

EVALUATING EVIDENCE FOR ASSOCIATION OF HUMAN BLADDER CANCER WITH DRINKING-WATER CHLORINATION DISINFECTION BY-PRODUCTS

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Exposure to chlorination disinfection by-products (CxDBPs) is prevalent in populations using chlorination-based methods to disinfect public water supplies. Multifaceted research has been directed for decades to identify, characterize, and understand the toxicology of these compounds, control and minimize their formation, and conduct epidemiologic studies related to exposure. Urinary bladder cancer has been the health risk most consistently associated with CxDBPs in epidemiologic studies. An international workshop was held to (1) discuss the qualitative strengths and limitations that inform the association between bladder cancer and CxDBPs in the context of possible causation, (2) identify knowledge gaps for this topic in relation to chlorine/chloramine-based disinfection practice(s) in the United States, and (3) assess the evidence for informing risk management. Epidemiological evidence linking exposures to CxDBPs in drinking water to human bladder cancer risk provides insight into causality. However, because of imprecise, inaccurate, or incomplete estimation of CxDBPs levels in epidemiologic studies, translation from hazard identification directly to risk management and regulatory policy for CxDBPs can be challenging. Quantitative risk estimates derived from toxicological risk assessment for CxDBPs currently cannot be reconciled with those from epidemiologic studies, notwithstanding the complexities involved, making regulatory interpretation difficult. Evidence presented here has both strengths and limitations that require additional studies to resolve and improve the understanding of exposure response relationships. Replication of epidemiologic findings in independent populations with further elaboration of exposure assessment is needed to strengthen the knowledge base needed to better inform effective regulatory approaches.

BACKGROUND

Disinfection by-products (DBPs) in drinking water were discovered more than 40 years ago

(Rook 1974; Bellar et al. 1974) in the form of trihalomethanes (THMs), including chloroform, bromodichloromethane (BDCM), dibromochloromethane (DBCM), and bromoform.

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This transformational discovery was followed by findings that chloroform was carcinogenic in rodents (National Cancer Institute [NCI] 1976), leading to the banning of chloroform use in a wide range of consumer products such as cough syrups, antihistamines, decongestants, and cosmetics (National Toxicology Program [NTP] 2013). The first THM regulatory initiative under the 1974 Safe Drinking Water Act appeared in 1979, specifying a limit of 100 $\mu\text{g/L}$ for a mass concentration sum of the 4 THMs (THM4) as a running annual average of 4 quarterly samples, citing a concern for long-term cancer risk (U.S. Environmental Protection Agency [EPA] 1979). Crump and Guess (1982) summarized the early epidemiologic evidence exploring chlorinated drinking water and cancer risk.

Following the initial discovery of THMs in drinking water, more than 600 new DBPs with varying degrees of toxicity and potential cancer risk have been discovered (Richardson et al. 2007; 2008). DBPs are produced to some degree by any chemical disinfection process, but those produced by disinfection processes involving chlorine, including chloramination, attracted the most attention. In this review, DBPs produced by disinfection processes involving chlorine (excluding chlorine dioxide) are collectively termed chlorination DBPs (CxDBPs) because these chemicals are formed by chlorine-based disinfectants. Although chloramination is known to yield lower THM4 production, the impact is highly dependent on free chlorine contact time before ammonia addition and overall water quality such as levels of total organic carbon and/or bromide. CxDBPs do not necessarily contain chlorine and include non-halogenated nitrogenous DBPs like nitrosamines.

Numerous cancer sites have also been evaluated for association with chlorinated drinking water (Mills et al. 1998), including two IARC reviews of the evidence for the following specific CxDBPs: chloramines, chloral, chloral hydrate, dichloroacetic acid, trichloroacetic acid, and MX (IARC 2004), and bromochloroacetic acid, dibromoacetic acid, and dibromoacetonitrile (IARC 2013). To date, epidemiologic evidence related to

urinary bladder cancer exhibited the greatest consistency (U.S. EPA, 2006a), and bladder cancer was a key aspect of the cost–benefit analysis for the Stage 2 DBP Rule (U.S. EPA 2006b).

PUBLIC HEALTH RELEVANCE OF BLADDER CANCER IN THE UNITED STATES

Bladder cancer is an important public health issue, causing the fifth highest number of estimated new U.S. cases of cancer in 2015 (American Cancer Society 2015) for both genders, accounting for about 74,000 new cases out of about 1.66 million new cases for all cancer sites. Bladder cancer accounted for approximately 16,000 estimated deaths out of the total of about 590,000 U.S. deaths for all cancer sites in 2015. The risk for bladder cancer rises exponentially with age over 40 yr. The U.S. median age at diagnosis is 73 yr, with 91% of new cases incident after age 55, 72% after 65, and 45% after 75 (SEER 2015). In the United States, bladder cancer is currently threefold more common in men than women.

Risk factors for bladder cancer include exposures to tobacco smoke, occupational chemicals, certain cancer treatments including drugs such as cyclophosphamide, radiation therapy to the abdomen or pelvis, and arsenic in drinking water. Other risk factors are listed as family history of bladder cancer and personal history of bladder cancer for possible recurrence (NCI 2004).

OBJECTIVES

The Water Research Foundation and the American Water Works Association commissioned an interdisciplinary panel review to evaluate the scientific evidence concerning the association of chlorination disinfection by-products (CxDBPs) and human bladder cancer. The panel was asked to address three fundamental questions regarding DBPs:

1. What are the qualitative strengths and limitations of the evidence for known and/or unidentified CxDBPs that inform causality of human urinary bladder cancer?

2. What critical knowledge gaps exist for explaining human urinary bladder cancer risk in relation to drinking water chlorine and/or chloramine-based disinfection practice(s) in the United States?
3. Does the available evidence support a quantitative and qualitative analysis of CxDBP risk management strategies that will be more accurate and informative for guiding risk management than previously possible with evidence available for developing the current Stage 2 DBP Rule?

The primary outcome of the expert panel workshop was an evidence review, published as a Water Research Foundation research report that incorporated inputs from the workshop, with a comprehensive summary of supporting evidence, what knowledge is lacking, and a summary of viable options for gaining the missing evidence (Interdisciplinary Expert Panel 2015).

METHODS

A draft background paper was prepared by Hrudehy, outlining the issues and available evidence addressing the objectives of the project, and was provided to the expert panel (Backer, Hrudehy, Humpage, Krasner, Michaud, Moore, Singer, and Stanford) electronically 10 days before an in-person, 2-day workshop, March 24 and 25, 2014, in Washington, DC. The background paper for the panel workshop was developed from a comprehensive literature search of major databases including Web of Science, Medline, EMBASE, Global Health, Toxline, Pollution Abstracts, Water Resource Abstracts, Cochrane Library, BIOSIS previews, and Scopus. Manual searching of bibliographies of the relevant papers identified was performed. Tracking citations of major relevant papers using Web of Science was also pursued. In addition to the literature search results, the background paper review was built upon and compared with coverage from several earlier reviews that included some of the objective topics (Hrudehy 2008; Hrudehy et al. 2003; IARC 2004; 2013; Mills et al. 1998; Sinclair et al. 2002; U.S. EPA 2006). The evidence available

to deliberations for the Stage 2 DBP Rule was briefly reviewed and taken as the starting point for reviewing new, relevant evidence. This provides the framework for this current paper.

Classification of exposure to CxDBPs for epidemiologic studies was considered in some detail because of the inherent challenges posed by estimating CxDBP exposures among individuals over several decades to address an estimated bladder cancer latency period, which typically exceeds 30 years. A summary of exposure assessment challenges for drinking water epidemiology was provided in Table 3 of the paper by Villanueva et al. (2014). Several aspects of estimating DBP exposures were previously suggested by Arbuckle et al. (2002), which generated numerous recommendations for improved assessment that might be useful for short-term DBP exposures mostly meaningful for studying effects like adverse reproductive outcomes. Arbuckle et al. (2002) suggested few practical options, beyond improved DBP occurrence models, for enhancing long-term, retrospective exposure assessments required for cancer epidemiology.

As long as associations are large enough to be demonstrated, epidemiologic study results suffering from exposure misclassification or quantitative inaccuracy can still provide useful evidence toward an evaluation of consistency of association and potentially for informing causal inference. However, from the perspective of those who must regulate exposures to minimize potential health risks in drinking water, quantitatively inaccurate exposure assessment has serious implications if used for quantitative risk assessment to inform regulation. If true DBP exposures are under- or overestimated, the level of CxDBPs associated with elevated cancer risk may not accurately reflect the actual exposure levels associated with the health outcome. These issues are developed further in the following for the purposes of interpreting epidemiologic studies in our discussion of Amy et al. (2006). Further, for the purposes of supporting regulation, the most common quantitative measures of DBP exposure in epidemiologic studies may only be a surrogate that correlates with an

unknown causal agent(s) (e.g., THM4 serving as a surrogate for unknowns). Therefore, without developing further knowledge, it is unclear whether mitigation measures, such as treatment for a surrogate marker, will reduce exposures to causal agent(s) when the strength of correlation between them and/or relative amenability to removal may vary. For example, if the focus is on brominated compounds and THM4 is a surrogate, many of the bromine-containing DBPs will have different amenability to formation or removal than THM4, which is primarily chloroform. Conventional coagulation and advanced treatment processes such as granular activated carbon remove total organic carbon but not bromide (Summers et al. 1993; Watson et al. 2015), so the proportion of brominated DBPs (brominated THMs, brominated HAAs, etc.) might increase even though total THM4 may be reduced. If the focus is on HAAs, removal of the semivolatile THM4 by aeration may not accurately represent removal of the nonvolatile HAAs because they have different physical-chemical properties. In either illustration, the behavior through treatment processes of brominated DBPs or HAAs might not be accurately represented by class sums like THM4.

RESULTS AND DISCUSSION

Epidemiologic Evidence

Previous Studies and Findings Published (1974–2004) The U.S. EPA relied primarily upon five incident case-control studies focused on bladder cancer for assessing the health benefits of the Stage 1 DBP Rule (Cantor et al. 1987; 1998; McGeehin et al. 1993; King and Marrett 1996; Freedman et al. 1997). For the final Stage 2 DBP Rule health benefits assessment, the U.S. EPA (2006) also included consideration of a meta-analysis by Villanueva et al. (2003a) performed on six case-control studies (Cantor et al. 1987; 1998; McGeehin et al. 1993; Vena et al. 1993; King and Marrett 1996; Koivusalo et al. 1998) and two cohort studies (Wilkins and Comstock 1981; Doyle et al. 1997), and a pooled analysis by Villanueva et al. (2004) that included six case-control studies (Lynch et al.

1989; Cordier et al. 1993; King and Marrett 1996; Cantor et al. 1998; Koivusalo et al. 1998; Porru 2003 [an unpublished Italian study]).

The U.S. EPA (2006a) determined for its evidence review for setting the Stage 2 DBP Rule:

Based on a collective evaluation of both the human epidemiology and animal toxicology data on cancer and reproductive and developmental health effects discussed below and in consideration of the large number of people exposed to chlorinated byproducts in drinking water (more than 260 million), EPA concludes that (1) new cancer data since Stage 1 strengthen the evidence of a potential association of chlorinated water with bladder cancer and suggests an association for colon and rectal cancers, (2) current reproductive and developmental health effects data do not support a conclusion at this time as to whether exposure to chlorinated drinking water or disinfection byproducts causes adverse developmental or reproductive health effects, but do support a potential health concern, and (3) the combined health data indicate a need for public health protection beyond that provided by the Stage 1 DBPR.

This finding was expanded to explain:

Human epidemiology studies and animal toxicology studies have examined associations between chlorinated drinking water or DBPs and cancer. While EPA cannot conclude there is a causal link between exposure to chlorinated surface water and cancer, EPA believes that the available research indicates a potential association between bladder cancer and exposure to chlorinated drinking water or DBPs.

The U.S. EPA concluded: “Overall, bladder cancer data provide the strongest basis for quantifying cancer risks from DBPs.” These findings are reasonable for the evidence base that was available for precautionary regulatory purposes.

In performing its review of the epidemiologic data, the U.S. EPA referred to causal criteria, including consistency, strength and specificity of association, temporality (exposure precedes outcome), a biological gradient (dose-response relationship), biological plausibility, and coherence with multiple lines of evidence—criteria that can be traced to those originally proposed by Bradford-Hill (1965). In addition, the U.S. EPA also considered the reliability of exposure data, statistical

power and significance, and freedom from bias and confounding. These factors and criteria were considered in our following review of the more recent epidemiologic evidence for its strengths and limitations.

Recent Studies (Years 2004–2014) Our panel focused primarily on evidence that became available after publication of studies just cited. Amy et al. (2006) performed an updated exposure assessment for two of the previous studies (Cantor et al. 1998; King and Marrett 1996) by applying expert knowledge regarding formation of CxDBPs to use available water quality and DBP data to better model past exposures, improve exposure assessment for THMs, and add estimates for selected CxDBPs. Amy et al. (2006) noted that exposure misclassification is inevitable in studies that attempt to estimate exposures that occurred decades earlier, but that such misclassification may likely be nondifferential (i.e., no difference between cases and controls) unless an unrecognized bias has occurred.

Amy et al. (2006) expanded the scope of the exposure assessment, making better-informed predictions of historical THM4 occurrence for each of these studies, based on greater water quality detail from accessible site-specific monitoring databases. These approaches provided for significantly more discrimination at higher estimated exposures in the Cantor et al. (1998) study. In the original Cantor et al. (1998) investigation, the assigned maximal average THM4 exposure was 74 $\mu\text{g/L}$, whereas in the reanalysis the maximal lifetime average exposure was 154 $\mu\text{g/L}$. In the historical monitoring database, which was collected following the 1979 THM rule, the 75th and 95th percentile and maximum THM4 concentrations were 46, 96, and 219 $\mu\text{g/L}$, respectively (Amy et al. 2006). In the original analysis, the odds ratio (OR) values were significant for the two highest assigned exposure categories, which included the 90th and 95th percentiles (32.6–46.3 $\mu\text{g/L}$ and >46.4 $\mu\text{g/L}$) for males. However, using the same cutoff points for the categories, but based on more detailed and better-informed exposure estimates, only the highest category (>46.4 $\mu\text{g/L}$) remained statistically significant (Table 1).

In the reanalysis that provided a new distribution of exposure, the 97.5th percentile (exposure categories were assigned for defined percentiles of the controls) of lifetime average THM4 was >96.1 $\mu\text{g/L}$ (Table 2), and it was only at this level that there was a significant OR. By comparison, as noted earlier, in the reanalysis based on the original cutoff points of exposure (Table 1), only lifetime average THM4 exposure above 46.4 $\mu\text{g/L}$ showed a significant OR. This is an important distinction, given that the current U.S. regulatory level for THM4 is a maximum locational annual average of 80 $\mu\text{g/L}$ and there is an operational target¹ THM4 average of 80% of that limit, that is, 64 $\mu\text{g/L}$ (Roberson et al. 1995). The reclassification in Table 2 resulted in a wider range for estimated exposure, which, being based on the monitoring database, is more representative of the true THM4 exposure. The cutoff points used in Table 2 resulted in a smaller number of cases in the higher exposure categories, which also led to more imprecision in the point estimates for these categories.

The Amy et al. (2006) reanalysis of the King and Marrett (1996) study demonstrated lower bladder cancer risks, with wider confidence intervals (CI), and without a consistent exposure-response pattern for THM4 exposure (Table 3). These data are for men and women. Unlike Cantor et al. (1998), women showed higher bladder cancer risk than men in King and Marrett (1996).

Four “newer” studies include Chevrier et al. (2004), Villanueva et al. (2006, 2007), Bove et al. (2007), and Cantor et al. (2010). Chevrier et al. (2004) performed a somewhat unique study addressing bladder cancer because they assessed ozone disinfection, either alone or in combination with chlorination, an important distinction because ozone produces substantially different DBPs than chlorination. They used data from an incident, hospital-based, case-control study in France performed almost

¹An operational target is a value that governs normal operations of the water treatment plant so this is the highest concentration of THM4 that any treatment plant should normally produce.

TABLE 1. Comparison of Risk Estimates (ORs) of Bladder Cancer for Males in Cantor et al. (1998): Original Analysis Versus Reanalysis (Amy et al. 2006)

Lifetime average THM4*	Cases/controls	Original analysis	Cases/controls	Reanalysis
≤0.7	269/501	1.0 (reference)	347/592	1.0 (reference)
0.8–2.2	244/314	1.27 (1.00–1.60)	107/157	1.02 (0.80–1.40)
2.3–8.0	123/188	1.14 (0.85–1.50)	156/212	1.09 (0.80–1.40)
8.1–32.5	133/194	1.11 (0.80–1.50)	150/230	1.02 (0.80–1.30)
32.6–46.3	53/54	1.67 (1.10–2.60)	24/28	1.32 (0.70–2.40)
≥46.4	53/57	1.53 (1.00–2.40)	83/85	1.46 (1.03–2.10)

*Exposure categories based on 35th, 60th, 75th, 90th, and 95th percentiles in distribution among controls of lifetime average THM4 as estimated in the original analysis.

TABLE 2. Risk Estimates (ORs) of Bladder Cancer for Males in Cantor et al. (1998): New Exposure Categories in Reanalysis (Amy et al. 2006)

Lifetime average (50 yr) THM4*	Cases/controls	Reanalysis
≤0.5	294/510	1.0 (reference)
0.6–8.4	341/442	1.13 (0.91–1.40)
8.4–36.1	179/264	1.04 (0.80–1.30)
36.2–58.0	38/47	1.15 (0.70–1.80)
58.1–96.1	46/51	1.31 (0.80–2.10)
>96.1	30/26	1.80 (1.02–3.20)

*Exposure categories based on 35th, 70th, 90th, 93.75th, and 97.5th percentiles in distribution among controls.

20 years earlier (1985–1987). Some important qualifiers noted by Chevrier et al. (2004) included having a “high” percentage exclusion (63%) from the initial sample as a result of missing data, a “modest” total sample size (281 cases, 240 male, 41 female), and a need for more detailed knowledge of the characteristics of raw water before their findings might be generalized to any type of water source. However, Chevrier et al. (2004) yielded some intriguing results with small case numbers (total available for all exposures of 281), suggesting that exposure to ozonated water

(91 cases) exhibited a lower bladder cancer risk than exposure to chlorinated water (112 cases).

Bove et al. (2007) published a case-control study based on data originally reported by Vena et al. (1993) for cases and controls collected between 1979 and 1985, which reported only fluid consumption data. Residential address was used to estimate individual THM4 exposure levels. Bove et al. (2007) found significant associations for three of four THMs (chloroform, bromodichloromethane, and bromoform), but given the low relative carcinogenic potency in animal studies of these THMs and low THM exposure levels measured, these agents do not provide plausible explanation of the cancer risk observed in this study. The main limitations of Bove et al. (2007) are the small sample size, limited explanation of how the retrospective exposure assessment was conducted, and difficulty in reconciling OR observations for bromoform (except as a surrogate for uncharacterized brominated DBPs) and chloroform with what is known regarding their low cancer risks.

A series of papers by Villanueva and colleagues (2003b; 2006; 2007) was published on a comparatively large (1219 cases, 1271

TABLE 3. Comparison of Risk Estimates (ORs) of Bladder Cancer for King and Marrett (1996): Original Analysis Versus Reanalysis (Amy et al. 2006)

Duration of exposure	Cases/controls	Original analysis, peak THM4 >50 µg/L	Cases/controls	Reanalysis, mean THM4 >40 µg/L
≤10 years	253/650	1.0 (reference)	593/1310	1.0 (reference)
10–19 years	226/519	1.10 (0.87–1.38)	23/51	1.21 (0.71–2.05)
20–34 years	163/297	1.36 (1.05–1.76)	30/68	1.01 (0.63–1.60)
≥35 years	54/79	1.63 (1.08–2.46)	43/65	1.36 (0.90–2.07)

controls) hospital-based incident case-control study on bladder cancer (1998–2001), also referred to here as the Spanish Bladder Cancer Study (SBCS). This study provided a number of important advances, including additional exposure assessment, explicit consideration of inhalation and bathing exposure, and inclusion of some water supplies with high proportions of brominated THM species.

In the SBCS exposure assessment, 200 local authorities and 150 water companies were contacted to obtain relevant data. Annual average THM4 levels and water source history since 1920 were determined and/or estimated for 78.5% of the total study person-years of exposure. New THM4 data were obtained for 113 samples between September and December 1999 to cover the geographic regions in the study (Villanueva et al. 2003b). Historical THM4 levels were assumed to remain unchanged for treated water from a given water source, and, on this basis, THM4 exposures were extrapolated back to 1920. The levels of THM4 exposure presented for many of the chlorinated water supplies are likely to be a serious underestimation. Actual THM4 exposures were most likely higher before the discovery of THMs in chlorinated drinking water and their reported adverse health effects, findings that led to lowering of THM4 concentrations to satisfy new drinking-water guidance (Health and Welfare Canada 1978; U.S. EPA 1979; World Health Organization [WHO] 1984) from the early 1980s followed by a European Union [EU] Directive in 1998 (EU 1998).

A major finding of this study was a clear, significant association of male bladder cancer with estimated average residential THM4, expressed as micrograms per liter (Table 4). The evidence of an association based on average exposure of THM4 via ingestion, expressed as micrograms per day, for men did not exhibit a stronger association than was found when using THM4 concentration or duration of exposure to chlorinated drinking water. Showering/bathing duration data, weighted by average residential THM4 level (min/d × µg/L), also produced significant exposure-related increasing OR for men.

TABLE 4. Bladder Cancer Odds Ratio in Relation to Residential THM4 Exposure (Villanueva et al. 2007)

Average THM measure	Cases/controls	Odds ratio (95% CI)	<i>p</i> Trend
≤8 µg/L	137/172	1.0 (reference)	
>8.0–26.0 µg/L	140/158	1.53 (0.95–2.48)	
>26.0–49 µg/L	183/160	2.34 (1.36–4.03)	
>49 µg/L	158/180	2.53 (1.23–5.20)	<i>p</i> < .01

Because the toxicological evidence does not support an association between cancer risk and chloroform at or below current U.S. regulatory THM4 levels from drinking water ingestion (Bull et al. 1986; Butterworth and Bogdanffy 1999; U.S. EPA 2000), the possible carcinogenic contributions from brominated THMs may be critical if THMs are to be considered important with respect to cancer risk. Moreover, in waters with a majority of brominated THMs, there will be higher levels of brominated species of other classes of DBPs (Krasner et al. 2006). Further, for U.S. cities with moderate (~0.1 mg/L) to high (~0.5 mg/L) levels of bromide in their source waters (Amy et al. 1994), the proportion of bromine species is generally not as high as that detected in certain parts of Spain (e.g., 0.5–1.2 mg/L in the Llobregat River, a major source of water for Barcelona, Spain; GE Power & Water 2010) because the bromide concentrations in the United States are typically lower than those found in certain cities in Spain that were used in Villanueva et al. (2007). In Barcelona, Alicante, and Tenerife, Spain, the weight percentages of THM4 containing bromine were 69, 84, and 95%, respectively, and weight percentages of the sum of 9 HAAs containing bromine were 61, 55, and 79%, respectively (Villanueva et al. 2007). The average concentration of dibromoacetic acid in these three Spanish cities was 6.5, 5.2, and 1.3 µg/L, in contrast to a major U.S. nationwide survey (McGuire et al. 2002) in which the median occurrence of dibromoacetic acid was zero. Further, many high-bromide waters in the United States use chloramines as a secondary disinfectant, whereas free chlorine is still the final disinfectant in Spain. Because of these factors, much of the THM4 occurrence in

some of the Spanish cities exceeds typical post-Stage 2 DBP Rule THM4 levels in the United States.

Genotyping Applied to Epidemiologic Studies

Cantor et al. (2010) reported one of the first evaluations of THM exposure and bladder cancer risk to determine whether susceptible subpopulations could be identified by nine polymorphic variants in five genes using exposure data from the SBCS case-control study. Genes that encode these enzymes are polymorphic in human populations, resulting in modified enzyme activity. Comparatively, bladder cancer risk might be higher or lower among a subgroup of individuals carrying a certain genotype upon exposure to CxDBPs when compared to the group lacking this genotype. Variants in *NAT2*, *GSTM1*, *GSTT1*, *GSTZ1*, and *CYP2E1* were selected for identification of susceptible subpopulations in this study using current knowledge of their role in CxDBPs metabolism, bladder cancer risk, and variant allele prevalence among population controls. Cantor et al. (2010) used all exposure assessment data and exposure modeling previously reported by Villanueva et al. (2007) among a subset of subjects (680 cases/714 controls) who were successfully genotyped.

The conceptual model guiding selection of *GSTT1* as one of the genes that may contribute to variability in the susceptibility to bladder cancer evaluation undertaken by Cantor et al. (2010) was based on brominated THMs being mutagenic, carcinogenic, and prevalent in chlorinated drinking water containing bromide. Briefly, this concept (Richardson et al. 2007) focused on dermal absorption or inhalation, whereby these CxDBPs escape first-pass hepatic metabolism and reach target tissues in the urinary tract where *GSTT1*-mediated activation may be favored over other metabolism (Landi et al. 1999; Ross and Pegram 2003; 2004). Leavens et al. (2007) showed that dermal exposure to bromodichloromethane (BDCM) resulted in blood levels 25- to 130-fold higher than those from oral exposure.

GSTT1 metabolizes small halogenated compounds, among many others, and *GSTT1* conjugation by the active variant is required for bio-activation into reactive metabolites (Ginsberg et al. 2009).

Considering only the THMs, the concepts proposed for this model suggest that dermal and inhalation exposure routes for DBP exposure may result in higher exposures of urinary-tract target tissue than exposure via ingestion. This finding is consistent with Villanueva et al. (2007), who found that a stronger association was observed with estimated individual residential THM4 concentration ($\mu\text{g/L}$) than with individual ingestion of THM4 ($\mu\text{g/d}$) as estimated by volume consumed multiplied by tap-water THM4 concentration.

In the subset of genotyped individuals from the SBCS in Cantor et al. (2010), bladder cancer risks for average THM level (Table 5) were similar to those reported in the original study (Villanueva et al. 2007). Main effects for genotypes demonstrated elevated bladder cancer risk among subjects carrying *GSTM1* null versus present, *GSTT1* active versus null, and *NAT2* slow versus fast acetylator genotypes. After consideration of THM exposure (Table 6), significant bladder cancer risks increased with exposure only among subjects with the *GSTT1*² active genotype, the *CYP2E1*³ rs2031920 CC, and the *GSTZ1*⁴ rs1046428 CT/TT groups. The rs2031920 C>T transition, also referred to as the *CYP2E*5B* allele, is associated with enhanced gene transcription that would promote activation of low-molecular-weight compounds and procarcinogens to reactive intermediates (Wang et al. 2009). In contrast, the *GSTZ1T* allele has lower enzymatic activity compared to the C allele to conjugate and excrete dichloroacetic acid and related metabolites (Blackburn et al. 2001). No evident relationship between bladder cancer and THM exposure was observed among the low-risk variant groups (Table 6). In instances where gene-environment interactions were found, *p*

²<http://www.ncbi.nlm.nih.gov/gene/2952>

³<http://www.ncbi.nlm.nih.gov/gene/1571>

⁴<http://www.ncbi.nlm.nih.gov/gene/2954>

TABLE 5. Bladder Cancer Odds Ratio in Relation to Residential THM4 Exposure (Cantor et al. 2010 for Genotyped Cases, and Control Drawn From Villaneuva et al. 2007)

Average THM measure	Cases/controls	Odds ratio (95% CI)	<i>p</i> Trend
≤8 µg/L	156/175	1.0 (reference)	
>8.0–26.0 µg/L	153/174	1.2 (0.8–1.9)	
>26.0–49 µg/L	197/169	1.8 (1.1–2.9)	
>49 µg/L	174/196	1.8 (0.9–3.5)	.029

values for interactions were generally significant at the *p* < .05 level.

Cantor et al. (2010) found the most striking relationships with bladder cancer risk and THM4 among a subset of 195 cases and 192 controls with the *GSTT1* Present (active) and *GSTZ1* CT/TT genotypes. The subset of subjects with combined genotype is small and statistically underpowered for a study of gene–gene–exposure interaction. The large OR found, not surprisingly, attracted attention and commentary (Freeman 2010). If similar associations are replicated in other study populations,

these would indicate the presence of genetically susceptible subpopulations that have comparatively higher bladder cancer risk upon exposure to substances/compounds indicated by THM4 exposure levels. Polymorphisms in other pathways linked to metabolism of CxDBP and similar chlorinated hydrocarbons need to be included in such studies (e.g., cysteine beta-lyase; Moore et al. 2010). However, with the exception of the *GSTT1* enzyme, genotyping is not necessarily representative of functional enzymatic activity in specific tissues. Functional assays relating genetic variants with enzyme activity will also be required.

In summary, Cantor et al. (2010) provided some important new findings that variation in the genotypes of some enzymes involved in CxDBP metabolism may identify subpopulations of individuals exposed to CxDBPs (mostly brominated compounds) who may be susceptible to bladder cancer. Interpretation (and/or extrapolation) of this evidence needs to be done with caution until replicated in additional exposed populations.

TABLE 6. Bladder Cancer Odds Ratio in Relation to Residential THM4 Exposure (Cantor et al. 2010) for Specified Genotype Comparisons

Average THM4 measure	Odds ratio (95% CI) GSTT1 Active	<i>n</i> (cases/controls)	Odds ratio (95% CI) GSTT1 Null	<i>n</i> (cases/controls)	<i>p</i> Interaction
≤8 µg/L	1.0 (reference)	121/141	1.0 (reference)	34/34	
>8.0–26.0 µg/L	1.2 (0.7–1.9)	116/136	1.2 (0.5–2.5)	36/37	
>26.0–49 µg/L	2.0 (1.2–3.4)	160/126	1.2 (0.5–2.5)	37/41	
>49 µg/L	2.2 (1.1–4.3)	145/147	1.0 (0.4–2.5)	29/48	
<i>p</i> Trend	.0072		.28		.021
	<i>GSTZ1</i> rs1046428 CT/TT		<i>GSTZ1</i> rs1046428 CC		
≤8 µg/L	1.0 (reference)	52/62	1.0 (reference)	95/86	
>8.0–26.0 µg/L	1.4 (0.7–2.7)	47/54	1.1 (0.7–1.9)	100/102	
>26.0–49 µg/L	2.2 (1.1–4.2)	73/62	1.5 (0.9–2.7)	116/97	
>49 µg/L	2.9 (1.3–6.7)	72/64	1.3 (0.6–2.8)	94/117	
<i>p</i> Trend	.0043		.28		.018
	<i>CYP2E1</i> rs2031920 CC		<i>CYP2E1</i> rs2031920 CT/TT		
≤8 µg/L	1.0 (reference)	125/132	1.0 (reference)	15/9	
>8.0–26.0 µg/L	1.3 (0.8–2.0)	133/141	0.98 (0.4–2.5)	10/14	
>26.0–49 µg/L	2.1 (1.2–3.5)	176/134	1.1 (0.4–3.1)	9/11	
>49 µg/L	2.0 (1.0–4.1)	156/162	0.6 (0.1–2.7)	3/8	
<i>p</i> Trend	.0014		.33		.035

Secondary Studies

Salas et al. (2013) evaluated several other metrics of exposure based on the original data from the SBCS (Villanueva et al. 2007; Cantor et al. 2010). The proportion of THM4 that was brominated ranged from 35 to 84%, and an estimated overall average value, weighted according to the number of subjects reported for each geographic region, demonstrated that brominated THMs comprised almost 60% of THM4 across 4 geographic regions of the 5 analyzed for bladder cancer risk in Villanueva et al. (2007). Evidence indicated that the original THM4 comparisons, rather than any measure more specific to brominated DBPs, yielded the strongest exposure-response relationship with bladder cancer risk.

Costet et al. (2011) undertook a meta-analysis to explore whether there was any evidence of a different bladder cancer association with THMs for European versus North American studies. Despite a substantial contribution of the SBCS to the meta-analysis, data demonstrated no marked difference in bladder cancer risk for North American men in relation to European men. The North American studies involved brominated THM levels that are lower (e.g., Cantor et al. 1998: average THM4 for the Iowa surface waters studied was 56 $\mu\text{g/L}$, whereas the average sum of the bromine-containing THMs was only 10.4 $\mu\text{g/L}$, 19 wt%) than what was estimated in Villanueva et al. (2006; 2007: 35–95 wt% in 4 Spanish cities, including Asturias, which was low in bromide impact relative to other Spanish cities but still higher than in Iowa), such that the Costet et al. (2011) comparisons are not consistent with a mechanistic model based upon a major role for brominated THMs or other brominated compounds for which they may serve as surrogates.

Nieuwenhuijsen et al. (2009) provided a qualitative review of epidemiology and possible mechanisms for adverse human health outcomes that focused primarily on adverse reproductive outcomes. However, epidemiologic evidence (including Villanueva et al. [2007], but not Cantor et al. [2010]) indicated for drinking-water exposure, measured as

THMs, an “association” with bladder cancer that was “good,” but notably less consistent epidemiologic associations for other cancer sites. Limitations of these studies for causal inference (vs. association) were not directly addressed in the brief discussion of epidemiologic studies addressing cancer outcomes.

Experimental, Laboratory, and Shorter Term Study Approaches

An interdisciplinary collaboration among analytical chemists, toxicologists, and epidemiologists explored what might be learned from 11 European water samples with respect to epidemiologic and toxicologic responses. Detailed chemical analyses were used to characterize a wide range of DBPs and a suite of short-term toxicology tests (Jeong et al. 2012). Chemical analysis found more than 90 different DBPs among the 11 samples, with THM4 and the sum of 9 HAAs (HAA9) dominant on a mass concentration basis, as would be expected. A clear difference was found in the genotoxic responses among the samples, but these differences in genotoxic results did not correlate well with chemical analyses to reveal any explanation of genotoxicity in terms of specific CxDBP(s) being present.

Given the challenges posed by retrospective exposure assessment and latency for studying human bladder cancer association with CxDBPs, the use of intermediate markers of effect may prove useful. One option that has been evaluated for determining exposure to carcinogens, including arsenic, has been human biomonitoring to measure micronucleus (MN) formation (Warner et al. 1994; Moore et al. 1996). This has particular appeal for studying bladder cancer because bladder epithelial cells are exfoliated in urine, providing a feasible sampling approach. Similarly, current exposures are easily measured and subjects may be selected to include a large variation in exposure using biomonitoring (Kogevinas et al. 2010).

Ranmuthugala et al. (2003) reported an experimental study using MN that was well conceived in terms of comparative CxDBP

exposure scenarios. Although 348 males completed the full protocol, no significant association between MN prevalence and any measure of THM exposure was found. However, the higher occurrence of smokers in the community having no THM4 exposure effectively reduced study sensitivity, as smoking may have confounded the results.

Villanueva et al. (2007) included a sub-study of a sample of 44 female controls that had THM4 exposure data, but significant associations were not observed. The study was likely statistically underpowered. Further, female exfoliated cells in urine consist of only a small percentage of transitional cells associated with transitional-cell carcinoma (i.e., bladder cancer) and are predominantly squamous cells of the bladder trigone (Schulte et al. 1963; Tyler 1962).

Subsequently, Kogevinas et al. (2010) studied a volunteer cohort of 49 adult, nonsmoking swimmers from a group of 17 males and 33 females in Barcelona, who were monitored for individual THMs in exhaled breath. Changes in MN prevalence and DNA damage (comet assay) were measured in peripheral blood lymphocytes (PBL) before and 1 h after swimming. Urine mutagenicity (Ames assay) was measured before and 2 h after swimming, and exfoliated MN were measured before and 2 wk after swimming. The last analysis was likely too soon after swimming to expect urothelium to change and shed MN. Kogevinas et al. (2010) found significant increases in genotoxicity in PBL, but no significant associations with changes in MN urothelial cells. Having females as 66% of the study group was also a limitation of a study focused on urothelial cells.

A major series of experimental studies was undertaken by the U.S. EPA to evaluate complex mixtures of DBPs. Simmons et al. (2008) outlined the scientific rationale used for planning the experimental protocols used in what was described as the Four Lab Study. Richardson et al. (2008) described the comprehensive chemical analyses of the treated drinking-water concentrates developed for these experiments. Claxton et al. (2008) evaluated *Salmonella* mutagenicity and Crosby

et al. (2008) evaluated the gene expression in rat hepatocytes for these Four Lab Study concentrates. Rice et al. (2009) and Bull et al. (2009) assessed the ability to compare the composition of DBP mixtures and Schenk et al. (2009) evaluated correlation of DBP mixture composition with *Salmonella* mutagenicity.

Cancer Risk Assessment

Bull (2012) considered Cantor et al. (2010) and argued why those results alone cannot establish THMs as being responsible for the observed bladder cancer risk. Bull (2012) also argued against the Cantor et al. (2010) data necessarily implicating THMs based on comparison of *CYP2E1* and *GSTT1* activity. However, the Bull (2012) analysis considered *GSTT1* enzyme activity in rodents derived from Ross and Pegram (2004), which cannot necessarily be extrapolated to humans.

The conceptual inhalation–dermal exposure model that was proposed by Pegram and described in Richardson et al. (2007) received support from a number of studies (Ross and Pegram 2003; 2004; Leavens et al. 2007; Pegram et al. 1997; DeMarini et al. 1997; Kundu et al. 2004; Landi et al. 1999). However, there are no apparent human data for the metabolic activity of *CYP2E1* and *GSTT1* for THMs that bypass the liver (via inhalation or dermal exposure) for other tissues, such as bladder, lung, or possibly kidney. Without these human data, the Bull (2012) critique of this model cannot be resolved. The Pegram (personal communication 2014) model also did not consider other routes of exposure or other, non-volatile CXDBPs, such as HAAs that may be metabolized by *GSTZ1*. The conceptual mechanistic model in Cantor et al. (2010) provides a different risk perspective that needs further investigation. Detailed insights are limited by the reality that there are no long-term cancer bioassays suitable for supporting quantitative cancer risk assessments that evaluate inhalation or dermal exposure to any of the brominated CxDBPs. Physiologically based pharmacokinetic (PBPK) modeling may provide some additional insights for these issues.

Cancer risk assessment using animal bioassays studying carcinogenic agents follows an entirely different paradigm based on entirely different evidence than the prediction of human cancer cases by means of human epidemiology studies. However, attribution of observed human cancers to agents like CxDBPs in drinking water, whether individually or in some combination, would be strengthened by finding some concordance in the cancer risk predictions by these disparate approaches.

Bull (2012) and Bull et al. (2011) tackled this comparison, including cancer risk predictions for more potent mutagenic DBPs like brominated dihaloacetic acids and dihaloacetoneitriles, and 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX) with brominated analogues and *N*-nitrosodimethylamine (NDMA) to conclude that the latter summation for all-cancer site risk is 100-fold lower than the epidemiologic, site-specific bladder cancer risk estimates. These discrepancies in cancer risk predictions, notwithstanding the differences in approaches, are indicative of the evidence gaps and quantitative uncertainties inherent in the current evidence base.

CONCLUSIONS

The evidence for associations between bladder cancer and CxDBPs are primarily reliant on 10 case-control studies, with the most recent original case data collection from prior to 2001 (Villanueva et al. 2007; Cantor et al. 2010). The other case-control studies have cases mostly from a decade earlier, which may reflect effects of earlier, higher exposures prior to implementation of THM regulations. Replication in other populations with exposures reflecting more recent levels with current exposures, and utilizing other study designs (e.g., cohort studies), may provide additional evidence for judging a causal association between CxDBP exposure and bladder cancer under current exposure scenarios. Improved exposure and genetic susceptibility assessments would ideally be a part of these newer studies.

To specifically evaluate the impact of the Stage 2 DBP Rule on bladder cancer incidence in the United States, future studies are required that account for both the lower CxDBP levels associated with the rule implementation, and passage of sufficient time to account for bladder cancer latency.

Strengths and Limitations of the Evidence for Linking CxDBPs and Bladder Cancer

Strengths are:

- There are 2 cohort studies and 10 case-control studies using various indicators of exposure to CxDBPs or exposure to chlorinated surface water. Among the case-control studies, 8 suggested an association with bladder cancer, with significant ORs for men ranging from about 1.4 to 2.5. Table 7 summarizes these studies and shows the consistency of associations noted with bladder cancer.
- There are two meta-analyses (Villanueva et al. 2003a; Costet et al. 2011) of case-control studies that provide some consistency and support for an association of CxDBPs with bladder cancer. A pooled analysis (Villanueva et al. 2004) found similar associations.
- The Villanueva et al. (2007) study based on the comparatively large Spanish case-control study from 1998 to 2001 included explicit exposure categories of showering/bathing and swimming. Observed associations support a conceptual model that suggests that inhalation/dermal exposure to CxDBPs may be at least as important for bladder cancer risk as ingestion.
- The Villanueva et al. (2007) study also included explicit exposure to CxDBPs with a majority proportion of brominated species (Villanueva et al. 2003b). Observed associations are stronger than found previously in North American studies, suggesting that brominated species might be more important than their fully chlorinated counterparts.

TABLE 7. Analytical Epidemiology Studies Addressing Urinary Bladder Cancer (Adapted and Expanded From IARC 2013)

Study/design	Observation dates/location(s)	Exposure	Sample characteristics and size	Exposure categories and metrics	Risk estimate (95% CI); * significant	Comments
Drinking-Water Cohort Studies						
Wilkins and Comstock (1981): Prospective cohort	1963–1975 Washington County MD USA	Chlorinated surface water (average chloroform 107 µg/L vs. nonchlorinated deep well water)	30,780 persons 14,553 Male 16,227 Female ≥ 25 yr of age 52 cases	Male Female	RR 1.8 (0.8–4.8) 1.6 (0.5–6.3)	Adjusted for differences between cohorts in age, marital status, education, smoking history, church attendance, housing, persons per room. Kidney and liver cancer also evaluated but no Significant RR observed
Doyle et al. (1997): Prospective cohort	1986–1993 Iowa, USA	1108 municipal water supplies (1979, 1986–1987). THM4 and chloroform 1986–1987 Surface water sources THM4 geo mean 56 µg/L THM4 max 315 µg/L Chloroform geo mean 46 µg/L Chloroform max 287 µg/L	Incidence 41,836 Female 55–69 yr 42 cases	Chloroform (µg/L) < limit of detection 1–2 3–13 14–287 p for trend	RR 1.0 0.9 (0.4–2.0) 1.2 (0.6–2.7) 0.6 (0.3–1.6) .46	Adjusted for age, education, smoking, physical activity, fruit and vegetable intake, calorie intake, body mass index, waist-to-hip ratio Chloroform comprised approximately 80 to 90% of THM4 Significant RR found: Females in this study: Colon 1.72 (1.10–2.70) Breast 1.35 (1.03–1.76)
Koivusalo et al. (1997): Retrospective cohort	1971–1993 56 towns Finland Only 32% of resident population included	Estimates of mutagenic potency of drinking water—3000 net Ames assay revertants/L increase in average exposure to mutagenicity	Incidence 313,464 Male 613 cases 307,967 Female 223 cases	Male Female	RR 1.03 (0.82–1.28) 1.48* (1.01–2.18)	Record linkage study; adjusted for age, time period, urbanization and social status; including cancers of ureter and urethra. Estimates of mutagenic potency of drinking water, 20 of 56 towns estimated at zero mutagenicity with chlorinated supplies increased from 0 to 6905 net revertants/L in average exposure to mutagenicity according to an empirical equation applied to water source questionnaire data. Significant RR also found in this study: Females: esophagus 1.90 (1.02–3.52), rectum 1.36 (1.03–1.85), breast 1.11 (1.01–1.22), Males: lung 1.21 (1.07–1.36)

(Continued)

TABLE 7. (Continued)

Study/design	Observation dates/location(s)	Exposure	Sample characteristics and size	Exposure categories and metrics	Risk estimate (95% CI); * significant	Comments
Drinking-Water Case-Control Studies						
Cantor et al. (1987): Incident case	December 1977 to December 1978	Duration of consumption of chloraminated surface water for subjects consuming more than a median of 1.44 L/d	incident cases Male 2116 cases 3892 controls Female 689 cases 1366 controls	Male (years) 0 1-19 20-39 40-59 ≥60 p for trend Female (years) 0 1-19 20-39 40-59 ≥60 p for trend	OR 1.0 1.1 (0.7-1.6) 1.1 (0.7-1.5) 1.2 (0.8-1.7) 1.2 (0.7-2.1) .44	Adjusted for age, smoking, high-risk occupation, population size of normal residence, reporting center
Population controls	All persons 21-84 newly diagnosed with histologically confirmed urinary bladder cancer 10 geographic regions of the USA	Information on water source (surface water or groundwater) and chloramination status used to develop a personal exposure profile for each respondent			2.2 (1.0-4.8)* 3.2 (1.2-8.7)* .02*	Survey of 1102 water utilities to determine water sources (surface water or groundwater), treatment and distribution areas dating back to 1900. These data were geocoded in the same manner as the residential histories to allow individual linkage for establishing exposure history for U.S. regions studied: Atlantic, Connecticut, Detroit, Iowa, New Jersey, New Mexico, New Orleans, Seattle, San Francisco, Utah.
McCeehin et al. (1993): Incident case	1990-1991 Colorado USA	Lifetime exposure to chloraminated water from individual histories of residence and water source. Study also included about half the total population that was exposed to chloraminated vs. chloraminated water	incident 327 cases 261 cancer controls Male and Female All subjects white	Duration (yr) of exposure to chloraminated water 0 1-10 11-20 21-30 >30	OR 1.0 0.7 (0.4-1.3) 1.4 (0.8-2.5) 1.5 (0.8-2.9) 1.8 (1.1-2.9)*	Adjusted for coffee consumption, smoking, tap-water intake, family history of bladder cancer, sex, medical history of bladder infection or kidney stone. Cancer controls excluded lung and colorectal When compared with persons with no recorded exposure to trihalomethanes, persons with up to 200, 201-600, and greater than 600 trihalomethane-years had OR of 1.8, 1.1, and 1.8 respectively —p for trend = .16 Risk of bladder cancer decreased with increased duration of exposure to chloraminated surface water. Persons who consumed chloraminated water for 21-40 years had OR = 0.7 (0.4 -1.1) and for >40 years the OR was 0.6 (0.4-1.0)
Cancer controls						

King and Marrett (1996): Incident case Population controls	1992–1994 21 months Ontario, Canada	Consumption of chlorinated surface drinking water THM years THM concentration in drinking-water supply	696 incident cases 1545 population controls Male and female	Duration (yr) 0–9 10–19 20–34 ≥35 Quartiles (µg/L-yr) 0–583 584–1505 1506–1956 1957–6425 THM concentration (µg/L) 0–24 25–74 ≥75 p for trend	OR 1.0 1.04(0.7–1.5) 1.2 (0.9–1.5) 1.4 (1.1–1.8)* 1.0 1.2 (0.9–1.6) 1.08(0.8–1.4) 1.4 (1.1–1.9)*	Adjusted for age, sex, log pack-yr smoking, current smoking, calorie intake. THM levels were modeled for chlorinated surface water sources and were validated against Ontario monitoring data.
Freedman et al. (1997): Incident case Population control	1975–1992 Washington County MD USA	Duration of residence with municipal water source Nearly all municipal sources in 1975 were supplied by surface waters that had been chlorinated for more than 30 years. Only one municipal source in a small town serving 279 households was chlorinated for less than 30 years, and that was for 10 years. In contrast, only 6% of nonmunicipal sources were chlorinated.	293 incident cases Male 209 Female 84 2308 population controls	Duration (yrs) Male 0 1–10 11–20 21–30 31–40 >40 Female 0 1–10 11–20 21–30 31–40 >40	OR 1.0 1.1 (0.6–1.9) 1.1 (0.6–1.9) 1.3 (0.7–2.5) 1.5 (0.6–3.3) 2.2 (0.8–5.1) 1.0 0.7 (0.3–1.7) 0.7 (0.3–1.8) 0.6 (0.2–1.6) 0.7 (0.2–2.2) 0.6 (0.2–2.2)	Adjusted for age, sex, smoking, urbanization. Households reporting municipal sources were treated as receiving chlorinated drinking water from surface waters, and thus, as having relatively high exposure to chlorination by-products. Households with nonmunicipal water sources were characterized as having low exposure. There was limited information on levels and composition of chlorination byproducts in the drinking water during the exposure period (prior to 1975). Only the water source for the single city in this County had been monitored for THMs. Study authors acknowledge that water treatment changes made in 1979 likely decreased THMs levels so that levels prior to 1975, during the study's exposure period, were greater than more recent levels.

(Continued)

TABLE 7. (Continued)

Study/design	Observation dates/location(s)	Exposure	Sample characteristics and size	Exposure categories and metrics	Risk estimate (95% CI); * significant	Comments
Cantor et al. (1998): Incident case Population control	1986-1989 Iowa	THM4 total lifetime exposure estimated from lifetime residential history, water utility survey and water sample analyses. Cases and controls had data relating to at least 70% of their lifetime drinking-water source. Surveyed all water utilities serving > 1,000 (345) 66% of state population. Most of remaining population used private wells. THM4 measured by study in 1987 found for 20 Cl ₂ surface water plants, geo. mean = 73.9 µg/L.	1123 incident cases 1983 population controls	THM lifetime (g) Male ≤ 0.04 0.05-0.12 0.13-0.34 0.35-1.48 1.49-2.41 ≥ 2.42 p for trend Female ≤ 0.04 0.05-0.12 0.13-0.34 0.35-1.48 1.49-2.41 ≥ 2.42 p for trend	OR 1.0 1.3 (1.0-1.7) 1.1 (0.8-1.7) 1.2 (0.9-1.6) 1.3 (0.8-2.0) 1.8 (1.2-2.7)* .05*	Eligible cases were residents of Iowa, ages 40-85 years, newly diagnosed with histologically confirmed bladder cancer in the years 1986-1989, and without previous diagnosis of a malignant neoplasm. Controls under 65 years of age were selected from computerized state driver's license records and controls 65 years old and older from U.S. Health Care Financing Administration listings. Persons with a previous cancer diagnosis were excluded. Adjusted for age, study period, education, high-risk occupation, cigarette smoking (6 levels) of cities with substantial chemical, pulp and paper or agricultural workers, socioeconomic status.
Koivusalo et al. (1998): Incident cases Population controls	1991-1992 Finland	Ames mutagenic potency estimated by historical exposure according to residence, water source, water quality and treatment.	732 incident cases 552 Male 180 Female 914 population controls 621 Male 293 Female	Exposure tertiles for subjects ≥ 30 yr exposure (net revertants/L) Male Unexposed Low (1-999) Medium (1000-2499) High (≥ 2500) Female Unexposed Low (1-999) Medium (1000-2499) High (≥ 2500)	OR 1.0 1.2 (0.8-1.6) 0.97 (0.7-1.4) 1.4 (0.9-2.0) OR 1.0 1.2 (0.7-2.0) 1.3 (0.7-2.4) 1.2 (0.6-2.2)	

Chevrier et al. (2004) Incident cases Hospital-based controls	1985-1987 France	Average level of THM in a 30 yr exposure window from 5 to 35 yr before interview – Analysis restricted to subjects with known exposure of at least 70% of the exposure period Based on treatment process descriptions, plants were assigned average THM levels ranging from 10 µg/L for chlorinated groundwater through 4 levels of chlorinated surface water, 27.4, 31.8, 64.9, and 78 10 µg/L	281 incident cases 240 Male 41 Female 272 controls 233 Male 39 Female	Average THM4 level Male (231 cases, 314 control) <1 µg/L 1–5 µg/L 6–50 µg/L >50 µg/L Female (38 cases, 38 control) <1 µg/L 1–5 µg/L 6–50 µg/L >50 µg/L cumulative THM exposure Male (231 cases, 314 control) 0 µg/L-yr 1–150 µg/L-yr 151–1500 µg/L-yr >1500 µg/L-yr Female (38 cases, 38 control) 0 µg/L-yr 1–150 µg/L-yr 151–1500 µg/L-yr >1500 µg/L-yr	OR 1.0 1.32 (0.7–2.6) 1.97 (0.8–5.2) 3.73 (1.2–11)* – 1.0 1.97 (0.2–18) 1.55 (0.1–32) 1.0 1.20 (0.6–2.3) 1.82 (0.7–4.8) 3.49 (1.2–10.6)* 1.0 0.45 (0.1–3.6) 1.04 (0.0–22.8) 4.79 (0.1–219)	Adjusted for hospital, age, socioeconomic status, smoking status, coffee consumption, high risk occupations, tap water consumption. Controls were randomly selected from hospital patients and did not have cancer, respiratory disease, or symptoms suggestive of bladder cancer. A protective duration response was observed for exposure to ozonated water (whether or not used in combination with chlorine) Men 231 cases 0 yr OR = 1.0 1–9 yr OR = 0.58 (0.3–1.3) 10–30 yr OR = 0.27 (0.1–0.6)*
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(Continued)

TABLE 7. (Continued)

Study/design	Observation dates/location(s)	Exposure	Sample characteristics and size	Exposure categories and metrics	Risk estimate (95% CI); * significant	Comments
Bove et al. (2007): Incident case Cohort-based controls	1979-1985 Western NY State Monroe County, NY Buffalo, NY	Individual THM level at the last known residence about 20 yr after recruitment to the study. Cases and controls were derived from Vena et al. (1993), which originally used only the volume of water consumed.	Total incident cases 129 Male white controls 256 Male ^a , white	Chloroform ^b ≤ 17.14 µg/d 17.42–25.72 µg/d 26.15–38.61 µg/d 38.46–192.52 µg/d Bromo-dichloromethane ^b ≤ 9.35 µg/d 9.40–13.31 µg/d 13.35–18.75 µg/d 18.80–78.93 µg/d Dibromo-chloromethane ^b ≤ 4.67 µg/d 4.68–6.89 µg/d 6.90–9.35 µg/d 9.37–35.62 µg/d Bromoform ^b ≤ 0.43 µg/d 0.44–0.73 µg/d 0.75–1.14 µg/d 1.16–41.88 µg/d	OR 1.00 1.79 (0.81–3.09) 1.76 (0.91–3.35) 2.55 (1.25–4.66)* 1.00 1.89 (0.95–3.59) 2.20 (1.12–4.26)* 2.49 (1.19–4.48)* 1.00 1.29 (0.77–2.83) 1.34 (0.78–2.85) 1.17 (0.84–3.03) 1.00 2.12 (1.05–4.17) 2.34 (1.18–4.57) 3.05 (1.51–5.69)	Adjusted for daily tap-water consumption, cigarette smoking (pack-yr), carotene, water consumption from foods, dietary fibre, alcohol. Controls were disease-free white men, age between 35 and 90 years, from a diet study cohort. Controls were matched to cases by county of residence and were drawn from studies of cancer of the colon, esophagus, larynx, lung, oral cavity, and stomach cancers, but controls from the rectal cancer study were excluded. ^a Table 1 of Bove et al. (2007) presenting covariates adjusted for shows controls as being 129; on p. 43, the combination of Monroe County. ^b The Table 4 title in Bove et al. (2007) shows data as µg/L, but the note to Table 4 explains that “Readings for quartiles are total µg/d consumption calculated as total daily tap water consumption x calculated trihalomethane value” (emphasis added).

Villaneuva et al. (2007): Incident case Hospital-based controls	1998–2001 5 geographic regions, Spain: Barcelona, Valles/Bages (including the cities of Sabadell and Manresa), Alicante, Tenerife, and Asturias	Years of exposure to chlorinated surface water in the residences from 16 yr until the time of interview. Inclusion limited to subjects with known exposure for at least 70% of the exposure window.	1219 incident cases 1067 male 152 female 1271 controls 1105 male 166 female	THM exposure Level— $\mu\text{g/L}$ Male $\leq 8 \mu\text{g/L}$ $> 8\text{--}26 \mu\text{g/L}$ $> 26\text{--}49 \mu\text{g/L}$ $> 49 \mu\text{g/L}$ p for trend Female $\leq 8 \mu\text{g/L}$ $> 8\text{--}26 \mu\text{g/L}$ $> 26\text{--}49 \mu\text{g/L}$ $> 49 \mu\text{g/L}$ p for trend Duration of exposure-years Male 0–3 yr $> 3\text{--}25$ yr $> 25\text{--}30$ yr > 30 yr p for trend Female 0–3 yr $> 3\text{--}25$ yr $> 25\text{--}30$ yr > 30 yr p for trend	OR 1.0 1.53 (0.95–2.48) 2.34 (1.36–4.03)* 2.53 (1.23–5.20)* <.01 1.0 0.40 (0.13–1.27) 1.14 (0.31–4.10) 1.50 (0.26–8.61) .20 OR 1.0 2.26 (1.19–4.29)* 2.58 (1.33–5.01)* 2.21 (1.17–4.20)* < 0.01 1.0 2.72 (0.56–13.26) 2.32 (0.44–12.13) 2.33 (0.51–10.55) .62	Adjusted for age, smoking status, education, urbanization of longest residence until 18 yr of age, overall quality of interview, geographic area. Controls were patients admitted to participating hospitals with diagnoses thought to be unrelated to the main risk factors for bladder cancer, such as tobacco use Average THM level in the residences from 16 yr until the time of interview. Inclusion limited to subjects with known exposure for at least 70% of the exposure window. See also Cantor et al. (2010) because this study is drawn from the same study population.
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(Continued)

TABLE 7. (Continued)

Study/design	Observation dates/location(s)	Exposure	Sample characteristics and size	Exposure categories and metrics	Risk estimate (95% CI); * significant	Comments
Cantor et al. (2010): Incident case Hospital-based controls	1998–2001 5 geographic regions, Spain: Barcelona, Valles/Bages (including the cities of Sabadell and Manresa), Alicante, Tenerife, and Asturias	Average THM level in the residences from 16 yr until the time of interview. Inclusion limited to subjects with known exposure for at least 70% of the exposure window.	680 incident cases 595 male 85 female 714 controls 622 male 92 female	GSTT1 present (542 cases) ≤8 µg/L >8–26 µg/L >26–49 µg/L >49 µg/L p for trend GSTT1 null (136 cases) ≤8 µg/L >8–26 µg/L >26–49 µg/L >49 µg/L p for trend GSTZ1 rs1046428 CT/TT (244 cases) ≤8 µg/L >8–26 µg/L >26–49 µg/L >49 µg/L p for trend GSTZ1 rs1046428 CC (405 cases) ≤8 µg/L >8–26 µg/L >26–49 µg/L >49 µg/L p for trend CYP2E1 rs2031920 CC (590 cases) ≤8 µg/L >8–26 µg/L >26–49 µg/L >49 µg/L p for trend	OR 1.0 1.2 (0.7–1.9) 2.0 (1.2–3.4)* 2.2 (1.1–4.3)* .0072* 1.0 1.2 (0.5–2.5) 1.2 (0.5–2.5) 1.0 (0.4–2.5) .28 1.0 1.4 (0.7–2.7) 2.2 (1.1–4.2)* 2.9 (1.3–6.7)* .0043* 1.0 1.1 (0.7–1.9) 1.5 (0.9–2.7) 1.3 (0.6–2.8) .28 1.0 1.3 (0.8–2.0) 2.1 (1.2–3.5)* 2.0 (1.0–4.1)* .014*	Adjusted for age (continuous), sex, smoking status (never/former/current), size of municipality of longest residence until 18 yr of age, education (3 levels), geographic area (6 categories), overall quality of interview. Controls were patients admitted to participating hospitals with diagnoses thought to be unrelated to the main risk factors for bladder cancer, such as tobacco use. Average THM level in the residences from 16 yr until the time of interview. Inclusion limited to subjects with known exposure for at least 70% of the exposure window. See also Villanueva et al. (2007) because this study is drawn from the same study population.

CYP2E1 rs2031920	OR
CT/TT (37 cases)	1.0
≤8 µg/L	0.98 (0.4–2.5)
>8–26 µg/L	1.1 (0.4–3.1)
>26–49 µg/L	0.6 (0.1–2.7)
>49 µg/L	.33
p for trend	
GSTT1 null and GSTZ1 CC	
n (cases/controls)	1.0
≤8 µg/L (19/17)	1.1 (0.4–3.0)
>8–26 µg/L (25/24)	1.1 (0.4–3.1)
>26–49 µg/L (21/29)	1.5 (0.4–5.4)
>49 µg/L (17/26)	.57
p for trend	
GSTT1 [present] and GSTZ1 CC/TT	
n (cases/controls)	1.0
≤8 µg/L (40/51)	1.5 (0.7–3.5)
>8–26 µg/L (35/45)	3.4 (1.4–8.2)*
>26–49 µg/L (61/49)	5.9 (1.8–19.0)*
>49 µg/L (59/47)	.0012*
p for trend	
GSTT1 [present] vs. null	
≤8 µg/L	0.9 (0.5–1.5)
>8–26 µg/L	0.9 (0.5–1.6)
>26–49 µg/L	1.5 (0.9–2.5)
>49 µg/L	1.8 (1.1–3.1)*

(Continued)

TABLE 7. (Continued)

Study/design	Observation dates/location(s)	Exposure	Sample characteristics and size	Exposure categories and metrics	Risk estimate (95% CI); * significant	Comments
Cantor et al. (2010) continued				GSTZ1 rs1046428 CT/TT	OR	
				vs. CC		
				≤8 µg/L		0.7 (0.4–1.2)
				> 8–26 µg/L		1.0 (0.6–1.6)
				> 26–49 µg/L		1.0 (0.6–1.6)
				> 49 µg/L		1.6 (1.0–2.6)*
				CYP2E1 rs2031920		
				CC vs. CT/TT		
				≤8 µg/L		0.8 (0.5–1.5)
				> 8–26 µg/L		1.2 (0.7–2.0)
				> 26–49 µg/L		1.9 (1.1–3.3)*
				> 49 µg/L		2.0 (1.1–3.9)*
				NAT2, slow vs. rapid /intermediate		
				≤8 µg/L		1.3 (0.8–2.0)
				> 8–26 µg/L		1.5 (0.9–2.5)
			> 26–49 µg/L		1.1 (0.7–1.8)	
			> 49 µg/L		1.4 (0.9–2.2)	
			GSTM1, null vs. present			
			≤8 µg/L		1.8 (1.1–2.9)*	
			> 8–26 µg/L		1.6 (1.0–2.5)*	
			> 26–49 µg/L		1.8 (1.2–2.8)*	
			> 49 µg/L		1.9 (1.2–2.9)*	

- A conceptual model involving *GSTT1* developed around several toxicological investigations (Richardson et al. 2007; Pegram personal communication 2014), combined with the findings of Villanueva et al. (2007), involves a number of plausible elements that collectively raise the conceptual model above that of simply being a possibility. However, the model is currently short of providing a probable explanation of the epidemiologic observations.
- Cantor et al. (2010) evaluated a subset of the Villanueva et al. (2007) subjects according to a number of candidate genes with variation in several single nucleotide polymorphisms (SNPs), some of which were selected with the conceptual model of Pegram (personal communication 2014; Richardson et al. 2007) in mind. These results provide a possible mechanistic basis for the association with bladder cancer; however, the specific tie to THMs or other volatile DBPs needs to be established and the genetic susceptibility results require replication in an independent population.

Limitations are:

- Of the 10 case-control studies already mentioned, 2 (Cantor et al. 2010; Villanueva et al. 2007) are different analyses of the same case-control study, and 3 (Bove et al. 2007; Chevrier et al. 2004; McGeehin et al. 1993) have size or methodological issues that reduce the weight that can be placed on their findings as sources of replication for the purposes of judging consistency.
- The two meta-analyses (Villanueva et al. 2003; Costet et al. 2011) involve six case-control studies in common. The pooled analysis (Villanueva et al. 2004) includes three of the case-control studies among the six used in the two meta-analyses.
- For the genetic factors studied by Cantor et al. (2010), the specific tie to THMs or other volatile DBPs needs to be established and there is an absolute need for replication of results in another setting with independent populations before confidence can be placed in these findings.
- The differing views between Bull (2012) and Pegram (personal communication 2014) on the causal role of THMs according to the conceptual inhalation/dermal exposure model underlying Cantor et al. (2010) will not be resolved without some additional direct human evidence.
- The impact of a high proportion of brominated DBPs involved in Villanueva et al. (2007) was not noted in the meta-analysis of Costet et al. (2011), although two other European studies reported no information on brominated DBPs. The North American epidemiologic studies examined waters without such high levels of bromide and brominated DBPs as found in the Spanish study (Villanueva et al. 2006; 2007). THM4, chloroform in particular, may be serving as a surrogate for an as-yet-unidentified causal agent.
- CxDBP epidemiologic studies that used estimates of past THM levels, with the exception of the CxDBP exposure reanalyses performed by Amy et al. (2006), estimated CxDBP exposure levels in the distant past by extrapolating back in time based on current or recent measurements. These extrapolations did not take into account changes in treatment practices over time, such that past CxDBP exposures are likely underestimated. As a result, where associations with bladder cancer are found, those associations will be reported to occur for lower CxDBP exposures than were likely experienced.
- There is a quantitative discrepancy between epidemiologic site-specific risk estimates for bladder cancer from THM4 and what can be estimated by summing all the upper-bound, all-cancer-site risk predictions from application of cancer slope factors for individual genotoxic CxDBPs. This analysis has not been conducted with all the emerging CxDBPs that may be of higher health concern than the regulated CxDBPs because most of the former do not have estimated cancer slope factors. Two genotoxic CxDBPs (MX, NDMA) that were included have cancer slope factors that are orders of magnitude more potent than the THMs or HAAs but they also occur at orders of magnitude lower concentrations than THM4 or HAA9.

Knowledge Gaps

- THM4 has been evaluated in many epidemiologic studies because of the availability of these monitoring data; however, it is not possible using these studies to determine whether THM4 or some correlate is an etiologic factor associated with bladder cancer.
- For the genetic factors studied by Cantor et al. (2010), there is an absolute need for replication of results in another setting with independent populations before confidence can be placed in these findings.
- The differing views between Bull (2012) and Pegram (personal communication 2014) on the causal role of THMs according to the conceptual inhalation/dermal exposure model underlying Cantor et al. (2010) will not be resolved without some additional direct human evidence (see discussion on cancer risk assessment).

Evidence Supporting Better Risk Management

The available new evidence does not clearly or adequately indicate what changes to DBP quantitative limits would be beneficial for the United States. The evidence opens possibilities of new hazard identification (e.g., brominated compounds, many of the other nonregulated DBPs, inhalation/dermal exposure). There are opportunities to inform a better understanding of CxDBP exposures, but any causative CxDBP agents for bladder cancer have not yet been identified.

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