The Cooperative Research Centre for
Water Quality and Treatment

WATER SERVICES ASSOCIATION
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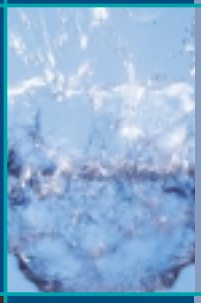
Disinfection
By-Products and Health Effects



Occasional Paper 5: Disinfection By-Products

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Disinfection By-Products and Health Effects

Report from a Seminar and Workshop
Conducted by the
Cooperative Research Centre for
Water Quality and Treatment
and the
Water Services Association
of Australia

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Melbourne Australia

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Disinfection By-Products and Health Effects: Report from a Seminar and Workshop Conducted by the Cooperative Research Centre for Water Quality and Treatment
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Introduction

This report summarises the proceedings of a three-day seminar and workshop on disinfection by-products and health effects held in Melbourne, Australia in October 2001. The meeting was organised by the Cooperative Research Centre for Water Quality and Treatment as a component in the development of a research strategy to guide its work on this topic over the next several years. The event was co-sponsored by the Water Services Association of Australia, and also supported by Alberta Health and Wellness, and the Natural Sciences and Engineering Research Council of Canada.

The first day of the meeting consisted of a public Seminar attended by 65 delegates from the water industry, regulatory agencies, and public and environmental health fields. The seminar was opened by Professor John McNeil, Program Group Leader of the Health and Aesthetics Program of the CRC for Water Quality and Treatment. Professor McNeil outlined the historical significance of drinking water chlorination as a major advance in public health, leading to a substantial reduction in deaths and illnesses from waterborne pathogens. The discovery of disinfection by-products (DBPs) during the 1970s led to concerns over the potential health effects of these chemicals, and stimulated investigations of their chemical and biological properties.

Six speakers then presented overviews of various aspects of the DBP issue including:
- the historical recognition of DBPs, and their formation and removal during drinking water treatment
- the behaviour of DBPs in water distribution systems
- epidemiological study methods and the evidence linking DBPs and cancer outcomes
- the evidence linking DBPs and adverse reproductive outcomes
- the toxicological evidence and limitations of past research approaches
- new developments in analytical methodology

The second and third days of the meeting consisted of a workshop for CRC researchers, representatives of CRC industry parties, and regulatory and public health bodies. The objective of this specialist workshop was to develop a suggested research strategy for investigation of the health effects of disinfection by-products (DBPs).

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DRINKING WATER DISINFECTION BY-PRODUCTS: WHEN, WHAT AND WHY?

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BRIEF HISTORY OF DISINFECTION BY-PRODUCTS (DBPS) AND DRINKING WATER

Trihalomethanes (THMs), primarily chloroform, were first reported in drinking water by Rook (1974) in Holland, soon followed by Bellar et al. (1974) in the US. THMs were shown to be produced by chlorination reactions with natural organic matter in water such as humic and fulvic acids. The existence of THMs had been missed by earlier analytical schemes which used solvents of similar volatility to, and including chloroform, for extracting organic compounds. The discovery of THMs did not change what we were being exposed to in drinking water but changed what we know we are being exposed to.

The discovery of trace organics in drinking water was a major revelation to the water industry, which had come to believe that conventional water treatment was "proven" for decades because of the control of microbiological quality and this was all that was needed for "safe" drinking water. As analytical techniques advanced, the pattern of identifying contaminants and then looking for health effects was established, and this pattern has repeated in the succeeding decades. In other words, the focus of public health research on particular classes of DBPs has been driven largely by advances in detection capabilities, rather than by rational assessment of their potential toxicological importance.

CLASSES OF CURRENTLY KNOWN DBPS

DBPs are, by definition, the result of a reaction between a disinfecting agent (chemical or physical) and a precursor chemical in the source water. Therefore, DBP formation will depend on factors such as the disinfectant used, the precursors present and the reaction conditions provided.

Major classes of DBPs include halogenated organic compounds such as trihalomethanes, haloacetic acids, haloacetonitriles, chlorophenols, chloral hydrate and chloropicrin. Significant inorganic compounds reported include chlorate, chlorite, bromate, iodate, hydrogen peroxide, HOBr, nitrate, nitrite and hydrazine. Other non-halogenated DBPs reported include aldehydes, ketoacids, ketones, carboxylic acids, maleic acids, nitrosamines, alkanoic acids, benzene. Table 1 lists the individual DBP species of the various classes (adapted from Krasner 1999, Froese et al 1999).

Table 1 Classes of currently known DBPs

DBP Class	Individual DBPs	Chemical Formula
Trihalomethanes	Chloroform	$CHCl_3$
	Bromodichloromethane	$CHCl_2Br$
	Dibromochloromethane	$CHClBr_2$
	Bromoform	$CHBr_3$
Haloacetic acids	Monochloroacetic acid	$CH_2ClCOOH$
	Dichloroacetic acid	$CHCl_2COOH$
	Trichloroacetic acid	CCl_3COOH
	Bromochloroacetic acid	$CHBrClCOOH$
	Bromodichloroacetic acid	$CBrCl_2COOH$
	Dibromochloroacetic acid	$CBr_2ClCOOH$
	Monobromoacetic acid	$CH_2BrCOOH$
	Dibromoacetic acid	$CHBr_2COOH$
	Tribromoacetic acid	CBr_3COOH
Haloacetonitriles	Trichloroacetonitrile	CCl_3CN
	Dichloroacetonitrile	$CHCl_2CN$
	Bromochloroacetonitrile	$CHBrClCN$
	Dibromoacetonitrile	$CHBr_2CN$
Haloketones	1,1-Dichloroacetone	$CHCl_2COCH_3$
	1,1,1-Trichloroacetone	CCl_3COCH_3
Miscellaneous	Choral hydrate	$CCl_3CH(OH)_2$
chlorinated organics	Chloropicrin	CCl_3NO_2
Cyanogen halides	Cyanogen chloride	$ClCN$
	Cyanogen bromide	$BrCN$
Oxyhalides	Chlorite	ClO_2^-
	Chlorate	ClO_3^-
	Bromate	BrO_3^-
Aldehydes	Formaldehyde[1]	$HCHO$
	Acetaldehyde[2]	CH_3CHO
	Glyoxal	$OHCCHO$
	Methyl glyoxal	CH_3COCHO
	Isobutyraldehyde[3]	$(CH_3)_2CHCHO$
(odorous)	Isovaleraldehyde[4]	$(CH_3)_2CHCH_2CHO$
	2-Methylbutyraldehyde[5]	$(CH_3)(C_2H_5)CHCHO$
	Phenylacetaldehyde[6]	$(C_6H_5)CH_2CHO$
Aldoketoacids	Glyoxalic acid	$OHCCHO$
	Pyruvic acid	$CH_3COCOOH$
	Ketomalonic acid	$HOOCCOCOOH$
Carboxylic acids	Formate	$HCOO^-$
	Acetate	CH_3COO^-
	Oxalate	$OOCCOO^{-2}$
Maleic acids	2-*tert*-Butylmaleic acid	$HOOCC(C(CH_3)_3):CHCOOH$
Chlorphenols	Chlorophenol	C_6H_5Cl
(odorous)	Dichlorophenols	$C_6H_4Cl_2$
	Trichlorophenols	$C_6H_3Cl_3$
Chloroanisoles		
(odorous)	Trichloroanisoles[7]	$CH_3OC_6H_3Cl_3$

[1]formed from glycine
[2]formed from alanine
[3]formed from valine (Hrudey et al. 1988)
[4]formed from leucine (Hrudey et al. 1988)
[5]formed from isoleucine (Hrudey et al. 1988)
[6]formed from phenyalanine (Hrudey et al. 1988)
[7]biotransformation of trichlorophenols

Trihalomethanes and haloacetic acids are the most prevalent compounds in chlorinated drinking water and form the largest groups in terms of quantity. Reported DBP concentrations ranges for trihalomethanes in drinking water supplies range from a minimum 3.1 µg/L to a maximum of 1280 µg/L and for haloacetic acids from <0.5 µg/L to 1230 µg/L (IPCS 2000). Reported ranges for other classes include:

Haloacetonitriles:	(0.04 µg/L – 12 µg/L)
Haloketones:	(0.9 µg/L – 25.3 µg/L)
Chlorophenols:	(0.5 µg/L – 1 µg/L)
Chloral hydrate:	(1.7 µg/L – 3.0 µg/L)
Chloropicrin:	(<0.1 µg/L – 0.6 µg/L)

Although chlorination disinfection by-products have undergone the most investigation, it is important to recognise that all disinfectants will generate disinfection by-products because effective disinfectants will be chemically reactive. Ozonation for instance produces a vastly different profile of disinfection by-products than chlorine, producing oxygenated species such as bromate, iodate, chlorate, aldehydes and ketoacids rather than THMs, HAAs or HANs.

Table 2 lists various disinfection by-products that have been determined for chlorine, chlorine dioxide, chloramine and ozone.

Table 2 Disinfectants and Disinfection By-products (adapted from ICPS 2000)

Disinfectant	Significant organohalogen DBPs	Significant inorganic DBPs	Significant non-halogenated DBPs
Chlorine	THMs, HAAs, HANs, CH, CP, CPh, N-chloramines, halofuranones, bromohydrins	chlorate (mostly from hypochlorite use)	aldehydes, cyanoalkanoic acids, alkanoic acids, benzene, carboxylic acids
Chlorine dioxide		chlorite, chlorate	unstudied
Chloramine	HANs, cyanogen chloride, organic chloramines, CH, chloramino acids, haloketones	nitrate, nitrite, chlorate, hydrazine	aldehydes, ketones, nitrosamines
Ozone	bromoform, MBA, DBA, dibromoacetone, cyanogen bromide	chlorate, iodate, bromate, hydrogen peroxide, HOBr, epoxides, ozonates	aldehydes, ketoacids, ketones, carboxylic acids

PHYSICAL AND CHEMICAL PROPERTIES OF DBPS

The basic physical and chemical properties of individual compounds are important in determining their fate in water treatment processes, distribution systems and at the point of supply to consumers. An understanding of these properties is also needed for assessing human exposure routes.

Two important properties are the Henry's Law Constant (K_H) and the log Octanol – Water Coefficient (K_{OW}). The value of the Henry's Law Constant provides an indication of likely partitioning from water to air (i.e. a measure of volatility). The Log Octanol – Water Coefficient is a measure of the preference of the compound for the organic phase (lipophilic compounds) or the water phase (hydrophilic compounds).

Available data on K_H and K_{OW} for disinfection by-products is limited. However, values for trihalomethanes indicate that volatilization is significant for these compounds and that they are slightly lipophilic; indicating that human exposure to these compounds is strongly influenced by inhalation/vapour-phase and dermal routes of exposure with activities such as bathing and showering being important. Haloacids are known to be very hydrophilic with negligible volatilization. Exposure to haloacids is therefore likely to be limited to ingestion uses of drinking water. Thus significantly different exposures to various DBPs from the same water supply will occur at an individual level depending on the varying water use activities undertaken by each person.

FORMATION OF DBPS IN DRINKING WATER

Since the discovery of DBPs in drinking water there has been a concerted effort to understand how DBPs are formed and how they can be avoided. Most research was initially directed at THMs and variations on chlorination. Initially to avoid formation of THMs and other halogenated DBPs, alternative disinfectants have been pursued and continued research has shown that varying levels and types of DBPs are produced by all disinfection methods and that DBPs may be reduced but not eliminated all together.

The formation of DBPs in water treatment is influenced by several factors:

- contact time
- disinfectant dose
- pH
- temperature
- total organic carbon (TOC)
- ultraviolet absorption (UV_{254})
- bromide

At the treatment plant, THMs and HAAs follow similar patterns of formation with rapid and curvilinear increases with both increasing contact time and increasing disinfectant dose. Both have shown rapid formation in less than five hours, with 90% being formed in the initial 24 hours with concentrations levelling off after a prolonged period. Bromate formation (a function of ozone residual and bromide) is also shown to have a curvilinear increase with increasing contact time with most bromate forming in less than five minutes. With increasing ozone concentration, bromate indicates a linear increase following TOC demand and then levels off after ozone residual disappearance. Increasing contact time also increases concentrations of aldehydes and chlorate/chlorite providing a residual is present. Increase in disinfectant dose has a similar effect depending on the dose applied.

Increasing pH tends to favour the formation of THMs (up to pH 9.5) and decrease formation of HAAs. Maximum concentrations of DCAA have been shown to occur at pH 7-7.5. For DBPs such as DCAN and trichloroacetone, higher formation occurs at low pH while concentrations of chlorate/chlorite and bromate show a positive effect with increasing pH. Aldehydes, which form mostly through molecular ozone, indicate a negative effect with increasing pH with a 25% decrease in concentration for pH 7-8.5.

Generally, temperature shows a positive effect for most DBPs with increasing temperature (10 to 30 degrees Celsius) resulting in an 15 – 25 % increase in concentration. For aldehydes, terminal products such as carbon dioxide increase with increasing temperature and total aldehydes slightly decrease. Concentrations of THMs and HAAs also tend to increase with increasing TOC and UV_{254}. However precursor content and character are important; humic acids are more reactive than fulvic acids. Aldehydes also show a positive effect with increasing TOC and UV_{254} but bromate and chlorate/chlorite have been shown to decrease with increasing TOC. Again, precursor content and character are important; humic acid is more reactive with ozone.

The presence of the bromide ion shifts THMs and HAAs towards the more brominated species rather than the chlorinated species. Aldehydes are independent of bromide at concentrations less than 0.25 mg/l but at greater concentrations, aldehydes can decrease due to ozone-bromide oxidation demand. In hypochlorite solutions, the presence of bromide shifts chlorate/chlorite towards more toxic bromate. For ozonation, there is a curvilinear increase in bromate dependent upon the ozone residual.

There are various minimisation strategies that can be used to reduce DBP formation in drinking water, such as TOC removal, pH control, alternative disinfectants, minimising chlorine residual and contact time, minimising and optimising ozone residual, etc. Granular activated carbon (GAC), electron beam, biofiltration, ferrous sulfate are some removal strategies for specific DBPs. Competing risks must be considered in evaluating DBP minimisation strategies. For instance, minimising chlorine residual and contact time will lead to less effective disinfection and increased risks from microbiological contaminants. All alternatives will be limited by their effectiveness, the generation of other water quality problems (including other DBPs in some cases) and their overall cost for the benefit achieved.

RECENT AND EMERGING DBPS

As analytical power increases in the search for DBPs, new compounds continue to be reported. Major gaps in our knowledge exist particularly for the more water soluble, non-volatile and thermally labile fractions because analytical capabilities for such compounds are currently more limited. Alternative chemical disinfectants may produce new types of DBPs. Non-chemical modes of disinfection such as UV irradiation are also likely to produce DBPs although little research has been carried out in this area to date.

For halogenated DBPs, mass balance calculations (based on total organic halides, TOX) suggest that less than fifty percent of total halogenated organics have been identified. It is not possible, by mass balance, to determine the quantity of non-halogenated DBPs that remain unidentified because there is no means of estimating the total amount.

New analytical approaches are necessary to assess the full spectrum of possible DBPs. However, there is difficulty in finding unknowns because some knowledge of the chemical properties of the target compound is required in order to develop the necessary analytical capabilities.

Some recent described and emerging DBPs are listed in Table 3.

Table 3 Recent and Emerging DBPs (Arbuckle et al. 2002)

Haloacids	**Haloaldehydes**
3,3 Dichloropropenoic acid	Dichloroacetaldehyde
	Bromochloroacetaldehyde
Halomethanes	**Haloacetates**
Bromochloroiodomethane	Bromochloromethyl acetate
Dichloroiodomethane	
Halonitromethanes	**Haloamides**
Dibromonitromethane	2,2-Dichloroacetamide
Haloacetonitriles	**Aldehydes**
Bromochloroacetonitrile	2-Hexanal
Dibromoacetonitrile	Cyanoformaldehyde
Haloketones	**Nitrosamines**
1,1,3-Trichloropropanone	Nitrosodimethylamine
1,1-Dibromopropanone	(NDMA)

Halogenated Furanones

3-Chloro-4-(bromochloromethyl)-5-hydroxy-2(5H)-furanone (BMX-1)
3-Chloro-4-(dibromomethyl)-5-hydroxy-2(5H)-furanone (BMX-2)
3-Bromo-4-(dibromomethyl)-5-hydroxy-2(5H)-furanone (BMX-3)
3-Chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX)

HISTORICAL PERSPECTIVE: THE CHLOROFORM STORY

Chloroform was once a widely used chemical in consumer products ranging from cough syrup to toothpaste, in addition to being used as an anaesthetic for over 50 years. In 1976, chloroform was banned from drugs and cosmetic products by the US FDA after the National Cancer Institute declared it to be a carcinogen based on a rodent cancer bioassay.

Several epidemiological studies over the past 25 years have suggested an elevated cancer risk among consumers of "chlorinated water supplies" and a few recent epidemiological studies suggest an association between THM exposures and adverse reproductive outcomes.

The concerns over adverse health risks from THMs in drinking water led to a variety of regulations and guidelines being developed for chloroform and other THMs (Table 4) The original rationale for these numbers was based on the cancer risk derived from rodent bioassays that was calculated using cancer slope factors based on linearized low dose models.

The premise underlying these models is that the carcinogen is genotoxic (directly DNA-reactive) and that there does not exist a threshold level of exposure below which no adverse response is expected. Effectively, the no-threshold hypothesis assumes that any exposure above zero for a genotoxic carcinogen may produce some risk above zero.

Table 4 THM Regulations and Guidelines

Australia:	250 µg/L TTHMs (maximum) set in 1996
USA:	100 µg/L TTHMs (annual average) in 1979 changed to 80 µg/L L TTHMs (annual average) in 1998
Canada:	350 µg/L TTHMs (maximum) set in 1978 changed to 100 µg/L TTHMs (annual average) in 1996
WHO:	30 µg/L chloroform (annual average) in 1984 changed to 200 µg/L chloroform (annual average) in 1993 100 µg/L bromoform (annual average) in 1993 100 µg/L dibromochloromethane (annual average) in 1993 60 µg/L bromodichloromethane (annual average) in 1993

The differences in guideline and regulatory values reflect the differences in how the risk has been evaluated and the cancer slope factors used which are typically obtained by extrapolating through zero on a dose-response curve. However, the meaning of the cancer slope factors based on the linearized low dose models is now widely recognised as demanding close scrutiny (Krewski et al. 1993).

In the chronic animal feeding studies from which cancer slope factors are determined, the potential response (proportion of test animals developing cancer) is limited to two orders of magnitude (as only 50 to 100 animals are tested for each dose), while the dose (daily intake of chemical under test) may vary by nine orders of magnitude (due to the wide range in acute toxicity of different chemicals). Because of these constraints, the cancer slope factor calculated by extrapolating through zero is highly dependent on the dose, which is in turn highly dependent on the acute toxicity. Thus cancer slope factors calculated by this method show an extremely high degree of correlation (r= 0.941) with the maximum tolerable dose (a measure of acute toxicity), and are unlikely to reflect the chronic carcinogenic potency of the chemical.

Moreover, consistent evidence now exists that the carcinogenic effects of chloroform are related to regenerative cell proliferation in response to cellular toxicity which occurs only at high exposure levels. This mode of action is non-genotoxic, making inappropriate the use of cancer slope factors and the no-threshold hypothesis. More appropriate methods of risk estimation for such compounds are based on the No Observable Adverse Effect Level (NOAEL).

Depending on which method is pursued, very different risk estimates result. For example using the no-threshold assumption, the Risk Specific Dose (RSD) at 10^{-5} lifetime cancer risk for chloroform using the default linearized multi-stage (LMS) model is 60 parts per billion. Using the threshold approach, the NOAEL for B6C3F1 mice drinking water studies was 1,800,000 parts per billion indicating a Reference Dose (RfD) using uncertainty factors of 100 of 18,000 parts per billion. For Osborne-Mendel rat drinking water studies, the NOAEL was 900,000 parts per billion and the RfD (Uncertainty Factors of 100) estimated at 9,000 parts per billion. Thus to obtain a value equivalent to RSD of the LMS model, the RfD would need Uncertainty Factors in the order of 15,000 to 30,000. (Butterworth et al. 1995). This illustrates the extreme divergence in risk estimates derived from the two different approaches to the same toxicological data.

The US EPA policy for carcinogens in drinking water calls for a maximum contaminant level goal (MCLG) of zero. However, the mounting toxicological evidence on the mode of action of chloroform resulted in a US EPA expert review panel recommending the abandonment of the MCLG of zero and replacement with a limit based on an estimated threshold. Thus in 1998, the US EPA proposed to raise the MCLG to 0.3 mg/L in accordance with this expert advice. However, the US EPA Final Rule withdrew the proposal to change the MCLG for chloroform from zero as many intervenors protested this precedent-setting measure (Pontius 2000).

The Chlorine Chemistry Council sought a court review of the US EPA decision as the Safe Drinking Water Act requires the US EPA to use the best available science in setting standards and regulations. Although the US EPA acknowledged that the best available science called for raising the MCLG above zero, it had nevertheless decided to retain the zero MCLG. On March 31 2000, the US District Court ruled that the US EPA had violated the Safe Drinking Water Act by failing to use the best available science. The court found the zero MCLG to be 'arbitrary and capricious' and in excess of statutory authority.

The changing fortunes of chloroform over the years illustrate some of the problems in risk management for DBPs in the presence of uncertainty and incomplete evidence, and the difficulty in revising entrenched regulatory measures as scientific knowledge improves.

CONCLUSIONS

We are left with somewhat of a chicken or egg dilemma: DBP monitoring data is based on what drinking water guidelines require to be monitored and until now, they have been based on animal toxicology risk assessments (mainly cancer) for only a few compounds. It is now clear that the DBPs being measured for regulatory purposes are not sufficiently toxic to account for the possible human health effects suggested by epidemiological studies. Retrospective epidemiology can only test causal hypothesis using available monitoring data for assessing exposure. The problem still remains however 'exposure to what?' and we can never really know 'what' in drinking water actually causes disease in humans without meaningful epidemiological data.

Countless DBPs have been identified in drinking water and more will continue to be identified. Any disinfected water supply will contain a complex mixtures of DBPs including both halogenated and non-halogenated compounds, volatile and non-volatile compounds, and brominated compounds. Risk assessment has been blind to the majority of DBPs and has focused on only a small fraction of what has been identified. Similarly, drinking water guidelines and regulations have focused on a small proportion of DBPs representing the most abundant and readily assayed classes. Given the limited evidence on adverse health effects the regulatory levels set for DBPs have been precautionary in nature.

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DBPS IN THE WATER SUPPLY SYSTEM

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INTRODUCTION

Since the discovery of trihalomethanes (THMs) in drinking water and subsequent concern over human health effects of these compounds, various guidelines and regulations have been established which limit the levels of DBPs in drinking water as well as defining monitoring requirements. Much of the focus has concentrated on THMs as these are generally the most predominant DBPs arising from chlorination of drinking water.

However, as evidence has emerged on the occurrence of other DBPs and their potential health effects, guidelines and regulations have extended to other DBPs or to individual compounds. Based on toxicological evidence, these limits have been set to minimise the potential cancer risk associated with chronic DBP exposures suggesting that long-term average DBP exposure is the relevant measure. As such, they are often based on annual running averages taken from quarterly samples.

More recently however, the nature of health concerns has expanded to include adverse reproductive outcomes. Adverse reproductive outcomes provide a vastly different temporal profile for exposures and outcomes, shifting focus from chronic exposure to acute exposures with short-term peak exposures being more relevant. Thus understanding the general kinetics and how concentrations change in the distribution system is important in order to:

- accurately assess exposure to DBPs, and
- establish appropriate guidelines to minimise that exposure.

Furthermore, the problem still remains 'exposure to what?': in the absence of knowing which agents, if any, are causal, understanding the relationships and correlations between DBPs is imperative for accurate exposure classification.

DBP KINETICS IN THE DISTRIBUTION SYSTEM

Formation and evolution of DBPs in distribution systems is not well understood and reflects the complex nature of the reactions involved. Factors influencing the formation of DBP levels in water distribution systems include:

- water distribution and storage system hydraulics
- pH
- water temperature and season
- nature and concentration on NOM, and
- chlorine residual.

Trihalomethanes

Much of the research and analysis on DBP formation and evolution in distribution systems has focused on chlorination DBPs and more specifically THMs as they are commonly the most prevalent DBPs found in drinking water. Limited data exist for other classes of DBPs.

Many laboratory bench-scale and Simulated Distribution Tests have shown that most THM formation occurs at a fast initial rate followed by a slower rate suggesting that in a distribution system, the concentration of THMs will increase steadily with increasing residence time providing a chlorine residual persists. The distribution system however is very dynamic, and field tests in real distribution systems have shown more inconsistent results.

Studies of THM occurrence in distribution systems have indicated that THMs can significantly vary both spatially and temporally. Spatially, many field studies have confirmed the general change in THMs with increasing residence in the distribution system with concentrations being shown to increase to up to three fold compared with concentrations at the treatment plant. Other research however has shown that there can be a wide variation across the distribution system even laterally, indicating that concentrations in distribution systems are not always directly correlated with total residence time.

Temporally, research has shown that THM concentrations can vary significantly hourly, daily as well as during a year (seasonally). For example, one study showed THM concentrations in samples collected every four hours from a continually running tap fluctuated as much as 44%.

Field data has also consistently shown significant variation in seasonal concentrations of THMs with levels generally much greater in the summer than in the winter due to an increase in temperature of the water and precursor concentrations. Other important parameters that have been shown to influence THM levels in distribution systems include the pH of the water (with higher concentrations at higher pH) and the persistence of a chlorine residual.

For the limited studies done on treatment practices other than chlorination such as ozone-chlorine and chlorine-chloramine, similar trends have been shown; however, THM levels are usually present at lower concentrations than with chlorination.

Haloacetic Acids

More recent studies have extended the investigation in the distribution system to other classes of DBPs such as haloacetic acids (HAAs) - generally the second predominant class of DBPs found in chlorinated drinking water. Some evidence has suggested HAAs exhibit different spatial and temporal variations compared with THMs. With increasing residence in the distribution system, more inconsistent results have been reported than THMs.

While some studies have shown HAAs to follow a similar trend to THMs with increasing concentrations through the distribution system or occasionally levelling out, recent studies have also shown that concentrations of HAAs can significantly decline with increasing residence time, with the highest levels of HAAs observed at the site nearest the treatment plant. It has been speculated that these decreases in HAAs are attributable to biological degradation and/or photodegradation occurring more frequently at higher temperatures.

Increase in temperature has also been shown to increase HAA concentrations with some studies reporting higher levels in summer months versus winter months. However, it has been suggested that HAA formation is not as affected by temperature as THMs and that seasonal differences are more apparent with THMs. Particularly in winter months, concentrations of HAAs have been reported to be greater than those of THMs. In some instances, surveys have indicated that HAA concentrations (consisting predominantly of di- and tri-chloroacetic acid) are often present in concentrations equal or greater to those of THMs. This is also reported to be attributable to a lower pH of chlorination practiced. Compared to THM formation which increases with increasing pH, HAA formation increases with decreasing pH. Higher levels of free chlorine residual have also been reported to favour the formation of HAAs over THMs.

Other DBP classes

Haloacetonitriles, haloketones, chloropicrin and chloral hydrate have also been detected in most chlorinated drinking water; however, concentrations are usually an order of magnitude lower that THM or HAA levels.

There is limited research on these other classes of DBPs in the distribution system but in the few studies that have been conducted, they have been reported to form rapidly upon chlorination but then level off or further degrade through distribution system, resulting in a decrease in concentration with increasing contact time as a result of hydrolysis and continuing reactions with chlorine. These classes have also been reported to diminish in concentration at high pH.

CORRELATIONS BETWEEN DBPS

The finding that there is great variability in the distribution system and across different systems results in major implications regarding exposure to these DBPs depending on proximity to the treatment plant, as well as implications for the sampling scheme needed to determine maximum concentrations.

THMs have long been thought of as an indicator of total by-products in chlorinated drinking water and are often used as a surrogate for other DBPs in estimating exposure. Although assessment of human exposure to DBPs ideally requires estimating concentrations of the compounds at the consumer's tap, most studies have estimated DBP exposure in terms of estimating exposure to THMs at the treatment plant or at a few points in the distribution system.

For THMs to serve as a viable surrogate measure of exposure to other chlorination DBPs, they must a) be correlated with these other DBPs after creation, and b) follow similar patterns of change in the distribution system. Therefore understanding the correlations between DBPs is important because if there is poor correlation between THMs and these other DBPs, it will cause inaccurate exposure classification possibly resulting in lower risk estimates than would be achieved by a direct estimate of exposure to the causal agent if known (non-differential misclassification of the exposure variable).

A number of studies have used linear regression techniques to correlate THMs with other DBPs. Several of these studies have reported that there is often good

correlation at the treatment plant between THMs and total DBPs as well as THMs and HAAs; thus indicating at the treatment plant, THMs may serve as a potential surrogate for other DBPs. In the distribution system, however, correlations between DBPs are much more inconsistent.

For instance, analysis of correlations between THMs and HAAs (the most prevalent DBPs in chlorinated drinking water) at the treatment plant and in the distribution system of two Canadian drinking water systems to determine if there was sufficient consistency in DBP formation or degradation behaviour to use THMs as a reliable surrogate for other DBPs resulted in 2 very distinct observations (Rizak et al. 2000).

Monitoring data were viewed from the perspective of being able to use upstream data for THMs at the treatment plant effluent to predict values for HAAs firstly at the same site and then at a downstream site in the distribution system. System 1 practices conventional water treatment (coagulation, flocculation, sedimentation and filtration with pre- and post-disinfection). For two years of data (1997-1999), there was an excellent correlation between HAA and THM concentrations at the discharge of the treatment plant. The coefficient of determination was 0.90 indicating that 90% of the variation in HAA levels can be attributed to variation in THM levels at the treatment plant (Figure 1).

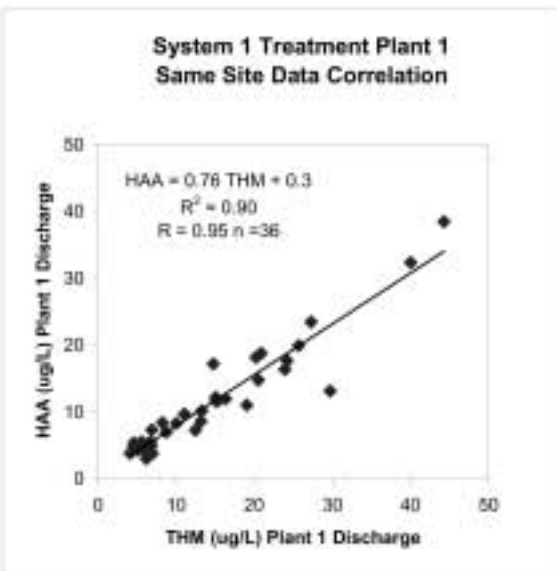

Figure 1 Haloacetic Acids (DCAA+TCAA) in Relation to Trihalomethanes (TCM+BDCM) at Treatment Plant of System 1 (1997 – 1999)

Figure 2 for the same system shows an excellent correlation between HAA and THM concentrations at a site in the distribution system approximately 10 km from the treatment plant (r^2 = 0.94). The correlation observed in Figure 3 would be the type that is commonly used in epidemiological studies whereby THM monitoring data at the treatment plant is used to estimate DBP exposures in the distribution system. Figure 3 looks at the effect of not only the different parameters (THM versus HAA) but also different locations of sampling (sampling at treatment plant versus the distribution system. The correlation remains good (r^2 = 0.87).

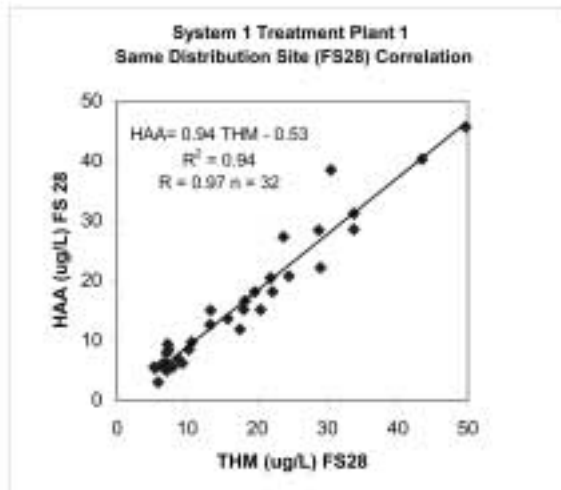

Figure 2 Haloacetic Acids (DCAA+TCAA) in Relation to Trihalomethanes (TCM+BDCM) at Distribution Site (~10 km) of System 1 (1997 – 1999)

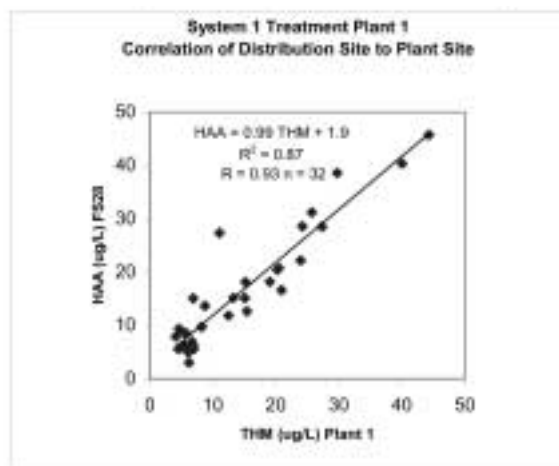

Figure 3 Haloacetic Acids (DCAA+TCAA) at Distribution Site (~10 km) in Relation to Trihalomethanes (TCM+BDCM) at Treatment Plant of System 1 (1997 – 1999)

For System 1 and the time period covered, these results indicate that THMs measured at the treatment plant (or in the distribution system) could act as an excellent surrogate for HAA exposure in the distribution system if the samples were only analysed for THMs.

System 2 has water disinfected at three points along the system which is characterised by very long travel times and extended storage of chlorinated water. The correlation (r^2) between total HAA and THM concentrations at the inlet to a large open reservoir is 0.52 indicating that about 50% of the observed variation in total HAAs can be attributable to the observed variation in total THM levels in the inlet water (Figure 4). The DBP levels observed here are created during an approximate 30 hour travel time through a 160 km aqueduct from the intake. Figure 5 shows the correlation at the outlet of the reservoir rather than the inlet: the correlation is much weaker than that observed at the inlet (r^2 = 0.11).

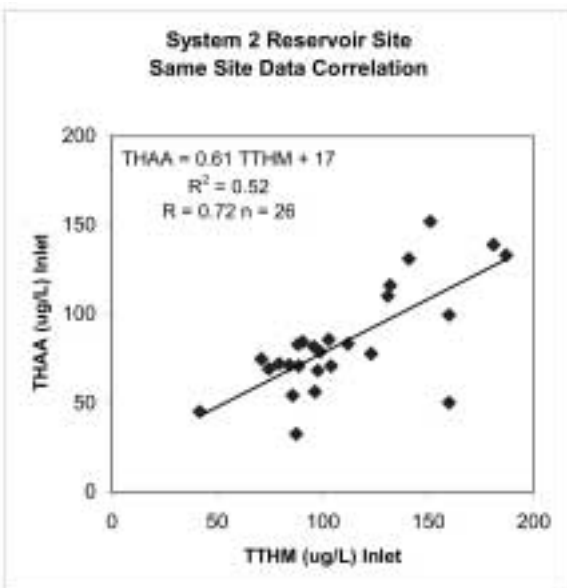

Figure 4 Total Haloacetic Acids in Relation to Total Trihalomethanes for an Open Reservoir Inlet Site of System 2 (1997 – 1999)

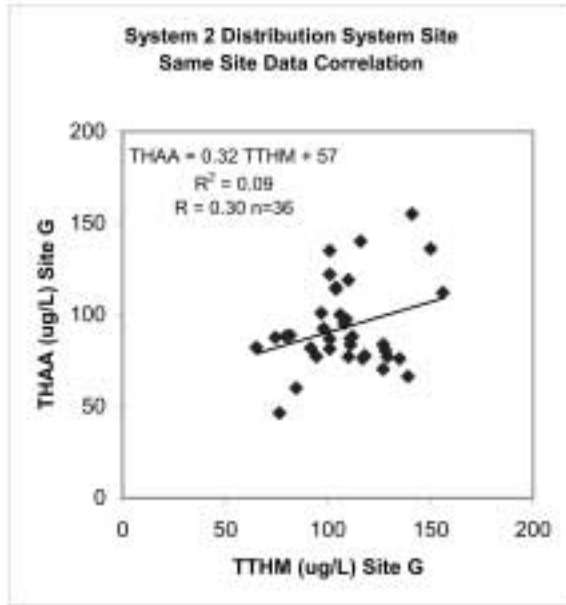

Figure 5 Total Haloacetic Acids in Relation to Total Trihalomethanes for an Open Reservoir Outlet Site of System 2 (1997 – 1999)

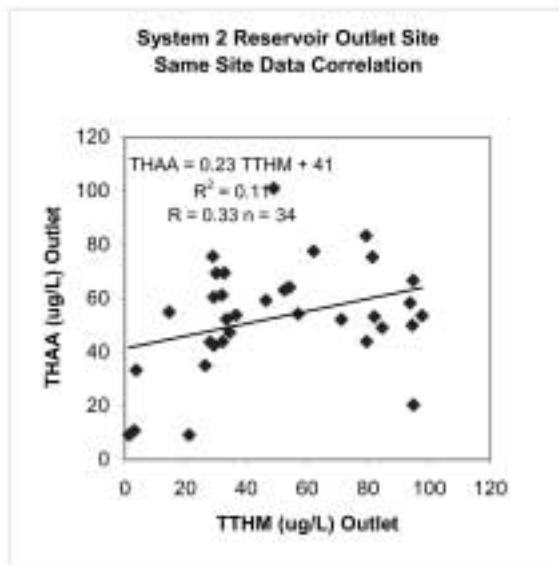

Figure 6 Total Haloacetic Acids in Relation to Total Trihalomethanes for a Distribution System Site of System 2 (1997 – 1999)

Figure 6 presents correlation for total HAA and THM concentrations in the distribution system. There is essentially no correlation between total HAAs and THMs (r^2 = 0.09). In contrast to observations for System 1, the correlations observed in System 2 are substantially weaker indicating that the use of total THMs as a

surrogate for total HAAs would be of limited practical value. Overall, the analysis of data from these two distinctly different systems has shown that the ability to use monitoring data such as THMs as surrogates for other DBPs has ranged from promising to almost negligible.

An additional study which looked at variations within classes of DBPs as opposed to between classes analysed correlations between total THMs and individual THMs in several water zones throughout a system. Results indicated that there was a strong correlation between chloroform (TCM) and total THMs (r^2 = 0.98), a moderate correlation between total THM and bromodichloromethane (BDCM) (r^2 = 0.62), and a poor correlation between total THM and dibromochloromethane (DBCM) (r^2 = -0.09). It was also found that between zone variation was larger than within zone variation (Keegan et al. 2001).

CONCLUSIONS

The distribution system is very dynamic and there is substantial variability in DBP formation and degradation within the distribution system and across different systems. These findings have highlighted the importance of performing a site specific analysis to understand the characteristics of the individual water supply system in order to accurately evaluate exposure to DBPs and to establish proper monitoring programs. Furthermore, the current practice of quarterly sampling at the locations with maximum residence time may not provide an accurate indication of the maximum DBP concentrations in the system.

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DISINFECTION BY-PRODUCTS AND CANCER OUTCOMES: A SUMMARY OF THE EPIDEMIOLOGICAL EVIDENCE

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EPIDEMIOLOGICAL METHODS

A range of methods are used in epidemiology to study health outcomes. These methods differ in complexity, rigour of design, cost, and in the strength of evidence they provide. Generally, investigations of possible adverse health effects from environmental exposures begin with simple low cost studies, then progress to more complex and costly designs.

Ecological studies compare rates of disease in two or more populations. The populations are defined on geographic boundaries which are inferred to correspond to differences in exposure level to the factor of interest. The incidence of disease (new cases diagnosed in a given time period) or mortality rates in each area are measured and then compared. In this type of study, the actual exposure levels of individuals are unknown, and it is not possible to measure other important factors that may affect disease (eg smoking, diet). Ecological studies are rapid and relatively inexpensive, as they often use existing data sources. However, such studies are considered to be hypothesis generating, not analytical in nature.

Case-control studies involve the identification of people with a particular disease (cases), and otherwise similar people from the same population who do not have the disease (controls). In order to assess whether particular factors may be associated with the disease, the past exposures of cases and controls are compared. If an exposure is associated with the disease, then it should occur more commonly among cases than among controls. Exposures may be determined from existing records (eg employment history), or by interview with cases or proxy respondents (eg a close relative of a deceased case). In case-control studies the accuracy of retrospective estimates of exposure is variable, and the selection of an appropriate control group may sometimes be difficult. These studies are of moderate cost and complexity, and provide a moderate strength of evidence of the association between an exposure and the disease in question. For rare diseases, case-control studies are often the only practicable study design.

In cohort studies a group of people without the disease of interest are identified and followed over a period of time until some people develop the disease. Data is collected on suspected risk factors and protective factors, and the exposures of people who developed disease are compared with those of people who did not. In this type of study, exposures may be measured retrospectively or prospectively. If the disease under study is rare, it may be necessary to observe many people over a long time, and therefore costs can be high. Cohort studies also provide a moderate strength of evidence of the association between an exposure and the disease in question.

The clinical trial design is most commonly applied to trials of medication, where the efficacy of different drugs is compared. In a clinical trial a group of people are recruited and randomly assigned to different treatments (exposures). Health outcomes are assessed in terms of the rate or severity of disease, or mortality if applicable. In order to minimise potential bias in reporting of symptoms on the part of both participants and researchers, "blinding" (concealment of the type of exposure) is often used. Clinical trials are considered to be the "gold standard" in epidemiological study designs, and provide the greatest strength of evidence of the association between an exposure and the disease in question. However they are also the most expensive and complex in design, and their applicability restricted by ethical considerations such that participants cannot be exposed to increased risks beyond everyday life. Therefore this study design is seldom applicable for assessing the effect of environmental exposures.

MEASURING THE STRENGTH OF ASSOCIATIONS

Two different measures are used in epidemiology to describe the strength of the association between an exposure and a health effect:

The Odds Ratio is used in case-control studies. It compares the occurrence of the exposure in cases and controls:

$$OR = \frac{\textbf{Odds of Exposure in Cases}}{\text{Odds of Exposure in Controls}}$$

OR = 1.0 – no association between exposure and disease
OR > 1.0 – exposure is associated with disease (ie exposure is more common in cases than in controls)
OR < 1.0 – inverse association between exposure and disease (ie exposure is less common in cases than in controls)

For cohort studies, the Relative Risk is used. It compares the rates (incidence) of disease in exposed and unexposed groups:

$$RR = \frac{\textbf{Incidence in exposed group}}{\text{Incidence in unexposed group}}$$

RR = 1.0 – no association between exposure and disease
RR > 1.0 – exposure is associated with disease (incidence in exposed group is greater than in unexposed group)
RR < 1.0 – inverse association between exposure and disease (incidence in exposed group is less than in unexposed group)

For rare diseases, the Odds Ratio and the Relative Risk are essentially similar. For common diseases, the Odds Ratio will overestimate the Relative Risk.

ASSOCIATION VS CAUSATION

The epidemiological studies described above measure associations between a particular exposure and disease, however the finding that an association is present does not mean that a cause and effect relationship exists. In order to conclude that a causative link exists, an additional body of evidence is also required. The criteria for causation (Bradford-Hill 1965) may be summarised as follows:

- *consistency of association* - has the same association been found in other studies?
- *strength* - a strong association provides greater evidence of possible causation that a weak association.
- *specificity* - an association that is specific to a single exposure, particularly when there is no association with various other exposures, provides better evidence of causation than a situation where the disease is apparently associated with many factors.
- *appropriate time relationships* - some diseases require a period of time to develop and become apparent after exposure to a causative agent. An association is more suggestive if the strongest effect is seen for exposures focussed on the appropriate time interval.
- *coherence with other information* - a finding from an epidemiological study is more convincing if it is supported by other evidence such as animal toxicity /carcinogenicity studies. Such studies should suggest a plausible biological mechanism whereby the exposure exerts its effect.
- *dose response* - differing strengths of association may be evident for different lengths/intensities of exposure (assuming that studies are large enough to allow this type of statistical analysis). Generally speaking, the risks for a disease should increase as the level or duration of exposure increases.

Evidence on every aspect listed above may not be available for a particular exposure-disease combination, however a substantial and generally consistent body of evidence is needed before a causative relationship can be considered established.

DIFFICULTIES IN EPIDEMIOLOGICAL STUDIES OF CANCER

The study of risk factors for cancer has particular difficulties. Firstly, most cancers have a long latency period; for example cancers of the blood and lymphoid system (leukemia and lymphoma) tend to develop two to five years after exposure to harmful agents, while solid tissue tumours (cancers of the internal organs) may take from 10 to 40 years to develop after exposure. Assessing exposure to suspected risk factors and lifestyle factors such as smoking over a period of several decades before cancer diagnosis occurred is extremely difficult. In addition, people diagnosed with cancer or another potentially fatal disease often spend time thinking about the possible causes of their illness, and as a result, may report some exposures in great detail. This introduces a bias between cases and controls, who do not have the same degree of motivation to recall past exposures.

Studies which rely on measures of cancer mortality may be inaccurate as mortality depends not only on the incidence of cancer but also on the availability of treatment and access to medical care. For types of cancer which have good survival rates, those who die of the cancer may be a subgroup who differ from the majority of cases, and their exposures and other risk factors may not be representative. Cancer incidence is considered a

more accurate measure of disease than mortality but incidence figures may sometimes be affected by changes in diagnostic techniques, classification of diseases, and access to medical services.

STUDIES OF CANCER RISKS AND DBPS

DBPs from chlorination were first described in the literature in 1974. Shortly thereafter a number of ecological studies were published comparing cancer mortality rates or cancer incidence rates in regions with different water supplies. The results were variable, with some studies reporting associations between DBP exposure and bladder, colon or rectal cancer, while others did not.

The ecological studies were followed by case-control studies examining cancer mortality.

Comparisons were made of mortality rates in areas with different water supplies including: surface and ground water sources, chlorinated and non-chlorinated water supplies, and areas served by the Mississippi river or other sources. These studies used residential address at the time of death to assign exposure to water sources. During the analysis, adjustments were made for age, sex, and urbanicity but because of the lack of information about the exposures of individual cases, no adjustments could be made for other important factors such as smoking. The results of these studies were again variable, but some supported an increased risk of some cancers. These studies are now considered weak in design because of their inability to assess individual exposures.

Improvements in design were incorporated in later case-control studies including the use of individual residential history to assess exposure to water sources, adjustment for confounders such as smoking, and use of cancer incidence instead of mortality statistics. Some studies also used THM levels recorded by water companies to assess exposure levels, rather than utilising the treatment type. Despite these improvements in methodology, the findings of the studies remained somewhat variable.

A meta-analysis assessed 10 studies and 11 cancer sites to give an overall estimate of risk (Morris et al. 1995). Statistically significant associations were observed for bladder cancer (RR = 1.21) and rectal cancer (RR = 1.38). The authors concluded that if a causal link exists between these cancers and DBP exposure, then about 9% of bladder cancers and 18% of rectal cancers in the US may be attributable to drinking chlorinated surface water. This would equate to about 4,200 cases of bladder cancer, and 6,500 cases of rectal cancer per year.

The application of the meta-analysis technique to observational studies has been criticised (Egger et al. 1998). This method of analysis was developed to combine the results of randomised controlled trials, where variation between the results of individual studies can be attributed to chance, and therefore the results can be legitimately combined to give an overall estimate of effect with higher statistical precision. For observational studies, however, the differences between the results of individual studies may represent chance, confounding or bias. Therefore it may not be valid to simply combine the studies without assessing the potential reasons for the differences. Some statisticians are of the opinion that if the results of different observational studies are strongly inconsistent, reviewers should not attempt a numerical summary of risk through meta-analysis.

A specific review of the Morris et al. 1995 meta-analysis by Poole and Greenland (1999) confirmed the existence of strong inconsistencies between the individual studies, and concluded that the derivation of overall RR values was not valid. This review also used a graphical technique known as a "funnel graph" to illustrate that individual studies with higher statistical precision clustered around the null estimate (suggesting no association between DBP exposure and cancer risks), while less precise studies gave either weakly positive or weakly negative associations. The funnel graph also showed some evidence of publication bias against negative studies.

The following tables summarise some of the more recent studies that have examined bladder, rectal or colon cancer risks and DBP exposure (adapted from Mills et al. 1998). NS = not statistically significant. N/A = not applicable.

Bladder cancer studies

First Author (Year)	Exposure Measure	OR (CI)	Association	Dose response	Duration response	Outcome measure
Cantor (1998)	THM	1.5 (0.9-2.6)	positive (NS)	yes	yes	incidence
Freedman (1997)	municipal water	1.4 (0.7-2.9)	positive (NS)	N/A	no	incidence
King (1996)	THM	1.6 (1.08-2.46)	positive	yes	yes	incidence
McGeehin (1993)		THM 1.8 (1.1-2.9)	positive	no	yes	incidence
Zierler (1988)	chlorine vs chloramine 1.4	(1.2-2.1)	positive	N/A	yes	mortality
Cantor (1987)	chlorinated surface water	1.8 (N/A)	positive	N/A	N/A	incidence
Gottlieb (1982)	surface vs groundwater	1.2 (N/A)	positive (NS)	N/A	yes	mortality
Young (1981)	chlorine dose	1.04 (0.43-2.5)	positive (NS)	N/A	N/A	mortality
Wilkins (1981)	surface vs well water	2.2 (0.71-9.39)	positive (NS)	N/A	N/A	mortality
	males	1.8 (0.80-4.75)	positive (NS)			incidence
	females	1.6 (054-6.32)	positive (NS)			incidence
Brenniman (1980)	chlorinated groundwater	0.98 (N/A)	negative (NS)	N/A	N/A	mortality
Alvanja (1978)	chlorinated water	1.69 (N/A)	positive	N/A	N/A	mortality

Rectal cancer studies

First Author (Year)	Exposure Measure	OR (CI)	Association	Dose response	Duration response	Outcome measure
King (2000)	THM	<1.0	negative (NS)	no	no	incidence
Hildesheim (1998)	THM	1.7 (1.1-2.6)	positive	yes	yes	incidence
Marrett (1995)	THM	0.99 (0.5-1.4)	negative (NS)	no	no	incidence
Zierler (1986)	chlorinated water	0.96 (0.89-1.04)	negative (NS)	N/A	N/A	mortality
Gottlieb (1982)	surface vs ground	1.79 (N/A)	positive	N/A	N/A	mortality
Wilkins (1981)	surface vs well	1.42 (0.70-3.16)	positive (NS)	N/A	N/A	mortality
Young (1981)	chlorine dose	1.39 (0.67-2.86)	positive (NS)	N/A	N/A	mortality
Brenniman (1980)	chlorinated groundwater	1.22 (N/A)	positive (NS)	N/A	N/A	mortality
Alvanja (1978)	chlorinated water	1.93 (N/A)	positive	N/A	N/A	mortality

Colon cancer studies

First Author (Year)	Exposure Measure	OR (CI)	Association	Dose response	Duration response	Outcome measure
King (2000	THM					
	males	1.53 (1.13-2.09)	positive	yes	yes	incidence
	females	<1.0	negative (NS)	no	no	incidence
Hildesheim (1998)	THM	1.13 (0.7-1.8)	positive (NS)	no	no	incidence
Marrett (1995)	THM	1.5 (1.0-2.2)	positive (NS)	yes	N/A	incidence
Young (1987)	THM	0.73 (0.44-1.21)	negative (NS)	no	no	incidence
Zierler (1986)	chlorine vs chloramine	0.89 (0.86-0.93)	negative	N/A	N/A	mortality
Cragle (1985)	chlorinated water	3.36 (2.41-4.61)	positive	N/A	yes	incidence
Gottlieb (1982)	surface vs ground	1.01 (N/A)	positive (NS)	N/A	N/A	mortality
Wilkins (1981)	surface vs well	0.89 (0.57-1.43)	negative (NS)	N/A	N/A	mortality
Brenniman (1980)	chlorinated groundwater	1.11 (N/A)	positive (NS)	N/A	N/A	mortality
Alvanja (1978)	chlorinated water	1.61 (N/A)	positive	N/A	N/A	mortality

Despite improvements in methodology which should have provided more accurate exposure assessment, the results for colon and rectal cancers remain rather inconsistent. For bladder cancer, more consistently positive associations have been seen. However there have also been some contradictory or unexpected observations. For example, some early studies suggested bladder cancer risks were higher in non-smokers than in smokers (Cantor et al. 1987), while in a more recent study this finding was reversed (Cantor et al. 1998). Gender differences in cancer risks have been observed in some studies but not others. Studies which have been able to assess dose-response relationships have also shown somewhat inconsistent results.

While the epidemiological evidence seems to suggest an association between bladder cancer risks and DBP exposure, current toxicological data do not provide support for this hypothesis. None of the DBPs characterised to date have been observed to produce bladder cancer in animal studies, and thus there is no known biological basis for increased risk for this type of cancer. For other cancers, animal studies have shown that exposure to high levels of some DBPs can cause colon or rectal cancers. However extrapolation of the cancer risk to the low levels of exposure seen with drinking water produces estimates very much lower (up to one million-fold less) than the cancer rates suggested by epidemiological studies.

The disparity between the risk estimates derived from toxicology and epidemiology is illustrated by calculations performed by the US EPA in the risk-benefit analysis of Disinfectant /DBP Rule (Odom et al. 1999). Epidemiological estimates suggest up to 9,300 excess cases of bladder cancer per year might be due to current DBP levels, while toxicological estimates suggest up to 100 excess cancers (of all types) per year may be occurring. However because of the uncertainty in both estimates, it is not possible to exclude the possibility that no cases of cancer are attributable to DBP exposure.

TRENDS IN RESEARCH

In recent years there has been growing recognition that DBP profiles in different water supplies may vary greatly and that crude measurements of exposure such as disinfectant type or total THM concentrations are not adequate for epidemiological studies. Thus there has been a move to assess exposure in terms of specific DBPs, although available data on individual DBPs are limited mainly to THMs. Brominated compounds have come under increased scrutiny as some toxicological

evidence suggests they may be of greater health concern than their non-brominated counterparts.

Efforts are now being made to reassess some past cancer studies by reclassifying exposures on the basis of individual THMs and other classes of DBPs. In many cases, analytical data are absent, and modelling based on knowledge of raw water characteristics and treatment processes is being used to estimate past exposure levels.

Attempts are being made in new studies to incorporate measures of DBP exposure via dermal absorption and inhalation routes. Such improvements in exposure assessment require individual questionnaire-based approaches, however for cancer studies doubts remain about how accurate exposure assessment can hope to be given that data on individual behaviours spanning a 30 to 40 year time period is needed. There have also been some attempts to identify potential biomarkers of pre-cancerous cellular damage (eg micronuclei in bladder epithelial cells) but without reported success to date.

WHY IS THE EVIDENCE STILL INCONSISTENT DESPITE IMPROVEMENTS IN METHODOLOGY?

Three possibilities need to be considered when assessing the continuing lack of clarity in the evidence for the association between DBP exposure and cancer risks.

1. *That the observed associations are due to bias and confounding and they are not a real effect of water supply.*

The comparisons made in non-randomised epidemiological studies assume that all important extraneous factors affecting disease outcome have been identified, measured and adequately adjusted for in the analysis, so that differences in DBP exposure levels are the most probable reason for any observed difference in health outcomes. However the possibility exists that residual confounding may still occur even when efforts have been made to adjust for factors such as urbanicity (comparison of surface water and ground water often equates to comparison of large urban centres and small towns) or socioeconomic status (poor water quality supplies often equate with lower socioeconomic status). Urban living exposes people to many factors such as air pollution which may affect cancer risks, and low socioeconomic status is linked to many adverse health outcomes.

2. *That the observed association represents a real effect of a water component other than DBPs.*

In most epidemiological studies the water supplies being compared almost certainly differ in other components as well as DBP levels. If these other unmeasured components affect cancer risks, then an effect of DBP exposure may be falsely inferred. For example, an inverse relationship has been reported between water hardness and colon and rectal cancer in ecological studies (Yang et al. 1997, 1999), although no plausible biological basis for this observation has yet been proposed. If this relationship truly exists, it may explain the apparent association between DBPs and cancer risks seen when surface water and groundwater supplies are compared, as groundwaters tend to be harder than surface waters. Alternatively, it may be feasible that an unidentified component in some surface water supplies is responsible for increased cancer risks, and that DBPs are merely "innocent bystanders" in the relationship.

3. *That the observed association represents a real effect of a DBP (or DBPs), but occurrence of this causative substance does not correlate well with the prevailing measures of DBP exposure.*

The levels of different DBPs formed by the disinfection process depends on many factors including the nature and amount of the NOM component, disinfectant type, dose, temperature, pH, and bromine levels in the water supply. Exposure assessment in epidemiological studies has been based on crude measures of water treatment type or THM levels, and water supplies classified as similar on this basis may in fact differ markedly in their DBP profiles. If the putative cancer-causing DBP or DBP class has a different occurrence pattern to THMs, then exposure classifications would be subject to considerable error, leading to inconsistent results in epidemiological studies.

CONCLUSIONS

On present evidence the existence of a causative link between DBPs in water supplies and elevated cancer risks remains unproven. However if such a link exists, it may represent a significant public health burden of avoidable disease and mortality. There is a need for further epidemiological research on this topic, however simple repetition of past methodology is unlikely to produce resolution of the issue.

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DISINFECTION BY-PRODUCTS AND REPRODUCTIVE EFFECTS

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The reproductive effects of DBP exposure are a relatively recent area of concern with publications on human epidemiological studies on this topic mostly confined to the last decade. As noted previously, DBP formation is influenced by a number of factors including water source (ground vs surface water), water treatment (disinfectant type), pH, temperature (season), chlorine levels, bromide levels, organic content (fulvic and humic acid) and residence time in the distribution system. The trihalomethanes are usually the most abundant class of DBP found in drinking water supplies, followed by the haloacids. Other DBP classes including aldehydes, haloacetonitriles and haloketones occur at lower concentrations. Regulatory monitoring for DBPs is generally based on measurement of THMs in a few samples (typically four) from each water quality zone each year.

People may be exposed to DBPs through a range of water use activities including drinking tap water, showering/bathing, boiling water, dish washing and swimming. Differences in the patterns and duration of these activities mean that a large degree of variability in exposure is possible for one individual over time and also between individuals. There are three routes of uptake: ingestion, inhalation and skin absorption. All three routes are significant for THM uptake, however significant HAA uptake occurs only by ingestion.

Biomonitoring has been used in some studies to assess DBP uptake. THMs may be measured in exhaled breath or in serum, however their biological half-life is short (approximately 30 minutes). HAAs, specifically TCAA and DCAA, may be measured in urine. DCAA has a short biological half-life (< 2 hours), while TCCA has a longer half-life (70-120 hours).

Epidemiological studies have used several methods to characterise differences in DBP exposure. Some studies have compared water supplies with different sources or different treatment methods, while others have used routinely collected THM data (based on water zone mean concentrations) to estimate exposure levels. A few studies have used water colour (an indirect measure of humic and fulvic acid content) as a surrogate for DBP levels. Personal exposure measures (mainly for water ingestion) have sometimes been combined with routinely collected THM data in an effort to provide more accurate estimates of individual exposure. Only one study to date has assessed exposure to DBPs other than THMs.

A wide range of reproductive outcomes have been examined including congenital malformations (neural tube defects, cardiac defects, respiratory cleft, and urinary tract defects), spontaneous abortions, still birth, pre-term delivery (<37 weeks) and low birth weight (<2,500 g). Study designs have included case-control studies and retrospective cohort studies, and there has been one prospective cohort study. Some studies have included assessment of potential confounding factors which may affect reproductive outcomes including maternal age, smoking and socioeconomic status. Table 1 summarises the published studies on reproductive outcomes of DBPs.

Table 1 Studies on reproductive outcomes and DBPs

First Author, Year	Design	Size	Country	Outcomes examined
Source/treatment				
Aschengrau 89	C-C	1677	Massachusetts, US	Spontaneous abortion
Aschengrau 93	C-C	2348	Massachusetts, US	Congenital malformations
Kanitz 96	RC	676	Italy	Pre-term delivery, birth weight
Magnus 99	RC	141077	Norway	Congenital malformations
Jaakkola 01	RC	137145	Norway	Pre-term delivery, birth weight
Kallen 00	RC	114484	Sweden	Birth weight characteristics, congenital malformations
Yang 00	RC	18025	Taiwan	Pre-term delivery, birth weight
Routinely collected trihalomethanes in water zones				
Kramer 92	C-C	4028	Iowa, US	Pre-term delivery, birth weight
Bove 95	RC	81602	New Jersey, US	Pre-term delivery, birth weight, congenital malformations
Gallagher 98	RC	1244	Colorado, US	Pre-term delivery, birth weight
Dodds 99	RC	49842	Canada	Birth weight, stillbirth, congenital malformations
King 00	RC	49756	Canada	Still birth
Dodds 01	RC	49842	Canada	Congenital malformations
Routinely collected trihalomethanes in water zones and personal characteristics				
Savitz 95	C-C	1003	North Carolina, US	Pre-term delivery, birth weight, spontaneous abortion
Waller 98	PC	5144	Californa, US	Spontaneous abortion
Klotz 99	C-C	360	New Jersey, US	Neural tube defects

C-C = case-control, PC = prospective cohort, RC = retrospective cohort

The findings of studies in relation to individual reproductive outcomes and DBP exposure are summarised in Tables 2 to 12.

Table 2 Neural tube defects

Study	Exposure	Risk estimate
Magnus 99	Chlorin. vs non chlorin.	1.26 (0.61-2.62)
Kallen 2000	Chlorin. vs non chlorin.	1.0
Bove 95	<=20 vs >80 µg/l	2.96 (1.26-6.62)*
Dodds 99	0-49 vs > 100 µg/l TTHM	1.18 (0.67-2.10)
#Klotz 99	< 5 vs 40+ µg/l TTHM	2.1 (2.1-4.0)
	<0.5 vs 3.0+ haloacetonitriles	1.3 (0.6-2.5)
	<3 vs 35+ haloacetates	1.2 (0.5-2.6)
Dodds 01	<5 vs => 20 µg/l BDCM	2.5 (1.2-5.1)
		*90% CI

#Inclusion of ingestion, showering, bathing and swimming made little difference.
#Vitamin intake did: 0.5 (0.1-1.7) vs 2.6 (1.2-6.0) (High exp.)

Table 3 Major cardiac defects

Study	Exposure	Risk estimate
Magnus 99	Chlorin. vs non chlorin.	1.05 (0.76-1.46)
Kallen 2000	Chlorin. vs non chlorin.	1.1 (0.9-1.3)
Bove 95	<= 20 vs >80 µg/l	1.83 (0.97-3.29)*
Dodds 99	0-49 vs > 100 µg/l TTHM	0.77 (0.57-1.04)
Dodds 01	< 5 vs => 20 µg/l BDCM	0.3 (0.2-0.7)
		*90% CI

Table 4 Respiratory defects

Study	Exposure	Risk estimate
Achengrau 93	Chlorin. vs chloramination	3.2 (1.1-9.5)
Magnus 99	Chlorin. vs non chlorin.	1.07 (0.52-2.19)

Table 5 Oral cleft or cleft palate defects

Study	Exposure	Risk estimate
Magnus 99	Chlorin. vs non chlorin.	0.94 (0.64-1.42)
Kallen 2000	Chlorin. vs non chlorin.	1.1 (0.8-1.6)
Bove 95	<= 20 vs >100 µg/l	3.17 (1.18-7.26)*
Dodds 99	0-49 vs > 100 µg/l TTHM	1.01 (0.55-1.86)
Dodds 01	< 5 vs => 20 µg/l BDCM	0.6 (0.2-1.9)
		*90% CI

Table 6 Urinary tract defects

Study	Exposure	Risk estimate
Achengrau 93	Chlorin. vs chloramination	4.1 (1.2-14.1)
Magnus 99	Chlorin. vs non chlorin.	1.99 (1.10-3.57)

Table 7 All congenital malformations

Study	Exposure	Risk estimate
Achengrau 93	Chlorin. vs non chlorin.	1.0
	Chlorin. vs chloramination	1.5 (0.7-2.1)
Magnus 99	Chlorin. vs non chlorin.	1.14 (0.99-1.31)
Bove 95	<=20 vs >80 µg/l	1.57 (1.23-1.99)*
*90%		

Table 8 Spontaneous abortion

Study	Exposure	Risk estimate
Achengrau 89	Chloraminated vs chlorin.	0.9 (0.6-1.4)
	Surface vs ground water	2.2 (1.3-3.6)
Savitz 95	40.8-59.9 vs 81.1-168.8	1.2 (0.6-2.4)
	per 50 change	1.7 (1.1-2.7)
	including ingestion	0.9 (0.6-1.3)
Waller 98	=> 75 TTHM + =>5 glasses	1.8 (1.1-3.0)
	=> 18 BDCM + =>5 glasses	3.0 (1.4-6.6)

Table 9 Stillbirth

Study	Exposure	Risk estimate
by water source or treatment		
Achengrau 93	Surface vs ground/mix	1.0
	Chlorinated vs chloraminated	2.6 (0.9-7.5)
Kallen 00	Chlorinated vs non-chlor.	0.8 (0.63-1.01)
by routinely collected THMs (µg/l)		
Bove 95		no association
Dodds 99	0-49 vs >100 TTHM	1.66 (1.09-2.52)
King 00	0-49 vs >100 TTHM	4.57 (1.93-10.77) for asphyxia related
	<5 vs =>20 BDCM	1.98 (1.23-3.49)

Table 10 Pre-term delivery

Study	Exposure	Risk estimate
by water source or treatment		
Kanitz 96	Chlorinated vs non-chlor.	1.1 (0.3-3.7)
Kallen 00	Chlorinated vs non-chlor.	1.09 (1.01-1.17)
Yang 00	Chlorinated vs non-chlor.	1.34 (1.15-1.56)
Jaakkola 01	Chlorinated vs non-chlor.	0.91 (0.84-0.99)*
		*high colour
by routinely collected TTHMs (µg/l)		
Kramer 92	0 vs =>10 (CH_3CL)	1.1 (0.7-1.6)
Bove 95		no association
Savitz 95	40.8-63.3 vs 82.8-168.8	0.9 (0.6-1.5)
	including ingestion	0.9 (0.6-1.3)
Gallagher 98	<=20 vs =>61	1.0 (0.3-2.8)
Dodds 99	0-49 vs >100	0.97 (0.87-1.09)

Table 11 Low birth weight

Study	Exposure	Risk estimate
by water source or treatment		
Kanitz 96	Chlorinated vs non-chlor.	6.0 (0.6-12.6)
Kallen 00	Chlorinated vs non-chlor.	1.15 (1.05-1.26)
Yang 00	Chlorinated vs non-chlor.	0.90 (0.75-1.09)
Jaakkola 01	Chlorinated vs non-chlor.	0.97 (0.89-1.06)*
		*high colour
by routinely collected TTHMs (µg/l)		
Kramer 92	0 vs =>10 (CH_3CL)	1.3 (0.8-2.2)
Bove 95	<=20 vs >100	1.42 (1.22-1.65)*
Savitz 95	40.8-59.9 vs 82.8-168.8	1.3 (0.8-2.1)
Gallagher 98	<=20 vs =>61	2.1 (1.0-4.8)
		5.9 (2.0-17.0)**
Dodds 99	0-49 vs >100	1.04 (0.92-1.18)
by routinely collected TTHMs (µg/l) and ingestion estimate		
(µg/l x number of glasses/day)		
Savitz 95	44.0-169.9 vs 330.9-1171.0	0.8 (0.5-1.3)

*50% confidence interval **term births

Table 12 Small for gestational age/intrauterine growth retardation

Study	Exposure	Risk estimate
Kallen 00	source	1.07 (0.96-1.19)*
Jaakkola 01	source	1.00 (0.91-1.10)**
Kramer 92	0 vs =>10 (CH_3CL)	1.8 (1.1-2.9)*
Bove 95	<=20 vs >100	1.5 (1.2-1.9)*
Dodds 99	0-49 vs >100	1.08 (0.99-1.18)***

***<-2SD, **<=10%ile *<5%ile

Comparing the studies done for each type of outcome, it is evident that there has been little consistency in methodology, with different cutoff levels being used to classify DBP exposures, and the "high" levels of exposure in some studies overlapping with "low" levels in others. The majority of studies have examined chlorinated or chloraminated water supplies, with only a few conducted on supplies disinfected with chlorine dioxide. Risk estimates for studies examining the same outcome have also been inconsistent with weakly positive or weakly negative effects reported by most studies, and the majority not reaching statistical significance.

Research on DBPs and reproductive outcomes is continuing with at least eight major projects in progress in five countries. Three studies are presently underway in the US: one will will examine associations between DBP exposure and factors affecting fertility (semen quality, menstrual cycle; Waller and Swann) while another will focus on spontaneous abortion (Savitz et al.), and the third on birth weight (Wright et al.). Two studies are also continuing in Canada: the first will examine congenital malformations, stillbirth and growth/maturity outcomes (Dodds and King), the second is a case-control study on stillbirth in relation to THM and HAA exposure (Dodds). Researchers in Norway are studying congenital malformations (Jaakkola et al.), while in Italy there is a cohort study on birth weight (Aggazotti et al.). In the UK, a large multistage study on birth weight, stillbirth and congenital malformations is being undertaken (Toledano et al.)

The UK study covers three large water supply regions served by different companies, and data on some confounders is being collected (maternal age, gender, socioeconomic status). The exposure assessment is based on annual estimates of total THMs, with three exposure categories: < 30, 30 to 60 and > 60 µg /litre. Preliminary results have suggested a possible increased risk for low birth weight in the lowest socioeconomic category for one water supply region but not for the other other two regions.

Overall, most of the studies performed to date have had a number of significant limitations in methodology and data quality. There have been only a small number of high quality studies, and these have produced apparently inconsistent results. The small sample size and small number of cases in many studies has led to reduced statistical power, as has categorisation of outcomes which often leaves very small numbers of cases in each group. Many factors are known to influence reproductive outcomes including maternal age, history of past pregnancies, smoking, exercise, nutrition, etc. Most studies of DBPs have assessed only a limited number of such confounders, particularly those studies using routinely collected data from perinatal registries.

Case ascertainment for reproductive outcomes is not always straightforward: for example spontaneous abortion rates can not usually be measured from routinely collected health data, and failure to account for elective pregnancy terminations will affect measured rates of congenital malformations. Grouping of congenital malformations into only a few major categories (for example combining all heart defects into a single category) is inappropriate as these conditions are generally heterogeneous with respect to both phenotype and presumed causes. Regional differences may occur in the ascertainment of reproductive outcomes because of differences in data collection methods, producing spurious differences in recorded occurrence rates.

Exposure assessment in reproductive studies has been limited mainly to THMs, with only one study including estimates of exposure to other DBPs (Klotz and Pyrch, 1999). Generally speaking, exposure assessment has not taken into account the considerable degree of temporal and spatial variability in THMs within water zones. Routine DBP monitoring provides only a limited number of measurements, although in some cases modelling has been used in an attempt to improve exposure estimates. Given that particular developmental defects may arise in short critical time windows (eg neural tube defects during first 12 weeks of pregnancy), it is important that exposure estimates be matched to the relevant time period for the outcome under study.

Most studies have also not considered the possibility of residential mobility during pregnancy, nor exposure to water sources outside the home. Lack of individual data has also meant that use of private water supplies, bottled water or boiling of water before drinking have not been included in exposure assessment. Thus the exposure level assigned to each pregnancy may have substantial inaccuracy. Comparisons between the results of studies is also complicated by the varying levels of exposure that have been examined in different countries. For example the studies done in Norway, Sweden and Italy have examined low levels of DBPs, while those in Canada have examined mostly high levels.

Routinely collected THM measurements for water supplies currently form the basis of exposure assessment, but are likely to be of limited accuracy. Levels of chloroform are often highly correlated with total THMs, but total THMs often do not correlate well with other classes of DBPs in the distribution system. The relationships between classes of DBPs need to be established empirically for each system under study.

Simple assessment of DBP concentrations in water does not account for the various exposure pathways and the three possible exposure routes. The relative importance of each route will vary for different DBPs. Studies with chloroform have shown that concentrations in serum increase two to four-fold after showering,

Figure A Schematic overview of the simulation of chloroform uptake

Chloroform concentration	Chloroform levels in tap water (microgram/l)	Chloroform levels in tap water (microgram/l)	Chloroform levels in tap water (microgram/l)	Chloroform levels in pool water (microgram/l)
	x	x	x	x
Activity	Ingested amount Level of tap water (L)	Frequency and duration of showering (mins?)	Frequency and duration of bathing (mins?)	Frequency and duration of swimming (mins?)
	x	x	x	x
Rate of uptake	Uptake of chloroform (microgram) per microgram/l tap water for ingestion	Uptake of chloroform (microgram/min) per microgram/l tap water for showering	Uptake of chloroform (microgram/min) per microgram/l tap water for bathing	Uptake of chloroform (microgram/min) per microgram/l pool water for swimming
	x	x	x	x
Uptake	Chloroform uptake through ingestion (microgram)	Chloroform uptake through showering (microgram)	Chloroform uptake through bathing (microgram)	Chloroform uptake through swimming (microgram) Total uptake (microgram)

illustrating the importance of inhalation and dermal routes of exposure for this compound. In contrast, only the ingestion route appears to be important for the haloacetic acids.

In order to assess the degree of correlation between chloroform levels in water and total uptake of chloroform, UK researchers have carried out a simulation experiment modelling exposure and uptake in a population of 300,000 women (Nieuwenhuijsen et al. unpublished data). The components of the model are outlined in Figure A.

This simulation exercise has demonstrated the relative importance of different water use behaviours for chloroform exposure, and the influence of different behaviours on the accuracy of exposure assessments based solely on water chloroform levels. In turn this will help to identify areas where efforts at improving exposure assessment would be most productively targeted.

In summary, current epidemiological evidence on reproductive outcomes and DBP exposure is generally inconsistent and inconclusive. Most of the studies performed to date have had significant limitations. There is a need for well designed analytical studies, with good case ascertainment, inclusion of relevant confounders, sufficient statistical power and in-depth exposure assessment. As a prerequisite for such studies, methods of exposure assessment need to be substantially improved. Efforts in this area need to address the spatial and temporal variability of DBPs in the distribution system, and the validity of using selected DBPs as surrogate measures of exposure to others. To determine individual exposures, better instruments are needed to assess water consumption and other water uses, and to identify the main determinants of exposure and uptake.

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Further reading

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ARE THERE SIGNIFICANT HEALTH EFFECTS ASSOCIATED WITH THE USE OF CHEMICAL DISINFECTION OF DRINKING WATER?

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INTRODUCTION

The central issue in water disinfection is how to effectively control waterborne infectious disease and minimize risks from DBPs. It is acknowledged that control of microbial risks in water supply systems must have the highest priority, and that chemical disinfection is the least costly way to address such risks. There are a range of effective chemical disinfection options, each of which has benefits, but these processes produce many of the same DBPs and some may also produce unique DBPs. The quality of water being treated has major impacts on the type of DBPs formed, and problems with DBPs need to be put into the perspective of individual water systems.

In terms of possible health risks from DBPs, cancer has the longest history of study and therefore the largest accumulated body of evidence. Research has focused mainly on bladder and colorectal cancers. The epidemiological data are fairly consistent with an increased risk for bladder cancer associated with exposure to chlorinated water supplies, but the biological plausibility is weak as animal studies have not demonstrated induction of bladder cancers by any of the tested DBPs. Instead, animal carcinogenicity studies suggest liver or kidney cancer are more plausible risks from DBPs. However none of the DBPs characterised to date is a sufficiently potent carcinogen to explain the apparent risk levels seen in epidemiological studies.

The study of reproductive and developmental effects has been a relatively recent area of research, and the epidemiological data here are limited and inconsistent. Animal studies suggest that of the known DBPs those in the haloacetate class, perhaps the haloacetonitriles, are the most plausible agents for such effects. The haloacetate class is particularly interesting because a wide variety of halogenated organic acids and aldehydes are formed in chlorination of humic or fulvic acids. The aldehydes are metabolized to the corresponding acids, so the haloacetate class may be the tip of the iceberg of chemicals with similar toxicological properties.

THE NEED FOR A NEW APPROACH

In considering future directions for DBP research, elevation of cancer risks has the greatest weight of evidence and the largest potential public health burden if a causal relationship with DBP exposure truly exists. However toxicological evidence indicates that the most prominent chlorinated by-products (chloroform, trichloroacetate, dichloroacetate) are not likely to be causal for these endpoints.

While the major research focus to date has been on screening and identification of DBPs and potential risks, efficient resolution of the cancer issue requires a hypothesis-driven approach within a qualitative and quantitative framework. A similar approach should be taken with other endpoints (eg reproductive effects) if they reach a similar threshold of concern.

This new strategy needs to recognise that:

- this is not a single compound problem - DBPs are complex mixtures.
- the direct approach of epidemiology is unlikely to identify "the" responsible DBP, however it is also critical to finding "the" responsible DBP. There is a need to develop epidemiological exposure measures that distinguish between mixtures of DBPs.
- toxicology can identify responsible entities and key events leading to disease. The use of animal models (eg gene knockouts) of appropriate sensitivity that reflect sensitivities in humans may be a critical approach.

The almost exclusive focus of DBP research and regulation on halogenated products to the neglect of non-halogenated compounds can also no longer be justified. The limitations of this approach have been demonstrated by the recent finding that levels of the potent non-halogenated carcinogen N-nitrosodimethylamine (NDMA) may be higher in water supplies treated by chloramination in comparison to those treated by chlorination. It is possible that other carcinogenic nitrosamine compounds may be formed if the source water contains secondary amines (if it receives significant waste water discharges). Switching

from chlorination to chloramination is one strategy commonly adopted by water authorities to achieve lower levels of the regulated DBP classes (THMs and HAAs). It is open to speculation whether this change may have the effect of increasing cancer risks in some water supplies by formation of nitrosamines rather than decreasing risks as is the intent of regulation.

The figure below illustrates the traditional approach to understanding health risks. In this model consideration of variations in individual sensitivity is a minor and often overlooked aspect of the problem (represented by dashed arrows). However increasing understanding of human genetic variation is likely to reveal the importance of individual sensitivity in disease development, and make this a central aspect of risk assessment rather than an afterthought. Genetic polymorphisms in key enzymes involved in DBP metabolism could feasibly lead to large differences in susceptibility to adverse effects.

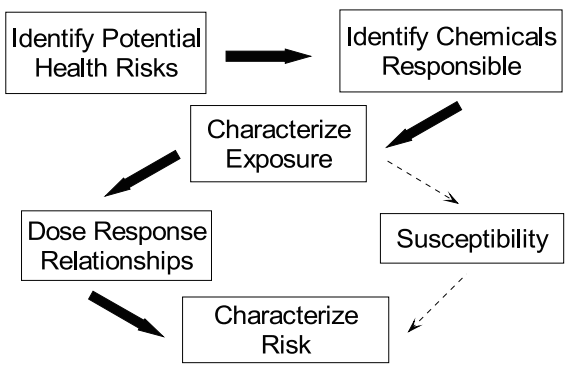

Figure 1 Path to understanding risks

A MODEL FOR CHARACTERISING THE NATURE OF HEALTH RISKS

While different disinfectants may tend to produce characteristic DBP profiles, the nature of the source water is also critical in determining which DBPs are produced. Therefore it is not possible to simply choose the "safest" disinfectant and apply it to all water sources and circumstances. To address health risks the focus must be on water conditions that influence DBP formation both qualitatively and quantitatively.

Differences in the types of DBPs present in a mixture will determine which health effects are likely to be important, while the amounts of DBPs will determine the level of risk. The qualitative difference in risk is what can be meaningfully addressed now, while quantitative risk assessment will require an understanding of individual sensitivity and distributions in the population.

The major determinants of DBP formation are the disinfectant used, bromide concentration, pH, ammonia, and total organic carbon. Consideration of how these factors influence DBP formation, and the toxicological evidence on known classes of DBPs allows the construction of a model that relates water quality parameters and DBP profiles to potential health risks (see Figure 2). For example:

- TOX (total organic halogens) reflects Br and Cl substitution of known DBPs
- Low pH favors halogenation reactions
- Low pH favors formation of potent mutagens
- High pH can favor formation of oxidized inorganics, e.g. chlorate and bromate
- Increasing pH favors THMs, decreases other X_3-C-
- Chemistry favors formation of one nitrosamine, precursors for others may also be present
- TOC quantity increases DBPs in general therefore it is not a discriminator of risks
- TOC quality determines formation of different DBPs

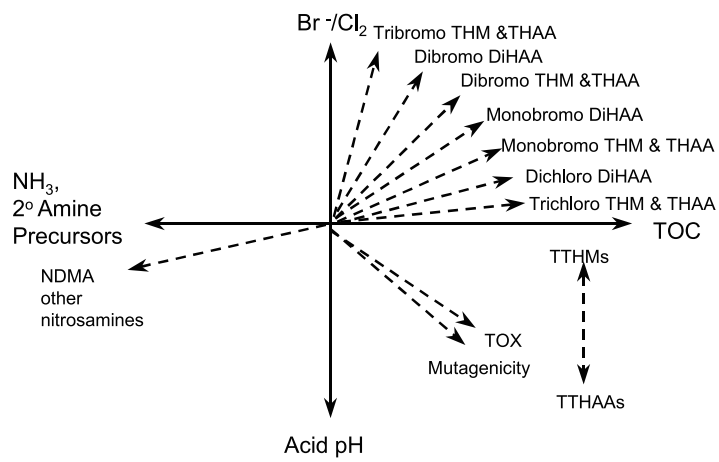

Figure 2 Variables that affect formation of chlorination by-products

In turn, this model can be used to focus epidemiological investigations on groups of candidate DBPs relevant to different health outcomes (see Figure 3). For example, some brominated DBPs induce colon cancer in animals. While none of the compounds evaluated to date are sufficiently potent to account for risk levels suggested by epidemiological studies, it is plausible that other as yet unidentified compounds of this type could be the causative agent. Thus it would be rational for human studies of colon or rectal cancer to assess exposure on the basis of brominated compounds.

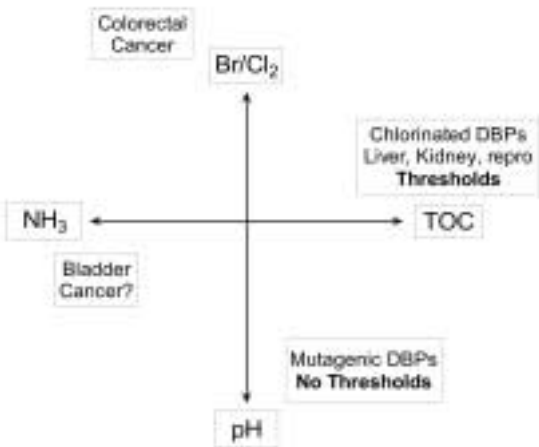

Figure 3 Potential health outcomes associated with DBP groups

Given that only a small number of individual DBPs can actually be measured in water supplies, exposure assessment under this model would need to incorporate generic measures reflecting DBP formation potential as well as specific assays. A number of suggested parameters for characterising water quality and DBP profiles are shown in Figure 4.

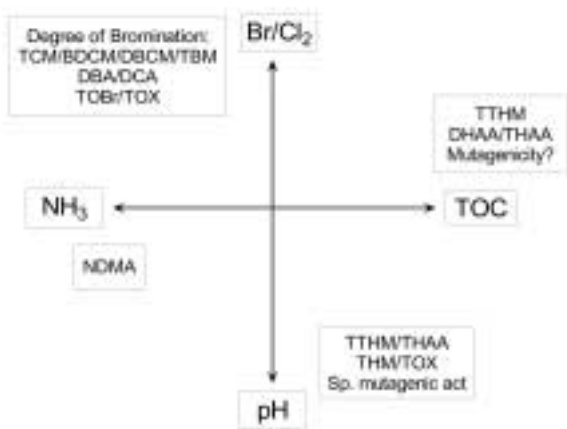

Figure 4 Suggested parameters for characterising water supplies

This model is drawn from a synthesis of current knowledge and provides a logical basis for characterising the probable nature of health risks in different water supplies. It offers a framework for exposure assessment in epidemiological studies that is likely to be more useful than the past practice of assessing exposure by simplistic measures of DBPs which are of little toxicological relevance to the outcomes under study.

TOXICOLOGY'S CONTRIBUTION TO RESOLVING HEALTH ISSUES

In addition to contributing to the understanding of the nature of risks from DBPs, toxicology can contribute to resolving health issues via:

- characterization of exposure - in particular the use of pharmacokinetics to characterise exposure at the target organ
- provision of evidence on health endpoints identified by epidemiology, such as defining the probable causes or key events leading to cancer, or providing evidence of mechanisms of action from animal studies
- exploring the possible role of genetic susceptibility

An understanding of the mode of action of a given chemical is fundamental for translating the risks seen in high dose animal studies to the real world situation where exposure levels are much lower. As previously discussed by Professor Hrudey, the default assumption of no threshold for cancer risk resulted in setting of an MCLG of zero for chloroform. However, knowledge of the mode of action for carcinogenesis by this chemical leads to the conclusion that levels encountered in drinking water are not a health risk. Similarly, consideration of their modes of action indicates that several other DBPs do not pose plausible health risks at the levels found in drinking water.

Describing the dose-response relationships for DBPs and health effects requires consideration of the following aspects in humans:

- Dosimetry - the metabolism and pharmacokinetics of the compound
- Mechanisms and modes of action - risks predicted from default models may be significantly modified by this knowledge
- Susceptibility - genetic variation may have a major influence on health risks (perhaps as much as 1000-fold), while age and gender probably have a smaller influence (perhaps only two to three fold)
- Lifestyle factors

Biomarkers can play an important role in understanding and confirming health effects, although their identification and validation may be difficult. Three types of biomarkers are recognised:

- Biomarkers of exposure - the best measures are obtained as close to the site of action as possible.

- Biomarkers of effect - this requires identification of key event(s) in disease causation.
- Biomarkers of susceptibility - these were most frequently identified empirically in the past. Now such markers can be developed based upon knowledge of the mechanism of action and factors that will enhance effects.

NDMA AS AN EXAMPLE

An example of the complexity of interactions and the possible mechanisms of disease causation can be seen by considering N-nitrosodimethylamine (NDMA). This compound is a semivolatile organic chemical with a variety of industrial uses. NDMA is also found in some foods and alcoholic beverages, and in tobacco smoke. The compound may also be generated in the bladder in association with certain bacterial infections when nitrate is present. NDMA is known to cause liver, kidney and lung cancers in animals, and is classified as a probable human carcinogen by the US EPA. While the EPA does not yet regulate NDMA levels in drinking water, it has been estimated that concentrations in the order of 0.7 to 2 nanograms/litre equate to a lifetime cancer risk of 1 in 10^6. Cured and smoked foods have been reported to contain up to 32,000 nanograms/ kg and the average concentration in US beer is 8,000 nanograms/litre, with a reported maximum of 70,000 nanograms/litre.

Recent concerns over NDMA in drinking water supplies were initially raised by its detection as a groundwater contaminant in an aerospace facility in California in 1998 (NDMA was an impurity in some rocket fuels produced from the 1950s to the 1970s). Subsequently it was found that NDMA could be formed at levels in excess of 100 nanograms/litre during chloramination of tertiary filtered waste water. In California, such waste waters are used (after reverse osmosis filtration) to recharge groundwaters, leading to concerns over the potential long term effects. NDMA can also be formed by chloramination of some drinking waters, but is not generated when the same waters are chlorinated (Najm and Trussell 2001).Living organisms have a number of repair and defence mechanisms which can combat genetic and cellular damage. Consideration of the effects of NDMA illustrates a number of ways in which exposure to a toxic compound may eventually result in cancer. The metabolism of NDMA is summarised in Figure 5. NDMA is not itself carcinogenic but is converted by cytochrome CYP2e1 to a toxic metabolite which forms DNA adducts, in particular O6-methylguanine. The presence of this modified form of guanine may then lead to genetic mutation.

Once damage to the DNA has occurred, the cell may be able to successfully repair the damage, restoring the original DNA sequence and returning the cell to a normal state. Alternatively, the damaged cell may be recognised as abnormal by surrounding cells and sent a series of chemical signals causing it to enter the state of apoptosis.

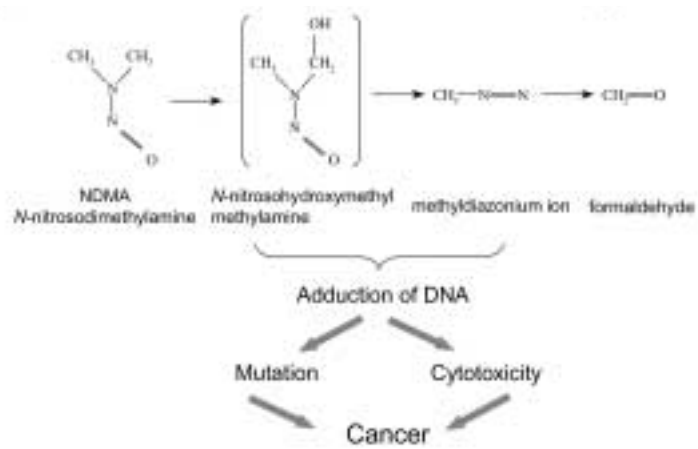

Figure 5 Metabolism of NDMA and its toxicological effects

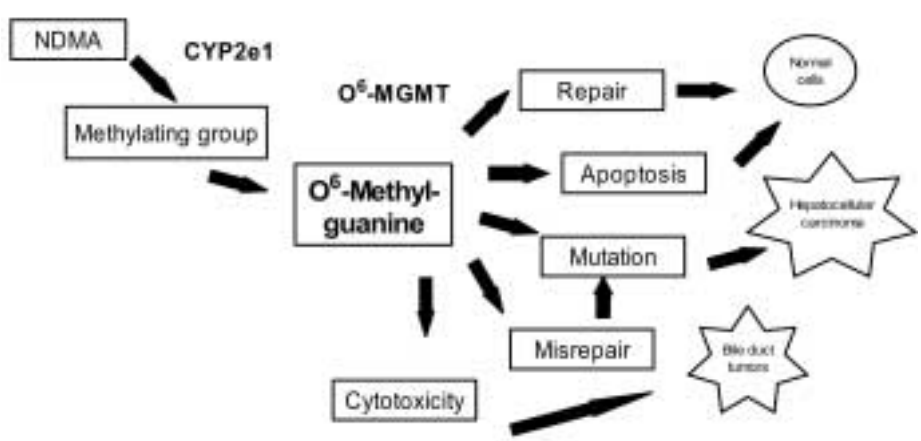

Figure 6 Mechanisms in NDMA-induced Cancer

Apoptosis (also known as programmed cell death) results in the cell essentially "committing suicide", leaving the unaffected healthy cells alive to grow and function normally. In this instance, the normal state of the relevant organ or tissue is also restored.

The process of DNA repair is not always successful, and the induced mutation may persist and be passed on to daughter cells, perhaps eventually leading to cancer. The toxic chemical may not act directly on the DNA, but may cause damage to the cell (cytotoxicity). Chronic cytotoxicity can cause an abnormal growth response (proliferative response) by the organ or tissue to repair the damage, increasing the risk that mutation will occur during DNA replication. Again this may eventually lead to cancer.

The process through which a normal cell gives rise to a malignant cancer involves multiple steps. The early steps in this pathway are now known to occur quite frequently in healthy organisms, but progress to cancer is rare because of the defence mechanisms that are in place to suppress or destroy abnormal cells. There is increasing recognition that toxins and carcinogens may act not only by directly causing genetic or cellular damage, but by interfering with normal defence and repair mechanisms thus increasing the chance that abnormal cells will escape control.

DEVELOPMENTAL AND REPRODUCTIVE EFFECTS

Some DBPs (eg chlorate, bromate, HAAs) have endocrine effects and therefore may have the potential to affect development in utero or early childhood. Some are capable of tumour promotion, and therefore may also have the potential to affect development. Animal studies show that HAAs can affect male reproduction, and some epidemiological studies have suggested a link with spontaneous abortion. Overall the epidemiological evidence on reproductive effects and DBPs is less extensive and more inconsistent than the evidence for bladder cancer.

THE ROLE OF GENETICS

Genetic variation between individuals undoubtedly affects cancer risks, although the specific basis for differences may not be well understood. For a compound such as NDMA which requires metabolism to convert it to an active form, a person lacking the required enzyme or possessing a variant enzyme with low activity may be less susceptible to cancer if exposed to this compound. Conversely where an evironmental contaminant itself is toxic but its metabolites are less so, a person who metabolises the compound slowly would be predicted to be at higher risk.

When considering the possible role of genetic susceptibility to adverse effects of DBPs, some candidate genes with involvement in DBP metabolism are already known. For example, a family of enzymes called glutathione S-transferases play an important role in the detoxification of potentially harmful compounds. These enzymes appear to be heavily involved in metabolism of DBPs, and are also highly polymorphic in humans. Cytochromes of the P450 family are known to be involved in the metabolism of THMs and NDMA. The glutathione S-transferases metabolize the THMs to reactive intermediates, but appear to very rapidly detoxify the HAAs, HANs and MX.

Considering more broadly the genetic risk factors for chronic disease, it is estimated that at least 40% of human cancers involve genetic factors, and 80-90% of this fraction arises from multi-gene interactions. A number of common chronic diseases involve a genetic predisposition but are also partially dependent on environmental factors. Some of these illnesses are also associated with increased cancer risks, diabetes is associated with a high risk for liver cancer. If some of these relatively common genetic variations involve a significantly decreased ability to detoxify DBPs or repair cellular damage, this may put a subgroup of the population at higher risk. To date however, no specific relationship between cancer risks from DBPs and particular genetic variants has been identified.

CONCLUSIONS

The major question to be resolved regarding the health effects of DBPs is whether the bladder cancer risk suggested by epidemiological studies is real. While none of the DBPs tested to date in animal studies can induce bladder cancer, the recent discovery of NDMA formation in drinking water has raised the possibility that other nitrosamines may also be formed. This would provide an element of biologically plausibility as some nitrosamines are documented bladder carcinogens. Establishing the extent to which NDMA and other nitrosamines occur in drinking water supplies, and the factors affecting their formation should be a priority for investigation in this area.

Any future research program on DBPs should build more effectively on what we already know. This will require a more creative use of epidemiology, particularly with respect to the focus of exposure assessment. Relevant water quality and DBP variables need to be reparameterized to better characterize differences between systems. The model presented here provides a framework which integrates current knowledge.

Better focus in the use of toxicological tools is also required. More full characterization of the modes of action of DBPs is needed, and issues of susceptibility

should be more broadly addressed. Careful, hypothesis-driven studies of mixtures and interactions on meaningful endpoints are also required.

The past decade has seen too little effort invested on the fundamental chemistry of disinfectants. In particular there has been unwarranted focus on halogenated by-products to the almost total exclusion of the spectrum of non-halogenated products. The potential importance of nitrosamine formation and the influence of organic nitrogen precursors on this process must be explored. Until we have accounted for the bladder cancer question, we are not finished. Other endpoints such as developmental and reproductive effects appear to be of lower priority as they are presently supported by a less extensive body of evidence, however studies in this area should also be guided by a more innovative approach building on existing knowledge.

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ANALYSIS OF DISINFECTION BY-PRODUCTS

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ANALYSIS TECHNIQUES

The techniques available for DBP analysis have changed over time, with most current methods based on Gas Chromatography (GC) and /or Mass Spectroscopy (MS). The evolution of Gas Chromatography-Mass Spectroscopy (GC-MS) methodology has been built around the analysis requirements of clinical and environmental scientists. Significant developments in MS and high-resolution MS were driven by concerns over the environmental effects of the organochlorine /POP (persistent organic pesticides) /PBT (persistent bioaccumulative and toxic) classes of compounds including:

- the discovery of DDE causing egg shell thinning (1970's)
- detection of dioxins in milk and paper products (1980's)
- PCBs and pesticides in the Great Lakes, USA (1970's, 1980's)

As MS improved in sensitivity and resolution, more and more environmental samples produced positive test results for contaminant chemicals. Evaluation of potential adverse environmental or health effects through toxicology and risk assessment led to regulation and legislation for some chemicals, and in turn this provided the impetus for further lowering of detection thresholds.

There has been significant focus on non-polar compounds (particularly volatiles and semi-volatile PBTs), as these are relatively simple to analyse by GC-MS methods. This circular process has led to improved knowledge of PBTs, but has also focused resources on a narrow band of contaminants dictated by the development of suitable environmental MS techniques, and the availability of funding for specific environmental monitoring and health protection inititatives (such as the U.S. Superfund sites).

There are a range of validated routine methods for DBP analysis (EPA, Standard Methods). These are largely adequate for regulatory monitoring purposes for a handful of well characterised target compounds, but mostly inadequate for identifying and characterising unknown compounds. Routine methods and monitoring programs based on regulatory requirements are also inadequate for exposure assessment for both epidemiological and toxicological studies. There is a need for the ability to analyse a broader range of DBPs using faster, more sensitive techniques.

There are several challenges for quantitative exposure assessment of DBPs:

- the routes of exposure are complex and varied
- DBP profiles (relative ratios) are likely to change with different exposure routes
- potential confounders are not well characterised
- human pharmacokinetics are not characterised for the wealth of DBP compounds and mixtures which are known to exist
- little is known about the metabolites of DBPs

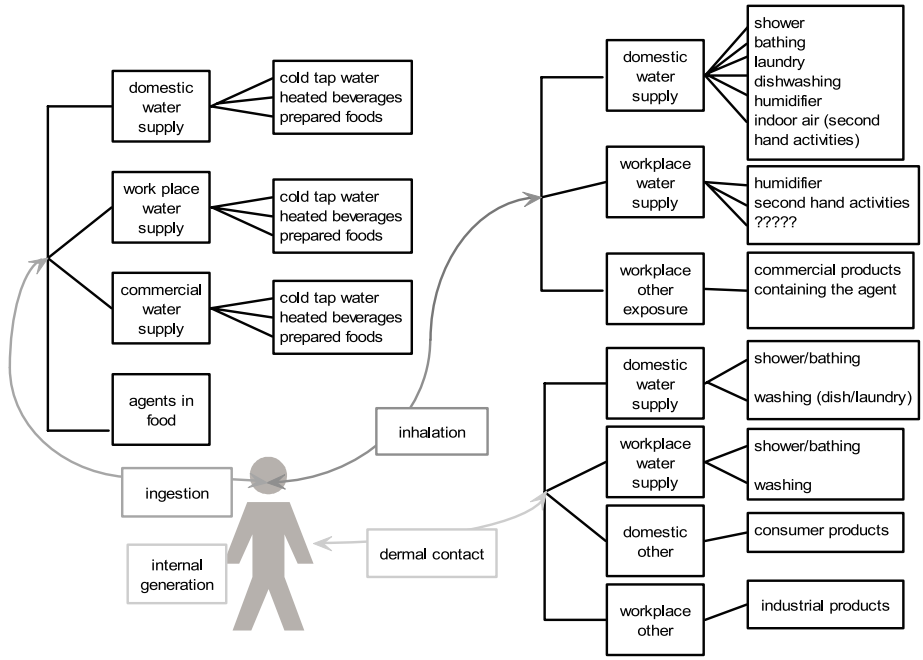

Figure 1 Exposure routes and DBP sources

Figure 1 summarises the three DBP exposure routes (ingestion, dermal absorption and inhalation) and the numerous sources of DBPs which may be encountered in daily life (figure reproduced by permission of S. Hrudey).

DBP analytical methods are also many and varied:

- sample preparation:
  - LLE; purge & trap; closed loop striping; headspace; SPME; small volume techniques (<< 1mL)
  - numerous derivitisation methods for polar DBPs

- instrument methods: quantitative and qualitative
  - GC-MS (includes HRMS and CI-MS); GC-IR; LC-MS; ESI-FAIMS-MS; CE; purge&trap GC; headspace SPME-GC; fast GC; IC
  - membrane introduction mass spec (MIMS): also used in conjunction with GC-MS for THMs (low ppt)

Assay methods may need substantial modification for detection of DBPs in water and bio-fluid matrices.

ANALYSIS OF HAAS

HAAs have attracted interest as potential biomarkers of DBP exposure via the ingestion route. A study of women of reproductive age found a significant correlation between HAA levels in urine, and estimated ingested dose of HAA from drinking water in the previous 24 hours (Weisel et al. 1999). Thus the feasibility of using HAAs in large epidemiological studies has been examined.

The routine assay methods (US-EPA Method 552.2 or Standard Methods 6251) involve solvent extraction, derivatization (methylation by acidic methanol or diazomethane), followed by GC-ECD. This process is time and labour intensive and susceptible to analyte losses.

Estimates of throughput and costs suggest that analysis of 120 samples would require two technicians working for one week, at a cost of $250/sample. The use of HAA as a biomarker in a reproductive epidemiology study involving 2000 couples, with two biological samples per person and samples of home/workplace water is likely to require analysis of over 11,000 samples. This would require funds of $2 million and two years of analysis time. Therefore, the limitations of current analytical methods preclude their use for individual exposure assessment in large scale epidemiological studies.

There is some scope to enhance routine assay methods by using GC based methods with improvements in sample preparation and or chromatographic separation. Alternative methods such as HPLC, ion-chromatography, capillary electrophoresis (CE), LC-MS (ESI-MS), or CE-MS also exist. These methods do not require derivatisation prior to analysis, but usually some kind of sample preparation is needed. Separation times are sometimes lengthy, and the methods often lack sufficient sensitivity for ultra-trace analysis in the nanogram to picogram range.

SPME-GC

- Solid phase microextraction (Pawlizsyn, University of Waterloo)
- Absorption of organic compounds into a polysiloxane coating on a fused silica needle
  - different functional groups on the fibre coating target different groups of analytes
  - can sample directly from headspace to avoid matrix influences on chromatography
- Follow by thermal desorption of compounds from the fused silica needle into GC injector and capillary column

Fast GC

- Rapid GC has been under development since the 1950's, but limitations in electronics (detection), injector design and sample preparation have restricted application.
- Use a narrower bore, shorter capillary column to achieve the same separation in a much shorter time.
- For example, go from a 30m x 0.25 mm i.d. column to 10m x 0.10 mm i.d.
  - Reduces chromatographic time from >35min to <7 min

ESI-FAIMS-MS

This novel analytical technique combines direct injection electrospray ionization mass spectrometry with ion separation based on gas-phase ion mobility in an electric field (ESI-FAIMS-MS). The technique was developed by Guevremont & co-workers at the Institute for National Measurement Standards, NRC, Ottawa (Ells et al. 1999).

The technique is based on difference in ion mobility (K)

- in low vs. high electric fields (E).
- In low E, K is constant for an ion.
- In high E, K becomes non-constant (K_h).
- K_h is compound-dependent

Figure 2 illustrates how the mobility of different ions may vary according to electric field strength. For Type A the mobility increased as field strength increases, while for Type C the ion mobility decreases. For Type B, mobility increases at first then decreases again as field strength increases.

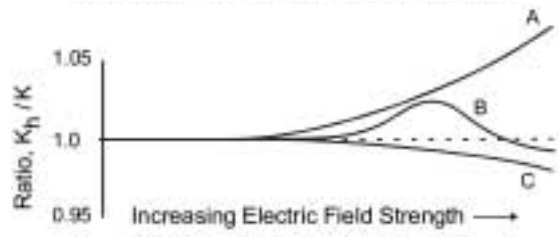

Figure 2 Ion mobility for three types of ions

The principles behind the FAIMS system are summarised in Figure 3. Ions are transported by a gas stream between two parallel plates. The lower plate is kept at ground potential while an asymmetric waveform consisting of a brief high voltage component (t_{high}) and a longer low-voltage component of opposite polarity (t_{low}) is applied to the top plate. The integrated voltage-time product of one complete cycle of the waveform is zero, however if the high field portion of V(*t*) is large enough the distance travelled by the ions during t_{high} will be larger than the distance travelled in the opposite direction during t_{low}. Therefore the ions will experience a net displacement toward the lower plate (shown by dashed line in figure).

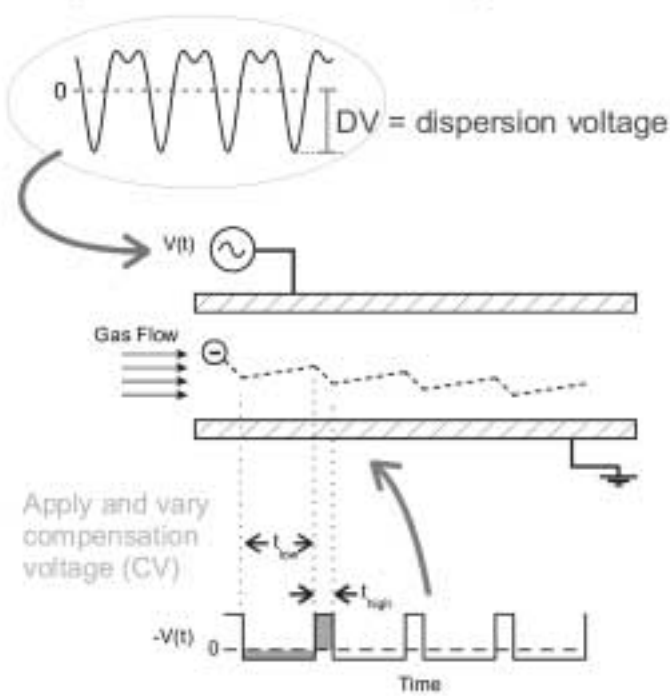

Figure 3 Schematic of FAIMS operation

The movement of the ions towards the lower plate can be counteracted by applying a constant DC voltage to "compensate" for the drift, so that the ions move through the apparatus parallel to the plates. If two types of ions in a mixture have different responses to changes in electric field strength, then each will require a different compensation voltage to prevent the drift effect. Therefore mixtures of ions can be separated and analysed by varying the compensation voltage.

Further modifications to the design of the apparatus to improve sensitivity have been made using an arrangement of concentric cylinders instead of parallel plates.

The ESI-FAIMS-MS technique offers the ability to rapidly separate and assay a wide array of compounds including DBPs, and to differentiate structural isomers of the same compound. The table below summarises the main advantages of the ESI-FAIMS-MS technique, and the differences from other assay methods.

The advantages of ESI-FAIMS-MS over alternative methods include:

- electrospray background reduction - this yields sensitivity one to three orders of magnitude better than direct ESI-MS.
  For example, detection limit for HAAs: < 0.1 – 0.4 mg/L
- provides a new dimension in separation, orthogonal to traditional chromatography and orthogonal to mass - this may assist the identification and characterization of new structures and stereoisomers.
- rapid throughput - "direct" sample analysis results in vastly improved sample through-put of approximately three min/sample.
  this opens the potential to apply the technique to large exposure studies, and to allow rapid environmental toxin assessment screening with fewer false positives

Table 1 Comparison of Analytical Techniques

	LLE (cf. EPA 552.2)	**SPE** (cf. Benanou et al. 1999)	**SPME** (cf. Sarrion et al. 1999)	**ESI-FAIMS-MS** (cf. Ells et al. 1999; Ells et al. 2000)
Extraction	MTBE	AG 1-X8 (Solid phase resin)	MTBE	None
Concentration Factor	10	10	40	0.1 (Mixed 9:1 with Methanol)
Derivatization	10 % Acidified Methanol	30% Acidified Methanol	33% Acidified Methanol (50mL H_2SO_4, 100 mL Methanol)	None
Injection	Liquid Injection	Liquid Injection	SPME	Electrospray
Analysis	ECD	ECD	ECD or MS	FAIMS-MS
Sample Prep and Analysis Time	3 hours	2.5 hours	1.25 hours	5 min
Matrices tested	Water Urine	Water Urine	Water Urine	Water
HAAs detected (standards)	MCA, MBA, DCA, BCA, TCA, DBA, BDCA	MBA, DCA, BCA, TCA, DBA, BDCA	MCA, DCA, TCA, MBA, BCA, DBA, BDCA	HAA9
LOD ug/L (water)	0.05-0.2	0.07-0.2	0.01-0.2	0.06-0.4
Method Precision - TCAA (average % sd)	4.8 water (n=11)* 8.5 urine (n=17)	na	7.8 water (n=3) 5.7 urine (n=5)	2.8 (TCAA) water (n=18)

In summary, while the ESI-FAIMS-MS methodology is still in the prototype phase, this new technique appears to hold great promise for rapid and sensitive analysis of DBPs and other chemicals of environmental concern. The availability of such a method would greatly enhance the possibilities for more accurate individual exposure assessment in large scale epidemiological studies.

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DISINFECTION BY-PRODUCTS AND HEALTH EFFECTS WORKSHOP

Tuesday 30 October & Wednesday 31 October 2001

The Workshop commenced with a welcome by Dr Tony Priestley, Deputy CEO of the Cooperative Research Centre for Water Quality and Treatment, who provided an overview of the history, structure and purpose of the Centre. Professor Steve Hrudey then outlined the objectives of the workshop.

This was followed by a series of presentations from CRC researchers to provide an indication of the skills, resources and experience of the participating organisations relevant to development of the DBP research program. Brief presentations were then made summarising two health-related DBP projects undertaken in the public health program of the CRC.

The delegates then developed a range of questions to be addressed during the remainder of the workshop. Three working groups were formed, each group having members from backgrounds in public health and water supply. For each of the questions addressed, representatives reported the details of the group discussion, and there was a general discussion session. Finally the overall outcomes of the workshop were summarised by Professor Hrudey.

OVERVIEW OF THE CRC FOR WATER QUALITY AND TREATMENT

Dr Tony Priestley
National Manager - Water Supply, CSIRO

The Cooperative Research Centre for Water Quality and Treatment was established in 1995 under the Australian Government's Cooperative Research Centres Program to provide a national strategic research capacity for the Australian water industry. The research program conducted by the Centre has the two underlying themes of health risk reduction and water quality improvement. In July 2001, the Centre commenced a second funding cycle that will extend to June 2008.

The activities of the centre are structured into three major program groups:

- Program Group 1: Health and Aesthetics
  Including the programs Epidemiology, Toxicology and People's Perspectives.
- Program Group 2: Catchment to Customer
  Including the programs Catchments, Reservoirs, Measurement, Water Treatment Technology, Distribution, and Sustainable Water Sources.
- Program Group 3: Policy, Regulations and Stakeholder Involvement
  Including the programs Strategic Directions, Policy and Regulation, Regional and Rural Water Supplies, Education and Training, Commercialisation, and Communication.

Research expertise available within the Centre covers a wide range of disciplines including epidemiology, toxicology, risk assessment, ecology, genetics, microbiology, microbial physiology, reservoir hydrology, water chemistry, water treatment technology, systems modelling, and social sciences.

The Centre comprises 29 Parties including water suppliers, universities, research organisations, government departments responsible for environmental and health regulation, consultants, and a major industry association:

CRC Participants

- ACTEW Corporation
- Australian Water Quality Centre
- Australian Water Services Pty Ltd
- Brisbane City Council
- CSIRO
- Curtin University
- Department of Human Services (VIC)
- Department of Natural Resources (QLD)
- Egis Consulting Australia Pty Ltd
- Griffith University
- Melbourne Water Corporation
- Monash University
- NQ Water
- Orica Australia Pty Ltd
- Power & Water Authority (NT) Queensland Health Pathology & Scientific Services
- RMIT University
- South Australian Water Corporation
- South East Water Ltd
- Sydney Catchment Authority
- Sydney Water Corporation
- The University of Adelaide
- The University of New South Wales
- United Water International Pty Ltd

- University of Queensland
- University of South Australia
- Water Corporation (WA)
- Water Services Association of Australia

The Centre also has an Associates program designed to allow smaller water authorities and other organisations with an interest in water supply to benefit from the activities of the Centre. The Centre currently has nine Associates.

OBJECTIVES OF THE WORKSHOP

Professor Steve Hrudey
Department of Public Health Sciences, University of Alberta

The previous Seminar presentations provided a global overview of the DBP problem which can defined briefly as follows:

- DBPs ***may*** cause cancer, and/or reproductive effects. The evidence for both areas of health risk is not conclusive, but due to the widespread exposure of the population adverse effects from DBPs could represent a significant public health burden.
- DBPs in water supplies are diverse, variable and complex. This presents particular difficulties in characterising and defining exposure that are probably not equalled by any other class of water contaminants.
- Toxicology, alone, cannot answer the questions that have been raised. The issue of human health effects requires confirmation in human populations.
- Epidemiology, alone, cannot make much progress on the issues without better insights from toxicology. Epidemiology requires meaningful measures of exposure, but most available data relate to compounds of little toxicological concern.
- Drinking water guidelines and associated monitoring requirements remain, at best, tenuously associated with adverse health effects. Regulation has focused on the most abundant and easily measured DBPs, but the evidence suggests these compounds are not of significant health concern and therefore limiting the concentrations of these classes may not lower health risks.
- Consumers and suppliers are confused and some are concerned. Continuing uncertainty over health risks, and differing regulatory levels in different jurisdictions foster anxiety and misunderstanding.

In developing a research agenda for the CRC for Water Quality and Treatment, the following factors need to be considered:

- the potential resources and capabilities required to address DBP research
- defining the problem(s) for Australia
- identifying research needs that address the problems for Australia
- developing a strategy to implement projects that address the DBP problems identified for Australia

In particular, the participants in this workshop should:

- seek to identify unique questions worthy of research answers
- critically assess the feasibility of studies
- ensure the capacity of researchers to perform the required studies

We recognise that the issue of DBPs and health effects is one of the most complex research questions relating to drinking water quality. In this field, it is unlikely that any single breakthrough will yield a "knock out" punch, however significant advances should be achievable with an integrated approach combining the strengths offered by the range of skills within the CRC.

CAPABILITY STATEMENTS BY CRC PARTICIPANTS

National Research Centre for Environmental Toxicology

Dr Glen Shaw
Senior Research Fellow, University of Queensland

NRCET was established in 1991 under an initiative of the National Health and Medical Research Council to provide an Australian centre of expertise in the study of the health effects of exposure of biological systems and human populations to environmental contaminants. The Centre is located within the Brisbane laboratories of Queensland Health Scientific Services, and operates in collaboration with the University of Queensland, Griffith University and Queensland Health. The laboratory complex was recently extended to include the centres of Public Health, Environmental Health, and Forensic Sciences from Queensland Health Scientific Services. NRCET joined the CRC in 2001 and coordinates the Toxicology program.

NRCET has available a wide range of skills in analytical toxicology including analysis of algal toxins, organic micropollutants, metals, DBPs, and byproducts of treatment of algal toxins. The centre has toxicopathology expertise, a large biological research facility, and established linkages with international research institutes providing access to additional techniques such as DNA microarray and biosensor technology.

Research services include cell culture facilities and *in vitro* assays, and chromosome damage assays (eg assays for micronuclei formation, chromosal abberations, sister chromatid exchange). In addition to carrying out extensive research on algal and dinoflagellate toxin

production and purification, NRCET has developed methods for the isolation and purification of byproducts of water treatment of algal toxins. This work has also included the development of facilities for production of radiolabelled algal toxins and pharmacokinetic/ pharmacodynamic studies.

NRCET also has available a range of techniques which are potentially applicable to biomarker development for DBP research. These include genotoxicity assays such as the identification of DNA adducts, gene mutations, oxidative DNA damage and DNA strand breakages.

Sydney Water Corporation

Dr Trung Tran
Water Quality and Treatment Specialist, Sydney Water Corporation

Sydney Water Corporation supplies drinking water to nearly four million people in Sydney, the Blue Mountains and the Illawara. Water is drawn from eight different surface raw water supplies, and treated at 10 water filtration plants (eight with direct filtration, one with two stage filtration and one with DAF/contact clarifier followed by Granular Activated Carbon). There are 14 long water distribution systems with either chlorination or chloramination as the primary disinfection process.

Sydney Water is conscious of international trends to reduce regulatory levels for DBPs, and has been actively working on ways to reduce DBP levels while maintaining adequate control of microbial water quality to meet licensing requirements. Consumer satisfaction with aesthetic water quality has also been an important consideration. Conversion of some systems from chlorination to chloramination has been undertaken to achieve these multiple goals. As part of its planning for management of DBPs, Sydney Water has established analytical methods for THMs, HAAs, and HANs. Research has included the isolation and analysis of DBPS, studying the effect of inhibitors on the analysis of DBPs, and the effect of pH and pre-oxidation on kinetic formation of DBP in filtered water.

Pre-oxidation has a variable effect on THM formation in filtered water at different pH values. At pH 6.5 and pH 8.5, pre-oxidation results in an increase in THM production at reaction times up to 240 minutes. However at pH 7.5, THM production is reduced by pre-oxidation. The differences in THM formation with and without preoxidation become smaller as reaction time increases (analogous to water in a long distribution system).

Real distribution systems are dynamic systems, and DBP formation may not be accurately predicted from static models in the laboratory. Sydney Water's research has shown that formation of THMs in the pipeline is fairly similar to predictions from laboratory tests, however for HAAs the curves are considerably more divergent with lower levels formed in the real system than would have been predicted from the laboratory situation. While HAA levels continue to increase up to reaction times of at least 250 minutes in the laboratory, in the real system levels plateau at about 150 minutes then begin to decline again.

The commissioning of one typical water filtration plant in the Sydney system reduced THM levels in finished water to about 30% to 50% of the levels seen with unfiltered water, while HAA levels were reduced to about 10% to 25% of previous levels. While total dissolved organic carbon levels were reduced to 40% to 60% of pre-filtration levels, UV absorbance at 254 nm has been reduced to 20% to 35% of previous levels. These observations illustrate how the different components of DBP precursors present in raw water may be differentially removed by water treatment processes. In the Sydney system, control of iron and manganese levels is also a significant consideration, thus the operation of water treatment processes can not be aimed solely at reduction of DBPs, but must also must take other water quality parameters into consideration.

Department of Epidemiology and Preventive Medicine, Monash University

Associate Professor Malcolm Sim
Head of Occupational and Environmental Health Unit, Department of Epidemiology and Preventive Medicine, Monash University

DEPM has been responsible for coordinating the public health research program of the CRC since its establishment in 1995, initially under the Public Health Risk Assessment Program and now under the Health and Aesthetics Program Group. DEPM also coordinates the Epidemiology program of the CRC. The core skills of the department relate to epidemiology and its application to problems in clinical medicine and public health. DEPM plays a prominent role in public health medicine in Australia, and the expertise of senior academic staff covers a wide range of specialist areas.

Epidemiology has been defined as the "study of the distribution and determinants of disease in human populations". This discipline is research question driven, and is guided by information from other research fields such as toxicology. To outsiders, epidemiology may appear to be simple as it lacks the tangible "high tech" hardware associated with laboratory disciplines, however the methodology is complex and a considerable range of skills and knowledge are required to carry out high quality research in this field.

When designing human studies to assess the health effects of DBPs, epidemiologists must consider a range of factors including the choice of the appropriate study design for the question being addressed, the availability of a study population of adequate size with a range of exposure levels to allow effective comparisons, and defining good operational measures of health outcomes which are valid and repeatable. In studies of DBPs, the definition and measurement of cancer outcomes is relatively straightforward due the existence of good quality cancer registries in most developed nations, however reproductive outcomes may be more difficult to define and ascertain.

Exposure measures are critical, and it is important to consider the differences between objective and subjective data, to develop appropriate markers of exposure which are as close as possible to the target organ, and to consider all exposure routes and kinetics. Ideally, quantative measures of exposure should be the aim. Care must be taken in data collection, quality control and data management to produce high quality data for analysis.

Bias and confounding must be considered in the design phase, and efforts made to reduce and/or control for them throughout the study. Analysis and interpretation are complex, and a range of approaches are possible. The presentation of results should include the illustration of uncertainties (the use of confidence intervals to show (im)precision), and the degree of consistency of trends or patterns. Care and judgement are needed in interpreting isolated positive findings.

DEPM is well placed to conduct studies of DBPs and health effects, with diverse academic and clinical strengths, and a strong track record in environmental epidemiology. The Australian Centre for Human Health Risk Assessment is soon to be established in the department, and there is extensive experience in the conduct of large observational studies and a high degree of expertise in exposure assessment methodology.

Australian Water Quality Centre

Mr Keith Hayes
Manager Research & Chemistry, Australian Water Quality Centre

AWQC is a business unit of the South Australian Water Corporation (SA Water) which is the State Government organisation responsible for the provision of public water supplies and sewerage services in South Australia. In addition to providing analytical services to SA Water, the centre also undertakes consultancy and grant funded research work. The expertise of the centre includes the chemistry, microbiology, biology and ecology of waters, wastewaters, sediments and sludges. AWQC was a founding participant in the CRC for Water Quality and Treatment in 1995, and is the location of the administrative headquarters of the CRC.

AWQC began routine analysis for THMs in Adelaide drinking water supplies in 1976, and initiated research into the identification and formation of chlorination DBPs during the 1980s. A wide range of analytical methods for DBPs are available at AWQC including those commonly used in routine testing by water authorities, as well as more specialised and sensitive techniques for research purposes.

Major research projects on DBPs undertaken recently have included:

- Occurrence and Removal of the Highly Mutagenic DBP 'MX' in Disinfected Drinking Water. (1990-93) Funded by the Land and Water Resources Research and Development Council.
- Drinking Water DBPs Relevant to the 1996 NHMRC/ARMCANZ Australian Drinking Water Guidelines. (1994 - 96) Funded by Urban Water Research Association of Australia.

The latter project required the establishment and evaluation of analytical methods for all 23 DBPs listed in the ADWG. The stability of DBPs was examined under a range of storage conditions, and a DBPs survey of Australian drinking waters from 16 locations was carried out (Simpson KL and Hayes KP, *Water Research* **32**(5):1522-1528, 1998, and UWRA Research Report No 115).

DBP research at AWQC has continued following formation of the CRC with involvement of AWQC in three projects in the public health and water treatment areas:

- 1997: *CRC Project 1.3.5.1:* "Chlorine, Trihalomethanes and Micronuclei (genotoxic change) in Bladder Epithelial Cells"
  This project examined the rates of occurrence of micronuclei in bladder cells from human populations exposed to drinking water supplies with different levels of chlorination DBPs. (See summary later in this report).
- 1997: *CRC Project 3.2.3* "Alternative Disinfection Regimes"
  This project examined the generation and nature of the precursors leading to formation of assimilable organic carbon and DBPs in water treatment processes involving ozone or ozone/chlorine, and the formation of brominated DBPs in ozonated water.
- 2000: *CRC Project 1.3.5.3* "DBP Exposure Assessment" - Australia & Canada.
  This collaborative project with the University of Alberta examined the potential utility of urinary trichloroacetic acid (TCAA) as a biomarker for ingestion exposure to DBPs. (See summary later in this report).

School of Applied Chemistry, Curtin University of Technology

Professor Robert Kagi
Director, Centre for Petroleum and Environmental Organic Geochemistry, Curtin University of Technology

The School has extensive experience in petroleum geochemistry and environmental geochemistry, particularly in relation to the petrolchemical industry in Western Australia. In the area of drinking water, the School has a long history of work on the analysis of micropollutants in groundwater, including industrial contamination and identification of non-point sources of solvents, pesticides and endocrine disruptors such as drainage systems, septic systems and market gardens. Curtin University joined the CRC in 2001 and coordinates the Measurement program.

The investigation of taste-and-odour problems in drinking water is also an area of expertise. Compounds responsible for these problems are often highly potent, and even ultratrace amounts in the nanogram or picogram range per litre may exert strong tastes or odours. Work on the formation of dimethyltrisulfide (odour threshold 10 ng/L) in distribution systems required specialised analytical techniques for DMTS and for precursor compounds. The School recently worked on the challenging problem of identifying the cause of intermittent plastic tastes in the Perth water supply which were generated only when water was boiled. This problem was traced to the formation of halophenols (taste threshold 0.5 ng/L) in some types of household kettles. This ongoing investigation involved the development of a new technique for analysis of sub-nanogram per litre levels of phenols.

The spectrum of specialised analytical work undertaken has also required development of the capability to synthesize surrogate standards for improved analytical performance. Trace analysis by GC techniques requires the use of internal standards that are chemically similar to the analytes of interest. However most compounds cannot be purchased, so the capability for in-house synthesis is necessary. Studies on DBPs undertaken by the School include correlations between structure of NOM (as determined by Py-GC-MS and TCM) and THMFP and SUVA, and identification of unusual and novel chlorination by-products in WA country water supplies (e.g. hexahalocyclopentadienes; benzyl chloride and other halogenated aromatics; bromal; perhaloethylenes, etc)

The range of specialised analytical tools and techniques available in the School offer new possibilities for the detection and characterisation of DBPs that may be applicable to studies across a number of programs within the CRC. For example, the measurement of stable carbon and hydrogen isotopes at natural abundance levels in specific compounds within complex mixtures is now possible. This enables the ratios of $^{13}C/^{12}C$ and $^{2}D/^{1}H$ to be used to identify the source of organic molecules and trace their movement. This method has been applied for tracing algal sterols in reservoir food web studies.

Novel approaches to the characterisation of NOM are also available including molecular characterisation to elucidate chemical structures, correlation of structural characteristics with source environments, and correlation of structural characteristics with treatability. Existing techniques based on flash pyrolysis and thermochemolysis can be extended, for example use of new instrumentation such as MSSV-GC-MS for sealed pyrolysis, or use of hydropyrolysis. Another potential approach is the development of new techniques based on chemical modification of NOM.

HEALTH-RELATED DBP PROJECTS UNDERTAKEN BY THE CRC

Biomarkers in Bladder Epithelial Cells and DBPs

Dr Martha Sinclair
Senior Research Fellow, Department of Epidemiology and Preventive Medicine, Monash University

This project was undertaken by Dr Geetha Ranmuthugala, Dr Louis Pilotto, Dr Wayne Smith and Professor Bob Douglas at the National Centre for Epidemiology and Population Health (NCEPH) in Canberra. NCEPH was a participant in the CRC for Water Quality and Treatment from 1995 to 2001.

The project was an epidemiological study examining the occurrence of micronuclei in bladder cells in three human populations exposed to different levels of chlorination DBPs in drinking water supplies. Micronuclei are fragments of chromosome which arise by chromosome breakage, and can be visualised in appropriately stained cells as small bodies of genetic material separate from the main nucleus. Micronuclei in bladder epithelial cells can be detected in cells shed into the urine, providing a convenient, non-invasive method of specimen collection. Exposure to some bladder carcinogens (eg cigarette smoke, arsenic) is associated with increased rates of micronuclei formation. If increased levels of micronuclei were also associated with drinking chlorinated water this would add to the weight of evidence for cancer risk, and would provide a surrogate marker for risk assessment which could be measured prospectively.

This study was therefore designed to compare THMs in water supplies, in urine, and the number of micronuclei in bladder cells in people exposed to different levels of DBPs. The initial design was for a randomised trial of chlorinated and unchlorinated bottled water, but

technical problems were encountered with this approach. The design was therefore revised to a comparison of people in Adelaide (chlorinated water supply with 110 to 150 micrograms /L TTHMs), Canberra (chlorinated water supply with 50 to 60 micrograms /L TTHMs), and Bungendore (undisinfected water supply with 0 TTHMs).

The eligibility criteria for people enrolled in the study were:

- Males aged 30 to 65 years of age - bladder cancer is more common in older age groups and in males rather than females.
- Resident at current address for at least six months - to provide relatively stable recent exposure levels to DBPs from drinking water.
- Do not swim regularly (once or more in a week) in a chlorinated pool - to exclude a major source of exposure to THMs not related to drinking water.
- Never been diagnosed as having cancer, not including skin cancer - to exclude the possibility of secondary bladder cancers being present.

Data collection for the study included a residential history, a questionnaire recording risk factors, urine samples for micronuclei testing, and a fluid intake and showering diary for two weeks. A total of 342 people completed the study.

No significant differences in micronuclei rates were found between the three groups, and there was no correlation of micronuclei rates with THM levels in water or urine. Thus the study did not provide evidence in support of cancer risks from a mechanism involving chromosome breakage. However, the negative result does not rule out a possible effect of DBPs on bladder cancer risk, as it is possible that carcinogenesis may occur by a mechanism not involving chromosome breakage.

TCAA as an Exposure Biomarker for Drinking Water Disinfection By-Products (DBPs)

Dr Ken Froese
Assistant Professor, Department of Public Health Sciences, University of Alberta

Efforts are being made to develop biomarkers of DBP exposure in order to improve the accuracy of exposure assessment in epidemiological studies of health effects. THMs do not provide a good measure for ingestion exposure because of the first pass effect through the liver which rapidly removes most ingested THMs from the bloodstream. Therefore measurement of THMs in blood or breath is only useful to assess recent inhalation or dermal exposure (within about one hour prior to sampling). In a preliminary study, TCAA in urine was reported to be a more promising biomarker (Weisel et al. 1999) showing a reasonable degree of correlation with ingested dose from drinking water in the previous 48 hours.

A pilot study was carried out in Adelaide, Australia to evaluate the utility of urinary TCAA as biomarker (Adelaide Water Exposure Trial, AWET). The project was carried out jointly by the University of Alberta, AWQC, and Monash University. The five-week study involved 10 participants who provided urine samples and household water samples for analysis, and filled in diaries covering fluid consumption and water use activities. Participants drank either normal tap water or TCAA-free bottled water at different stages of the study, but were also permitted to drink other beverages as desired (Froese et al. in press).

Over 400 samples were analysed for TCAA using modified EPA Method 552.2. The method was found to have low variability with the average standard deviation for TCAA analysis being 0.40 µg/L or 8.5% using 17 urine triplicates, and 0.19 µg/L or 4.8% using 11 water triplicates. The results of the study suggested that TCAA may be a useful biomarker for ingested dose of DBPs with a urinary excretion half-life in the range of 2.3 to 3.7 days.

The study revealed a very high degree of day-to-day variability in exposure:

- Inter-individual exposure/ingestion variability - Concentrations of HAAs varied significantly in the water distribution systems, dependent on source water. Concentrations also fluctuated substantially from day to day in any single supply system. The average per cent standard deviation across locations was 100%, while the average per cent standard deviation over time was 39%. Individuals also showed large variation in the quantities of water or tap-water based beverages ingested each day.
- Intra-individual variability - Individuals consumed water from different sources and in different amounts each day. On average, 34% of total daily beverage volume consumed was from "other" sources (ie not from work or home water supplies), meaning that TCAA levels in such beverages were unknown.

A second study was carried out in Edmonton, Canada with five participants (Winnipeg-Edmonton Trial, WET study). In this study beverages were limited to drinking water only, and participants were requested to ingest one litre of water after 17:00 each day. Participants were supplied with Winnipeg tap water (high TCAA levels) for two weeks, then with bottled water (TCAA-free) for two weeks. From the experience gained in the Adelaide study, improvements were made in the diary design for recording water consumption and other exposures (showering, swimming, dry cleaning).

Again, over 400 samples were analyzed for TCAA with good method performance. The average standard deviation for TCAA analysis was 3.6% using three urine triplicates, and 5.7% using five water triplicates. The results of the study showed stronger correlations

between ingested and excreted TCAA dose compared to those seen in the Adelaide study, presumably due to reducing confounding from fluctuations in water ingestion volume and TCAA from other beverage sources.

These observations give improved confidence in the validity of TCAA as a biomarker for DBP exposure. Estimates of urinary excretion half-life in the Edmonton study ranged from two to six days, consistent with published studies of TCAA as a metabolite of TCE and other literature. The range of values indicates significant inter-individual variability in TCAA metabolism and excretion. The study also showed that FMU samples (first morning urine) appear to be representative of daily TCAA excretion. Creatinine-normalization of excretion is likely not required, but accurate recording of the time since last urine void is required to calculate the excretion rate.

It is notable that TCAA in urine did not reach non-detect levels even after 14-21 days of TCAA-free bottled water. This may suggest that there are sources of TCAA in the diet or that TCAA in endogenously generated. Alternatively, it may be that the observation time on TCAA-free water was simply too short to achieve complete washout in these studies.

There is still need for a larger study over a range of concentrations to test the "dose-response" relationship of the biomarker. The experience from these small volunteer studies indicates that future studies will still be primarily limited by logistics and analytical costs and capacities.

REFERENCES

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DEFINING A RESEARCH AGENDA

Following the presentations from CRC researchers, the workshop participants defined four aspects of the problem to be considered by working groups:

1. characteristics of Australian water industry that differ from US/Europe and that may affect DBPs
2. characteristics of Australian water supplies, consumers or water uses differing from US/Europe that may affect DBPs
3. aspects of DBP issues that may be common to all, but are not being addressed elsewhere and may be important to Australian industry
4. aspects of DBP issues that Australia may be uniquely suited to research

Each question was considered in turn, and there was also general discussion on research and management issues relating to DBPs. International research projects of relevance and information to be sought from them were identified. In this report the issues raised in these general discussions are summarised first, followed by brief descriptions of the international projects, then the discussions relating to the four aspects listed above. Finally a range of potential research approaches was developed for further consideration and development by the CRC.

A. GENERAL ISSUES

There are a number of differences in by-products formed by chlorination or chloramination that should be considered in assessing risks. There are differences in the toxicity of brominated and chlorinated compounds that make the former more probable causes of certain types of effects. Nevertheless, this does not carry across all endpoints and non-brominated compounds should not be discounted as possible causes for other health risks.

Formation of halogenated compounds

There is a common misconception that mono-, di-, and tri- halogenated by-products (eg members of the haloacetic acids) arise from sequential halogenation of common precursors. In fact, there is considerable evidence that in large part, different levels of halogenation involve different precursors. Thus, the ratios between mono-, di- and tri- halogenated by-products in a given chemical class can vary widely between water supplies. The unusually high formation of monohaloacetic acids in Australia, discussed later, relative to the dihaloacetic and trihaloacetic acids which predominate this class in Europe and North America is a case in point. This pattern should be reflected, at least in part, in most other classes where multiple substitutions of halogen are possible, such as haloacetaldehydes and haloketones. This is extremely important in the development of exposure measures in epidemiology studies.

Brominated Compounds

Brominated compounds are chiefly of concern in chlorinated drinking water because members of the classes that have been studied include compounds that produce colon cancer in rats. In the history of the National Toxicology Program of the US, brominated compounds, in addition to disinfection by-products, have been the most common chemicals to induce colon cancer in rats. Colon cancer is one of the cancers that have been associated with chlorinated drinking water.

Bromate, MX, and NDMA are the most potent carcinogens yet found in drinking water. MX occurs at such low concentrations that it does not appear to be a great concern. The occurrence of NDMA has not been widely examined, so it is difficult to assess its importance today. However, ozonation of water containing significant amounts of bromide frequently results in concentrations of bromate above the 10^{-4} lifetime risk level. The increasing use of ozonation in the US has brought problems with bromate formation in some groundwater supplies, and a number of ozone plants in California have been abandoned due to high bromate levels in treated water.

Significance of nitrosamines

The discovery that NDMA is formed when chlorinated or chloraminated water has significant inputs of wastewater has raised concerns in those parts of the U.S. where groundwater recharge of reclaimed water is practiced. In the same water, chloramination yields greater amounts of NDMA than chlorine. It is believed that other nitrosamine compounds may also be formed during disinfection depending on the abundance of secondary amines in the water source. Some nitrosamines are potent carcinogens. Their presence in drinking water would lend biological plausibility to cancer risks.

To date there has not been an extensive survey of the occurrence of NDMA or other nitrosamines in US drinking water supplies, but those with significant wastewater input are likely to be of most concern. Limited data suggest that levels in surface water supplies are generally in the range of 10-20 parts per trillion (0.010 to 0.020 micrograms/litre). However levels up to 1 microgram/litre have been reported in sources receiving wastewater. Applying the default linear extrapolation model for cancer risks to this figure suggests the associated lifetime risk could be as high as 1 in 10^4 to 1 in 10^3.

Detection of NDMA requires a fairly difficult assay, and it is not the only compound of concern. For the purposes of risk analysis it may be better to develop a means to characterise the potential of a water supply to form nitrosamines rather assaying individual species.

There are also some industrial sources of NDMA including the computer chip and rubber industries, and work in Canada has suggested some pesticides may be converted to the related chemical dimethylamine.

Effects of changes in water treatment methods

Current regulations are based primarily on THM levels, with limits for HAAs now also being phased in by some regulators. Many water authorities are changing treatment methods to meet licensing requirements relating to these classes of DBPs. These changes will continue to occur despite the knowledge that reduction of THMs and HAAs may not necessarily reduce other classes of DBPs and may not reduce health risks (if they truly exist).

Across Australia a range of approaches are being adopted including switching from chlorination to chloramination, conventional treatment and filtration, aeration, microfiltration, reverse osmosis, and introduction of new treatment techniques such as Miex® DOC resin. More stringent licensing requirements for the handling of chlorine gas are causing water authorities to switch to hypochlorite for some smaller supplies and suburban rechlorination points. This may also affect the DBP profile since aging of the hypochlorite solution leads to the conversion of significant amounts of hypochlorite to chlorate.

Changes in treatment methods may also be motivated by regulations affecting other water quality parameters or by aesthetic considerations. Many of these changes are likely to affect the levels of DBPs and some may result in the generation of novel DBPs. Given the complexity of the factors affecting DBP formation, we can not be sure that the effect of a particular change in treatment method will be the same in all water supplies.

At present there is no systematic effort to document the effect of these changes on DBP formation other than for those classes which are regulated. It was felt that the CRC was well placed to undertake a role in collecting and collating information on the effect of such water treatment changes on DBP profiles. In the event that more definitive evidence emerges on the association of adverse health effects with particular classes of DBPs or with parameters affecting DBP formation, this information will indicate whether certain treatment chains may be problematic.

Public communication issues

Differing Standards and Guideline Values for DBPs (and many other water contaminants) have been set by regulators/advisory bodies in different countries. This leads to confusion in the eyes of the public and a perception that some jurisdictions are more stringent in their public health protection goals than others.

In fact many of the differences are due to the use of different assumptions and modelling methods applied to the same data. For example, the permissible daily exposure level for a toxic substance may be calculated using the "milligram/kilogram body weight" method (WHO) or the "body surface area" method (US EPA). This choice can lead to a 15 to 20 fold difference in the calculated exposure value, with neither method being intrinsically "better" in the scientific sense.

Many people perceive the numerical guideline value to be an absolute limit of safety, and do not appreciate the degree of uncertainty surrounding risk estimates, nor the fact that large safety margins are built into guidelines to account for uncertainty. There is also little appreciation of the influence of economic pragmatism and policy decisions on the regulatory process. Standards and guidelines are generally assumed to be based purely on scientific certainty, and thus any transitory exceedence is thought to represent a significant health risk, when in fact this is not the case.

There is a need for better communication on these issues with the public and those in the community who are opinion leaders and health advisers. In particular a plain language explanation of how guidelines values are developed is needed, as well as specific information on DBPs and other water quality issues.

Studies of pregnancy outcomes

While many developed nations maintain perinatal registries to record adverse pregnancy outcomes and congenital anomalies, there is evidence that only a minority of such outcomes are recorded in registries.

For example, a current study in the UK (not related to DBPs) has found that the rate of congenital anomalies detected by direct data collection from hospitals is two to three-fold higher than that reported in the national registry. A similar degree of underreporting has also been described in the US. This complicates the interpretation and comparison of such data sources.

Exposure assessment

This is a very complex area and much better measures of exposure are needed to advance epidemiological studies.

To adequately assess individual exposure reliable information is required on water consumption, showering, swimming, and spa use (with information on pool and spa chemicals). Dermal exposure may lead to endogenous DBP production from reaction of disinfectant with skin lipids. Better information is also required on the effect on DBP levels of water standing before use, point-of-use filters, boiling, refrigeration, overnight storage in hot water tanks and so on. Health Canada is undertaking some work in this area.

For many individual DBPs it is not known whether they are uniquely formed by disinfection or whether some may be generated endogenously by metabolic processes. It is also unknown to what extent DBPs may occur naturally in food or as a consequence of mixing/contact with chlorinated water during food processing or cooking. Not much is known about DBP levels in bottled waters.

Biomarkers such as urinary TCAA measurement may be of use to assess exposure but costs and logistical restraints are unlikely to permit their large scale use in epidemiological studies. More likely such biomarkers may serve to validate exposure assessments from questionnaires in a subgroup of subjects.

Regulation

Consideration of dermal and inhalation pathways of exposure may impact greatly on water quality standards for some DBPs if these routes are more important than ingestion. This would represent a paradigm shift in regulations.

The Guideline Values in the Australian Drinking Water Guidelines are applied differently in different states. In some states, regulations are based on an annual average that does not exceed the GV, while in others the GV is taken as a maximum allowed value for individual samples. A more uniform and scientifically justified approach would be preferable.

B. RELEVANT INTERNATIONAL RESEARCH

A number of organisations are undertaking studies relevant to the health effects of DBPs. The CRC will seek information on the progress of these studies to build on their experience and outcomes. Opportunities for development of collaborative projects will also be sought.

Occurrence of Drinking Water Disinfection By-products

The US EPA has identified a list of approximately 50 currently unregulated DBPs considered to represent those with highest potential toxicity. This assessment was based on consideration of mechanism-based structure-activity relationships, and any available toxicological and occurrence data. Assay methods have been developed and standardised, and reference standards purified or synthesised for these substances.

A survey for these compounds has been carried out at 12 water treatment plants using a range of disinfectants. The preliminary findings of this survey will be presented in November 2001 at the Water Quality Technology Conference of the American Water Works Association in Nashville (paper entitled *The occurrence of a new generation of DBPs (beyond the ICR).* SW Krasner et al.).

US National Birth Defects Prevention Study

The Centers for Disease Control and Prevention are conducting a large study (National Birth Defects Prevention Study) on birth defects in seven states. The study will examine the environmental, genetic and behavioural factors that cause or contribute to birth defects. Some states will include disinfection by-products in their exposure assessment.

Reassessment of past cancer studies

Exposure data from past cancer studies is being reassessed by looking at estimated exposures to brominated DBPs rather than total THMs. If this yields higher risk estimates, it may suggest that one or more brominated DBPs is implicated in carcinogenesis.

Characterisation of water parameters influencing DBP formation

A number of techniques to characterise raw water NOM characteristics are being developed including online monitoring of SUVA.

AWWARF research projects on DBPs

Dr Bull provided a summary of a number of potential projects developed by the American Water Works Research Foundation. Some of these are already established or have been approved for funding.

C. DISCUSSION TOPICS

1 Characteristics of the Australian water industry that differ from North America/ Europe and that may affect DBPs

- **Regulatory limits relatively high compared to other countries**
  The present Guideline Values for DBPs in the Australian Drinking Water Guidelines are relatively high in comparison to the US and Europe.

  Australia: 250 mg/L TTHMs (maximum) set in 1996

  USA: 100 mg/L TTHMs (annual average) in 1979 changed to
  80 mg/L L TTHMs (annual average) in 1998

  Canada: 350 mg/L TTHMs (maximum) set in 1978 changed to
  100 mg/L TTHMs (annual average) in 1996

  WHO: 30 mg/L chloroform (annual average) in 1984 changed to
  200 mg/L chloroform (annual average) in 1993
  100 mg/L bromoform (annual average) in 1993
  100 mg/L dibromochloromethane (annual average) in 1993
  60 mg/L bromodichloromethane (annual average) in 1993

  This means that the upper end of the exposure spectrum in Australia is likely to be higher than in other countries, presenting a greater range of contrasts in exposure.

- **Flexible regulatory environment**
  The Australian Drinking Water Guidelines (1996) are used as the basis for regulation in states and territories, however DBP guideline values may be applied differently. In some states significant exceedence of the Guideline Value may be tolerated, while in others the water supplier would be required to take action to lower DBP levels.

  Again, this means that higher exposure scenarios are likely to exist in Australia.

- **Different approaches to the management of small water systems**
  In some Australian states, small water supplies are operated by a large number of independent operators such as local councils. However in several states, a few large utilities operate many small systems. This has distinct logistical advantages for studying a large range of water types and treatment methods.

- **High degree of consumer trust**
  Australian consumers generally express a high level of trust in water authorities, and the overall level of concern over health risks from public drinking water supplies is low. However if health concerns are raised, the adverse public response may be immediate and very strong.

- **Water industry is not experienced in dealing with health concerns**
  Partly due to the low level of public interest and advocacy over drinking water safety issues in Australia, the water industry is less experienced than its international counterparts in dealing with consumer concerns about health. In situations where community concerns are raised, there may be credibility problems for advice given by water authorities, and information for the public needs to come from health regulators and health professionals.

- **Low price of water**
  On an international scale, water prices for Australian consumers are low and per capita water use is relatively high. If control of DBPs requires significant capital expenditure, this will impact on the cost to consumers.

- **Dual function authorities**
  Many Australian drinking water suppliers are also responsible for sewage treatment, and reuse schemes for wastewater are expanding rapidly. This provides an opportunity for studying DBPs in wastewater applications.

2 Characteristics of Australian water supplies, consumers or water uses differing from North America / Europe that may affect DBPs

- **NOM profiles may be significantly different from other countries**
  The report summarising the 1993-96 survey of DBPs in Australian water supplies was tabled at the meeting (Hayes and Simpson, 1996). The absolute concentrations and relative ratios of the chlorinated haloacetic acid species reported in this survey are substantially different from those published for North American and European supplies.

  In the US the total chloroacetic acid level (mono-, di- and trichloro) is usually about 50% of the total THM

level, but in the Australian systems surveyed in this report they were similar or only slightly less. Secondly, the ratios of mono-, di- and tri-chloroacetic acids were different. In Australian waters the mono-chloro species was the most abundant, while the di- and tri-chloro species were low. In the US, the mono-chloro species is generally lowest and the di- and tri-chloro species are higher.

The survey was based on only three samples per water system, taken mainly during the warmer months, and during discussions the analytical difficulties associated with measurement of the mono-halogenated species were raised. However if these observations can be confirmed, this would suggest that there may be significant differences in the nature of NOM compared to North American and European supplies. This may be related to the unique native vegetation (eg eucalypts) in catchments. Such differences would present an opportunity to examine DBP exposure scenarios that contrast with those under study in other countries.

- **Many unfiltered surface water supplies**
  Many surface water supplies in Australia are treated only by disinfection, so NOM levels are likely to be generally higher than in countries where filtration is more widely practiced. Lack of filtration contributes to higher DBP levels.

- **Long detention times in reservoirs**
  Rainfall patterns in Australia are very variable, and prolonged droughts are not uncommon. As a consequence, many large water storages have been built, some with average detention times of several years. This may affect the quality of NOM and could potentially impact on DBP formation.

- **Above ground pipelines at high temperatures**
  In several states water is transported via above ground pipelines for several hundred kilometres to reach remote communities. Water temperatures at the end of such pipelines may exceed 50°C. Chloramine is generally used as the disinfectant in these systems, and DBP levels are high due to the high temperature and prolonged contact time.

- **Long distribution systems**
  Australian cities tend to have a greater geographic spread than centres of equivalent population size in North America or Europe. Thus distribution systems are long, and supplementary disinfection points may be used to maintain chlorine residuals. This may result in different DBP exposure profiles in different geographic areas of one city.

- **Algal blooms in storage/streams**
  Algal blooms are a common occurrence in the warmer regions of Australia. These blooms will contribute to the NOM component, perhaps including intracellular NOM fractions. This may influence the nature of DBPs formed.

- **Rapid changes in water quality**
  Extreme weather events (eg rapid filling of reservoirs after a prolonged dry spell) can lead to rapid changes in NOM content and other parameters such as temperature and turbidity that affect DBP formation.

  Many Australian systems use multiple water sources, changing or blending them on a seasonal basis. This also is likely to affect NOM content and DBP formation.

- **Relatively high winter temperatures**
  The reticulation system is relatively shallow in Australian cities (about 0.6 metres below ground level) so seasonal changes in water temperatures tend to be greater than in countries where mains are buried deeper to avoid freezing in winter (for example in Canada water mains are about 3.0 metres below ground level). The generally warmer water temperatures and lack of freezing in winter are likely to permit higher activity of biofilms all year round. This may affect DBP formation and degradation.

- **Higher fluid consumption per capita**
  Some regions of Australia are warm all year round, and even the cooler regions experience warm summers with maximum temperatures exceeding 40°C. Average fluid intakes are therefore likely to be higher than in many North American and European countries.

- **Higher exposure from swimming pools, bathing, frequent showering**
  Australia's climate leads to a high rate of use of public and private swimming pools, in addition to ocean swimming. The hot weather also results in more frequent showering and bathing than in colder climates.

  In some states concerns over growth of *Naegleria fowleri* requires high levels of disinfection in swimming pools, and in some instances brominated chemicals are used.

  These differences may present particularly high exposure scenarios for some groups in the population.

- **Bromide-rich waters**
  Elevated bromide levels are not uncommon in Australian groundwaters, and elevated levels also occur in some Australian surface water supplies, perhaps due to land salinity in catchments. Thus relatively high exposure scenarios for brominated DBP species are likely to exist here.

- **Uncommon methods of water treatment**
  The Miex® treatment system using magnetic ion exchange resin was developed in Australia. This treatment method is now beginning to be applied in drinking water supplies in some states. Miex® removes large molecular weight and highly charged DOC, and thus is likely to alter the profile of DBPs generated by subsequent disinfection. A project to characterise these changes is currently underway at Curtin University of Technology.

  Systems using Miex® may present atypical exposure scenarios for study.

3. Aspects of DBP issues that may be common to all, but are not being addressed elsewhere and may be important to the Australian industry

- **Importance of disinfectant residual in the distribution system**
  Disinfectant residuals in distribution systems are advocated as means of providing some protection against pathogenic microbes that may enter the system, however there is legitimate debate over the degree of health protection that is actually conferred by this practice. Maintenance of residuals contributes to higher DBP formation in the distribution system. Delivery of water containing free chlorine to consumers will also result in DBP formation whenever the water contacts foodstuffs – this is an unexplored area of exposure that may be significant.

  Taste and odour problems associated with high disinfectant residuals are one of the most common sources of consumer complaints to water suppliers. The question needs to be asked whether the residual taste and its consumer aesthetics implications, and impact on DBP levels are worth the benefit for the limited microbial protection provided.

4. Aspects of DBP issues that Australia may be uniquely suited to research

- **Unique exposure scenarios**
  As mentioned under Topic 2 above, it appears that Australian water supplies may have substantially different DBP exposure profiles than those found in North America and Europe. This offers the prospect of examining different exposure scenarios.

- **CRC research partnership**
  The CRC provides an umbrella organisation bringing together public health researchers and the water industry that has few parallels internationally. This provides excellent opportunities for collaborative research. The CRC also has good relationships with government regulatory bodies, ensuring effective communication of research knowledge into regulatory activities.

D RESEARCH STRATEGY – POTENTIAL PROJECTS

1. Knowledge and attitudes of public and health professionals

A survey is suggested to obtain an understanding of the public and health professionals' views on what is the nature of the health risks for DBPs. Specific target groups could include general practitioners, nurses, pharmacists, local council environmental health officers etc.

The findings of this survey would be used together with the current state of knowledge on DBPs to develop a critical primer to assist water authorities and health regulators. Delivery of the findings needs to be done through the public health community to have credibility with the public.

This project could be part of a broader study on knowledge and understanding of water quality issues, and development of information material to raise levels of knowledge.

2. Risk assessment based on current exposure data

The existing data on DBP levels in Australian water supplies should be assessed to judge the relative importance of the high levels observed for regulated classes of DBPs (ie are numbers driven by chloroform, TCAA and other DBPs which are unimportant from a health viewpoint).

3. National survey of exposure under the "Bull" model

An updated national survey is required to classify exposures relative to the model presented by Dr Bull as representing the parameters most relevant to different types of health effects (eg pH, Br, nitrogen, NOM). This could be done in two stages – initially by collection and collation of existing data, followed by targeted sampling to identify contrasting exposure scenarios. The second stage will require the establishment of some new laboratory methods with appropriate quality control, availability of standards etc.

Parameters to be assessed should include:

- DBP measurements – only THM and HAA data are likely to be available for most supplies, therefore other DBPs of interest will need to be measured at the sampling stage. NDMA, nitrosamines and/or nitrosamine forming capacity should be included.
- Disinfectant type, dose, C.t values etc
- Raw and finished water parameters relevant to DBP formation (pH, Br, etc)
- Characteristics of NOM – a range of techniques for characterising NOM are already established within the CRC
- Catchment characteristics – particularly land use, vegetation types, significant sources of animal or human waste.
- Storage and distribution system characteristics.
- Effects of alternative disinfectants – systems with chloramine, UV etc should be included.

The outcomes of this survey should enable the identification of contrasting exposure scenarios with respect to the model. It may also be possible to determine which characteristics of NOM are most important in determining the properties of the DBP mix under this model. Epidemiological studies could then be targeted to appropriate sites to examine the effects of these contrasts.

4. Natural experiment ecological studies

Changes in water treatment methods may provide an opportunity to examine the effects of large changes in DBP exposure by doing 'before and after' studies or comparing locations where one has changed water treatment while the other has continued unchanged. However these are the lowest grade of epidemiological study, and caution is needed in interpreting the findings.

5. Exposure assessment studies

There are a large number of possible studies that could contribute to improved exposure assessment for DBPs:
Next generation TCAA biomarker trial – Professor Hrudey's group at the University of Alberta is planning a larger scale trial in women of childbearing age. This will provide insight into the practical utility of urinary TCAA as a biomarker with a general community group (previous trials have been done with research laboratory personnel as volunteers).

Individual exposure patterns – there is little information on variations in water consumption, showering and swimming behaviours in the Australian population.

DBP exposures from other foods and beverages – very little is known about this. Preliminary work has already shown that TCAA is formed when coffee is made from chlorinated water (K Froese, unpublished observations).

Variability of household samples versus. routine monitoring locations – epidemiological studies have used routinely collected DBP data for exposure assessment. Individual exposure levels have been inferred from the annual average DBP level for the water quality zone or the nearest sampling tap to the residential address. A study could be carried out to test the validity of this assumption by assessing exposure by frequent household sampling versus routine monitoring results.

Assessment of high exposure groups – people who swim in chlorinated pools or use spas frequently are obvious high exposure groups.

Integrated exposure measurement – is there scope for integrated exposure measurement (eg composite samples for THMs), rather than individual measurement?

6. Reproductive outcomes

Preliminary work on the feasibility of a study on reproductive outcomes should be undertaken. Potential types of studies include:

- Registry-based study of congenital defects
- Registry-based study of birth weight/preterm delivery measures
- Prospective study of birth weight/preterm delivery measures

7. Cancer outcomes

We need to assess whether there is any value in undertaking cancer studies in Australia in addition to those already underway overseas.

8. Genetic variability

Work on characterisation of the genetic polymorphisms of the glutathione S-transferase enzyme family has been carried out by an Australian group in Canberra. There may be possibilities for collaborative work on genetic susceptibility.

9. Toxicology

Depending on the scale of exposure to brominated compounds determined from the survey described above, it may be of value to undertake some basic toxicological characterisation of selected compounds.

10. The disinfectant residual question

What is the real value of distribution system disinfectant residual for protection of public health? This question needs to be seriously examined and debated. Are there research projects that can address this issue?

FURTHER DEVELOPMENT OF THE RESEARCH AGENDA

This workshop has suggested some avenues for future research by the CRC. In considering which of these to incorporate into an overall research plan on DBPs and health effects, the following selection criteria are of relevance:

- capability/feasibility of carrying out the research within the CRC
- linkage with other CRC programs and projects (eg Water Treatment Technology)
- priority in the Australian context
- strategic value to the water industry
- ability to bring useful or significant advance (perhaps even closure) to an issue
- improvements and refinements on approaches taken elsewhere
- building on existing strengths/capacity within the CRC
- cost and benefits of the project
- possibilities for leveraging, synergy and collaboration on a national or international scale.

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CRC for Water Quality and Treatment

CRC AUSTRALIA

The Cooperative Research Centre (CRC) for Water Quality and Treatment is Australia's national drinking water research centre. An unincorporated joint venture between 29 different organisations from the Australian water industry, major universities, CSIRO, and local and state governments, the CRC combines expertise in water quality and public health.

The CRC for Water Quality and Treatment is established and supported under the Federal Government's Cooperative Research Centres Program.

The Cooperative Research Centre for Water Quality and Treatment is an unincorporated joint venture between:

- ACTEW Corporation
- Australian Water Quality Centre
- Australian Water Services Pty Ltd
- Brisbane City Council
- Centre for Appropriate Technology Inc
- City West Water Ltd
- CSIRO
- Curtin University of Technology
- Department of Human Services Victoria
- Environmental Protection Agency Queensland
- Griffith University
- Melbourne Water Corporation
- Monash University
- Orica Australia Pty Ltd
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- Water Corporation
- Water Services Association of Australia
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