Biomonitoring Equivalents (BE) dossier for trihalomethanes

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1. Introduction

The benefits of disinfecting drinking water as a means of protecting the public from disease-causing microorganisms such as typhoid, hepatitis, Giardia and cholera are clear (USEPA, 2006a). At the same time, the reaction of drinking water disinfectants with naturally occurring organic matter in the water supply produces disinfection byproducts (DBPs). The most widely used disinfectant – chlorine – produces DBPs such as trihalomethanes (THMs, which for the purposes of this paper include chloroform [trichloromethane], bromoform [tribromomethane or TBM], bromodichloromethane [BDCM], and dibromochloromethane [DBCM]) as well as other classes of compounds (e.g., haloacetic acids) (USEPA, 2006a). Toxicological studies of THMs have consistently demonstrated that liver and kidney toxicity are the most sensitive endpoints in laboratory animals (USEPA, 2005). At doses above those producing liver and kidney toxicity, effects on reproductive and developmental endpoints and increases in tumor incidence in several target organs (liver, kidney, and large intestine) have been observed (USEPA, 2005). A variety of ecological and cross-sectional studies of human populations have examined possible associations between exposure to these compounds in drinking water and certain adverse health effects, such as specific cancers or reproductive effects (reviewed in USEPA, 2005; see also King et al., 2000; Nieuwenhuijsen et al., 2000; Dodds et al., 2004; Toledano et al., 2005; Villanueva et al., 2007a). The US Environmental Protection Agency (USEPA) has set limits on allowable levels of THMs in drinking water supplies, recognizing that DBPs are the unwanted but unavoidable consequence of treating drinking water, and the risks associated with exposures to DBPs must be considered in light of the important public health benefits associated with disinfection.

Numerous investigations have provided data on exposures to THMs from activities such as water consumption, showering, and bathing. However, because of the pharmacokinetic and chemical characteristics of THMs (rapidly eliminated, high volatility) and the fluctuating nature of human exposures (due to variation in human activities and to the variable concentrations of THMs in water at any given time), reliable dose estimates are difficult to obtain. Sophisticated exposure analysis coupled with physiologically based pharmacokinetic (PBPK) models has provided evaluations of the relative contributions of various exposure pathways to internal doses of THMs (Haddad et al., 2006; USEPA, 2006c). With the availability of analytical methods for measuring THMs in human blood (Bonin et al., 2005), researchers have sought to use biomonitoring data to better determine exposures to THMs on a population basis. However, there are no readily available methods for interpreting biomonitoring data in a public health risk context because the tolerable exposure guidelines established by USEPA and other agencies are based on external air concentration or daily dose.
Screening criteria for evaluation of biomonitoring data would ideally be based upon data from robust epidemiological studies that evaluate a comprehensive set of health endpoints in relationship to measured levels of chemicals in biological media. However, development of such epidemiologically-based screening values is a resource- and time-intensive effort. As an interim effort, the development of Biomonitoring Equivalents (BEs) has been proposed (Hays et al., 2007). A BE is defined as the concentration or range of concentrations of chemical in a biological medium (blood, urine, or other medium) that is consistent with an existing health-based exposure guideline. Chemical-specific pharmacokinetic data are used to estimate biomarker concentrations associated with the Point of Departure (PODs; such as No Observed Effect Levels [NOELs], Lowest Observed Effect Levels [LOELs], or Benchmark Doses [BMDs]) and to estimate biomarker concentrations that are consistent with the guidance value. BEs can be estimated using human or animal pharmacokinetic data. Guidelines for the derivation and communication of BEs are available in (Hays et al., 2008). BEs are designed to be screening tools to gauge which chemicals have large, small or no margin of safety compared to existing health-based exposure guidelines. BEs are only as robust as are the underlying health-based exposure guidelines that they are based upon and the underlying animal toxicology studies and pharmacokinetic data used to derive these health-based exposure guidelines. BEs are not designed to be diagnostic for potential health effects in humans, either individually or among a population.

The value of the development of BEs for THMs and other environmental chemicals is that the method directly addresses the problem noted by the National Research Council (NRC, 2006), that "We do not know how to convey the biomarker-predicence—does-not-indicate-health-effects message effectively." THMs present a case in which substantial data on mechanism of action and detailed pharmacokinetic models are available. Application of the BE approach, using forward dosimetry, provides a relatively straightforward method for providing a set of screening values for an initial evaluation of human biomonitoring data from a public health risk perspective. Reported concentrations of THMs in human blood can be compared to BEs to evaluate whether population blood concentrations are above, below, or close to blood concentrations that are consistent with exposure guidance values as determined by USEPA.

This BE dossier describes the scientific basis for and derivation of BE values for THMs and discusses issues that are important for the interpretation of biomonitoring data using BEs. This BE dossier is not designed to be a comprehensive compilation of the available hazard, dose–response or risk assessment information for THMs.

1.1. Current health-based exposure guidance values

Health-based exposure guidance and toxicity values have been established for many chemicals for the general population by the USEPA (Reference Doses or Reference Concentrations [RfDs or RICs]), the Agency for Toxic Substances and Disease Registry (ATSDR) (Minimal Risk Levels or MRLs), and various organizations outside the US including Health Canada and the World Health Organization (WHO) (Tolerable Daily Intakes or TDIs). Although these health-based guidance values have different labels and communication of the studies used, the most sensitive endpoints, and the identified POD used to estimate RfDs for each compound. The RfDs are based on hepatic effects for all four compounds, with the critical study being in rats for DBCM, BDCM, and TBM and from a study in dogs for chloroform.

USEPA has concluded that the carcinogenicity of chloroform observed in animal studies occurs as a result of repeated cytotoxicity and cytolethality resulting from high peak tissue concentrations and high rates of metabolism following repeated dosing (USEPA, 2006a,b). Based on this determination of mode of action for carcinogenicity, USEPA has concluded that exposures to chloroform below the RfD set for non-cancer endpoints will be protective for the cancer endpoint. EPA has not made a similar determination regarding the mode of action or cancer risks from DBCM, BDCM or TBM, relying instead on the default linear, non-threshold extrapolation approach to estimate cancer risks. In the recent assessment of brominated trihalomethanes conducted by the USEPA Office of Water, dose–response assessments of cancer bioassay data for the three brominated trihalomethane compounds were presented (USEPA, 2005). Table 2 gives an overview of the estimates of human equivalent benchmark doses for a 10% increase in tumor risk at the responding tumor sites and of the selected cancer slope factors (derived using the linearized multistage model and scaling to bodyweight to the 3/4 power) for the three THM compounds other than chloroform (USEPA, 2005).

1.2. Pharmacokinetics

The pharmacokinetics of THM compounds have been extensively investigated in laboratory rodents. The compounds are rapidly metabolized, volatile, and lipophilic, and toxicity is believed to arise due to the production of reactive metabolites (reviewed in Meek et al., 2002; USEPA, 2005). PBK models have been developed for all four compounds based on experiments in laboratory rats and mice (Corley et al., 1990; Lilly et al., 1997, 1998; Luciene da Silva et al., 1999) and extended to humans through the use of allometric scaling of metabolic parameters (Haddad et al., 2006). The pharmacokinetic models are discussed further in Section 2.1 below.

1.3. Biomarkers

The objective of using BEs is to provide a human health risk framework for screening-level evaluation of human biomonitoring data. The choice of the biomarker (analyte and medium) should be optimized to facilitate this objective. The key criteria for the choice of a biomarker are that it be as closely related to the appropriate dose to the target tissue as possible and that it be practical for collection in a biomonitoring study. This, in turn, means that the biomarker should be (i) the compound that causes the toxicity (parent or metabolite), or (ii) should be just upstream on the metabolic pathway from the toxic compound, and (iii) as closely related to the target tissue as possible.

Identification of relevant dose metrics depends upon the health endpoints that are the bases of the health-based screening values. The available health-based criteria presented in Table 1 focus on two health endpoints.

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1 See the definition of RfD at http://www.epa.gov/iris/gloss8.htm; definitions for ATSDR MRLs are included in ATSDR Toxicological Profiles at http://www.atsdr.cdc.gov/toxpro2.html. Definition of the TDI is available at http://ptcl.chem.oxy.ac.uk/MSDS/glossary/tolerable_daily_intake.html.
Exposures to THMs have been biomonitored by quantifying the parent compounds in blood, exhaled air, and to a limited degree in urine. The most common matrix has been blood, and thus BEs are derived for THMs in whole blood in this paper.

2. BE derivation

In this analysis, we utilize the underlying PODs for health-based exposure guidance values (RfDs) for the four THMs to derive estimated blood concentrations (BEs) consistent with the derivation of the RfD values. We also estimate BE values based on the cancer risk assessments conducted by USEPA. We then compare these BEs to available data on THMs in human blood, describe the uncertainties in the BE derivation for THMs, and discuss interpretation of biomonitoring data sets for these compounds in the context of the BEs and the risk/benefit considerations for water disinfection.

2.1. Models

The pharmacokinetics of chloroform has been studied extensively in animals and to a limited degree in humans, and a well-accepted PBPK model is available for chloroform (Corley et al., 1990). PBPK models for the other three THM compounds have been developed for rats (Lilly et al., 1998; Luciene da Silva et al., 1999; Haddad et al., 2006) through the use of allometric scaling of metabolic parameters. These are the same basic models used by USEPA in their examinations of exposure pathways for THMs (Teuschler et al., 2004) and by other researchers examining the relationship between biomonitored levels of THM compounds and external exposure patterns (Tan et al., 2006, 2007). PBPK models were implemented in Microsoft Excel® for all four THM compounds for humans and rats and for chloroform in dogs. Physiological and chemical-specific metabolic parameters for the human models were taken from Haddad et al. (2006). These models have been used to predict blood levels in humans associated with exposures.

2.1.1. Models

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Table 1

<table>
<thead>
<tr>
<th>Compound</th>
<th>Description of study used as the basis for RfD</th>
<th>Effects observed</th>
<th>POD</th>
<th>UF</th>
<th>RfD (mg kg⁻¹ d⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroform</td>
<td>Chloroform administered to dogs in a toothpaste base in gelatin capsules, 15 or 30 mg kg⁻¹ d⁻¹ 6 d/wk for 7.5 yrs (Heywood et al., 1979)</td>
<td>Reversible elevations in SGPT (ALT) during treatment at both doses; increased fatty cysts in liver</td>
<td>BMDL₁₀; 1.2 mg kg⁻¹ d⁻¹</td>
<td>100 10 for interspecies</td>
<td>0.01</td>
</tr>
<tr>
<td>DBCM</td>
<td>DBCM administered to rats via corn oil gavage, 0, 40, 80 mg kg⁻¹ d⁻¹, 5 d/wk, 104 wks (NTP 1985 as reported in USEPA 2005)</td>
<td>Fatty changes in the liver of male rats</td>
<td>BMDL₁₀; 1.6 mg kg⁻¹ d⁻¹</td>
<td>100 10 for interspecies</td>
<td>0.02</td>
</tr>
<tr>
<td>BDCM</td>
<td>BDCM administered to male rats in diet at 6, 25, or 138 mg kg⁻¹ d⁻¹, 24 months (Aida et al., 1992 as reported in USEPA 2005)</td>
<td>Fatty changes in liver of male rats</td>
<td>BMDL₁₀; 0.8 mg kg⁻¹ d⁻¹</td>
<td>300 10 for interspecies</td>
<td>0.003</td>
</tr>
<tr>
<td>TBM</td>
<td>TBM administered to rats via corn oil gavage, 12, 25, 50, 100, or 200 mg kg⁻¹ d⁻¹, 5 d/wk, 13 wks (NTP 1989 as reported in USEPA 2005)</td>
<td>Hepatocellular vacuolization</td>
<td>BMDL₁₀; 2.6 mg kg⁻¹ d⁻¹</td>
<td>100 10 for interspecies</td>
<td>0.03</td>
</tr>
</tbody>
</table>

RfD for chloroform from USEPA’s Integrated Risk Information System (USEPA 2006b); information on RfDs for brominated THM compounds from USEPA (2005).

Table 2

<table>
<thead>
<tr>
<th>Compound</th>
<th>Study description</th>
<th>Most sensitive endpoint</th>
<th>Human equivalent LEDₐ₀ (mg kg⁻¹ d⁻¹)</th>
<th>CSF b (mg kg⁻¹ d⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroform</td>
<td>&quot;A dose of 0.01 mg kg⁻¹ d⁻¹ (equal to the RfD) can be considered protective against cancer risk&quot;</td>
<td>Liver tumors in female mice</td>
<td>2.5</td>
<td>4.3 × 10⁻²</td>
</tr>
<tr>
<td>DBCM</td>
<td>NTP (1985, as cited in USEPA 2005) study in B6C3F1 mice; 0, 50 or 100 mg kg⁻¹ d⁻¹, 5 d/wk via corn oil gavage</td>
<td>Liver tumors in female mice</td>
<td>2.5</td>
<td>4.3 × 10⁻²</td>
</tr>
<tr>
<td>BDCM</td>
<td>NTP (1987, as cited in USEPA 2005) study in B6C3F1 mice; 0, 25, or 50 mg kg⁻¹ d⁻¹</td>
<td>Kidney tumors in male mice</td>
<td>3.0</td>
<td>3.5 × 10⁻²</td>
</tr>
<tr>
<td>TBM</td>
<td>NTP (1989, as cited in USEPA 2005) study in Fisher 344/N rats; 0, 100, or 200 mg kg⁻¹ d⁻¹, 5 d/wk via corn oil gavage</td>
<td>Tumors of the large intestine in female rats</td>
<td>22</td>
<td>4.6 × 10⁻²</td>
</tr>
</tbody>
</table>

LEDₐ₀: human equivalent (scaled using bodyweight¹⁴) lower bound on the estimated dose associated with a 10% increase in tumor occurrence. Cancer slope factor derived using the linearized multistage model and bodyweight¹⁴ scaling. Only the final CSF selected for each compound as assessed in the USEPA (2005) Drinking Water Criteria Document for Brominated Trihalomethanes is reported here. From USEPA IRIS, further discussion is provided: “...the RfD for non-cancer effects is derived from the most sensitive endpoint in the most sensitive species. The RfD is based on fatty cysts [sic] formation (fat accumulation) in the liver and elevation of SGPT in dogs (Heywood et al., 1979). Hepatic fat accumulation and elevated SGPT are considered early signs of impaired liver function resulting from chloroform-induced cytotoxicity. This effect occurs at doses at or below those that cause increased labeling index, morphological changes, or cellular necrosis, so protection against this effect is believed to protect against cytolethality and regenerative hyperplasia. Accordingly, the RfD of 0.01 mg kg⁻¹ d⁻¹ presented in Section I.A.1 can be considered protective against increased risk of cancer.”
to THMs via inhalation and ingestion (Tan et al., 2006, 2007) but have undergone limited validation against human experimental data. Parameters for the rat PBPK models for DBCM, BDCM, and TBM were taken directly from Luciene da Silva et al. (1999); for chloroform, the default uncertainty factor for animal to human pharmacodynamic differences in the human PBPK model. An equivalent human POD in terms of liver AUC was estimated by applying the default uncertainty factor for animal to human pharmacokinetic data. Finally, the rat and human PBPK models were extended to the dog by Meek et al. (2002) in order to assess the liver toxicity data from Heywood et al. (1979), which is the basis for the chloroform RfD. The dog model as parameterized by Meek et al. (2002) was implemented here to estimate blood levels in dogs at the POD used to derive the chloroform RfD. However, that model has not been validated against experimental pharmacokinetic data in dogs, but instead includes physiological and anatomical parameters specific for dogs in combination with metabolic parameters averaged from those used in the rat and human models.

2.1.2. Non-cancer

The approach to estimating blood concentrations consistent with the USEPA RfDs for the four THMs is illustrated in Fig. 1. The critical effects observed in animal studies in response to exposure to all four THMs were effects on liver (summarized in Table 1). Although the specific mechanism(s) of action for these effects are not known, they likely involve production of reactive metabolites that result in hepatotoxicity. The critical dose metric may be related to either rate of production or peak or average concentration of metabolites in liver tissue (USEPA, 2006c). For the purposes of the modeling conducted here, the following assumptions were made:

- Daily metabolite production in the liver (mg L\(^{-1}\) per day) is a relevant dose metric for the critical effect.
- Exposures in the range encountered in the environment will not result in saturation of the metabolic capability for individual compounds, nor are combined exposures sufficiently high to result in metabolic interactions/inhibitions among THM compounds (Tan et al., 2007; USEPA, 2006c).

As a consequence of the model structure, daily area under the curve (AUC) of the concentration of the parent THM compound in liver is directly proportional to daily metabolite production when metabolism is not saturated. Thus, hepatic AUC of the parent compound was selected as a biologically relevant dose metric for the critical non-cancer effects used as the bases for derivation of the RfDs. The relationship between the relevant dose metric, hepatic AUC, and average blood concentrations (the biomarker likely to be measured in humans) was investigated under different exposure scenarios (all oral, all inhalation, or mixed oral and inhalation) using the human PBPK models to confirm that blood concentration could be reliably used as a surrogate for hepatic AUC. Based on the results of that assessment, the following steps were taken:

- The 24-h hepatic AUC in the laboratory animals at the POD for each THM was estimated using the animal PBPK models.
- The 24-h hepatic AUC at the POD was extrapolated to the corresponding human equivalent hepatic AUC by applying an uncertainty factor of one-half an order of magnitude, representing the pharmacodynamic component of the interspecies default uncertainty factor (UFD). The pharmacokinetic component of the interspecies UF was not applied because the extrapolation is conducted using a relevant internal dose metric.
- The human PBPK models were used to evaluate the relationship between hepatic AUC and 24-h average blood concentration, and a value for 24-h average blood concentration consistent with the human equivalent hepatic AUC POD was estimated.

The resulting human 24-h average blood concentration at the POD was combined with the intraspecies uncertainty factor for pharmacodynamic variability (UFH-PD) and, where designated by USEPA, the uncertainty factor for database uncertainties selected by USEPA (UFD), to identify target average blood concentrations consistent with the derivation of the RfD. The pharmacokinetic component of the interspecies UF was not applied because measured blood concentrations in humans are the endpoint metric. Pharmacokinetically “sensitive” humans will display higher blood concentrations for the same external dose; thus, blood concentrations measured in a sample population will directly reflect this component of variability and no additional UF needs to be applied to the calculated target blood concentrations to account for this factor. In essence, humans are the perfect PBPK “model” and the pharmacokinetic variability present will be reflected in the sampling results.

2.1.3. Cancer

Fig. 2 illustrates the approach to the derivation of BE values based on the cancer risk assessment by USEPA. The starting point for the derivation is the lower bound on the estimated dose associated with a 10% increase in tumor frequency (LED\(_{10}\)) from the animal bioassays. Because increases in tumors were observed in several tissues and because the mechanism or mode of action for the tumor responses are not known, no specific internal dose metric could be selected as most relevant. However, blood concentrations are directly related to tissue concentrations throughout the body based on the organ-specific partition coefficients and are thus at least reflective of the relative magnitude of internal concentration within a given tissue. Because no data have been generated to validate mouse PBPK models for the two compounds (DBCM and BDCM) with cancer slope factors based on mouse tumor response data, animal PBPK modeling was not conducted for the POD. Instead, the human equivalent to the POD (the LED\(_{10}\) scaled

![Fig. 1. Flowchart of approach for deriving BE values for the THM compounds for non-cancer endpoints. Effects on hepatic tissue were the most sensitive endpoint observed in animal studies for each of the THM compounds. Based on this, for each compound, the hepatic area under the curve (AUC) resulting from the external dose point of departure (POD) was estimated using the animal pharmacokinetic (PK) model. An equivalent human POD in terms of liver AUC was estimated by applying the default uncertainty factor for animal to human pharmacokinetic differences (UF\(_{PK,R-PH}\)). The human PK model was used to estimate the average blood concentration associated with this hepatic AUC. Finally, remaining uncertainty factors for human variability in pharmacokinetics (UF\(_{1,R1-PH}\)) and database uncertainties (where designated by USEPA) were applied to estimate target average blood concentrations. See text for further discussion of uncertainty factors and approach.](image-url)
2.2. Results of modeling and identification of BE values

2.2.1. Relationship between hepatic AUC and average blood concentrations

Using the human PBPK model for each compound, the relationship between hepatic AUC and average blood concentrations was assessed under oral-only and inhalation-only exposure scenarios. The relationship between the two metrics was linear but route-specific (Fig. 3 for chloroform; other THMs give similar results). Under conditions of oral exposure, hepatic AUC is much greater for a given blood concentration than under conditions of inhalation exposure. This is consistent with the underlying physiology, as oral exposures result in direct absorption of compound from the gastrointestinal (GI) tract into the liver, with subsequent distribution to the venous blood supply. In contrast, inhalation exposures result in direct uptake to blood from the lungs, and subsequent distribution to liver is controlled by blood flow into the liver. For the purposes of using blood concentrations as a surrogate for liver AUC, an assumption of oral-only exposure results in the most conservative (i.e., lowest) estimates of blood concentration consistent with a target hepatic AUC, and this assumption was used in the BE derivation. Under conditions of mixed exposure routes or all inhalation exposure, higher average blood concentrations would be required to result in the target hepatic AUC.

2.2.2. Non-cancer endpoints

The hepatic AUC associated with the POD for each THM compound using the PBPK models for dogs (chloroform) and rats (the brominated THMs) are reported in Table 3. The corresponding human equivalent hepatic AUC POD and corresponding average human blood concentrations (estimated from the human PBPK models for each THM) are also reported. Finally, the estimated average blood concentration consistent with the derivation of the RfD (BE\textsubscript{RD}) for each compound is presented. Because of the rapid elimination kinetics and highly volatile nature of these compounds, blood concentrations at any given time point during a day can deviate substantially from the average estimate presented here and yet still be consistent with exposures not exceeding the critical hepatic AUC dose metric. For example, ingestion of a single dose of a THM equal to the RfD (through drinking water consumption, for example) can result in a rapid rise in blood concentration to a peak more than five times higher than the 24-h average concentration that would be associated with that same dose. Inhalation of THM compounds also results in a rapid rise and then fall in blood concentrations upon removal of the airborne exposure source.

Estimation of the magnitude of such peaks can be made using PBPK modeling by accounting for numerous sources of variability including variations in oral absorption rate and characteristics of the exposure episode. However, estimates of such peaks are far less certain than the 24-h average blood concentrations, which are relatively stable, and essentially insensitive to the oral absorption rate.

Table 3

<table>
<thead>
<tr>
<th>Compound</th>
<th>POD mg kg(^{-1}) d(^{-1})</th>
<th>BE\textsubscript{RD,animal} Animal avg. blood conc., pg ml(^{-1})</th>
<th>Animal hepatic AUC mg h L(^{-1}) d(^{-1})</th>
<th>UF\textsubscript{A/RD}</th>
<th>Human equivalent hepatic AUC mg h L(^{-1}) d(^{-1})</th>
<th>BE\textsubscript{RD,human} Corresponding human avg. blood conc., pg ml(^{-1})</th>
<th>UF\textsubscript{f16-h}</th>
<th>UF\textsubscript{f2}</th>
<th>BE\textsubscript{RD} pg ml(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroform</td>
<td>1.2</td>
<td>4,400</td>
<td>0.12</td>
<td>3.2</td>
<td>0.038</td>
<td>750</td>
<td>3.2</td>
<td>1</td>
<td>230</td>
</tr>
<tr>
<td>DBCM</td>
<td>1.6</td>
<td>2,200</td>
<td>0.056</td>
<td>3.2</td>
<td>0.019</td>
<td>270</td>
<td>3.2</td>
<td>1</td>
<td>80</td>
</tr>
<tr>
<td>BDCM</td>
<td>0.8</td>
<td>670</td>
<td>0.019</td>
<td>3.2</td>
<td>0.006</td>
<td>190</td>
<td>3.2</td>
<td>3</td>
<td>20</td>
</tr>
<tr>
<td>TBM</td>
<td>2.6</td>
<td>2,900</td>
<td>0.074</td>
<td>3.2</td>
<td>0.025</td>
<td>420</td>
<td>3.2</td>
<td>1</td>
<td>130</td>
</tr>
</tbody>
</table>
and exposure episode characteristics. But evaluation and interpretation of biomonitoring data for THMs must recognize that at any given time during the course of a day, concentrations in an individual several times higher than the target daily average concentration can occur without necessarily indicating exposures in excess of those considered to be tolerable. Conversely, concentrations below the daily average concentration would also be encountered depending on when sampling occurred in relation to exposure.

Fig. 4 illustrates the impact that varying only the oral absorption rate parameter in the PBPK model has on predicted blood concentrations following a single oral exposure. Because of the uncertainty associated with accurately predicting peak levels of biomarkers associated with a once daily exposure, experts at a recent workshop addressing technical and communications challenges in deriving biomonitoring equivalents recommended that for short-lived compounds, estimates of daily average blood concentrations were more reliable than estimates of peaks and are best used as a screening tool to evaluate average measured concentrations in a population (Hays et al., 2008). However, in individuals, the existence of transient peaks substantially above the BE should be recognized as potentially consistent with the BE values (Hays et al., 2008).

2.2.3. Cancer risk

Using the approach described in Fig. 2, the human equivalent POD and risk-specific doses associated with cancer risks of $1 \times 10^{-6}$ to $1 \times 10^{-4}$ were identified, and the daily average blood concentrations associated with daily exposure at these risk-specific doses were modeled (Table 4). The PBPK models implemented here were also used to predict peak blood concentrations associated with single daily dose exposures at the risk-specific doses to provide an indication of the degree of variation that might plausibly be attributed to different exposure scenarios (Table 4). As discussed above, transient peak blood concentrations several times the averages presented in Table 4 are also consistent with exposure at these risk-specific doses, but it is difficult to obtain a reliable estimate of such peaks. However, for rapidly metabolized compounds, it is important to recognize and reflect the substantial variation in measured blood concentrations that may result from equivalent daily exposures under differing temporal patterns (Hays et al., 2008).

2.3. Discussion of sources of variability and uncertainty

A number of issues affect the interpretation of, and confidence in, the BEs developed for the four THMs. These factors include the impact of temporal variations in exposure patterns for rapidly eliminated compounds, uncertainties regarding the pharmacokinetic models used, mechanistic considerations related to the carcinogenicity of the THMs, interpreting blood levels above the BEs, and methods for addressing simultaneous exposures to four THM compounds. These issues are discussed below.

2.3.1. Temporal variations: exposure, sampling, and short half-life

For compounds with very short elimination half-lives such as the THMs, both the variability in recent exposure behaviors and the longer term exposure profiles are relevant to interpreting blood levels with respect to BE values. Activities of an individual have implications for understanding whether measured levels in that individual represent a peak exposure (e.g., did the study participant shower immediately prior to sample collection? Were levels of THMs in the tap water at peak levels when the activity occurred?) or more typical exposures. Researchers are working to evaluate the relationships between exposure patterns and pathways and note the significant changes in blood concentrations that follow usage of THM-containing water (Backer et al., 2008; Ashley et al., 2005; Villanueva et al., 2007b). However, this type of information is not necessarily captured by questionnaire data or available for use in interpreting cross-sectional population-based biomonitoring data. Interpretation is further complicated by the fact that the concentrations of THMs in drinking water fluctuate (Ashley et al., 2005), with concentrations depending on such factors as the season, water temperature, amount of chlorine added by the treatment facility, and the amount of organic matter in the source water (USEPA, 2006a). The RfD and other health-based chronic exposure guidelines are set at levels designed to be protective for exposures over a specified period of time (e.g., one week, several months, or a lifetime), while biomonitoring data provide only a snapshot at a particular point in time. This will necessarily limit the interpretation of cross-sectional biomonitoring data for such compounds in terms of chronic health risks without substantial additional data on current and ongoing exposure characteristics.

### Table 4

Human equivalent LED$_{10}$ estimates for cancer endpoints (USEPA, 2005), corresponding average and peak blood concentrations at the LED$_{10}$ estimated using the human PBPK models, and extrapolated average and peak modeled human blood concentrations for the $10^{-6}$ to $10^{-4}$ risk range for three THM compounds.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Human equivalent LED$_{10}$ mg kg$^{-1}$ d$^{-1}$</th>
<th>Modeled human blood concentrations associated with LED$_{10}$ pg ml$^{-1}$</th>
<th>Range of modeled human blood concentrations (BEs) associated with $10^{-6}$ to $10^{-4}$ risk-specific doses, pg ml$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average</td>
<td>Peak</td>
<td>Average</td>
</tr>
<tr>
<td>BCM</td>
<td>2.5</td>
<td>16,000</td>
<td>120,000</td>
</tr>
<tr>
<td>BDCM</td>
<td>3.0</td>
<td>15,000</td>
<td>120,000</td>
</tr>
<tr>
<td>TBM</td>
<td>22</td>
<td>740,000</td>
<td>5,000,000</td>
</tr>
</tbody>
</table>

Chloroform is not included in this table because USEPA has determined that the non-cancer RfD is protective for cancer in humans.
and range of impacts of the bolus ingestion of chloroform. In contrast, an initial analysis of a recent data set published for BDCM (Leavens et al., 2007) suggests that the current published PBPK models may over-predict average blood concentrations associated with bolus ingestion of BDCM.

One consequence of the choice of target hepatic AUC following oral exposure as the critical dose metric is that many of the details of the PBPK models become irrelevant to the estimation of the human average blood concentration corresponding to the target hepatic AUC. That is, under conditions of linear kinetics (conditions which hold in the conceivable exposure ranges for humans exposed environmentally to THMs), the modeled relationship between daily hepatic AUC and daily average blood concentration depends solely on the partition coefficient between liver and blood. There are few published data on variability in partition coefficients among individuals. In one recent study, blood to air partition coefficients for six volatile organic compounds were relatively consistent across individuals, with coefficients of variation of less than 20% and less than 10% differences between males and females and between adults and children (Mahle et al., 2007). The model parameters for metabolism and oral absorption rate do not affect the relationship between daily hepatic AUC and estimated average blood concentration for compounds such as these that undergo nearly complete metabolism and elimination over the course of a day. In contrast, estimates of peak concentrations in liver or blood are highly sensitive to metabolic and absorption rate parameters. However, the BE values derived here for cancer endpoints, which translate external exposures at risk-specific doses directly to predicted average blood concentrations, are sensitive to the metabolic parameters of these models. Additional data sets involving controlled exposures should be conducted to refine and parameterize the human models for these compounds.

2.3.3. Mechanistic considerations related to the carcinogenicity of the THMs

The four THM compounds are considered by USEPA to be carcinogenic. However, USEPA has determined, based on well-studied mechanistic considerations, that chloroform is not likely to produce cancer in humans at exposures below the RfD (Schoeny et al., 2006). While no mode of action has been established for the other THMs (USEPA, 2005), a recent cancer bioassay for BDCM found no excess incidence of tumors in female mice and male rats exposed to BDCM via drinking water (NTP, 2006) at daily doses just below the lower end of the previous, positive, NTP gavage study dose range. The study protocol used three administered dose levels and a relevant route of administration (drinking water) at the maximum palatable concentrations of BDCM. No increases in tumors at any site in either species were observed, in contrast to the findings of liver, kidney, and large intestine tumors in the previous corn oil gavage bioassays (summarized in USEPA, 2005). Because the maximum doses used in this bioassay are approximately one half of the lowest doses used in the corn oil gavage studies, no clear conclusions regarding the effect of vehicle can be drawn. However, these data suggest the possibility that, as for chloroform, the carcinogenic response to BDCM in the corn oil gavage bioassay may be sensitive to peak concentrations resulting from bolus administration in corn oil gavage and perhaps of less relevance to human drinking water exposures.

2.4. Confidence assessment

Guidelines for the derivation of BE values (Hays et al., 2008) specify consideration of two main elements in the assessment of confidence in the derived BE values: Robustness of the available pharmacokinetic models and data, and understanding of the rela-

![Fig. 5](image-url)
tion between the measured biomarker and the critical or relevant target tissue dose metric.

2.4.1. Robustness of pharmacokinetic data and models

In the case of the THMs, the pharmacokinetic data and models for rodents are based on substantial data sets, while those available for humans have not been assessed against much experimental data. In the case of the non-cancer BE derivations, the most sensitive parameter within the human PBPK model is the partition coefficient between liver and blood. This value is dictated largely by the physical/chemical properties of these compounds, which are reasonably well understood (Gargas et al., 1989) and available data indicate relatively low variability in partition coefficients among individuals (Mahle et al., 2007); therefore, confidence in this aspect of the non-cancer BE values is high. The cancer risk-based BE values are more sensitive to metabolic parameters for the individual compounds, and as discussed above, the limited available human data sets suggest that the model predictions of average blood concentrations for a given risk-specific dose may be underestimate for chloroform and overestimate the actual average BDCM concentrations. In addition, the cancer risk-specific doses require extrapolation far below the range of observed data used to derive the basic model parameters in rodents and in humans. Thus, confidence in this aspect of the derivation of BE values for cancer is low.

2.4.2. Relationship of measured biomarker to critical or relevant dose metric(s)

The non-cancer endpoint of interest for all four compounds is liver toxicity, which may be related to production of reactive metabolites. Thus, the critical dose metric will be related to metabolite production and could be related to either cumulative or peak metabolite concentrations. Hepatic AUC of the parent compound is directly related to daily cumulative metabolite production when linear metabolism conditions hold; however, peak metabolite concentration is not directly related to hepatic AUC of parent compound. As discussed above, modeling of actual peak liver concentrations is highly uncertain. For this reason, daily cumulative metabolite production was selected as the relevant dose metric. Daily average blood concentration, the biomarker metric used in the non-cancer BE derivation, is directly related to daily liver AUC, so confidence in this element of the non-cancer BE derivation is high. For cancer, various target organ sites are of interest based on the results of laboratory rodent studies, and the mechanism of action is not agreed upon for THM compounds other than chloroform, although reactive metabolites are likely to be important. Despite a lack of detailed mechanistic information for all four THM compounds, average blood concentrations are likely to be relevant for critical internal dose metrics for most cancer target sites observed, except potentially the intestinal tumors observed following gavage administration of TBM, which might indicate a local, portal of entry response not directly related to blood concentrations. Based on this assessment, confidence in this element of the cancer risk-specific BE values is medium.

Overall, these assessments suggest high confidence in the non-cancer BE values, and low to medium confidence in the cancer risk-specific BE values.

3. Discussion and interpretation of BE values

The BE values presented here are screening values and can be used to provide a screening-level assessment of measured blood levels of THMs in population- or cohort-based studies. BE values do not represent diagnostic criteria and cannot be used to evaluate the likelihood of an adverse health effect in an individual or even among a population. BEs are not “bright lines” separating safe from unsafe blood levels. Chronic RfD values are set at levels that are designed to be health-protective for daily exposure for a full lifetime of exposure. The BE values identified here are tied to daily average blood concentration. For short-lived compounds, transient blood concentrations several times higher (and lower) than these target daily average concentrations would be expected in individuals resulting from episodic use of drinking water under conditions in which the target average daily concentration was not exceeded. Thus, a measured blood concentration exceeding the corresponding BE value in a single sample of blood from an individual may or may not reflect continuing elevated exposure.

As discussed above, for short-lived compounds, BEs should be used as tools to evaluate biomonitoring data on a population basis, rather than for assessment of an individual person’s biomonitoring levels. If the mean of the population-based biomonitoring data is below the BEs, then in general the population is experiencing exposures lower than those considered consistent with the RfD or a target cancer risk range, even though some of the blood samples may show higher concentrations. However, the potential for heterogeneous exposures within the population, combined with the transient nature of blood concentrations of these compounds, will limit the ability to interpret upper tails of the distribution of measured blood concentrations from cross-sectional studies. Fig. 6 illustrates the BE values derived for DBCM in this paper in a framework that can be useful for interpretation of biomonitoring data.
3.1. Implications of simultaneous exposures to four THM compounds

THMs are regulated in drinking water as a combined group of four compounds with an MCL (maximum contaminant level) of 80 μg L⁻¹ (USEPA, 2006a). However, as indicated by the range of RfD values and related BE₆₉ values, the toxic potency is not equivalent for these compounds, and equal exposures do not result in equal blood levels due to differences in metabolic rates, partition coefficients, etc. Thus, assessment of the concentration of THMs in blood by summing the concentrations of the four compounds may not be scientifically justifiable. One approach for combining available data for these compounds might involve a hazard quotient/hazard index approach, analogous to the approach used for risk screening across compounds in assessments of exposure to multiple chemicals at hazardous waste sites. In the case of THM compounds, all four compounds demonstrate liver toxicity as among the most sensitive non-cancer toxicological effects in animal studies, lending support to a hazard index approach. Specifically, the concentrations in blood of each THM could be related to that compound’s BE₆₉ value for an individual, with the resulting ratios summed to obtain the hazard index (HI):

\[ HI = \sum_{i=1}^{4} \frac{[\text{THM}_i]}{\text{BE}_{\text{RfD},i}} \]  

The current mixtures risk assessment paradigm implies no excess risks when the HI is below 1. This method requires examination of biomonitoring data on an individual-by-individual basis, and thus may be limited to situations where the biomonitoring data for all of the individual compounds are available on an individual-by-individual basis. However, as discussed above, blood concentrations in an individual are likely to fluctuate widely due to the rapid absorption and elimination of these compounds, and hazard indices of greater than 1 may occur in an individual without necessarily indicating daily or long-term exposures in excess of those consistent with the RfD. Assessment of the impact of combined exposure to THMs on cancer risk estimates could theoretically proceed in an analogous manner, with estimated compound-specific risks summed across the three brominated THM compounds, similar to approaches used in assessing cancer risks from mixtures on an external exposure basis. However, the risk assessment paradigm for both cancer and non-cancer endpoints incorporates an assumption of constant exposure for a full lifetime. Conclusions regarding either cancer or non-cancer risks based on biomonitoring data derived from cross-sectional studies for rapidly metabolized compounds such as THMs must be tempered by the recognition that such biomonitoring efforts may not accurately reflect long term average blood concentrations in individuals.

3.2. Risk/benefit considerations for drinking water disinfection

As noted by EPA (USEPA, 2006a), DBPs present a case in which there are obvious trade-offs between decreasing the potential risks associated with DBPs and increasing risk from exposure to pathogens in drinking water and “eliminating or significantly decreasing disinfection to stop disinfection byproduct formation would seriously compromise overall public health protection” (USEPA, 2006a). Complicating attempts to quantify the risk/benefit “trade-off” is the lack of commonly used methods for comparing two distinctly different types of risks, i.e., a potential risk of cancer from lifetime exposure to DBPs versus health benefits associated with protection against acute illness from exposures to pathogens in untreated waters. One attempt to perform such an analysis focused on reduction of risk of infection by Cryptosporidium parvum compared with risk of renal cell cancer from exposure to bromate in water disinfected by ozonation (Havelaar et al., 2000). The authors used the concept of disability adjusted life-years (DALYS) and found a net health benefit associated with disinfection of drinking water, even though estimated bromate levels were above World Health Organization guidelines (Ashbolt, 2004). These results cannot be applied directly to this current assessment because the method of water treatment, and therefore the DBPs formed, differ. However, it is believed that the potential risks associated with DBP exposure are insignificant compared to the microbial risks that would transpire without disinfection (Bull et al., 1995).

4. Conclusions

The BE values developed and described here provide quantitative tools that can be used in a screening-level assessment of biomonitoring data for chloroform, DBCM, BDCM, and TBM in human blood. These levels do not represent a bright line between safe and unsafe exposure levels, nor can they serve as diagnostic values for application to individual measured levels. Instead, the BE values presented here can provide a health-based context for evaluating biomonitoring data sets and assist in prioritization of further research, risk characterization, and risk management activities. BE values do not represent diagnostic criteria and cannot be used to evaluate the likelihood of an adverse health effect in an individual or even among a population.

Interpretation of measured blood concentrations of THMs will continue to pose challenges due to the rapid metabolism and elimination of these compounds. While these BE values refer to long-term average concentrations of THMs in blood, biomonitoring results for individuals are generally based on single, snapshot measurements of compounds and are highly influenced by whether an exposure (for example, due to showering or drinking water) has occurred recently. Thus, the BE values derived here, which correspond to 24-h average blood concentrations consistent with exposure guidance values, are most appropriately applied to assess the central tendency and overall pattern of results for a sampled population, rather than extreme values or individual measurements. In addition, these BE values should be used in combination with other tools and information to evaluate and interpret biomonitoring data for the THMs. Further discussion of interpretation and communications aspects of BE values is presented in LaKind et al. (2008).

**Conflict of interest disclosure statement**

The authors declare that they have no conflicts of interest.

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References


