylamine to form hydrazine, which in turn is oxidized by additional monochloramine to NDMA. A reaction model, which considers several important reactions, appears reasonably capable of predicting NDMA formation in this simple system (see Figure 1).

Reaction of monochloramine with humic type NOM may be an important loss pathway, which also results in the formation of several DBPs, especially HAAs. Reaction of some HAAs with pipe deposit material may be an important loss pathway in distribution systems.

The stability of nitrite in the presence of monochloramine indicates that they may coexist in drinking water. It is believed that formation of NDMA (and other nitrosamines) by reaction of monochloramine with appropriate precursor substances may be important in chlorinated and chloraminated water and wastewater. The hypothesized mechanism is in contrast to classical nitrosation reaction, which requires the presence of nitrite. NDMA should therefore be considered a "new" disinfectant byproduct.

Future work will focus on improving the model for monochloramine loss and HAA formation in the presence of NOM and nitrite. A special emphasis will be on improving the understanding of the newly proposed NDMA formation mechanism, and its potential significance in chlorinated and chloraminated waters.

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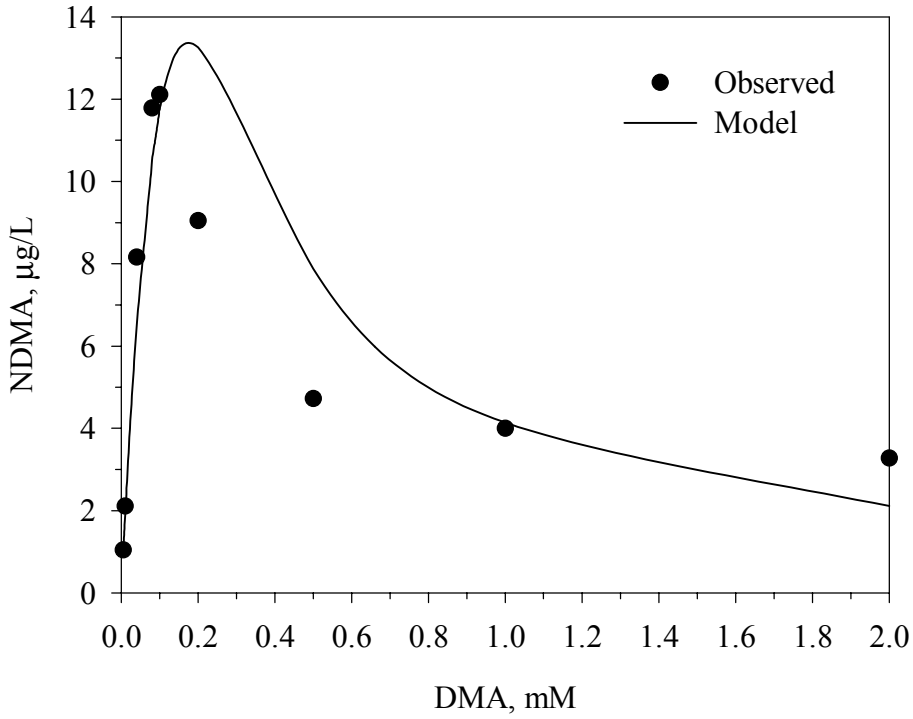

Figure 1. Formation of dimethylnitrosamine (NDMA) by reaction of monochloramine with dimethylamine (DMA). Reaction time: 24 hours. Monochloramine concentration: 0.1 mM. Temperature: 25°C. Air: saturated 1 mM bicarbonate buffer. pH: adjusted to 7.0 ± 0.1.

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Development of a New, Simple, Innovative Procedure for the Analysis of Bromate and Other Oxy-Halides at Sub-ppb Levels in Drinking Water

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This project developed a relatively simple methodology to provide the tools for assessing exposure to bromate in drinking water at the 10^{-6} cancer risk level of 0.05 µg/L that has hitherto been impeded by the lack of sensitivity of existing methodologies. This new analytical methodology provides the U.S. Environmental Protection Agency and the water monitoring community with the ability, using existing analytical equipment with simple add-on accessories, to monitor bromate, chlorite, and iodate in drinking water in a linear range from 0.05–100 µg/L without being impacted by the presence of higher levels of anions.

The postcolumn reaction converts bromate, chlorite, iodate, and to a lesser extent chlorate, eluting from an ion chromatographic column into the highly sensitive chromophore, the tribromide ion, which can be detected at levels as low as 0.05 µg/L in drinking water (see Table 1). This method was evolved with the express purpose of providing a tool for meeting the objective of evaluating bromate occurrence at the 10^{-6} cancer risk level resulting primarily from the use of ozone in drinking water treatment.

Coincidentally, the sensitivity of this method provided proof of the presence of bromate resulting from the use of hypochlorite as the agent of disinfection. Depending on the dosage and number of points of hypochlorite addition during treatment, the levels of bromate resulting from its usage are indicated in the range 0.1–3 µg/L.

Among 20 plants surveyed using this technique, the average level of bromate contributed by the use of hypochlorite was 0.94 µg/L. A mass balance between the levels of bromate in the hypochlorite feedstocks and finished water proved beyond doubt the source of the added contaminant. The results do suggest that the level of contamination of bromate in hypochlorite varies across the country with those plants sampled in Region 5 exhibiting the highest levels of contamination. With discussions towards future regulation of bromate in drinking water required to balance risk with the cost of alternative treatment, this finding seriously is impeding attempts to regulate bromate closer to 0.05 µg/L from its current regulated level of 10 µg/L in the United States.

Table 1. Statistics of analysis.

Detection	Analyte	PQL (µg/L)	N	Standard Deviation	RSD Percent	MDL (µg/L)
UV-PCR	Iodate	0.06	7	0.01	12	0.04
	Chlorite	0.10	6	0.02	18	0.08
	Bromate	0.05	7	0.004	7.8	0.01
	Chlorate	70.0	7	8.5	12.2	31.5
Conductivity	Bromide	10.0	7	1.5	15.4	5.6
	Chlorate	10.0	7	1.0	10.1	3.7

PQL = Practical quantitation limit.
RSD = Relative standard deviation.
MDL = Method detection limit.

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Formation and Stability of Ozonation Byproducts in Drinking Water

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Generally, it is perceived that disinfection byproducts (DBPs) produced by ozonation represent less of a health hazard than those produced by the chlorine-containing disinfectants, at least by the use of short-term bioassays. Also, it has been noted that the types of oxidation byproducts produced by ozonation of natural waters are in many cases the same as compounds produced by natural oxidation processes in streams, lakes, and reservoirs. The implication is that naturally occurring compounds will be safer than "unnatural" compounds such as trihalomethanes (THMs) and haloacetic acids (HAAs) produced by chlorination. However, there are fallacies associated with each of these arguments.

Short-term bioassays are not at a stage of development to use in relative risk assessments. Also, the preconcentration methods used to obtain extracts for bioassays may not efficiently trap polar byproducts from ozonation of natural waters. Natural waters themselves often are mutagenic, so the argument that ozone produces "natural" organics is not encouraging.

With less than 50 percent of the assimilable organic carbon generated by ozonation remaining unidentified, this project is investigating new methodologies for targeting unidentified byproducts. These together with refined existing techniques will be employed to study the impact of water quality parameters on the formation and stability of these compounds in distributed drinking waters.

Stage one of this study focused on evaluating methodologies for accurately targeting carbonyl-containing compounds tentatively identified in a variety of literature results combining field-, pilot-, and laboratory-scale ozonations. The species targeted in this initial approach include the following compounds: C_1-C_{12} monosaturated aldehydes, glyoxal, methyl glyoxal, dimethyl glyoxal, trans-2-hexenal, formic, acetic, and oxalic acids, and the three mixed functional pyruvic, glyoxylic, and ketomalonic acids.

Previous attempts to quantify these byproducts in aquatic matrices involving derivatization techniques may have suffered from inadequate recoveries. This conclusion was arrived at by synthesizing the relevant oximes and esters and comparing derivatization efficiency in the aquatic medium alongside the pure derivatized standard. Purification of the derivatized standards involved utilizing a stepwise thin layer chromatography with the isolated product assayed by proton nuclear magnetic resonance.

Stage two of the project involved the development of new analytical methods for determining three distinct groups of proposed ozonation byproducts that hitherto have not been identified in ozonated waters. These include organic peroxides, epoxides, and multifunctional carbonyl-containing organic byproducts. In the absence of many commercial standards for these compounds, these efforts have focused on evolving strategies for maximizing isolation from the aquatic matrix and enhancing detection for low molecular weight representative compounds from each of these classes.

The peroxides are separated on a C-18 column by high-performance liquid chromatography (HPLC). A postcolumn reaction then converts the individual species into hydroxyl radicals, which then react with para-hydroxyphenylacetic acid at pH 11 to produce a fluorescent dimer whose detector response is directly proportional to the concentration of organic peroxide in the original sample. Current detection limits are in the range of 10–30 μg/L, but these probably can be lowered by an order of magnitude using a preconcentration approach. Epoxides are extracted from water after derivatization with difluoroaniline, but the kinetics of the latter reaction are slow and are impacting a realistic minimum quantitation limit.

Carbonyl-containing compounds that have not been amenable to derivatization with pentafluorobenzylhydroxylam successfully are isolated from water using 2,4-dinitrophenylhydrazine, which produces a fingerprint diode-array spectrum following resolution of individual components by HPLC. Online preconcentration permits detection at the 10 nanomolar level, and the technique is compatible with electrospray mass spectrometric detection, which will be used to identify new byproducts. Laboratory-generated ozonated surface waters are being used to assess these methods using a semibatch operation to generate the byproducts (see Figure 1).

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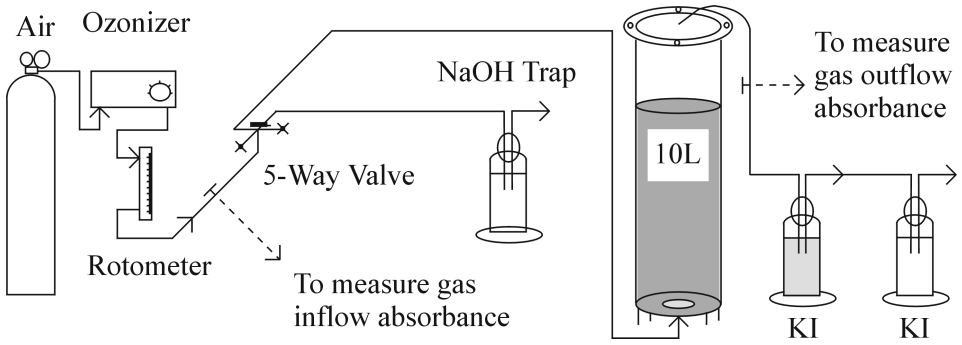

Figure 1. Scheme of the approach used in the laboratory for generating ozonated waters.

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Kinetic-Based Models for Bromate Formation in Natural Waters

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Ozone (O_3) is an effective disinfectant, but it can form byproducts (e.g., bromate). Bromate forms via oxidation of naturally occurring bromide through a series of steps involving O_3 and hydroxyl radicals (HO). There is a need to develop tools to understand and predict bromate (BrO_3^-) formation while still achieving high levels of microbial disinfection. The central hypothesis is that a kinetic-based understanding of natural organic matter (NOM) reactions with HO and aqueous bromine ($HOBr/OBr^-$) over a range of temperatures is necessary to develop mechanistic-based models for bromate formation in bulk waters.

The objectives of this project are to: (1) develop a comprehensive database of BrO_3^-, O_3, and HO radical concentrations; (2) determine rates of reaction between HOBr and OBr^- and NOM; (3) calibrate and verify a BrO_3^- formation mechanistic-based model that includes NOM; (4) simulate BrO_3^- control measures necessary to meet proposed and future maximum contaminant levels; and (5) link the numerical BrO_3^- formation model with hydraulic and concentration x time disinfection models.

Approximately one-half of the database including oxidant concentrations (O_3 and HO) and bromate data has been completed for ultrapure water and Colorado River Water. R_{ct} values have been calculated that represent the ratio of O_3 to HO concentrations. A correlation between R_{ct} and dissolved organic carbon (DOC), alkalinity, pH, and temperature has been developed from literature values.

After testing several experimental methods for determination of reaction rates between NOM and halogens (e.g., aqueous bromine), a colorimetric approach (ABTS) was adopted. A large experimental matrix of reaction rate constants have been determined for the reaction of HOBr and OBr^- with unozonated and ozonated NOM (see Figure 1). Although not directly related to bromate formation, companion experiments using HOCl and OCl^- were simultaneously undertaken to better understand the reaction mechanisms and provide insight into organohalogen formation.

As an example of representative results, the second order reaction between HOBr and CAP-NF for HOBr was 31 $M^{-1}s^{-1}$ and only 1.4 $M^{-1}s^{-1}$ for HOCl. Hence, HOBr reacts faster with NOM than HOCl, but the rate of reaction is quite low given the rates of reaction between important oxidants (ozone and HO radicals) and bromine species during the formation of bromate. The presence of NOM is likely to exert a larger effect on the amount of oxidant present than affect the intermediate bromine species during bromate formation.

A mechanist model has been developed to predict bromate formation as a function of oxidant pathways and key water quality factors (pH, ammonia concentration, alkalinity). The model handles the presence of DOC as a site-specific rate constant value.

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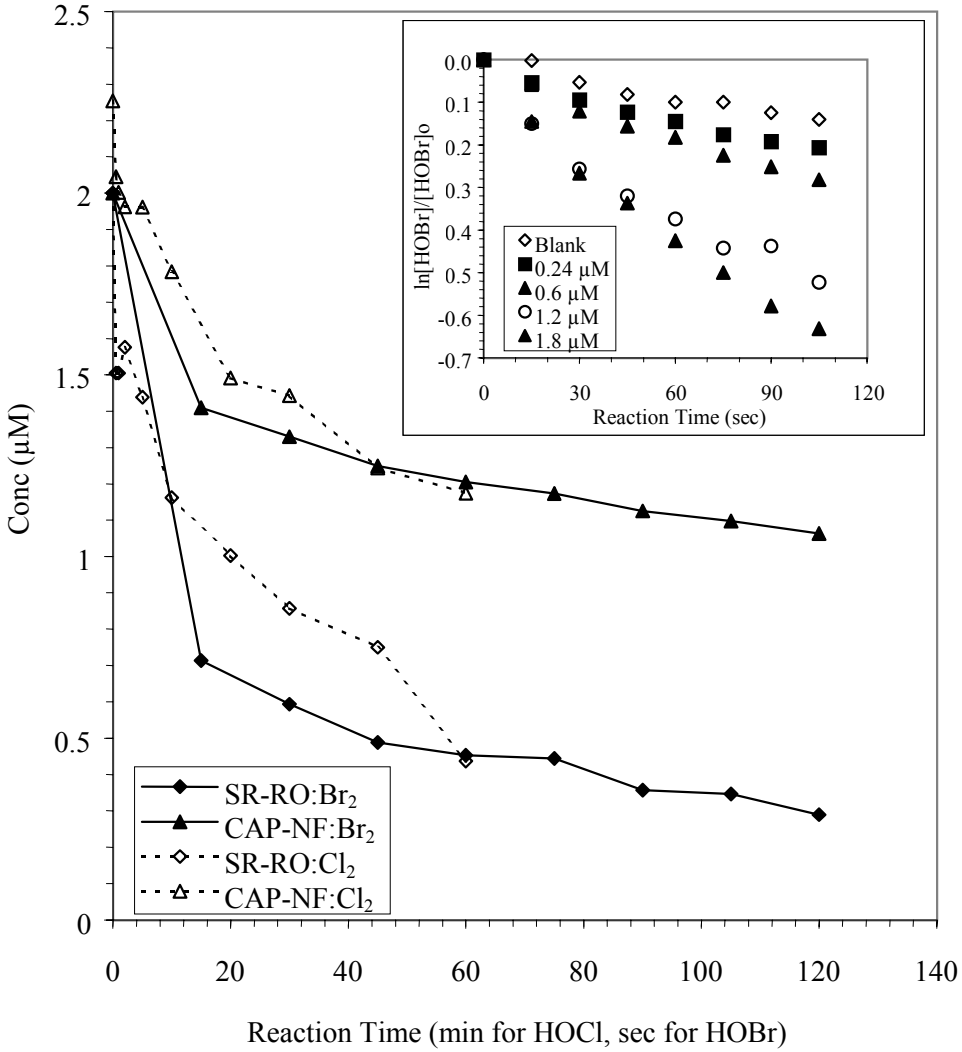

Figure 1. Consumption of HOBr and HOCl by a reverse osmosis isolate (SR-RO) from the Suwannee River or nanofiltration isolate (CAP-NF) from the Colorado River (pH = 5, DOC = 50 µM). The inset figure represents analysis for estimation of pseudo first-order rate constant for HOBr consumption by CAP-NF.

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Mechanistic-Based Disinfectant and Disinfectant Byproduct Models

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The water industry faces new challenges in understanding and controlling disinfection byproduct (DBP) formation as health concerns demonstrate a need for more stringent regulatory DBP requirements. Mechanistic tools for understanding and predicting the rate and extent of DBP formation are required to facilitate the evaluation of DBP control alternatives.

This research project involves the development of a mechanistic-based numerical model for chlorine decay and regulated DBP (trihalomethane [THM] and haloacetic acid [HAA]) formation derived from (free) chlorination; the model framework will allow future modifications for other DBPs and chloramination.

Predicted chlorine residual and DBP results will be compared against predictions from several other quasi-mechanistic models. It is anticipated that a significant improvement in prediction accuracy over existing empirical models will occur. The central modeling hypothesis is that a two-site reaction mechanism can be used to predict disinfectant decay in the presence of natural organic matter (NOM). It assumes that NOM contains both slow and fast disinfectant-reacting and DBP-forming sites. NOM site densities and concentrations are related to the concentration, size, structure, and functionality of NOM. The project is approximately 90 percent completed. Raw waters from the Colorado River (AZ), Lake Houston (TX), and Harwoods Mill (VA) were collected and subjected to laboratory treatment by the following processes: filtration only plus coagulation, ozonation, chemical softening, activated carbon sorption, and ultrafiltration followed by filtration. A total of 18 samples were produced and subsequently subjected to chlorination.

The amount and characteristics of the dissolved organic carbon were altered by these processes, and were quantified by UV absorbance, fluorescence, molecular weight, and hydrophobicity. Upon chlorination, the kinetic consumption of chlorine residual and DBP formation was monitored under different water treatment conditions (pH, temperature, chlorine dose, spiked bromide) over the timeframe of a few minutes to days.

Figure 1 represents typical THM species production (symbols), shown for raw Colorado River water. DBP production was simulated using a C+-language model that simultaneously solved differential equations (lines in Figure 1). A good correlation between experimental and predicted values has been obtained for chlorine residual, THM species, and dihalogenated HAAs.

The model has been encoded into Version 2 of the U.S. Environmental Protection Agency's Water Treatment Plant Simulation Model, which has the option of using either the mechanistic model developed herein, or the existing empirical models, for predicting DBP formation in water treatment plants and distribution systems.

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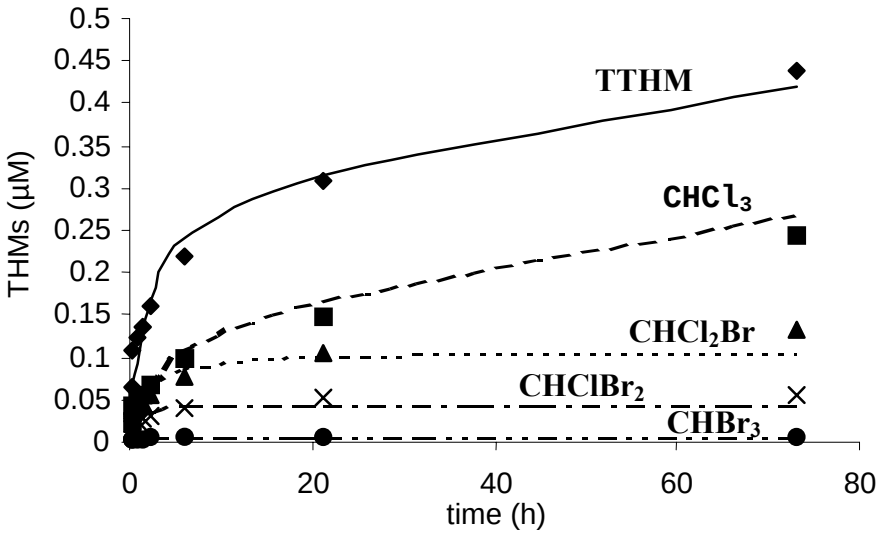

Figure 1. Kinetic production of THM species observed in laboratory batch tests (symbols) and simulated by the mechanistic model (lines) upon chlorination (38 µM) of raw Colorado River water (T = 15° C, pH=7.5, Br = 1.18 µM).

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Section 4.

Drinking Water Treatment Studies

Evaluation of the Efficacy of a New Secondary Disinfectant Formulation Using Hydrogen Peroxide and Silver and the Formulation of Disinfection Byproducts Resulting From Interactions With Conventional Disinfectants

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The objectives of this research project address two critical issues associated with the use of a new secondary disinfectant formulation utilizing hydrogen peroxide (H_2O_2) and silver (Ag^+): (1) the efficacy of the formulation to provide long-term residual disinfection, including the control of coliform bacteria, bacterial regrowth, and slime/biofilm control; and (2) the identification and quantification of disinfection byproducts (DBPs) that may result from interactions with conventional chlorine- and oxidant-based disinfectants. The research encompasses laboratory studies and demonstrations to evaluate the effectiveness of the alternative disinfectant in a range of source waters and utility system characteristics. The secondary disinfectant is one of the few nonchlorine-based disinfectants that can provide long-term residual disinfection in drinking water systems. By combining two or more disinfection agents, it may be possible to lower concentrations of each component, reduce exposures, minimize the formation of toxic and undesirable DBPs, and thus minimize health risks associated with disinfection.

The approach to this research includes the following: (1) laboratory evaluation of microbial disinfection efficacy, including optimal formulation of the secondary disinfectant and optimal doses of primary and secondary disinfectants; (2) laboratory evaluation of DBP formation resulting from interactions with various primary disinfectants; and (3) demonstration of the disinfectant's effect on biofilm formation and removal to provide "real world" results. These components are designed to provide a comprehensive evaluation of the microbial disinfection efficiency and DBP formation potential of the new disinfectant.

Preliminary findings indicate that the addition of the secondary disinfectant following the use of chlorine as a primary disinfectant produces very dramatic reductions in DBP formation (e.g., trihalomethanes [THMs] and haloacetic acids [HAAs]), as demonstrated in Figure 1 using local groundwater. This results from the reduction of chlorine to chloride by H_2O_2, which halts further reaction of chlorine with dissolved organic matter and other DBP precursors. When used with ozone, H_2O_2 quenches formation of THMs and also reduces, to an extent, the formation of inorganic byproducts (e.g., bromate), as demonstrated in Figure 2. These reductions result from several reactions that have been investigated in both empirical and mechanistic studies (e.g., Figure 3 shows the kinetics of bromate formation in a model system). The reduction in DBPs resulting from interactions among primary and secondary disinfectants applies to a wide range of temperatures, pH, bromide concentrations, and dissolved organic carbon levels. Sequential additions of chlorine, ozone, or other primary disinfectant (possibly with ammonia) and H_2O_2/Ag^+ as a secondary disinfectant may provide optimal performance. Aldehyde formation from reactions between H_2O_2 and ozone are being investigated.

The inactivation performance of the combined disinfectant, its individual components, and a commercially available stabilized formulation of H_2O_2 and Ag^+ have been evaluated for several bacteria and viruses. Laboratory studies indicate that the combined disinfectant exhibits a synergistic action on the viability of *E. coli*; however, no increased virucidal action was observed. The biocidal action generally increased with higher temperature and pH, and decreased in secondary and tertiary effluents. The H_2O_2 component induced a wide array of stress responses, and bacteria deficient in the ability to activate central cellular stress responses were hypersensitive to both H_2O_2 and Ag^+.

These studies suggest that the combined disinfectant may be appropriate for use as a long-term secondary residual disinfectant for relatively high-quality water. However, further experiments examining biofilm prevention showed that the bacteria surviving after 48 hours of disinfection had high catalase activity, hinting that the combined disinfectant may have limited effectiveness in continuous operation. Figures 4a and 4b show that biofilm prevention effectivity is largely due to H_2O_2, and that inactivation activity is higher at the first stages of biofilm formation and less effective at later ones.

Widespread use of the combined disinfectant, if practical, might result in potential for uptake in fish and humans. An ecological model was constructed to simulate partitioning between water and sediment, uptake by algae, invertebrates, and fish (trout and carp) as well as risks to humans from fish consumption. Monte-Carlo

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simulations were used to represent the uncertainty and variability of input parameters. The modeling effort used a variety of scenarios, including "worst case" conditions in which receiving waters provided small amounts of dilution and subsistence fishers consumed large amounts of high trophic-level feeders. The results suggest that risks are minimal under all likely scenarios.

Information is being developed regarding long-term disinfection efficacy in different source waters and environmental and utility conditions. Effects on DBPs of the primary disinfectants and any new byproduct formation are quantified, as are optimum dosages and pathogen inactivation. These results will be compared to the disinfection efficacy and DBP formation of conventional disinfectants. The research results will be suitable for use in exposure and risk assessment purposes to support future policies and decisions regarding disinfection approaches.

Laboratory tests on the DBP formation potential are being concluded with an investigation of aldehyde and other species resulting from interactions with H_2O_2 and ozone. The inactivation performance evaluation studies for poor water quality conditions (i.e., high total organic carbon-level water) are underway.

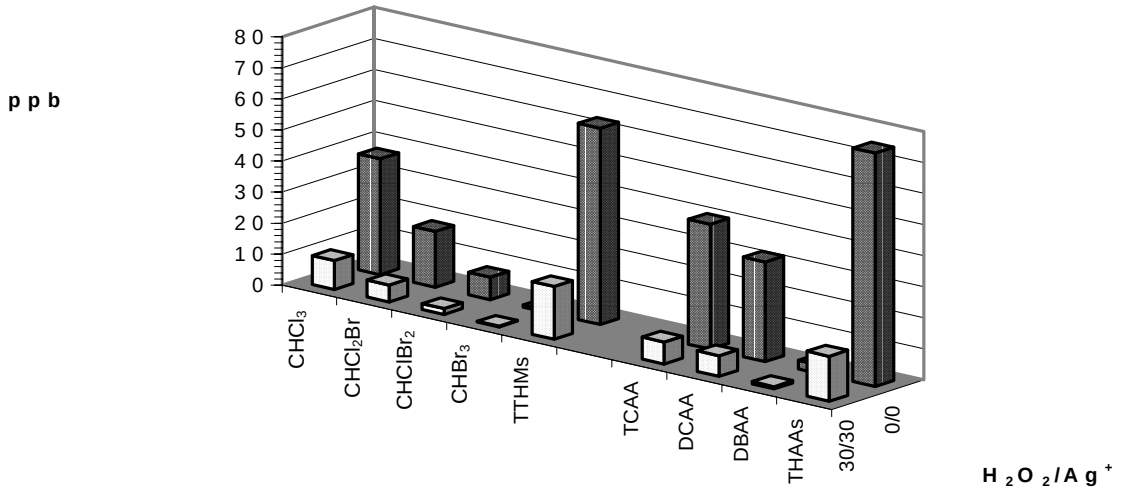

Figure 1. Reduction in THMs and HAAs as a result of the addition of a secondary disinfectant following the addition of chlorine as a primary disinfectant in laboratory tests. Mixed ground and surface water in the local city supply was used. The graph reflects DBPs resulting after 24 hours. The bars in the foreground reflect the secondary disinfectant, the bars in the rear reflect Cl alone. The basic water parameters were: Br^- = 0.081 mg/L, TOC = 3.1 mg/L; initial Cl = 5 mg/L; residual Cl = 1.85 mg/L, pH = 6.85 (buffered). TTHM=total THMs; THAAs=total HAAs.

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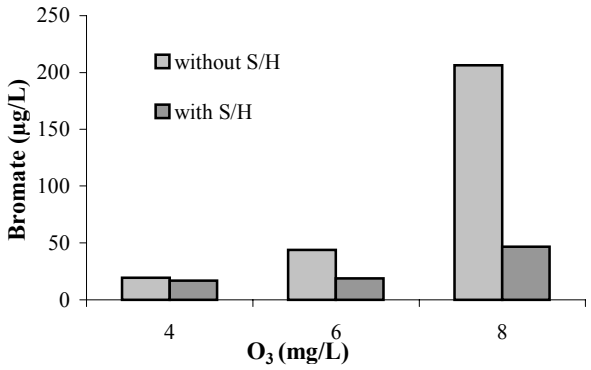

Figure 2. BrO_3^- formation with and without additions of Ag^+/H_2O_2 (S/H) at various initial O_3 concentrations. $[Br^-]$ = 600 µg/L; [DOC] = 3.0 mg/L; pH = 7.0; T = 30 C; t = 30 min.

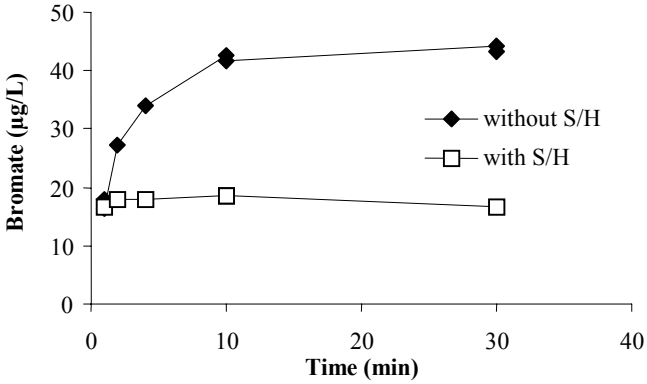

Figure 3. Time trend of BrO_3^- formation with and without Ag^+/H_2O_2 (S/H) addition. $[O_3]$ = 6.0 mg/L; $[Br^-]$ = 600 µg/L; [DOC] = 3.0 mg/L; pH = 7.0; T = 25; Ag^+/H_2O_2 (30 µg/L/30 mg/L) added 1 min after ozonation started.

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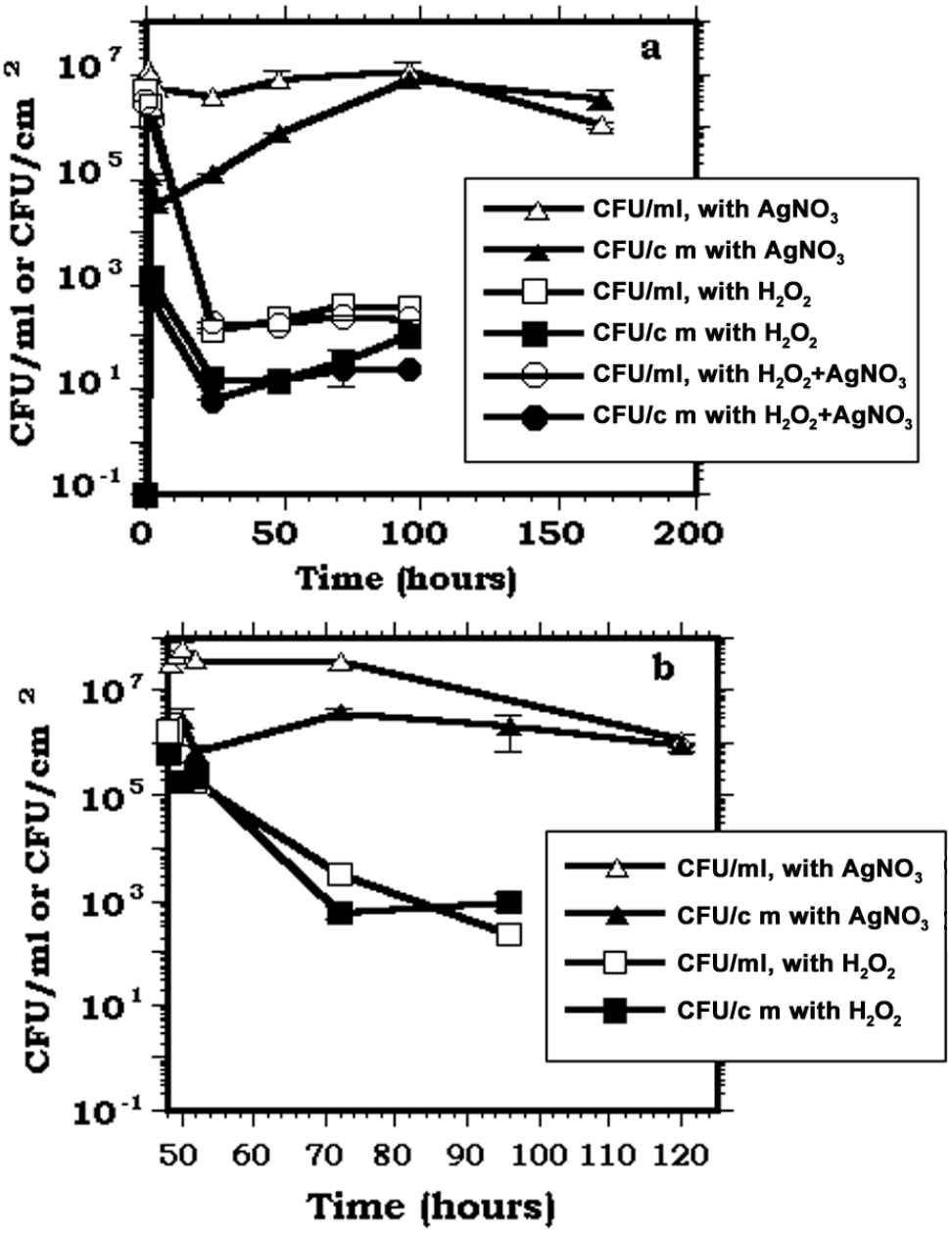

Figure 4a. Effect of $AgNO_3$ (30 ppb) and H_2O_2 (30 ppm) combined addition after 48 hours of biofilm formation. In this case, silver ions reduced biofilm bacteria by a half order of magnitude for short time (approx. 5 hours), then bacterial counts returned to previous values. A much sharper reduction was observed with free living and biofilm bacteria under the activity of H_2O_2. For 20 hours, biofilm count decreased by four orders of magnitude, while free living bacteria continued up to 95 hours to drop by four orders of magnitude.

Figure 4b. Effect of $AgNO_3$ (30 ppb) and H_2O_2 (30 ppm) on free living and adsorbed bacteria (biofilm) on galvanized iron coupons in tap water. Free living bacteria were reduced by 3.5 logs during 24 hours under combined $AgNO_3$ and H_2O_2. Biofilm bacteria were reduced under similar conditions by 2 logs for the same period of time. In both cases, bacterial numbers did not change and remained stable up to 100 hours (termination time of the experiment).

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Evaluation of Ozone Byproduct Formation Under Ozone Dose, Temperature, and pH Variation

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This project was one part of a comprehensive research plan (*Research Plan for Microbial Pathogens and Disinfection By-Products in Drinking Water*) established by the U.S. Environmental Protection Agency's Office of Research and Development in 1997. The principal goal of this study was to study the effects of ozone dose, temperature, and pH on the formation of ozonation byproducts (OBPs) as well as ozone's beneficial use for decreasing ultraviolet (UV) absorbing components and the precursors of chlorinated disinfection byproducts.

A main objective of this project was to examine bromate (BrO_3^-) formation resulting from low to moderate bromide levels (< 200 ppb) and higher than normal ozone dosages in accord with anticipated high ozone CT requirement for inactivation of *Cryptosporidium*. In addition, at the time the *Research Plan* was written, nonhalogenated organic OBPs still were receiving considerable scrutiny; therefore, another objective was to include the formation of aldehydes, carboxylates, and ketoacids in the analyses.

Finally, another objective was to examine the ability of ozonation to eliminate reactive sites within the dissolved organic matter that would otherwise react with chlorine to form chlorinated DPBs. Assessment was made by evaluating the loss of UV-absorbance and performing chlorination experiments and analyzing for trihalomethanes, haloacetic acids, and total organic halide formation. A natural water (East Fork Lake, OH) with a final dissolved organic carbon (DOC) concentration of 2 mg/L was used for the study. The water was spiked with bromide to either 100 ppb or 200 ppb.

Ozonation experiments were conducted in both a 1-L batch reactor and in a 2-L (14-minute theoretical hydraulic residence time) flow-through reactor, which consisted of a counter-current bubble column followed by a second reaction column and equipped with 10 sampling ports along the flow path.

The main experimental matrix included: (1) five O_3 doses ∝ 0.6–5 mg/L (pH 7.5, 22º C); (2) 4º C, 20º C, and 30º C (pH 7.5, high O_3); and (3) pHs of 6, 7.5, and 9 (22º C, high O_3). Chlorination studies were performed under "Uniform Formation Conditions."

To date, most of the experimental matrix is completed, although the data are not fully compiled. The very basic BrO_3^- formation results, however, show some very interesting trends.

Bromate formation was plotted against O_3-exposure ($[O_3]dt$; batch experiments) and O_3-CT (approximate exposure; column experiments) to attempt to compare BrO_3^- formation in both the batch and column reactors (see Figure 1).

In almost all column experiments, BrO_3^- formation increased somewhat linearly with O_3-CT; there was no apparent CT-threshold or Br^- limitations. In contrast, BrO_3^- formation in the batch reactors demonstrated a linear increase with O_3- exposure, but then leveled off despite the presence of Br^- and O_3. Quantitatively, BrO_3^- formation in the column experiment was significantly lower than in the batch reactor on an O_3-CT and O_3-exposure basis.

The analysis of BrO_3^- formation on an O_3-exposure or O_3-CT basis is a useful tool for comparing BrO_3^- formation to microbial disinfection levels. However, these very general findings have led to the question of whether a linear or nonlinear relationship between BrO_3^- formation and either O_3-CT or O_3-exposure should be expected, and furthermore, whether any empirical relationship can be formulated that enables a comparison between different reactor types.

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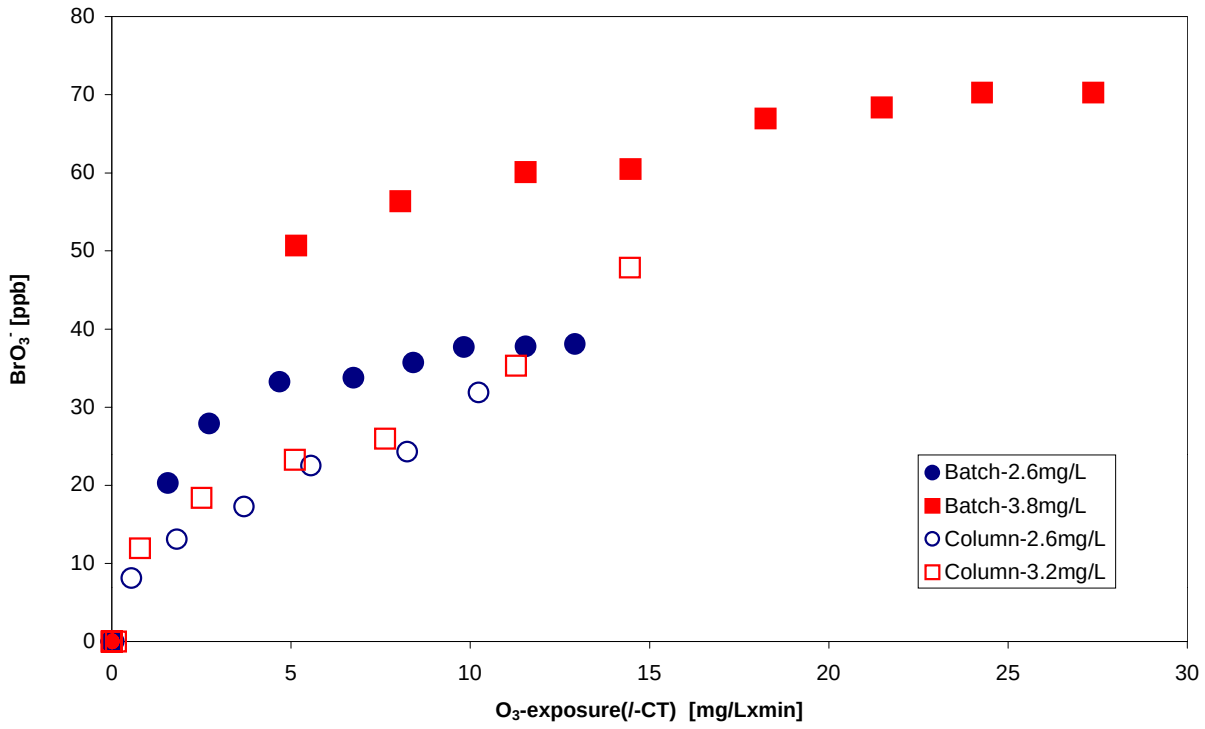

Figure 1. Bromate formation as a function of O_3-exposure and O_3-CT in the batch (solid symbols) and column (open symbols) reactors, respectively. Circles represent a low O_3 dose (2.6 mg/L) and squares a high O_3 dose (3.2 or 3.8 mg/L); 22° C, pH 7.5, 2 mg/L DOC.

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Integrated Approach for the Control of *Cryptosporidium parvum* Oocysts and Disinfection Byproducts in Drinking Water Treated With Ozone and Chloramines

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The overall goal of this project is the development of process design recommendations for the simultaneous control of *Cryptosporidium parvum* oocysts and disinfection byproducts (DBPs) during ozone/chloramines sequential disinfection of natural waters. Because the main objective of the study is to develop an integral control strategy, the scope of work focuses on a limited number of selected DBPs (bromate, formaldehyde, and cyanogen halides) associated with the ozone/chloramines sequential disinfection process.

Experiments have been performed to investigate the kinetics of *C. parvum* oocyst inactivation resulting from sequential application of ozone and monochloramine. Experiments with single disinfectants resulted in inactivation curves characterized by the presence of an initial lag phase, during which little inactivation occurred followed by pseudo-first order inactivation kinetics. The rate of *C. parvum* oocyst inactivation with monochloramine was enhanced when this chemical was used as secondary disinfectant. Ozone pretreatment resulted in the removal of the lag phase during the secondary inactivation with monochloramine. Furthermore, the rate of secondary monochloramine inactivation was faster than that observed for postlag-phase primary disinfection. This synergistic effect was more pronounced at lower temperatures.

Experimental results revealed that the CT (product of disinfectant concentration and contact time) required to achieve a certain level of inactivation was unique. No pH dependence was observed for primary inactivation with ozone in the pH range of 6–10, or primary and secondary inactivation with monochloramine at pH values of 8 and 10. The mechanism of cyanogen bromide (BrCN) formation during O_3/NH_2Cl sequential disinfection also is under investigation. BrCN can be formed through the reaction between HCHO and NH_2Br, the latter compound produced from the reaction between NH_2Cl and Br^- ion. Based on the reaction pathway reported for cyanogen chloride, potential intermediates in the formation of BrCN include *N*-bromoaminomethanol, *N*-bromodimethanolamine, *N*-bromomethanimine, and cyanide. The competing decomposition of NH_2Br involves many reactions with the overall rate affected by pH, $[NH_3]/[HOBr]$ ratio, initial NH_2Br concentration, and temperature.

An integrated model has been developed to simultaneously predict bromate formation and *C. parvum* oocyst inactivation during ozone treatment of natural waters. The model consists of elementary chemical reactions that are responsible for ozone decomposition and bromate formation as well as a delayed Chick-Watson kinetic expression for ozone disinfection of *C. parvum* oocysts. This model has been evaluated experimentally with a laboratory-scale batch reactor, and a laboratory-scale flow-through ozone bubble-diffuser contactor using synthetic solutions. In addition, semi-empirical expressions taking into account the effect of natural organic matter on relevant chemical and disinfection reactions have been incorporated into the model. The kinetic expressions were combined with empirical mass transfer correlations, and the axial dispersion model to simulate the gas transfer and hydrodynamic characteristics in pilot- and full-scale ozone bubble-diffuser contactors. Figure 1 shows an example of the integrated model application to simulate the full-scale ozone contactor located at the Los Angeles Aqueduct Filtration Plant.

Future research plans include the performance of additional experiments to assess the role of natural organic matter in the formation of bromate at temperatures in the range of 5–25^o C. The kinetic information obtained from laboratory-scale experiments will be incorporated into the computer model. Model evaluation with full-scale ozone contactors will be attempted using data available either in the literature or from water utilities.

Future efforts also will focus on the quantitative study of the fast equilibrium reactions and subsequent slower reactions between monobromamine and formaldehyde. Experiments also will be performed with various preozonated natural waters dosed with NH_2Cl and Br.

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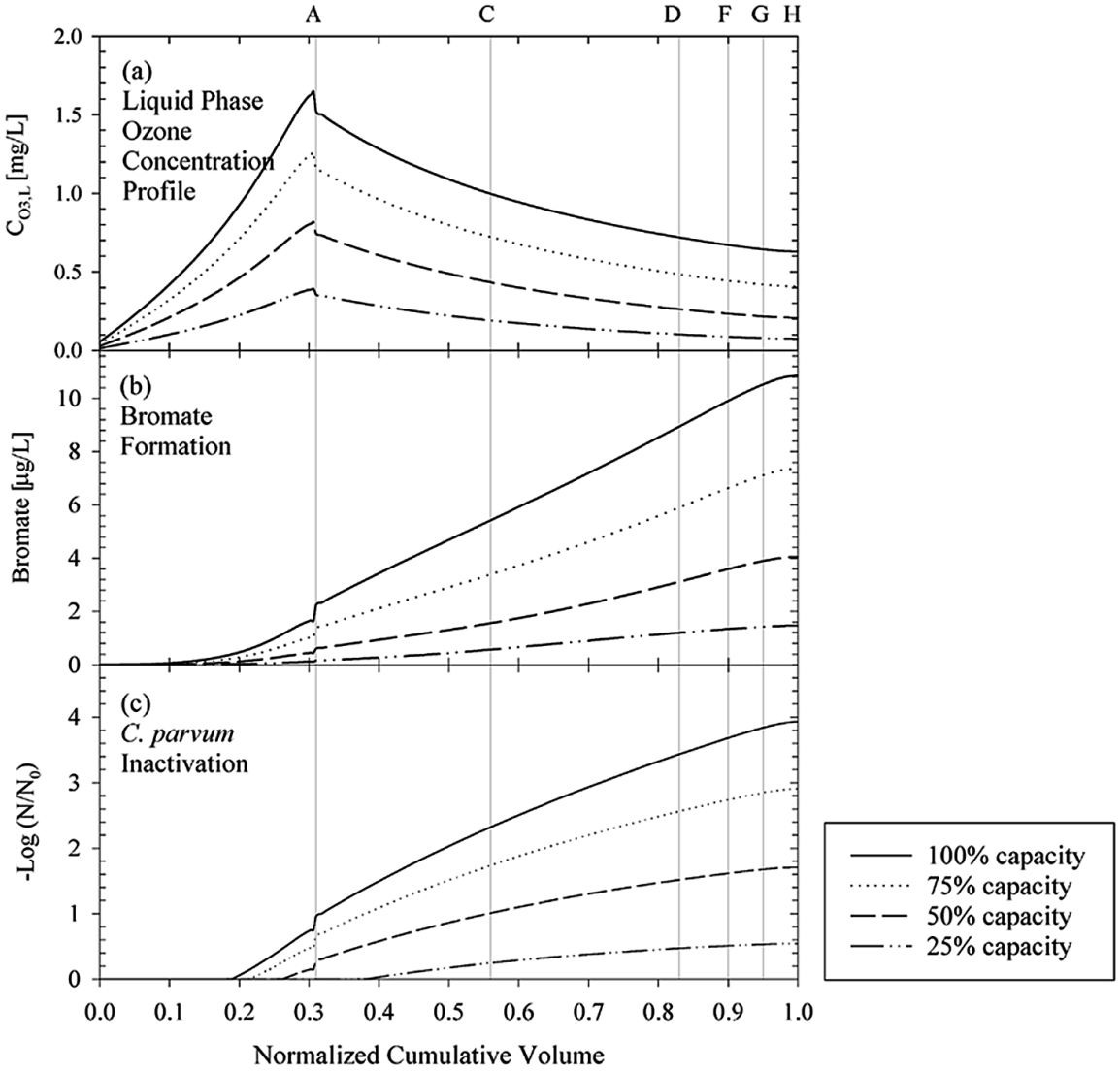

Figure 1. Simulation of dissolved ozone concentration, bromate formation, and *C. parvum* inactivation at the ADWP plant ozone contactor (water flow rate = 85 MGD; gas flow rate = 17.8 kscfh; temperature = 9° C; pH = 8.2; Br^- = 33 µg/L; DOC = 1.9 mg/L; ozone input capacity = 25–100%).

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Pilot Studies of an Ozonation/FBT Process for the Control of DBPs in Drinking Water

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Department of Civil and Environmental Engineering, Michigan State University, East Lansing, MI

A combined ozonation/biological fluidized bed treatment (FBT) for the removal of trihalomethane (THM) and other disinfection byproduct (DBP) precursors from drinking water is being investigated. This project also aims at developing design criteria for the proposed ozonation/FBT system. The goals of this project are being accomplished through bench-scale and pilot-scale studies using Lake Erie water collected at the Monroe Water Filtration Plant (Monroe, MI) and Lake Lansing water (Haslett, MI). Huron River water has a total organic carbon (TOC) concentration of 6–8 mg/L and is typical of rivers across the United States. Although Lake Lansing water does not provide source water to any treatment plant, it has been selected because of its high TOC concentration (9–11 mg/L). Future work will involve other source waters.

The effects of ozonation reaction pathways on the formation of both DBPs and biodegradable organic carbon (BDOC) were investigated using a bench-scale ozonation system. The study showed that the production of OH radicals relative to ozone dose adjusted for alkalinity was the greatest in water with the lowest TOC concentration (Lake Erie water) and the lowest in water having highest TOC concentration (Lake Lansing water). This suggests that organic matter in selected source waters acted more as a scavenger rather than as a promoter of radical reactions. Direct ozone reactions appear to favor the production of BDOC, whereas radical reactions result in the removal of organic carbon.

In biodegradation studies involving a bench-scale biodegradation system, several parameters were identified that described the kinetics of the removal of organic matter during biodegradation. These included: (1) the minimum empty bed contact time ($EBCT_{min}$), which represented the minimum EBCT required to remove rapidly BDOC ("fast" BDOC); (2) $BDOC_{slow}$, which represented the amount of BDOC that at least remained after biodegradation at $EBCT_{min}$; and (3) R_{max}, which was defined as the maximum rate of the biodegradation of "fast" BDOC. It was found that essentially all organic matter in Lake Erie water was refractory (i.e., not subject to biodegradation). Huron River water contained approximately 20 percent of potentially biodegradable organic matter, whereas nearly one-half of the organic matter in Lake Lansing could be biodegraded. The ozonation of Lake Erie water at doses of up to 3 mg/mg C did not result in the production of biodegradable organic carbon. Ozonation of Huron River water resulted in an increase of BDOC concentration from 1.2 mg/L in raw water to 2.8 mg/L in water ozonated at a dose of 1 mg/mg C. However, the concentration of $BDOC_{slow}$ also was increased after ozonation.

Unlike Huron River water, for which no significant changes in biodegradation kinetics were observed at doses greater than 0.5 mg/mg C, the biodegradation parameters for ozonated Lake Lansing water were affected by ozone doses to a much greater extent. Ozonation of Lake Lansing water at a dose of 0.75 mg/mg C resulted in an increase in BDOC concentration from 5.04 to 6.06 mg/L. Ozonation at ozone doses of 1.5 and 3 mg/mg C resulted in the formation of additional 0.8 and 1.33 mg/L BDOC, respectively. An increase in ozone dose resulted in an increase in R_{max} and a decrease in $EBCT_{min}$. The most striking difference between Huron River water and Lake Lansing water was observed with respect to "slow" BDOC. The concentration of $BDOC_{slow}$ in Lake Lansing water decreased with an increase in ozone dose compared to Huron River water, in which $BDOC_{slow}$ increased at a dose of 0.5 mg/mg C and leveled off at higher ozone dosages.

A pilot-scale study of the ozonation/FBT system has been initiated. The 1-gpm pilot-scale system has been installed at the Monroe Water Treatment Plant in Monroe, MI. Although all necessary kinetic data and operating information have been generated, bench-scale studies with selected source waters will continue. These studies will be directed towards further optimizing parameters for ozonation and biodegradation to increase the efficiency of the combined ozonation/FBT system.

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Section 5.

Microbial Contaminants

Prevalence and Distribution of Genotypes of *Cryptosporidium parvum* in United States Feedlot Cattle

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The overall goal of the proposed research project is to establish the prevalence and distribution of genotypes of *Cryptosporidium parvum* in western and central U.S. feedlot cattle. In addition, the fecal concentration of oocysts from each genotype will be quantified to estimate oocyst loading rates for this source of pathogen. A secondary objective is to determine management and animal risk factors that are associated with cattle shedding one or more genotypes of *C. parvum*. The original geographic focus for the multistate cross-sectional survey of feedlot cattle was California, Nebraska, Washington, South Dakota, and Colorado. Two additional states with large feedlot cattle populations have been added—Texas and Oklahoma—for an overall enrollment of seven states located throughout the central and western United States. As of December 31, 2000, approximately 240 cattle per feedlot have been sampled from feedlots located in six of the seven enrolled states, resulting in 1,440 tested samples. Using a direct immunofluorescent assay that reliably can detect 600 or more oocysts per gram of fecal material, four infected cattle have been identified out of the 1,440 tested. To confirm that the diagnostic method is not falsely classifying fecal samples as negative, 10 randomly chosen samples per feedlot are being tested using immunomagnetic separation of oocysts, followed by the standard direct immunofluorescent assay.

This research project has shown previously that this combined method can, on average, detect as few as one oocyst per gram of bovine fecal material. Of the 60 samples tested to date, none contained detectable levels of *C. parvum* oocysts. Using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique developed by the Centers for Disease Control and Prevention that targets the 18S small-subunit rRNA gene locus, three of these four presumptive *C. parvum* isolates have been confirmed as *C. parvum*, displaying the typical bovine PCR-RFLP pattern. DNA sequencing of the amplicon is in progress to improve the discriminatory power of this genotyping method and to detect minor polymorphisms in the genome. Preliminary inferences from the first year of research are that feedlot cattle located throughout central and western United States are not serving as an environmental source of *C. parvum*. It should be stressed that there still are more than 4,500 cattle to examine, so valid and precise conclusions should not be formed until the cross-sectional survey is completed. In particular, the survey data will be statically modeled to determine the oocyst loading rate from this cohort of adult cattle. It is this parameter, the oocysts loading rate, that is the primary parameter of public health interest and an ideal parameter for risk assessment applications, not the overall prevalence of fecal shedding.

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Development of Detection and Viability Methods for Waterborne Microsporidia Species Known To Infect Humans

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The proposed study targets the development of a strategy for the recovery and identification of the human microsporidia from natural waters, and it is divided into five major components: (1) sample collection, (2) sample concentration, (3) sample processing, (4) diagnostic assay, and (5) method validation in natural waters. The study design emphasizes the application of several biotechnologies that, when combined, offer the potential for the development of a highly sensitive and specific environmental monitoring tool.

The techniques proposed in the study (continuous flow centrifugation [CFC], flow cytometry/cell sorting, DNA amplification, and oligoprobe hybridization) were selected in part due to their successful application for the development of recovery methods for *Cryptosporidium* and *Giardia*. In addition, the proposed strategy offers the potential to detect low levels of microsporidia while simultaneously providing strain-specific identification. Continuous infections of model microsporidia (*E. intestinalis*, *E. hellem*, and *E. cuniculi*) were established in rabbit kidney cells, and a preliminary viability assay has been evaluated using coverslips according to a 24-well plate format. Sensitivity characterization suggests that fewer than 100 viable spores can be detected per coverslip in this format. A variety of monoclonal and polyclonal antisera have been produced and characterized for their binding characteristics against model microsporidia. Both primary and secondary labeling methods have been evaluated. Preliminary results indicate optimal fluorescence with fluorescein isothiocyanate-conjugated polyclonal antisera—improvements to indirect labeling methods with monoclonal antisera are underway. Spores labeled with fluorescent antibodies have been successfully sorted using automated cytometry technology.

Evaluation of sample collection methods during the first 3 months has focused on assessing the efficiency of filter/elution and CFC. Seeded challenge studies using reticulated foam filter units to capture spores indicate that recovery efficiencies as high as 60 percent may be achieved with idealized waters (reverse osmosis grade). Preliminary recovery experiments using CFC indicate that recovery efficiencies as high as 90 percent are possible.

Initial studies indicate that the viability of model microsporidia spores can be measured using cell culture-based techniques, and that an enumerative assay based on epifluorescence microscopy is possible. Continued improvements to these diagnostic procedures may make it possible to produce a finished method for the assessment of the fate, ecology, and distribution of infectious microsporidia in natural waters, and will assist in the effort to collect data for regulatory discussions on the contaminant candidate list of microorganisms.

The balance of the first year's effort will focus on the development of *in situ* labeling methods to improve the sensitivity and specificity of microsporidia detection using flow cytometry. The research also will focus on improving the sensitivity of the viability assay and will continue to optimize the immunological labeling procedures.

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Meaningful Detection of *Mycobacterium avium* Complex in Drinking Water: Are Disinfectant-Resistant Morphotypes Virulent?

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The bacterial pathogen Mycobacterium avium-intracellulare (MAC) is resistant to chlorine and difficult to eliminate from water supplies. The public health significance of MAC in drinking water is not well understood, and correlations between the occurrence of MAC in water and the incidence of MAC disease are not always perfect.

One source of variation is the unique propensity of the pathogen to segregate into "colony type" variants, also known as morphotypes, with differing characteristics of virulence, resistance to antimicrobial agents, and ability to survive in the environment. Typically, any MAC cell found in water is assumed to be a threat to public health, even though some MAC morphotypes are orders of magnitude more infectious than others. This research is aimed at identifying virulent morphotypes and the circumstances under which they come into contact with humans.

Four morphotypic variants of MAC were examined for virulence (ability to cause disease), sliding motility (ability to spread over solid surfaces), and sensitivity to chlorine. The morphotypes, distinguishable by colony opacity and ability to bind to Congo red, were: red-opaque (RO), white-opaque (WO), red-transparent (RT), and white-transparent (WT).

Virulence was assessed by using a cultured human blood cell model of disease, a mouse model of disease, and analysis of very fresh clinical isolates. Sliding motility was measured on soft agar plates. Chlorine sensitivity was measured in side-by-side and combined experiments, using hypochlorite as the chlorine source. WT was the only form that survived and grew within cultured human cells and mouse organs, suggesting that the other forms were weakly virulent or avirulent. Consistent with this, white was the predominant morphotype in 25 of the clinical isolates examined. RT and RO variants were capable of sliding motility, whereas WT and WO variants were not. RT cells were markedly more resistant to chlorine than the other three morphotypes.

In the isolates that were studied, only one morphotype, RT, was resistant to chlorine. This form was not virulent. Other forms, including the virulent WT form, were more easily killed by chlorine (see Figure 1). If this pattern applies to all MAC strains, it would suggest that virulent MAC might be easier to eliminate from drinking water than previously believed. This is an example of how colony morphotype can be a factor in determining the human health consequences of MAC in drinking water.

The role that morphotype plays in other characteristics important to the survival of MAC in water supplies will be examined, including the abilities to form biofilms and to grow on filters. The morphotypic characteristics and virulence properties of isolates taken directly from various public water supplies without extensive passage in the laboratory (which inevitably results in morphotypic switching) will be determined. If water distribution environments favor specific morphotypes, then the infectivity of those morphotypes must be taken into account when gauging their public health significance.

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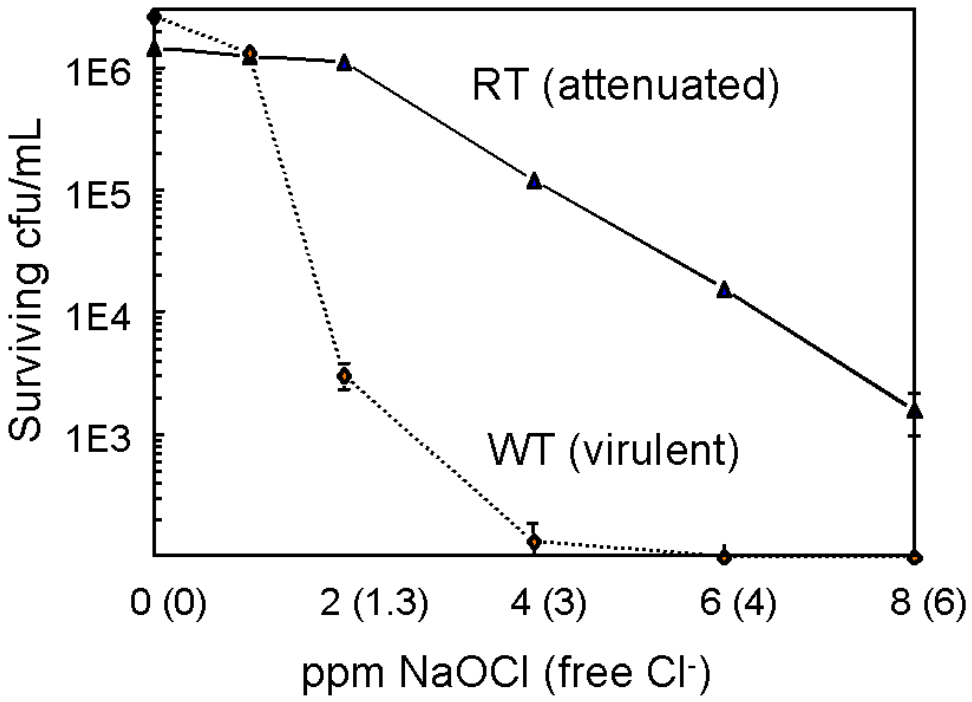

Figure 1. Killing of RT and WT variants of *M. avium* strain $HMCO_2$ by sodium hypochlorite.

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Detection of Emerging Microbial Contaminants in Source and Finished Drinking Water With DNA Microarrays

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DNA microarrays have not been applied to environmental samples (in general), and commercial array companies are focused solely on the high-throughput genotyping and drug discovery markets. With the potential to detect multiple genera of organisms and ability to incorporate live/dead discrimination via mRNA analysis within a single water sample, microarray technology may provide a significant technological advance in the pathogen monitoring of drinking water supplies.

The objective of this research project is to develop DNA microarrays for the detection of *Cryptosporidium parvum* and *Helicobacter pylori* and evaluate their sensitivity, specificity, and quantification ability relative to standard techniques for the detection of these pathogens in source and finished water. Genotyping arrays have been constructed for the vacA and cagA genes of *H. pylori* (36 oligonucleotide probes), and the hsp70 gene of *C. parvum* (68 oligonucleotide probes). Biotin-labeled polymerase chain reaction products are generated from genomic DNA and hybridized to their respective arrays (*Cryptosporidium* or *Helicobacter*). The signal from hybridized probes is generated using a strepavidin-alkaline phosphatase, ELF® chemiluminescent substrate system (Molecular Probes, Eugene, OR). The hybridized probes are visualized via ultraviolet transillumination using a BioRad Fluor-S imager.

Preliminary results from the *H. pylori* array demonstrate that reproducible single-base mismatch discrimination within vacA and cagA amplification products is possible. *C. parvum* hsp70 genes also have been successfully labeled, amplified, and detected on a prototype microarray.

This research project now is poised to investigate microarray sensitivity and matrix effects in an environmental context, and to assess the specificity of the microarrays on a collection of *Helicobacter* and *Cryptosporidium* (human versus nonhuman) isolates. Work during the next year will focus on: (1) optimizing hybridization conditions for the *Cryptosporidium* array to differentiate between human and non-human sources of *C. parvum*, (2) processing environmental water samples containing *C. parvum* to determine matrix effects on the sensitivity and specificity of the arrays, and (3) expanding the *H. pylori* arrays to detect additional bacterial pathogens (such as *E. coli* O157:H7) in source and finished water.

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Infectivity and Virulence of *Cryptosporidium* Genotype 1/H Oocysts in Healthy Adult Volunteers

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Cryptosporidium parvum is comprised of a genetically heterogeneous population, which can be divided into isolates that are transmitted among humans (genotype 1) and those transmitted between human and animal hosts (genotype 2). Experimental infection with genotype 2 oocysts has previously revealed significant variability in infectivity (ID_{50} range between 9–1,042 oocysts) and clinical outcome (asymptomatic to diarrheal illness). Further, a comparison of serologically negative versus serologically positive individuals showed a 20-fold increase in the ID_{50} in those persons with preexisting serum IgG. These antibody-positive individuals excreted much fewer oocysts than the antibody-negative individuals, suggesting that secondary infections would be less likely to occur with this population. Although the presensitized volunteers typically were resistant to low-level exposures, high challenge doses (>5,000 oocysts) resulted in infection and diarrheal illness in a number of the volunteers. Indeed, some illness measures indicated that the diarrhea in those with preexisting antibody was more severe than in serologically negative persons.

The overall goal of the present study is to generate dose-response curves in healthy volunteers using genotype 1 oocysts and to compare the resulting infectivity, clinical outcomes, and immune responses to previous genotype 2 results. Two different genotype 1 isolates have been proposed for testing. The specific objectives of the study are to: (1) establish the infectious dose (ID_{50}), clinical outcomes, and intensity of infection for two *Cryptosporidium* genotype 1 isolates in seronegative individuals; (2) investigate the antibody and cellular responses in volunteers to genotype 1 isolates; and (3) examine isolates for subtle genetic differences and determine the stability of DNA markers prior to and after passage in pigs and humans.

Year 1 activities have included: (1) identifying two genotype 1 isolates from HIV-negative donors that can be amplified in gnotobiotic (GNB) pigs; (2) establishing the genetic stability of the isolate in multiple pig passages; and (3) testing the first of several challenge doses in four healthy volunteers. The genotype 1 isolate used to date was obtained from an HIV-negative donor who developed a relatively mild, symptomatic illness with *C. parvum*. Genetic polymorphism studies revealed a banding pattern typical of genotype 1 isolates. Multiple passages were carried out in GNB pigs to examine the "fingerprint" of the amplified oocysts at each passage. All passages showed a stable pattern with no detectable changes in the mobility of PCR products of any of the loci studied.

After oocyst purification and safety testing for adventitious agents, 100 genotype 1 oocysts were ingested by each of four serologically negative volunteers. Volunteers were monitored daily for 14 days and three times per week for a total of 6 weeks. All four volunteers showed either clinical or parasitological evidence of infection, suggesting that the ID_{50} for this isolate (TU502) may be similar to the genotype 2 isolate, TAMU (ID_{50} = 9 oocysts). A diarrheal illness was noted in three of the volunteers, while the fourth volunteer had unformed stools and gastrointestinal symptoms, but did not meet criteria for diarrhea (see Table 1). Two of the four volunteers shed oocysts that were detectable by direct fluorescence assay and enzyme-linked immunosorbent assay. Peripheral blood mononuclear cells and serum samples, as well as saliva samples, have been collected at five time points (pre- and postchallenge) from volunteers, but evaluation of immune responses has not been completed. Continuing studies will test additional volunteers at 100 oocysts and other groups at lower dosages.

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Table 1. Outcome of *Cryptosporidium parvum* (genotype 1) challenge in serologically negative, healthy adult volunteers. Each volunteer received 100 oocysts of the TU502 isolate.

Volunteer Number	Diarrhea*	GI Symptoms†	Oocysts Detected
132	+	-	+
133	+	+	-
134	+	+	-
135	-	+	+

* Diarrhea is defined as the passage of unformed stools fitting any of the following criteria along with at least one additional gastrointestinal symptom: (1) ≥ 3 in 8 hours, (2) ≥ 4 in 24 hours, or (3) >200 grams per day.

† GI symptoms are defined as the presence of two or more gastrointestinal symptoms with one or more concurrent unformed stools.

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Mycobacterium avium Complex in Drinking Water: Detection, Distribution, and Routes of Exposure

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Organisms of the *Mycobacterium avium* complex (MAC) are an increasingly prevalent cause of clinical disease and are known to be widespread in drinking water supplies. However, there are significant gaps in current knowledge of MAC. At present, there are no validated methods for the detection of MAC in biofilms, a likely treatment-resistant reservoir for microorganisms in water distribution systems. Also, commonly used techniques in isolating MAC from water are relatively insensitive due to aggressive decontamination steps.

Objectives of the research project are to: (1) develop improved methods for detecting MAC in biofilms, (2) explore and implement more sensitive techniques for the detection of MAC in drinking water samples, and (3) determine the prevalence of MAC in municipal drinking water distribution systems and at sites of end-user exposure (drinking water, hot water, and toilet tank water).

MAC in biofilms will be quantified using powerful image analysis equipment and software. A microscope coupled with a digital camera will provide high-resolution images of the specimens. Florescence *in situ* hybridization, using highly specific labeled DNA probes and antibodies, will allow for the direct labeling of MAC on histological sections of biofilms. These biofilms will be obtained from cartridges (see Figure 1) that form part of a bypass apparatus (see Figure 2) integrated into the hot water recirculating system of a suitable large building. Also, it is proposed to investigate a novel method for selectively culturing MAC on paraffin wax slides. MAC's unique ability to utilize the wax as its sole source of carbon will be investigated, and modifications to the paraffin recipe to increase specificity will be explored.

Water and biofilms will be systematically sampled in the reservoirs, distribution systems, and end-user sites (commercial, institutional, and residential buildings) of four geographically separate communities in eastern Massachusetts. Samples will be collected over both space and time to investigate both physical and seasonal factors. Within end-user buildings, liquid and biofilm samples will be obtained from drinking water, hot water, and toilet tank water. Data on the presence of MAC will be correlated with water quality parameters.

To implement a bypass system, a suitable building water distribution system that is colonized by MAC first had to be identified. Water samples from 20 buildings were processed to selectively isolate MAC, and several have shown to be positive to varying degrees. In the interim, artificial MAC-*Pseudomonas aeroginosa* biofilms are being grown to develop and test the detection and image analysis methods.

This research should significantly improve the ability to detect MAC in environmental samples and provide information on the factors influencing the distribution of MAC in municipal water systems. Successful completion of this research will provide information on optimal methodologies for assessing MAC in drinking water. Designs for the bypass system have been finalized, and the construction and implementation stage will proceed in the coming 2–3 months. Also, in the next phase, collection of biofilm and water samples from the target list of municipal drinking water distribution systems and end-user exposure sites will begin.

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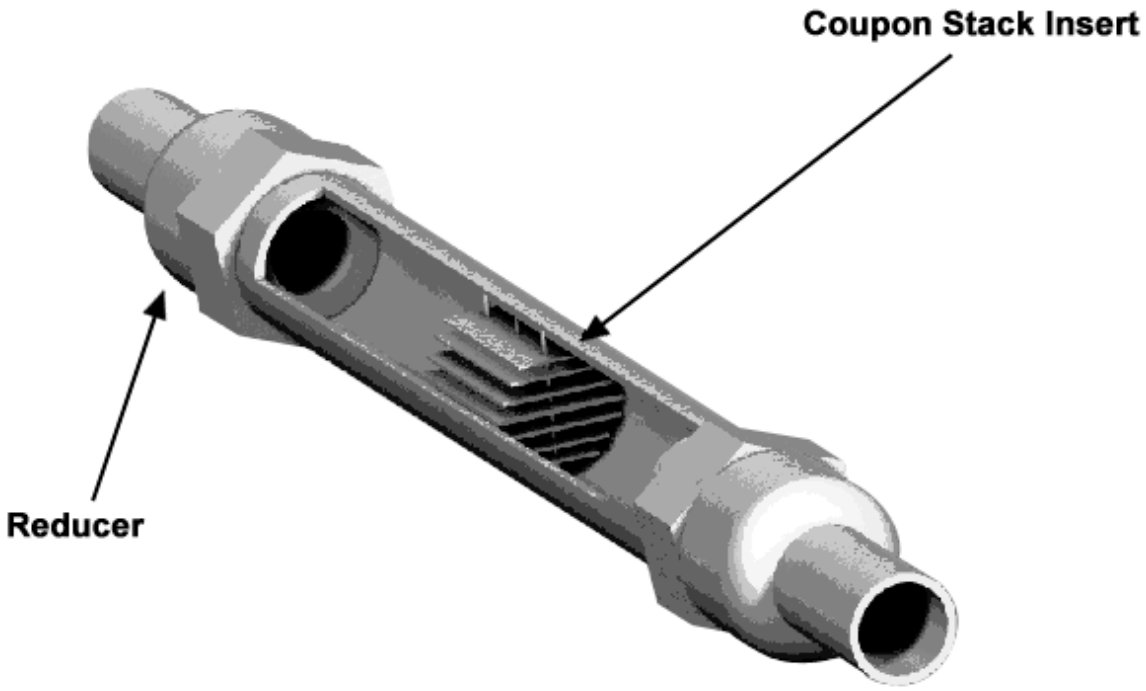

Figure 1. A three-dimensional cutout view of the Biofilm Cartridge that integrates into a bypass apparatus of a building hot water re-circulating system. Biofilms will form on the flat surfaces of the coupons in the insert. Several inserts may reside in the cartridge—these may be slid in and out by unscrewing the reducer.

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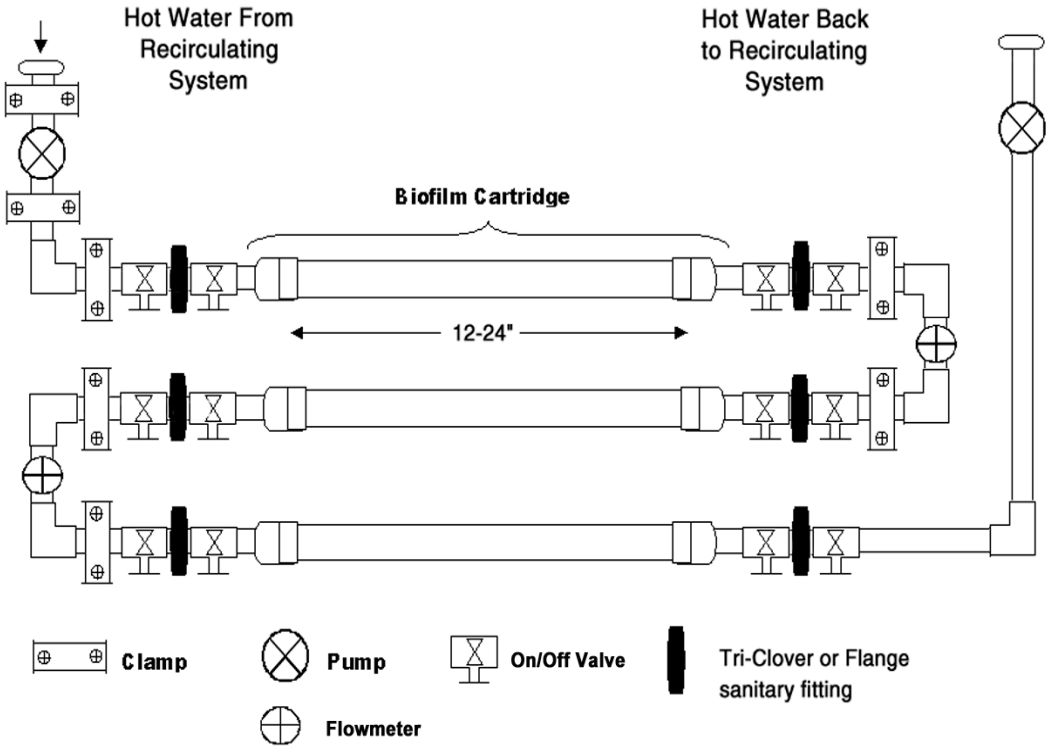

Figure 2. Schematic diagram of the bypass apparatus with three biofilm cartridges. The diagram shows the positions of the pumps, flowmeters, valves, and various other fittings that connect the system together.

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NERL Microbial Program
With Emphasis on Protozoan Methods

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The National Exposure Research Laboratory's facility in Cincinnati is engaged in a variety of microbiological research projects that include studies on bacteria, viruses, fungi, and protozoa. One of these was a protozoology project to determine the best way to evaluate methods reported for detecting *Cryptosporidium parvum* in water. The technology used in preliminary surveys to determine the incidence of this parasite in watersheds has had obvious shortcomings, notably low recovery of organisms and high inherent variability. To improve this technology, a large number of possible alternatives are available. Without a framework to compare these alternative methods, there is no way to select a method or battery of methods that will address the basic question of the underlying distribution of *C. parvum* in the environment.

Procedures for evaluating methods have inherent variability, and thus, it is difficult to determine whether the method, or the procedures for evaluating the method, is the source of variability. To meet this challenge, a set of criteria were developed and tested for use in methods evaluation. One of the primary tools, counting of absolute numbers of organisms by flow cytometry, has enabled more precise method evaluation. An example of an evaluation of four methods using this technique is presented in Figure 1.

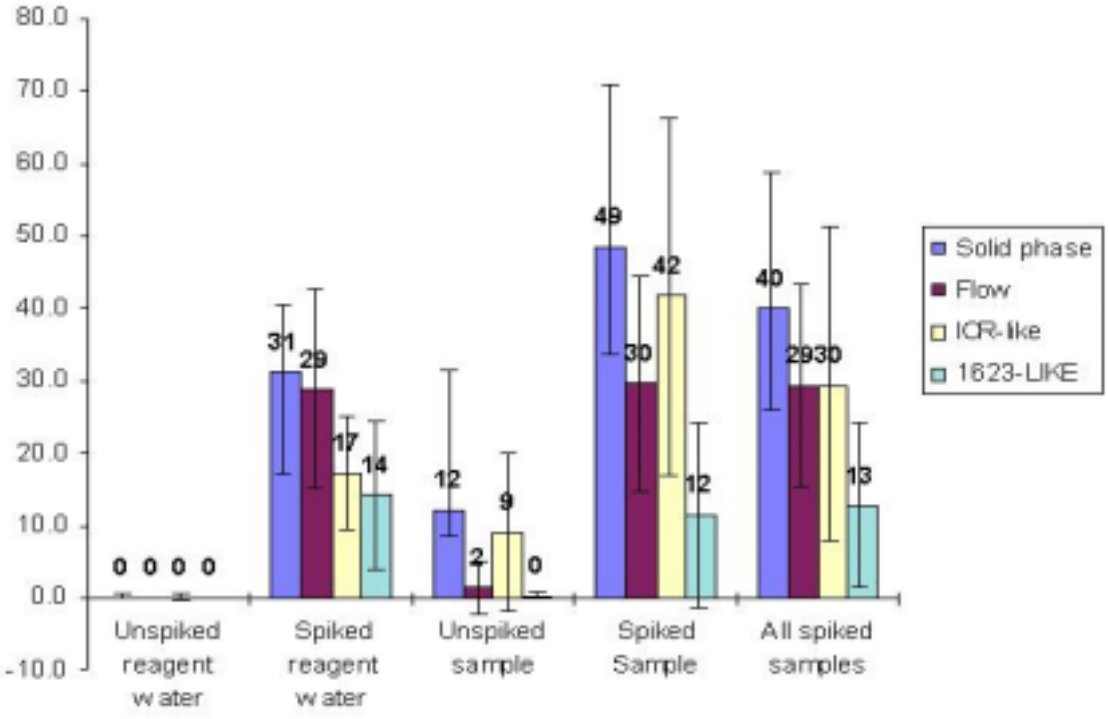

Figure 1. Presumptive recoveries.

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Studies of the Infectivity of Norwalk and Norwalk-Like Viruses

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The objective of this research is to develop the understanding of the risks associated with exposure to waterborne caliciviruses as a function of dose and host susceptibility factors. Specifically, this research will determine the infectious dose of three important human caliciviruses (HuCVs), a prototype Genogroup I virus (Norwalk virus [NV]), and two prototype Genogroup II viruses (Snow Mountain Agent [SMA] and Hawaii virus [HV]), that are recognized as major waterborne pathogens. The specific objectives are to: (1) identify the dose range of NV, SMA, and HV (ID_{10}, ID_{50}, and ID_{90}) in human volunteers with various levels of preexisting antibodies; (2) examine the immune response (serum and secretory antibodies) and determine the characteristics of volunteers that are susceptible to infection; and (3) evaluate the fit of several mathematical models of dose-infectivity to these data.

Double-blinded human challenge studies are being conducted to determine the dose-infectivity relationships for NV, SMA, and HV. These studies build on a previous pilot NV dose-ranging study also supported by the U.S. Environmental Protection Agency (EPA). The NV study focuses on infectivity in the critical low-dose region of the dose-response curve and provides more accurate information on the risks of NV infection associated with low levels of virus typical of the concentrations in water. The SMA and HV dose-ranging studies will examine a range of doses that approximate the ID_{10}, ID_{50}, and ID_{90}. In all studies, subjects are monitored for gastrointestinal symptoms for 5 days, and return for Day 8, 14, and 21 followup visits. Stool specimens are assayed for NV, SMA, and HV RNA by reverse transcription-polymerase chain reaction (RT-PCR). NV, SMA, and HV serum antibodies and secretory antibodies are measured by enzyme immunoassay. Infection is defined as excretion of NV, SMA, or HV or seroconversion.

The outcomes of interest in these studies are symptomatic and asymptomatic NV, SMA, and HV infection. Symptomatic infection is of concern because of the disease burden on the population, the effect on absenteeism, and the impact on the health care system. In terms of public health protection, asymptomatic infection also is of concern because of the potential for secondary transmission and the consequences of these infections for the immunocompromised population, including infants and the elderly.

Several mathematical models of dose-infectivity will be evaluated. A total of 76 subjects were challenged with NV, and 25 became infected. NV doses ranged from 1 x 10^{-1} to 1 x 10^{7} PCR detectable units (PDU). Seventy-two percent of the infected subjects had gastrointestinal symptoms. Asymptomatic infections occurred more frequently at low doses. Most subjects shed virus for at least 8 days postchallenge, and several continued to shed virus for 18–23 days postchallenge. A simple dose-infectivity relationship was not observed. The presence of anti-NV serum IgG in prechallenge sera was a significant predictor of infection, and subjects who were anti-NV IgG positive had consistently higher infection rates at all doses.

Assays for anti-NV salivary antibodies were developed and used to examine the salivary immune response. Ninety-one percent of challenged subjects developed an anti-NV salivary IgA response. However, the timing of this response was different for subjects who developed infection compared to subjects who did not develop infection, and suggests a mechanism of protective immunity to NV infection. The role of T-cell mediated immunity in NV infection currently is being evaluated. A two-population beta-Poisson model provided the best fit to the dose-infectivity data. Preliminary risk assessment using this NV dose-infectivity data indicates that the risk of waterborne NV is higher than previous EPA estimates based on rotavirus infectivity data.

SMA and HV inocula were extensively safety tested to ensure the absence of other pathogens and toxins. To date, two subjects have been challenged with the SMA inoculum (10^{5} PDU), and both developed symptomatic infections. Seroconversion could not be detected by recombinant NV antigen, so SMA excreted by these subjects was cloned and inserted into a Venezuelan Equine Encephalitis virus vector. This expression system then was used to make recombinant SMA capsid protein that self-assembled into particles. This recombinant protein will be used to examine the immune response in subjects challenged with SMA and HV, and to assess the preexposure immune status of the subjects in all of the challenge studies.

These findings indicate that NV is highly infectious. The low infectious dose, mild illness or asymptomatic infections, and prolonged shedding facilitate waterborne and secondary transmission of this virus. Humoral and mucosal immune responses are markers of host susceptibility to NV infection. These challenge studies measure HuCV infectivity in humans in terms of RT-PCR detectable units because this is the only avail-able method that can measure HuCVs in both clinical samples and environmental samples. These studies will provide the EPA with important data on the relationship between viral dose and host susceptibility

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to infection, clinical symptoms, and immune response. The results of these studies are valuable for estimating the risk of HuCV infection and gastroenteritis associated with exposure to contaminated water and to establish safe exposure limits for HuCVs in water to reduce waterborne disease. The next steps of this research are to: (1) challenge a small number of subjects with HV inoculum to determine whether it is infectious, (2) conduct dose-ranging studies with SMA and HV inocula and compare their dose-infectivity relationships to NV, and (3) examine host immune response to SMA and HV challenge.

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Development and Evaluation of Procedures for Detection of Infectious Microsporidia in Source Waters

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The microsporidia *Enterocytozoon bieneusi* and *Encephalitozoon* spp. are considered as emerging pathogens that represent critical threats to public health with potentially fatal consequences for immunodeficient individuals who become infected. Some microsporidia can lead to disseminated infection affecting almost every organ of the human body, and they also can infect immunocompetent individuals. Because many animals can carry microsporidia, it is possible that surface waters can be contaminated, and consequently, may serve as a route of transmission to humans. However, very little is known about the occurrence of microsporidia in environmental water sources, and there is a critical need to determine the role drinking water plays in the epidemiology of this group of parasites. Moreover, there are no routine methods for the detection of microsporidia in water.

The objectives of the proposed research project are to develop a method for detection of microsporidia in environmental waters, determine the viability and infectivity of detected spores, and use the methods to determine the occurrence of microsporidia in source waters in the United States. The recovery and purification methods that will be evaluated include filtration of water samples using a variety of different filter formats and porosities, immunomagnetic separation, and density gradient centrifugation (see Figure 1).

Optimized DNA amplification assays and microscopic methods will be used for detection and identification. Viability will be assessed using a spore germination assay coupled with a nucleic acid stain, a fluorescent dye exclusion assay, and phase contrast microscopy. Infectivity will be determined by inoculating spores into cell cultures and detecting infections using molecular and microscopic methods.

A variety of fluorescent agents have been evaluated for detecting spores. Staining procedures using calcofluor were useful for enumerating purified spore suspensions, but could not be used for detecting spores recovered from natural water samples due to the high level of staining of background material in the samples. Nonfluorescent stains, such as a modified trichrome method, only were practical for enumerating purified spore preparations. A commercially available fluorescently labeled antibody to *Encephalitozoon* spp. also was evaluated, but it demonstrated extensive nonspecific binding to sample debris and other microorganisms. Published amplification primers were evaluated, and those reported to be specific for *E. cuniculi* and *E. hellem* did not amplify DNA from other parasites. However, primers reported to be specific for *E. intestinalis* also amplified DNA from *E. hellem* and *E. cuniculi*. A cell culture-based spore propagation method has been developed for *E. intestinalis* using Madin-Darby canine kidney cells and will be used as the basis for an infectivity assay for spores detected in water.

The immediate future plans for the project include evaluation of different filtration formats for recovery of spores from environmental water samples. Custom manufactured capsule filters with pore sizes of 0.1–0.8 μm will be assessed along with compressed foam filters comprising stacks of 62–68 filter disks. The expected outcome of this research will be optimized methods for the detection of infectious microsporidia in environmental water samples. Information will be obtained on the prevalence of microsporidia in environmental waters, which will allow the water industry and public health officials to determine whether drinking water represents a significant route of transmission for these parasites.

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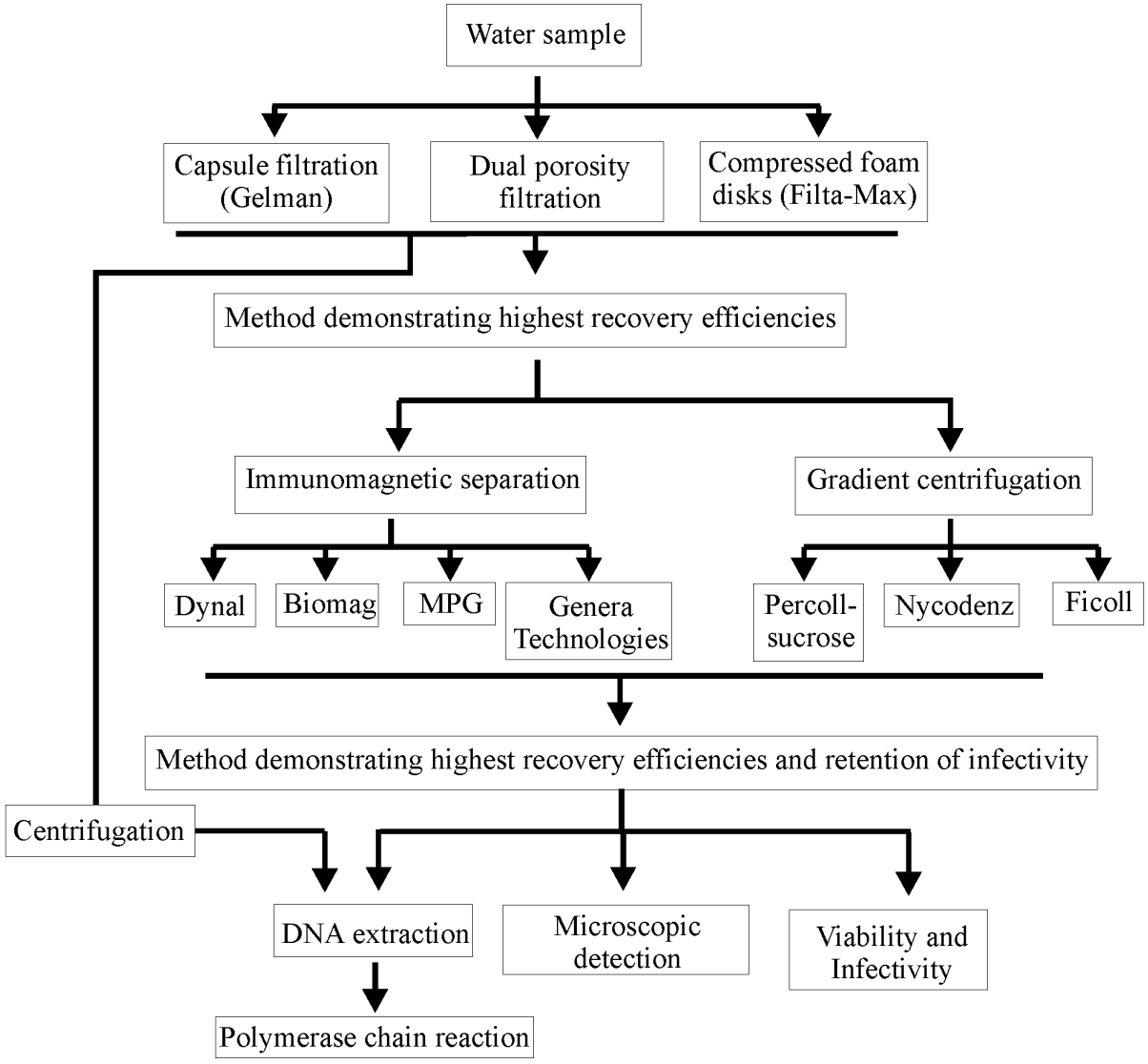

Figure 1. Outline of the approach for the development of a detection procedure for waterborne microsporidia.

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Attachment and Inactivation During Virus Transport in Groundwater

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[1]University of Colorado, Boulder, CO; [2]Yale University, New Haven, CT; [3]U.S. Geological Survey, Boulder, CO

The major objective of this research is to provide a better understanding of the processes that govern virus transport in groundwater. The specific goals are to investigate four key processes that are the source of much of the uncertainty in predicting virus transport and to develop a virus transport model that accounts for these processes. The four key processes, outlined as hypotheses, are: (1) organic matter will enhance virus transport in aquifers by adsorbing to positively charged grain surfaces and occupying these favorable virus attachment sites; (2) the reversibility of virus attachment to aquifer sediments is controlled by heterogeneity of aquifer grains and virus interactions with different mineral and organic matter surfaces; (3) the inactivation of viruses in groundwater is accelerated by strong, irreversible attachment, but not by weak, reversible attachment; and (4) the transport of viruses during long-term release will be enhanced by blocking of favorable attachment sites by attached viruses, but the extent of blocking will be diminished by accelerated inactivation of attached viruses.

The first task is to conduct virus attachment and release experiments in pulse-injection flow-through columns and a two-dimensional aquifer tank. These experiments are examining the effect of organic matter on virus attachment and release and the reversibility of virus attachment using the bacteriophage PRD1, synthesized ferric oxyhydroxide-coated quartz sand, various surfactants, two natural organic matter samples, and sewage-derived organic matter.

The second task is to determine the effect of attachment on virus inactivation. These experiments were conducted in static column experiments using the bacteriophages PRD1 and MS2, and ferric oxyhydroxide-coated sand and groundwater from the U.S. Geological Survey Cape Cod field site. The third task is to conduct virus attachment and release experiments in continuous-injection flow-through columns to test the dynamics of virus attachment. These experiments are being conducted in columns similar to those described in the first task.

The fourth task is to develop a two-dimensional virus transport model that incorporates physically and geochemically heterogeneous porous media, irreversible and reversible virus attachment, deposition dynamics, and surface inactivation.

Virus transport experiments in geochemically and physically heterogeneous sands have been conducted in flow-through columns and the two-dimensional aquifer tank. Geochemical heterogeneity, in the form of ferric oxyhydroxide coatings, dominates virus attachment in the aquifer tank (see Figure 1) and in a field experiment at the Cape Cod site. Virus transport results from column studies with porous media used to fill the aquifer tank have been to calibrate the virus transport model. Attachment of viruses to ferric oxyhydroxide-coated quartz sand accelerates virus inactivation relative to inactivation of viruses in the solution phase. The findings of this research currently indicate that geochemical heterogeneity and attachment-enhanced inactivation must be included in modeling and predictions of virus transport. The remaining work will focus on the effects of organic matter and deposition dynamics on virus transport.

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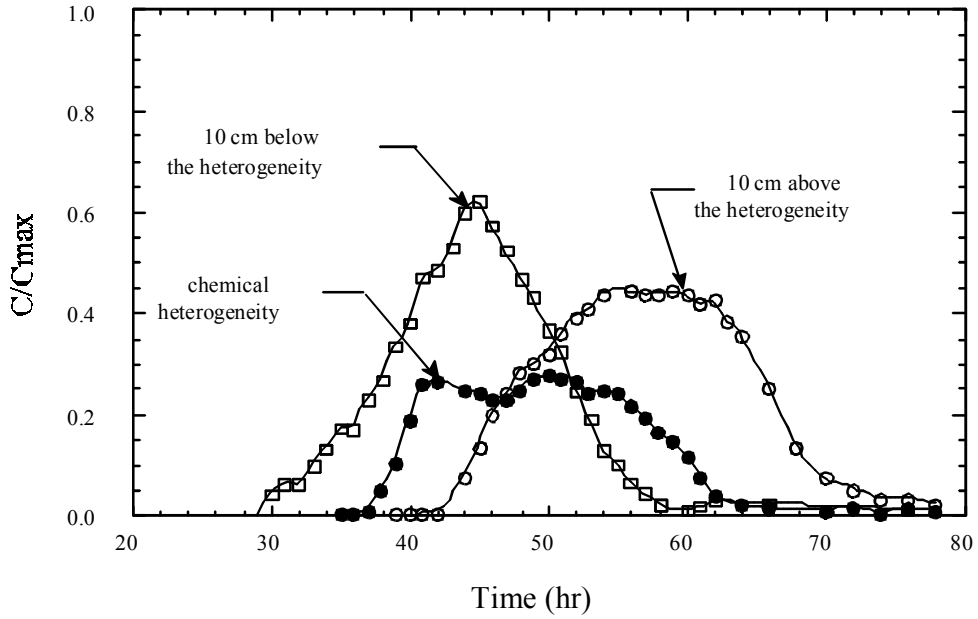

Figure 1. Breakthrough curves of bacteriophage PRD1 at a 6-m transport distance within, above, and below a "chemical heterogeneity" layer in a two-dimensional aquifer tank. The chemical heterogeneity layer is a partially ferric oxyhydroxide-coated sand. The breakthrough curves are presented as the concentration of PRD1 at a given time normalized by the maximum concentration of PRD1. The concentration of PRD1 was measured by radioassay of a P-32 label.

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Molecular Detection of Vegetative and Coccoid Forms of *H. pylori*

Manoucher Shahamat[1], C. Povlick[1], J. Hind[1], F. Robb[1], K. Sowers[1], M. Levin[1], and B. Bradley[2]
[1]University of Maryland Biotechnology Institute, Baltimore, MD; [2]Department of Biological Sciences, University of Maryland Baltimore County, Baltimore, MD

Helicobacter pylori is a major factor in the etiology of peptic ulcer disease and is the predominant cause of chronic gastritis, one of the major risk factors in the development of gastric cancer. More than 50 percent of the world's population may be infected with *H. pylori*. *H. pylori* has two morphological forms. In fresh culture and in the exponential phase of growth, most of the cells are curved, helical, rods; as the culture ages, or is environmentally stressed, the cells change to coccoid form (VBNC). The VBNC cells cannot be cultured on or in the media that support the growth of the vegetative form, although they can multiply if they encounter the appropriate environmental conditions. Coccoid *H. pylori* is suspected to be responsible for disease transmission or relapse of infections.

The basic goals of this project are to validate an accurate and sensitive molecular and/or immunodiagnostic method for the rapid detection of *H. pylori* in both the culturable and coccoid forms from environmental samples. The ability to do this is crucial for preventing underestimation of the number of bacteria in samples, developing epidemiological evidence, and understanding the ecological and public health significance.

Three laboratory approaches have been followed in the development of *H. pylori*-specific molecular and immunodiagnostic probes: (1) the application of monoclonal antibody specific to *H. pylori*—the monoclonal antibody must be capable of crossreacting with both helical and coccoid cells and be specific to *H. pylori;* (2) the evaluation and standardization of a polymerase chain reaction (PCR) based technique with specific oligonucleotide probes to detect vegetative and coccoid forms of *H. pylori;* and (3) the analysis of the entire protein output (proteome) of the organism for suites of proteins specific to each form and unique to *H. pylori* as a species. The monoclonal antibody has been applied using the immunofluorescence assay method, and the preliminary results using IgG are promising.

Work to identify variable regions that can be used for the design of strain-specific primers for PCR or the development of high throughput microarray screening has started. Sequencing of amplified fragments derived from eight strains has identified unique DNA in all eight strains as well as consensual primer sites. Evidence to date suggests that this variable region may provide a unique sequence that can be used to discriminate between individual strains of *H. pylori*. A PCR or microarray technique may be particularly suited to rapid screening and also to the detection of the coccoid form of *H. pylori* from environmental samples.

Proteomic analysis has started and several protocols have been tested. Homogenization methods specific to *H. pylori* have been developed to yield the optimum resolution of the proteins. The preliminary test results are promising—early analysis indicates that the 2D-PAGE protein pattern can be used to detect *H. pylori* in a mixed culture as well as differentiate specific strains. Further analysis will determine the protein expression signatures for morphological form.

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Detection and Occurrence of Human Caliciviruses in Drinking Water

Mark D. Sobsey
Department of Environmental Sciences and Engineering, University of North Carolina, Chapel Hill, NC

The general objectives of this research project are to: (1) develop improved methods to recover, concentrate, and purify Norwalk-like caliciviruses (NLVs) of both genogroups from water; (2) develop new and improved reverse transcription-polymerase chain reaction (RT-PCR) and oligonucleotide probe (OP) materials and methods to amplify and detect the recovered, concentrated, and purified NLVs; and (3) further evaluate these methods by applying them to the detection of field NLVs in environmental sewage and water samples, and thereby determine occurrence of NLVs in representative samples of sewage and raw and finished waters from both surface and ground sources. Lysine, glycine, and arginine eluants were evaluated at different pH levels with and without added nonionic detergents for efficient recoveries of Norwalk virus (NV) adsorbed to electropositive microporous filters from seeded tap water. These amino acid eluants were evaluated for inhibition of RT-PCR detection of NV compared to inhibition by beef extract-containing eluants.

Seven primer sets, representing four approaches to broad detection, were evaluated on a panel of eight samples representing seven distinct clusters within both genogroups, including Norwalk, Bristol, Hawaii, Desert Shield, Toronto, Gwynedd, and Cruise Ship (see Table 1). To confirm the presence of the desired amplicon, a subset of samples was dot-blot hybridized with a set of four oligoprobes, previously demonstrated to confirm NLV amplification.

Amino acid eluents efficiently eluted viruses adsorbed to electropositive microporous filters. Eluants were most compatible with RT-PCR at pH 9.5. At pH 8.5, glycine was the most compatible eluant. Eluants were least compatible with RT-PCR at pH 7.0. Initially, candidate detergents resulted in one $\log_{10}$ decrease in the detection limit by RT-PCR. Detergent concentrations and NV detection limits were not consistently correlated. Inhibition of RT-PCR by detergents was overcome by using them at low concentrations, and the detergents performed best when used at a concentration of 0.01 percent.

All of the primer sets evaluated were able to presumptively detect virus in the majority of samples. However, most of the primer sets also demonstrated some nonspecific amplification demonstrated by smearing or distinct bands of inappropriate size.

Although experiments to evaluate sensitivity to low copy number are not complete at this time, YGDD/GLPSG1 and YGDD/GLPSG2 primers seem to be the most promising for heat-release RT-PCR. However, initial results suggest differences in primer performance between heat-released and chemically extracted RNA.

The results of these studies suggest that NLVs can efficiently be recovered from water by adsorption-elution methods that are compatible with RT-PCR, and that the recovered viruses can be detected efficiently by RT-PCR and oligoprobe hybridization.

Additional experiments will be completed on the detection of low copy number of NLVs in environmental samples. Additional experiments to optimize recovery and RT-PCR compatibility of adsorbed NLV from positively charged filters will be completed. Additional experiments on the effect of RNA extraction on the performance of candidate primer pairs will be evaluated. Attempts will be made to propagate human NLVs in cell cultures.

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Table 1. Detection of NLVs by RT-PCR with confirmation by dot-blot hybridization is summarized for seven primer sets.

Primer Set	Gwynedd	Cruise Ship	Desert Shield	Bristol 1	Hawaii	Norwalk	Bristol 2	Toronto
Ando GI/GII	C	+	C	C	C	C	C	C
Vinje	C	P	–	+	C	+	C	C
LeGuyader	+	C	P	+	C	+	–	C
Wright	+	C	C	C	C	C	C	C
Green	C	C	C	C	C	C	C	C
Jiang	–	+	P	P	C	–	C	C
Monroe	+	+	+	+	+	+	+	+

+ = Presumptive positive, visible band failed probe.
– = Did not detect.
C = Confirmed positive, visible band probed positive.
P = Probed positive, no visible band.

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Development and Evaluation of Methods for the Concentration, Separation, and Detection of Three Protozoa From Large Volumes of Water

Saul Tzipori, Udi Zuckerman, Michael Buckholt, Giovanni Widmer, and Abhineet Sheoran
Division of Infectious Disease, Tufts University School of Veterinary Medicine, North Grafton, MA

The first phase of this project includes two aims. Aim 1 is the optimization and standardization of a portable continuous flow centrifuge (CFC) device. The CFC was modified and built from a blood separator into a compact portable unit for concentrating *C. parvum* oocysts, Giardia cysts, and Microsporidium spores from various volumes of water (10–1,000 L) and different matrices. Flow rate ranged from 0.5–1.4 L/min, with a maximum speed of 8,000 rpm (3,000 xg). After centrifugation, the residual volume was 250 mL, from which oocysts and cysts were dislodged by injecting a concentrated 10 mL elution buffer. The centrifuged residue was transferred for immunomagnetic separation, followed by reaction with specific monoclonal antibodies (MABs) and enumeration by fluorescence microscopy. The entire procedure, including parasite enumeration, takes approximately 2 hours. For *C. parvum* oocysts (90 oocysts) spiked into 10 L of filtered tap water, the average recovery (n = 12, flow rate 0.5, 0.75/ LPM) was 90.3 percent ± 49.8 percent. For the same dose spiked into 50 L (n = 11, flow rate 0.5–1.0/LPM), recovery was 82.7 percent ± 42 percent. For oocysts (250) spiked in 10 L of secondary effluent (turbidity = 2.8–4.6 NTU, n = 5, flow rate = 0.75/LPM), the average recovery was 56.5 percent. For a spike dose of 100 oocysts (80 ± 40 oocysts) in 1,000 L of tap water (turbidity = 0.5 NTU, n = 43, flow rate = 0.75/LPM), the average recovery was 57.33 percent ± 42.85 percent. *C. parvum* presence was confirmed by immunofluorescence microscopy and by polymerase chain reaction.

For Giardia cysts (95 and 1,080 cysts) spiked into 50 L (same matrix, n = 12, flow rate = 0.75 and 1/ LPM), the average recovery was 106.1 percent ± 24.4 percent. Aim two is the production of antibodies against Microsporidia. *Encephalitozoon intestinalis* and *E. cuniculi* were grown in rabbit kidney cells (RK13). Mouse anti-*E. intestinalis* MABs were obtained by immunizing BALB/c mice with *E. intestinalis* spores and subsequent fusion of spleen cells with Ag 8.653 myeloma cells. Hybridoma supernatants were tested by ELISA on *E. intestinalis* and *E. cuniculi* lysate-coated plates.

Positive supernatants also were tested on methanol-fixed spores by indirect immunofluorescence and in parallel by immunoblotting. Positive hybridomas were cloned twice and isotyped. Two clones, MABs CE5 and CG9, were derived following the screening of 1,000 wells from two fusion experiments. Both MABs react with *E. intestinal*is as well as *E. cuniculi* by ELISA and immunoblot. CG9 identifies proteins of about 48,000 and 44,000 daltons, whereas CE5 reacts with proteins of about 70,000, 41,000, and 36,000 daltons. CG9 exhibits strong surface staining of both *E. intestinalis* and *E. cuniculi* by indirect immunofluorescence, suggesting that a spore wall antigen located on the surface is detected, whereas CE5 shows no reactivity with either spore. Rabbit polyclonal antibodies also were raised by immunization with *E. intestinalis*, which crossreacts with *E. cuniculi*.

Currently, CG9 is being evaluated for its ability to inhibit invasion by mature spores, and is being characterized for its cross-reactivity with other bacteria, fungi, and protozoa. Also, two additional fusions have been performed to obtain *E. intestinalis*-specific MABs.

Enterocytozoon bieneusi spores for antibody production were purified from the stool of infected humans. *E. bieneusi* spores, detected by chromotrope stain, were votexed in suspension and filtered sequentially through four sieves of pore diameters 425, 280, 106, and 45 μm.

Density gradient centrifugation was performed with various concentrations of Percoll and Nycodenz. To eliminate or reduce bacterial and fungal load, the 30/37 percent band of Nycodenz was mixed with antibiotics. The sterile concentrate will be used to immunize rabbits and mice to produce polyclonal and monoclonal antibodies.

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Risk Factors for *Cryptosporidium parvum* Infection and Disease

Lucy. A. Ward, Y. Wang, S. Pereira, and S. Clarke
Food Animal Health Research Program, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, OH

The risk of transmitting the protozoan parasite *Cryptosporidium parvum* to humans via drinking water is multifactorial and includes not only the conditions under which *C. parvum* is introduced and survives in water, but also knowledge of the minimum infective dose, strain virulence variability, and a variety of individual host risk factors. A gnotobiotic (Gn) pig model is being used to assess risk factors to cryptosporidiosis.

Comparative studies in Gn pigs using two *C. parvum* genotype 2 (bovine) strains originating from humans (GCH1 and Ohio) suggest that host age is a significant determinant of risk to disease and death, but not infection. These Type 2 strains also demonstrated a 10- to 100-fold difference between their median diarrheal and lethal doses, but not median infective dose for neonatal pigs. However, upon deriving single oocyst "clones" of each strain, these differences were lost, with the cloned strains demonstrating significantly lower, albeit identical median diarrheal, lethal, and infective doses of less than five oocysts. In contrast to the parental strains, the phenotypic behavior of these "clones" has remained predictable and uniform between different pools and among multiple animal passages. This finding further suggests that the clones are more genetically homogenous (or clonal) compared to each parental strain.

Comparative morphologic and cytokine studies have demonstrated a positive correlation between cytokine mRNA levels and *C. parvum*-induced pathology, suggesting that the host immune response may be involved in disease expression, at least for genotype 2 strains. Comparative analyses of the different cytokine levels present in the intestine suggest a mixed Th1-Th2 cytokine response. Ongoing humoral and cellular immuneity studies suggest that blood lymphoproliferative responses to *C. parvum* develop sooner than serum antibody responses following infection. In fact, serum humoral responses do not develop until 3 weeks or more postinfection and loosely correlate with final clearance of the parasite from the intestinal epithelial surface.

Neonatal Gn pigs given 10^5–10^6 Type 2 oocysts typically begin shedding oocysts 2–3 days postinoculation and often shed intermittently or continuously up to 19 days postinfection. Hence, this apparent "delay" in serum antibody production (until 3 or more weeks postinfection) suggests that there may be a need for multiple or prolonged exposure to *C. parvum* to facilitate seroconversion, as has been suggested in studies of adult human volunteers.

This research recently has propagated three genotype 1 (human) *C. parvum* strains and one *C. meleagridis* strain (derived from an AIDS patient) in neonatal Gn pigs, and now is in the process of deriving single oocyst clones from each of these isolates. The mere availability of several Type 1 and 2 *C. parvum* clones as well as other non-*C. parvum* clones (such as *C. meleagradis*) for research purposes is an enormous advancement.

Preliminary studies demonstrate a uniquely different phenotypic behavior for the Type 1 strains compared to the Type 2 strains in the Gn pig model. Further studies will be conducted to determine whether differences also exist in the cytokine, antibody, and cellular responses of Gn pigs to Type 1 versus Type 2 strains.

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Development of a Rapid, Quantitative Method for the Detection of Infective Coxsackie and Echo Viruses in Drinking Water

Marylynn V. Yates, W. Chen, and A. Mulchandani
University of California, Riverside, CA

The objective of this research is to improve on the current analytical methods for quantitative detection of infective coxsackie and echo viruses in drinking water. The specific objectives are to: (1) improve the sensitivity and specificity of immunomagnetic separation polymerase chain reaction (IMS-PCR) for infective coxsackie and echo virus detection in water using polyclonal or monoclonal antibodies; (2) improve the efficiency and quantitation capabilities of IMS-PCR using molecular beacons; and (3) quantify the presence and viability of coxsackie and echo viruses in concentrated drinking water samples.

IMS-PCR will be combined with molecular beacons to increase the sensitivity and specificity of analysis of environmental water samples for coxsackie and echo viruses. The research approach includes: (1) optimization of IMS-PCR with polyclonal or monoclonal antibodies to the coxsackie and echo viruses; (2) development of molecular beacon-based, real-time reverse transcription PCR (RT-PCR) assays for the quantitative detection of target viruses; (3) analysis of virus-seeded water with the IMS-molecular beacon combination; and (4) testing of protocols on seeded drinking water concentrates to enable an assessment of the sensitivity of the method to inhibitors present in environmental waters. The specificity and quantitative capability of the protocols will be assessed by comparing results to quantitative cell culture analysis. To date, experiments have been conducted in which the quantitation of poliovirus 1, echovirus 11, and coxsackie virus B6 are compared using typical cell culture plaque assay on buffalo green monkey (BGM) kidney cells, direct RT-PCR, and IMS-RT-PCR. Results of these experiments indicate close agreement between the number of viruses detected using cell culture and IMS-RT-PCR (see Figure 1). The number of viruses detected using RT-PCR is 100 times higher than with the other two methods. This finding has been consistent for all three viruses studied. Primers to amplify a 149-base pair fragment of the conserved noncoding region of echovirus 11 and coxsackie virus B6 have been selected. Molecular beacons were designed to recognize a 25-base pair region on the amplicon. The selectivity and the specificity of the molecular beacon are being assessed. Detection and quantitation are carried out entirely in sealed tubes, enabling fast and direct measurements in a semiautomated format.

The results to date suggest that the use of IMS-RT-PCR allows for the detection of only infective virus particles. However, quantitation of virus particles using this method still is approximate. The use of the molecular beacon to quantify particles in combination with the IMS process to separate the infective particles should provide a rapid method for the enumeration of infective virus particles in water. After the methods have been refined, experiments will be conducted to assess the ability of the combined IMS-molecular beacon procedure to detect and quantify infective virus particles, using cell culture plaque assay results as a standard for comparison.

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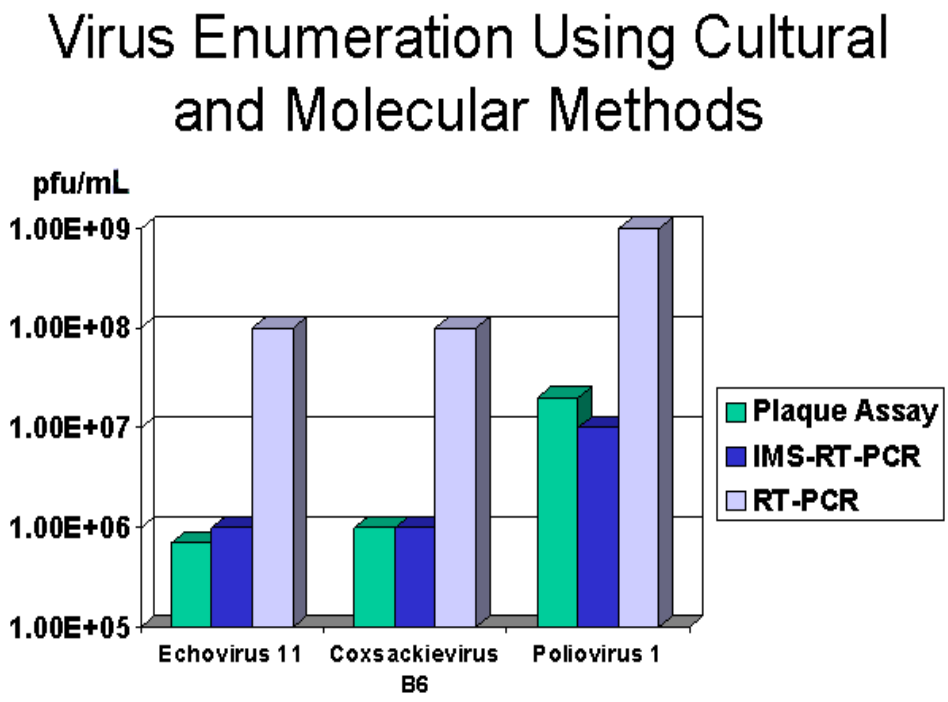

Figure 1. Virus enumeration using cultural and molecular methods.

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Section 6.

Contaminant Sources

Molecular Tracers of Contaminant Sources to Surface Water Drinking Supplies

Laurel J. Standley, Louis A. Kaplan, and J. Denis Newbold
Stroud Water Research Center, Avondale, PA

The objective of this research project is to develop a more quantitative method for apportioning the contribution of contaminants from point source effluents and nonpoint source runoff to surface waters that are drinking water supplies (e.g., rivers and reservoirs). In preliminary research, a suite of molecular tracers was developed for several potential contaminant sources that included wastewater treatment plants, agricultural runoff, urban/suburban runoff, and wildlife. Although accurate, the molecular tracer method is not yet quantitative. It is hypothesized that: (1) unique compounds (i.e., molecular tracers) that are constituents of runoff or effluent of contaminant sources reflect the contribution of these sources to contaminant budgets in drinking water supplies; (2) although the instream fate of contaminants and corresponding molecular tracers may differ, selection of more than one tracer can strengthen quantification of contaminant sources; and (3) threshold values for molecular tracers can be determined that are predictive for unacceptable levels of contamination (i.e., contaminant threshold values).

The approach is threefold: (1) concentrations and relative proportions of molecular tracers will be determined for contaminant sources; (2) the distribution of tracers in various riverine compartments (e.g., water column, suspended particles, and sediments) will be measured in receiving waters of study streams; and (3) transport and transformation processes that control the riverine fate of the molecular tracers will be investigated.

Results from this research will provide essential information regarding tracer occurrence and fate that is needed to develop a quantitative method for apportioning sources of contaminants in drinking water supplies. By quantitatively addressing fate and transport of the molecular tracers in streams and rivers, this work will provide the framework for future modeling efforts.

Beneficiaries of this work include the drinking water industry and all users of water resources by targeting remedial efforts where they will achieve the greatest improvement.

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Appendix

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U.S. Environmental Protection Agency
National Center for Environmental Research
STAR Drinking Water Progress Review Meeting
February 22-23, 2001
Holiday Inn Silver Spring
8777 Georgia Avenue
Silver Spring, MD

Agenda

Thursday, February 22, 2001

Lincoln Ballroom, 4th Floor

8:30 - 9:10 *Opening Remarks*
Dr. Peter W. Preuss, Director, National Center for Environmental Research, USEPA
Ms. Cynthia Dougherty, Director, Office of Ground Water and Drinking Water, USEPA
Ms. Cynthia L. Nolt-Helms, Program Manager, National Center for Environmental Research, USEPA

9:10 - 9:30 *Overview of Regulatory Context for Drinking Water Research*
Ephraim King, Director, Standards and Risk Management Division, OGWDW, USEPA

9:30 - 9:45 *Overview of ORD=s Drinking Water Research Program*
Fred Hauchman, National Drinking Water Program Manager, USEPA

Chemical Contaminants: Exposure, Effects, & Assessment

Moderator: Joyce Donohue, Office of Water, Office of Science and Technology, Health and Ecological Criteria Division, USEPA

9:45 - 10:15 *Genotoxicity and Occurrence Assessment of Disinfection Byproducts in Drinking Water*
Michael J. Plewa, University of Illinois at Urbana-Champaign, *1997 Recipient*

10:15 - 10:30 BREAK

10:30 - 11:00 *Assessment of Human Dietary Ingestion Exposures to Water Disinfection Byproducts via Food*
J.H. Raymer, Research Triangle Institute, NC, *1998 Recipient*

11:00 - 11:30 *The Toxicokinetics and Metabolism of Haloacids in Rodents: Effect of Chronic Exposure and Co-Administration*
Irvin R. Schulz, Battelle Memorial Institute - Pacific Northwest Division, *1997 Recipient*

11:30 - 1:00 LUNCH ON YOUR OWN

1:00 - 1:30 *Development of Biomarkers for Haloacetonitriles-Induced Cell Injury in Peripheral Blood*
Ahmed E. Ahmed, University of Texas - Medical Branch at Galveston, *1997 Recipient*

1:30 - 2:00 *NHEERL Research on Carcinogenic Contaminants in Drinking Water*
Douglas C. Wolf, National Health and Environmental Effects Research Laboratory, USEPA

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2:00 - 2:30 *Aluminum Toxicokinetics: Oral Absorption From Drinking Water and Brain Retention*
Robert A. Yokel, University of Kentucky and Purdue University, *1996 Recipient from Human Health Solicitation*

2:30 - 2:45 BREAK

2:45 - 3:15 *Overview of EPA=s Arsenic in Drinking Water Regulation*
Irene Dooley, Office of Water, USEPA

3:15 - 3:45 *Overview of ORD=s Arsenic Research Activities*
Herman J. Gibb, National Center for Environmental Assessment, USEPA

3:45 - 4:15 *Arsenic-Glutathione Interactions and Skin Cancer*
Catherine B. Klein, New York University School of Medicine, *1997 Recipient from Arsenic Solicitation*

4:15-4:45 *Trivalent Methylated Arsenicals: Novel Biomarkers of Arsenic Toxicity in Humans*
Miroslav Styblo, University of North Carolina at Chapel Hill, *1997 Recipient from Arsenic Solicitation*

4:45 - 6:00 POSTER SESSION

6:00 First Day of Meeting Adjourns

Friday, February 23, 2001

Session A: Potomac Room on the Lobby Level
Moderator: Jennifer Mclain, Office of Water, Office of Ground Water and Drinking Water, Standards and Risk Management Division, USEPA

Chemical Contaminants: Formation of DBPs

8:00 - 8:15 Introductions

8:15 - 8:45 *Development of a New, Simple, Innovative Procedure for the Analysis of Bromate and Other Oxy-Halides at Sub-ppb Levels in Drinking Water*
Howard Weinberg, University of North Carolina at Chapel Hill, *1997 Recipient*

8:45 - 9:15 *Kinetic-Based Models for Bromate Formation in Natural Waters*
Paul Westerhoff, Arizona State University, *1998 Recipient*

9:15 - 9:45 *Brominated DBP Formation and Speciation Based on the Specific UV Absorbance Distribution of Natural Waters*
Tanju Karanfil, Clemson University and James (Chip) E. Kilduff, Rensselaer Polytechnic Institute, *1999 Recipients*

9:45 - 10:00 BREAK

10:00 - 10:30 *Mechanistic-Based Disinfectant and Disinfectant Byproduct Models*
Paul Westerhoff, Arizona State University, *1998 Recipient*

10:30 - 11:00 *Use of Differential Spectroscopy To Probe Reactions Between Natural Organic Matter and Chlorinated Oxidants*
Gregory Korshin and Mark M. Benjamin, University of Washington, *1998 Recipient from Exploratory Research - Environmental Chemistry Solicitation*

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11:00 - 11:30 *Mechanisms and Kinetics of Chloramine Loss and Byproduct Formation in the Presence of Reactive Drinking Water Distribution System Constituents*
Richard L. Valentine, University of Iowa, *1998 Recipient*

11:30 - 12:30 LUNCH ON YOUR OWN

12:30 - 1:00 *Molecular Weight Separation and HPLC/MS/MS Characterization of Previously Unidentified Drinking Water Disinfection Byproducts*
Roger A. Minear, University of Illinois at Urbana-Champaign, *1998 Recipient*

1:00 - 1:30 *Formation and Stability of Ozonation Byproducts in Drinking Water*
Howard Weinberg, University of North Carolina at Chapel Hill, *1998 Recipient*

Drinking Water Treatment Studies

1:30 - 2:00 *Evaluation of Ozone Byproduct Formation Under Ozone Dose, Temperature, and pH Variation*
Michael S. Elovitz, National Risk Management Research Laboratory, USEPA

2:00 - 2:30 *Pilot Studies of the Ozonation/FBT Process for the Control of Disinfection Byproducts in Drinking Water*
Susan J. Masten, Michigan State University, *1998 Recipient*

2:30 - 3:00 *Integrated Approach for the Control of* Cryptosporidium parvum *Oocysts and Disinfection Byproducts in Drinking Water Treated With Ozone and Chloramines*
Benito J. Marinas, University of Illinois at Urbana-Champaign *1998 Recipient*

3:00 Adjourn

Friday, February 23, 2001

Session B: Lincoln Ballroom, 4th Floor
Moderator: Robin Oshiro, Office of Water, Office of Science and Technology, Health and Ecological Criteria Division, USEPA

Microbial Contaminants

8:00 - 8:15 Introductions

8:15 - 9:00 *General Overview of USEPA/National Exposure Research Laboratory Microbial Activities With Concentration on Protozoan Methods*
Bruce Mintz, NERL/USEPA, and Alan Lindquist, NERL/USEPA

9:00 - 9:30 Mycobacterium avium *Complex in Drinking Water: Detection, Distribution, and Routes of Exposure*
Timothy E. Ford, Harvard School of Public Health, *1999 Recipient*

9:30 - 10:00 *Meaningful Detection of* Mycobacterium avium *Complex in Drinking Water: Are Disinfectant-Resistant Morphotypes Virulent?*
Gerald Cangelosi, University of Washington and Seattle Biomedical Research Institute, *1998 Recipient*

10:00 - 10:15 BREAK

10:15 - 10:45 *Detection of Emerging Microbial Contaminants in Source and Finished Drinking Water With DNA Microarrays* (also a poster)
Darrell P. Chandler, Battelle Memorial Institute - Pacific Northwest Division, *1999 Recipient*

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10:45 - 11:15 *Development and Evaluation of Methods for the Concentration, Separation, and Detection of Three Protozoa From Large Volumes of Water*
Saul Tzipori, Tufts University, *1999 Recipient*

11:15 - 11:45 *Development of Detection and Viability Methods for Waterborne* Microsporidia *Species Known To Infect Humans*
David A. Battigelli, Wisconsin State Laboratory of Hygiene and University of Wisconsin - Madison, *1999 Recipient*

11:45 - 1:00 LUNCH

1:00 - 1:30 *Investigation of Secondary Transmission of Cryptosporidium parvum*
Mary E. Brown, National Center for Environmental Assessment, USEPA

1:30 - 2:00 *Infectivity and Virulence of* Cryptosporidium *Genotype 1/H Oocysts in Healthy Adult Volunteers*
Cynthia L. Chappell, University of Texas Health Science Center, *1999 Recipient*

2:00 - 2:30 *Attachment and Inactivation in Virus Transport in Ground Water*
Joseph N. Ryan, University of Colorado at Boulder, *1998 Recipient from Exploratory Research - Environmental Chemistry Solicitation*

2:30 - 3:00 *Studies of the Infectivity of Norwalk and Norwalk-Like Viruses*
Christine L. Moe, Rollins School of Public Health of Emory University, *1997 Recipient*

3:00 - 3:30 *Detection and Occurrence of Human Caliciviruses in Drinking Water*
Mark D. Sobsey, University of North Carolina at Chapel Hill, *1998 Recipient*

3:30 Adjourn

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Posters

Membrane Introduction Mass Spectrometry Studies of Halogenated Cyano By-Product Formation in Drinking Water
Terese M. Olson, University of Michigan, *1999 Recipient*

Haloacid Kinetics in Humans and Rhesus Monkeys
Irvin Schultz, Battelle Memorial Institute - Pacific Northwest Division, *1999 Recipient*

Detection of Emerging Microbial Contaminants in Source and Finished Drinking Water With DNA Microarrays
(also oral presentation by Darrell P. Chandler)
Timothy M. Straub, Battelle Memorial Institute - Pacific Northwest Division, *1999 Recipient*

Prevalence and Distribution of Genotypes of Cryptosporidium parvum in Feedlot Cattle in the Western United States
E. Robert Atwill, University of California - Davis, *1999 Recipient*

Development of a Rapid, Quantitative Method for the Detection of Infective Coxsackie and Echo Viruses in Drinking Water Marylynn V. Yates, University of California, *1999 Recipient*

Molecular Detection of Vegetative and Coccoid Helicobacter pylori
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