Case Study: Risk Assessment of Exposure to Trihalomethane Drinking Water Disinfection By-Products. Use of Biomonitoring Equivalents and Biomonitoring Data from NHANES

Prepared for:

Beyond Science and Decisions: From Problem Formulation to Dose-Response

Alliance for Risk Assessment Workshop
May 4-6, 2011

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Case Study: Risk Assessment for Exposure to Trihalomethane Drinking Water Disinfection By-Products. Use of Biomonitoring Equivalents and Biomonitoring Data from NHANES

Problem Formulation: How can the current USEPA THM non-cancer risk assessments be used to interpret human biomonitoring data for trihalomethanes (THMs)? How do alternative approaches to low-exposure extrapolation compare to the conventional Hazard Quotient approach for assessing non-cancer risks based on internal dose-response assessment in conjunction with the biomonitoring data? Assessment of these approaches and issues would be useful in the assessment and comparison of costs/risks and benefits of drinking water disinfection measures and assessment of regulatory options to reduce THM DBPs.

Method: This case study explores the application of the Biomonitoring Equivalents (BE) paradigm and population-representative biomonitoring data for THMs in blood from the National Health and Nutrition Examination Survey (NHANES) to risk assessment of non-cancer endpoints for THMs. Alternative approaches for low-exposure extrapolation of risk of non-cancer hepatic outcomes from THM exposure in the general US population based on the NHANES biomonitoring data and the BE approach are explored. Because THMs are rapidly absorbed and eliminated, issues in interpretation of biomonitoring data associated with the transience of the biomarker are discussed.

I.   Introduction

Quantitative dose-response assessments for trihalomethane (THM) drinking water disinfection byproducts (DBPs; chloroform, bromoform [TBM], bromodichloromethane [BDCM], and dichlororobromomethane [DCBM]) were recently conducted by the US EPA for both cancer and non-cancer endpoints, resulting in development of cancer slope factors (CSFs) (for all but chloroform) and reference doses (RfDs) (for all four compounds) (USEPA 2001, 2005).[1] Non-cancer RfDs for all four compounds are based on hepatic responses including fatty liver degeneration and hepatic vacuolization in laboratory rodents and dogs. Non-alcoholic fatty liver disease (NAFLD) is prevalent in adults in the US: prevalence estimates range from 3 to 24%, with most estimates in the 6 to 14% range (Clark 2006). Obesity and diabetes are significant risk factors for development of NAFLD. While many cases of NAFLD are clinically benign, of limited relevance to health, and potentially reversible, severe NAFLD may progress to liver inflammation and fibrosis with more serious health implications (Clark 2006).

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[1] A CSF for chloroform was not developed because the substance produces tumors via a threshold mode of action (Boobis et al. 2009)..

Exposure to THMs arising from disinfection of drinking water is widespread in the US, but variable and difficult to quantify using conventional external dose exposure assessments due to fluctuating water concentrations, complex water use patterns, and multiple exposure routes (ingestion, dermal, and inhalation). The biomonitoring component of the National Health and Nutrition Examination Survey (NHANES) includes measurement of trihalomethanes in blood samples collected from a statistically representative sample of the civilian, non-institutionalized US population. These measured concentrations provide an integrated reflection of exposures that may occur via multiple routes and pathways, and biomonitoring is increasingly being relied upon as a state-of-the-art tool for exposure assessment for environmental chemicals (Sexton et al. 2004). However, measured blood concentrations of THMs and other chemicals cannot be directly compared to RfDs to evaluate population risks because RfD values are expressed as applied doses (mg/kg-day) and biomonitoring results are expressed as concentration in blood, urine, or expired air.

In order to address this disconnect between exposures as reflected in biomonitoring data and exposure guidance values (such as RfDs) presented in terms of external exposures, the Biomonitoring Equivalents (BEs) method has been developed, and guidelines for the derivation and communication of these values have been published (Hays et al. 2007, 2008; LaKind et al. 2008). A BE is defined as the concentration or range of concentrations of a chemical or its metabolites in a biological medium (blood, urine, or other medium) that is consistent with an existing health-based exposure guidance value such as a reference dose (RfD) or Tolerable or Acceptable Daily Intake (TDI or ADI). BEs are intended to be used as screening tools to provide an assessment of which chemical biomarkers are present at levels well below, near, or at or above concentrations that are consistent with existing risk assessments and exposure guidance values, and thus can provide an evaluation of relative priority for risk assessment follow-up. BEs provide a translational tool allowing application of the conventional risk assessment paradigm to evaluation of exposure information provided by biomonitoring data. BEs for THM compounds have been previously published (Aylward et al. 2008) and the NHANES 2003-2004 data assessed in light of these BE values (LaKind et al. 2009).

Application of the BE methodology and interpretation of the NHANES blood concentration data for THMs requires consideration of the pharmacokinetic properties of THM compounds. These compounds are both rapidly absorbed and eliminated from the blood. Thus, THM blood concentrations within individuals within and across days are highly variable (>10-fold to 100-fold variation within a day in individuals) (Backer et al. 2000; Ashley et al. 2005). The BE methodology relies on estimation of 24 hour average blood concentrations consistent with the RfD. Blood samples as collected in the NHANES program provide only a snapshot in time of blood THM concentrations. Thus, the extremes of the population distribution of blood THM concentrations in NHANES datasets are unlikely to be reliable surrogates of the long-term 24-hour average blood concentration of these compounds in individuals. For this reason, BE values are most appropriately applied to assess the central tendency of population biomonitoring data

for transient compounds such as THMs (Aylward et al. 2008; Hays et al. 2008), and limitations in the ability to interpret the tails of the population distribution of blood concentrations for such transient compounds are acknowledged. Similarly, confidence in the tails of the estimated risks presented in this analysis is limited and these estimates must be interpreted with caution.

II. Methods
A. Summary of Derivation of BE Values for Systemic Toxic Effects

BE values for the THM compounds were derived using an internal dose-based risk assessment approach (Figure 1); the derivation is described in detail in Aylward et al. (2008). Briefly, the current USEPA RfD derivations were reviewed and summarized (Table 1, reproduced from Aylward et al. 2008). Fatty liver degeneration (or a related effect, hepatic vacuolization for TBM) was the critical endpoint for all four compounds, and benchmark dose modeling was used by USEPA to identify a $BMDL_{10}$ for use as a point of departure (POD) for the development of RfDs for all four compounds. Based on this information, hepatic AUC of the parent compound was selected as a relevant dose metric for each compound.

Previously-published PBPK models were used to estimate the hepatic AUC at the $BMDL_{10}$ for each THM in the relevant species. The hepatic AUC in the rodent or dog was extrapolated to a corresponding human-equivalent POD hepatic AUC by application of the interspecies uncertainty factor component for toxicodynamic extrapolation as used in the EPA RfD derivation ($10^{0.5}$). The toxicokinetic component of the interspecies UF was replaced by the use of a relevant internal dose metric. The human version of the PBPK model for each compound was used to estimate the steady-state average blood concentration consistent with the identified human-equivalent POD hepatic AUC. This quantity is termed the BE_{POD}. This estimate was dependent upon what route of exposure was assumed in the PBPK modeling. Assumption of 100% oral exposure produced the most conservative estimates of average blood concentration consistent with the target human-equivalent POD hepatic AUC for each compound; assumption of 100% inhalation exposure would have resulted in BE_{POD} values approximately 6-fold higher, with assumption of mixed exposure routes resulting in intermediate values. Finally, BE_{RfD} values were estimated via application of the toxicodynamic portion of the intraspecies UF and any database UFs applied in the RfD derivation (see Table 2 and Figure 1).

Table 2 (reproduced from Aylward et al. 2008) presents the modeled animal hepatic AUCs at the PODs, extrapolated human-equivalent POD hepatic AUCs, and the BE_{POD} and BE values for each of the four THMs. In the context of the quantitative risk assessment, the BE_{POD} values can be regarded as a conservative estimate of the average human blood concentrations associated with the POD (with interspecies toxicodynamic uncertainty factors applied), which in this case was set at a benchmark response of 10% risk of fatty liver degeneration.

For the purposes of this risk assessment case study, it will be assumed that the BE_{POD} (derived based on a $BMDL_{10}$) for each of the four compounds is a conservative estimate of the average human blood concentration associated with a 10% risk of fatty liver degeneration from chronic exposure to that compound. However, this interpretation should be discussed and considered further. Since each of the underlying RfDs have been derived to provide "an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime," each of the corresponding BE_{RfD}s reflect the same level of protection.

B. Description of NHANES THM Blood Concentration Data and Evaluation Using Biomonitoring Equivalents

NHANES data on the concentrations of THM compounds in blood in adults aged 20 to 59 from the 2003-2004 NHANES sampling cycle were downloaded from the NHANES web site (http://www.cdc.gov/nchs/nhanes/nhanes2003-2004/lab03_04.htm). THM levels in blood were measured by CDC using solid-phase microextraction coupled with gas chromatography and high-resolution mass spectrometry (Bonin et al., 2005). The 2003-2004 data have previously been described along with data from two earlier NHANES sampling cycles; weighted percentiles are presented in Table 3 (reproduced from LaKind et al. 2009).

The BE_{RfD} can be used analogously to the RfD for the purposes of estimating hazard quotients and hazard indices. Four chemical-specific hazard quotients (HQs) were calculated for each individual THM in the NHANES dataset by taking the ratio of the reported concentration to the chemical-specific BE_{RfD} values. Because the THMs produce similar liver pathology and are believed to act through a similar mode of action, it may be appropriate to assess the combined exposures using a hazard index (HI) approach. This is consistent with the IPCS *Framework for Assessment of Combined Exposures* (IPCS, 2009).[2] This approach assumes dose addition. So, for each individual in the NHANES dataset, a THM hazard index was also calculated, summing the HQs across the four THM compounds (i=1 to 4):

$$HI = \sum_{i=1}^{4} \frac{[THM_i]}{BE_{RfD\_i}} \tag{1}$$

The conventional interpretation of HQ and HI values is that values below 1 indicate no risk of adverse outcome.

C. Low-Exposure Extrapolation Approaches

The selection of a low-exposure extrapolation approach can be guided by consideration of the likely mode of action for development of clinically relevant fatty liver in laboratory animals due

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[2] http://www.who.int/ipcs/methods/harmonization/areas/aggregate/en/index.html.

to exposure to THMs as well as the mode of action for development of fatty liver in humans in the general population. The specific mechanism of action for fatty liver degeneration due to THM exposure in laboratory animals is unknown, but key events in the mode of action are likely similar to the early key events described for chloroform-induced liver cytotoxicity, regenerative proliferation, and high-dose carcinogenesis (summarized in Boobis et al. 2009). That is, key events are related to production of cytotoxic metabolites with subsequent cell damage and alteration of cellular lipid metabolism. In humans, the significant association of NAFLD with obesity, diabetes, and metabolic syndrome suggests that NAFLD results from a variety of alterations in lipid metabolism and liver function. The mode of action of THMs in laboratory animals is likely relevant to humans if the concentrations of the compounds and metabolites are sufficient to produce liver cell toxicity. However, if underlying cytotoxic processes require a minimal concentration of reactive metabolites to produce alterations in liver tissue, a threshold model may be most appropriate. Complicating the assessment of the appropriate response for low exposure extrapolation is the fact that the dose-response assessment based on the laboratory animal data was conducted using a quantal (liver designated as showing fatty degeneration or not) rather than a continuous (severity of fatty alterations and fat depositions) measure.

Given these uncertainties, two methods for low-dose extrapolation on the basis of blood concentrations were explored in this evaluation (Figure 2). Method 1 assumes an underlying threshold for pathologic fatty liver development. In this case, the RfD is defined as corresponding to zero risk of fatty liver development. Risks were thus linearly extrapolated from the BMR of 10% at the BE_{POD} to zero risk at the BE_{RfD}. Method 2 assumes that even small concentrations of reactive metabolites are associated with some increase in liver fattiness and thus occurrence of NAFLD. For method 2, the risk is assumed to be linear (arithmetic) from 10% risk at the BE_{POD} down to zero risk at zero exposure.

Note that other methods of extrapolation from the BE_{POD} could be selected, notably, application of the dose-response function selected in the derivation of the $BMDL_{10}$. However, the two methods presented here likely represent the bounds on other available approaches, and for simplicity in illustrating the use of the BE method and biomonitoring data, only these two methods of extrapolation are illustrated.

For each of the four THM compounds, a slope relating risk of fatty liver per pg/ml in blood was calculated according to each of the two methods:

Threshold Model (Method 1):

$$Slope1 = \frac{0.1}{BE_{POD} - BE_{RfD}} \text{ for blood concentrations} > BE_{RfD}, \text{ else, Slope1} = 0 \qquad (2)$$

No-Threshold Model (Method 2):

$$Slope2 = \frac{0.1}{BE_{POD}-0} \text{ for all blood concentrations} < BE_{POD} \qquad (3)$$

These slopes were applied to measured blood concentrations to estimate THM-specific risk for each individual in the NHANES dataset according to each of the two methods. Specifically, the risk, R, associated with blood concentrations of each THM in each individual in the NHANES datasets was estimated as:

$$R_{THMi} = Slope_{THMi} * Cblood_{THMi} \qquad (4)$$

where the slopes for the two methods are defined as above and applied in the specified concentration ranges. Weighted population percentiles of risks based on the calculated risks for each individual in the NHANES dataset were calculated using STATA IC10 (StataCorp LC, College Station, TX).

Because the critical endpoints and modes of action for all four THMS are likely shared, we also assessed combined risk from the THMs by summing the compound-specific risks for each method for each individual in the NHANES dataset, analogous to the calculation of a combined HI above. Weighted percentiles of population risk were then estimated for each method.

III.   Results

The distributions of chemical-specific systemic toxicity HQ values and combined THM HI values are presented in Figure 3. The population-weighted median HI based on the individual-specific summed HQ values is 0.15, and the 95th percentile HI is 0.8. Under typical interpretation of hazard quotients and hazard indices, these exposures would be considered to be acceptable. As noted above, the BE_{RfD} values are based on estimation of long-term average blood concentrations. For biologically transient compounds such as THMs, blood concentrations are expected to fluctuate widely in individuals both within and across days. Thus, interpretation of the tails of the distributions of concentrations from spot samples (and resulting HQ and HI values) is problematic, and the BE guidelines for biologically transient chemicals recommend focus on central tendency estimates for population sampling data and an acknowledgement of the limitations in interpretation of the tails of the distribution (Hays et al. 2008).

Slopes relating blood THM concentrations to risk of fatty liver calculated based on a threshold model (method 1) and linear no-threshold model (method 2) (as indicated by equations 2 and 3 and as illustrated in Figure 2) are presented in Table 4. The chemical-specific risks under each of the two methods were calculated for each individual in the NHANES dataset, and weighted percentiles of estimated risks under the two methods were calculated as described above. Table 5 presents the results of these calculations.

Under the threshold model (method 1), no risk of THM-related fatty liver due to combined THM exposure is predicted through at least the 95th percentile of the population based on the NHANES blood sampling and the slopes provided in Table 4. Under the linear no-threshold (arithmetic) model (method 2), estimated risks from combined THM exposure range from approximately 3 $\times 10^{-3}$ to 1.5×10^{-2} at the median and 95th percentiles, respectively.

As noted above, the extremes of the population distribution of THM blood concentration data in the NHANES program are unlikely to be reliable surrogates for the long-term 24-hour average blood concentration in any individual, and thus, the upper tail of the distribution of estimated risks should be regarded as likely to overestimate chronic risks for any individual. Given this limitation, focus on the central tendency of the estimated risks is most appropriate in the context of the BE paradigm and the biomonitoring data (Hays et al. 2008). Thus, this analysis suggests that at the central tendency based on a threshold method, no risk of NAFLD would be attributable to THMs. Under the no-threshold method, at the population median, a risk of 0.003 or 3 cases per 1,000 adults in the general population, might be attributable to THM exposure.

In a review of the literature on prevalence of NAFLD in the US, Clark (2006) noted that reported prevalence in adults in the US ranges from 3 to 24%, with most estimates in the range of 6 to 14%. For the purposes of this analysis, we will assume that a central tendency estimate of background risk of NAFLD in the US is approximately 10%, or 100 cases per 1,000 adults in the general population. Under method 2, the no-threshold approach, the median estimated population risk of 0.003 in the general population would translate to roughly 3 cases of NAFLD per 100 cases of NAFLD in the general population (assuming a population prevalence of NAFLD of 10%). That is, according to this analysis, the central tendency of THM exposures as reflected in the NHANES population biomonitoring data might be responsible for approximately 3% of the observed cases of NAFLD in the general US adult population.

Note that under method 2, risks associated with blood concentrations at the BE_{RfD} for each THM compound are NOT predicted to be zero (see Figure 2). The compound-specific risks predicted at the BE_{RfD} under this method (using equation 4) range from 0.01 (BDCM) to 0.03 (each of the other 3 compounds).

IV. Discussion

Biomonitoring data provide valuable information regarding chemical exposures because the measured biomarkers provide an integrated reflection of exposures from all routes and pathways and reflect actual rather than estimated exposure levels. The BE methodology provides a translational tool that allows assessment of biomonitoring data in the context of existing risk assessments using approaches such as estimation of hazard quotients, hazard indices, and margins of exposure and safety. This case study demonstrates application of the BE approach to THM drinking water disinfection by-products, but the method has its greatest utility when

applied to assess relative priority for risk assessment and risk management efforts across a range of chemicals. BE values corresponding to risk assessment-based tolerable intakes have been derived for a wide range of chemicals of interest in biomonitoring programs in the US and abroad (Table 6).

The transience of blood concentrations of THMs limits the usefulness of measured concentrations in single samples of blood for the characterization of long-term average blood concentrations, which is the dose metric of interest in the risk assessment paradigm. Because of this limitation, the BE methodology and guidance recommends focus on central tendency estimates for population biomonitoring data for chemicals that are short-lived and an acknowledgement of the limitations in terms of characterizing population distribution of exposures (Hays et al. 2008; Aylward et al. 2008). In a hazard quotient/hazard index framework using the chemical-specific BE_{RfD} values, the measured blood concentrations of the individual THM compounds as well as the assessment of combined exposures to all four THMs on an individual-by-individual basis result in HQs and an overall HI less than 1 at the central tendency and at higher percentiles in the NHANES 2003-2004 survey.

The results of low-exposure extrapolation of potential risks of hepatic responses similar to those observed as the critical effects in the bioassays underlying the USEPA risk assessments depends upon assumptions made regarding whether the responses can be assumed to have linear or threshold type behaviors in individuals and the population. Under a threshold assumption, no risk of NAFLD would be expected due to THM blood concentrations in NHANES. Under the most conservative assessment, the central tendency of blood concentrations in the NHANES dataset suggest a potential upper bound risk of 3 per 1,000 in the general population for NAFLD. Given the substantial prevalence of NAFLD in the general population (approximately 10%), if the no-threshold linear (arithmetic) method is biologically plausible, this risk suggests that up to 3% of the prevalent cases of NAFLD in the US population might be due to THM exposures.

A great number of assumptions are implicit and explicit in the analysis presented here. Some of these are enumerated in Table 7, along with some assessment of the potential impact on the risk assessment. The derivation of the BE_{POD} and BE values incorporate a number of exposure-related assumptions that tend to lead to conservative estimates of the BE values. Other uncertainties pertain to the extrapolation of fatty liver responses graded quantally (fatty vs. not fatty) in laboratory animal studies to diagnosable NAFLD in humans, which likely encompasses a range of severity in response and for which multiple risk factors contribute to development of the disease.

The NHANES data sets allow restriction of the evaluation to subsets of the general population which could allow examination of the risks in sensitive subpopulations. For NAFLD, obesity is a major risk factor for disease development. We conducted analyses of the estimated risks for individuals with BMI>=30. However, the distribution of THM blood concentrations in this group was not different than in the adult population as a whole, and so the estimated risks were

similar (details not shown). Because the prevalence of NAFLD in obese individuals is higher than in the general population as a whole, the *proportion* of NAFLD cases potentially attributable to the estimate of THM-associated risks using method 2 above would actually be lower in this group than in the population at large.

Because smaller changes in liver fat levels might lead to diagnosable disease in sensitive populations (such as those that are obese), it would be of interest to examine the dose-response for a "shift" in parameters related to NAFLD such as serum ALT or AST or the ratio of the two enzymes, although these are fairly unreliable markers of NAFLD in a clinical setting (Clark 2006). The NHANES database could be stratified on AST or ALT concentrations, and examination of THM concentrations and estimated risks in individuals with levels near or exceeding the top of the reference range for these enzymes could be conducted. Unfortunately, the current THM risk assessments focus on a quantal endpoint (yes or no for fatty liver) rather than a continuous endpoint such as severity of fatty liver or AST or ALT levels, and so evaluation of the "shift" that might occur in the population based on such endpoints cannot be conducted with the current risk assessments. However, it might be possible to retrieve the original data from the animal bioassays underlying the risk assessments and conduct a dose-response analysis for these enzymes. This dose-response assessment (on a blood THM concentration metric) could then be used to estimate the proportion of individuals whose THM exposure might be expected to alter their AST or ALT levels sufficiently to move them outside of the clinical reference range. We did not conduct such an analysis for this case study.

As this case study illustrates, the assumption of a linear (arithmetic) no-threshold model for extrapolating animal toxicity data to estimate human risks leads to a substantially different conclusion compared to the threshold method. The panelists who authored *Science and Decisions* (2009) recommend use of "linear conceptual models unless data sufficient to reject low dose linearity." While such a recommendation is conservative, a recently published review contradicts the recommendation of default low dose linearity for systemic toxicity, and concludes, "There is no compelling evidence-based justification for a general low-exposure linearity; rather, case-specific mechanistic arguments are needed" (Rhomberg et al. 2011). Given this, and our analysis in this case study, we recommend using, in lieu of a no-threshold default, the Key Events Dose Response Framework (Boobis et al. 2009) to analyze available data to evaluate the relationship between the quantal fatty liver endpoints identified in the animal bioassay data to human NAFLD and fatty liver responses of lesser severity, identify key events in the disease process, and to select the extrapolation method that best corresponds to the most up to date scientific understanding of the biological processes underlying the mode of action.

V.   References

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Table 1: Description of studies and endpoints used to establish the point of departure (POD) and the identified uncertainty factors (UFs) used in the derivation of the USEPA reference doses (RfDs) for four THM compounds. Table from Aylward et al. (2008).

Compound	Description of study used as the basis for RfD	Effects observed	POD	UFs	RfD (mg kg^{-1} d^{-1})
Chloroform	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)	Reversible elevations in SGPT (ALT) during treatment at both doses; increased fatty cysts in liver	BMDL$_{10}$[a]: 1.2 mg kg^{-1} d^{-1}	100 10 for interspecies 10 for intraspecies	0.01
DBCM	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)	Fatty changes in the liver of male rats	BMDL$_{10}$: 1.6 mg kg^{-1} d^{-1}	100 10 for interspecies 10 for intraspecies	0.02
BDCM	BDCM administered to male rats in diet at 6, 26, or 138 mg kg^{-1} d^{-1}, 24 months (Aida et al. 1992 as reported in USEPA 2005)	Fatty changes in liver of male rats	BMDL$_{10}$: 0.8 mg kg^{-1} d^{-1}	300 10 for interspecies 10 for intraspecies 3 to account for database uncertainty regarding potential reproductive effects	0.003
TBM	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)	Hepatocellular vacuolization	BMDL$_{10}$: 2.6 mg kg^{-1} d^{-1}	100 10 for interspecies 10 for intraspecies	0.03

RfD for chloroform from USEPA's Integrated Risk Information System (USEPA 2006b); information on RfDs for brominated THM compounds from USEPA (2005).
[a] BMDL$_{10}$: statistical lower bound on the benchmark dose associated with a 10 percent increase in the occurrence of the critical response.

Table 2: Estimated internal dose metrics and 24-h average human blood concentrations consistent with the derivation of the RfD for each THM (see Fig. 1). Table from Aylward et al. (2008).

Compound	POD mg kg^{-1} d^{-1}	BE$_{POD\_animal}$ Animal avg. blood conc., pg ml^{-1}	Animal hepatic AUC mg h L^{-1} d^{-1}	UF$_{A-PD}$	Human equivalent hepatic AUC mg h L^{-1} d^{-1}	BE$_{POD\_human}$ Corresponding human avg. blood conc., pg ml^{-1}	UF$_{H-PD}$	UF$_D$	BE$_{RfD}$ pg ml^{-1}
Chloroform	1.2	4,400	0.12	3.2	0.038	750	3.2	1	230
DBCM	1.6	2,200	0.056	3.2	0.019	270	3.2	1	80
BDCM	0.8	670	0.019	3.2	0.006	190	3.2	3	20
TBM	2.6	2,900	0.074	3.2	0.025	420	3.2	1	130

Table 3: Blood trihalomethane (THM) concentrations (pg/ml) and 95% confidence intervals (CIs) for selected percentiles for the three time periods 1999–2000, 2001–2002, and 2003–2004 from National Health and Nutrition Examination Survey data for individuals age 20 years and older (weighted data). Table from LaKind et al. (2009).

	Blood THM concentrations (pg/ml, 95% CIs[a]) by sampling period		
	1999–2000	2001–2002	2003–2004
Chloroform			
n	255	763	1238
% detected	100	97.2	92.1
10%	15.5 (13.9, 19.0)	5.14 (4.4, 5.9)	2.6 (2.3, 2.9)
25%	22.0 (18.8, 24.5)	9.05 (8.1, 10.2)	5 (4.4, 5.5)
50%	38.3 (26.7, 45.8)	16.10 (13.8, 17.7)	10 (9, 10.6)
75%	77.2 (61.7, 90.5)	31.90 (27.7, 36.6)	20 (19, 21)
95%	162.0 (59.0, 192.0)	74.60 (56.2, 83.9)	50 (43, 56)
Bromodichloromethane			
n	354	812	1322
% detected	96.6	98.2	73.4
10%	0.46 (0.38, 0.59)	0.50 (0.41, 0.58)	<LOD[b]
25%	0.87 (0.7, 1.0)	1.10 (0.98, 1.2)	<LOD[b]
50%	1.92 (1.5, 2.3)	2.28 (2.0, 2.5)	1.40 (1.2, 1.5)
75%	3.95 (2.7, 4.6)	4.65 (4.3, 5.1)	3.40 (3.1, 3.7)
95%	12.8 (9.2, 15.6)	12.40 (9.0, 13.9)	9.50 (8.0, 11.6)
Dibromochloromethane			
n	350	817	1333
% detected	87.2	76.3	47.7
10%	<LOD[c]	<LOD[c]	<LOD[c]
25%	0.47 (0.37, 0.56)	0.25 (0.20, 0.32)	<LOD[c]
50%	0.97 (0.66, 1.2)	0.78 (0.61, 0.92)	<LOD[c]
75%	2.52 (1.8, 3.0)	2.59 (2.1, 2.9)	1.3 (1.0, 1.4)
95%	8.50 (4.4, 10.4)	8.96 (7.7, 10.9)	7.2 (6.3, 9.1)
Bromoform			
n	330	807	1310
% detected	74.4	81.3	40.3
10%	<LOD[d]	<LOD[d]	<LOD[d]
25%	<LOD[d]	0.71 (0.62, 0.82)	<LOD[d]
50%	0.85 (0.7, 0.9)	1.34 (1.2, 1.5)	<LOD[d]
75%	1.73 (1.4, 2.1)	2.70 (2.4, 3.1)	1.8 (1.7, 1.9)
95%	5.14 (3.1, 6.2)	14.90 (3.5, 21.3)	6.4 (5.1, 7.8)

[a]Confidence interval.
[b]Median limit of detection (LOD) (range of LODs): 2003–2004, 0.62 (0.21–0.62).
[c]Median LOD (range of LODs): 1999–2000, 0.23 (0.21–0.24); 2001–2002, 0.23 (0.20–0.27); 2003–2004, 0.62 (0.21–0.62).
[d]Median LOD (range of LODs): 1999–2000, 0.52 (0.49–0.55); 2001–2002, 0.54 (0.49–0.59); 2003–2004, 1.5 (0.55–1.5).

Table 4: Calculated slopes for estimation of risks of fatty liver from THMs, calculated in accordance with equations 2 and 3.

Chemical	BE_{POD} pg/ml	BE_{RfD} pg/ml	Slope1 $(pg/ml)^{-1}$ [a]	Slope2 $(pg/ml)^{-1}$ [b]
Chloroform	750	230	0.00019	0.00013
DBCM	270	80	0.00053	0.00037
BDCM	190	20	0.00059	0.00053
TBM	420	130	0.00034	0.00024

[a] To be applied to estimate risks for blood concentrations in excess of the BE_{RfD}; risks associated with blood concentrations below the BE_{rfD} are defined as zero.
[b] For application to any blood concentration $<BE_{POD}$

Table 5: Estimated weighted population percentiles of risk of fatty liver due to THMs estimated via two methods of extrapolation from the POD, based on blood THM sampling data from NHANES 2003-2004. Risks were estimated for each individual due to each compound according to equation 4, and population-weighted percentiles were calculated as discussed in Methods.

	Percentile				
Chemical	25th	50th	75th	90th	95th
Method 1					
Chloroform	0	0	0	0	0
DBCM	0	0	0	0	0
BDCM	0	0	0	0	0
TBM	0	0	0	0	0
Sum of Four THMs[a]	0	0	0	0	0
Method 2					
Chloroform	0.0007	0.0013	0.0026	0.0046	0.0065
DBCM	<LOD	<LOD	0.0005	0.0013	0.0027
BDCM	<LOD	0.0007	0.0018	0.0033	0.005
TBM	<LOD	<LOD	0.0004	0.0008	0.0025
Sum of Four THMs, ND= LOD/sqrt(2)	0.0018	0.0031	0.0057	0.0096	0.015
Sum of Four THMs, ND=0	0.0014	0.0028	0.0055	0.0094	0.015

LOD: Limit of detection.

Table 6: Chemicals for which BE values corresponding to current risk assessment-based exposure guidance values have been derived.

Completed and Published	
2,4-D	n-Nonane
Cyfluthrin	1,1,1-Trichloroethane
Cadmium	1,1,2-Trichloroethane
Inorganic arsenic	n-Decane
Hexachlorobenzene	1,2,3-Trichloropropane
Bisphenol A	1,1,1,2-Tetrachloroethane
Triclosan	1,1,2,2-Tetrachloroethane
Diethyl phthalate	1,2-Dibromoethane
Dibutyl phthalate	Hexachloroethane
Benzyl butyl phthalate	1,1-Dichloroethene
Di-2(ethylhexyl) phthalate	cis-1,2-Dichloroethene
Dioxin TEQ	trans-1,2-Dichloroethene
Acrylamide	Trichloroethene
Chloroform	Tetrachloroethene
Bromoform	Benzene
Dibromochloromethane	Toluene
Bromodichloromethane	Styrene
Methylene chloride	Ethylbenzene
Carbon tetrachloride	Xylenes, mixed
Dibromomethane	Acrylonitrile
n-Hexane	Furan
1,1-Dichloroethane	Tetrahydrofuran
1,2-Dichloroethane	1,4-Dioxane
n-Heptane	Methyl-tert-Butyl Ether (MTBE)
n-Octane	Methyl isobutyl ketone
In Submission or Preparation	
Uranium	PBDE 99
Di-isononylphthalate	Deltamethrin
DDT/DDE/DDD	Hexabromocyclododecane

Table 7: Implicit and explicit assumptions in the analysis

Assumption	Impact on the assessment of risks[a]
Fatty liver changes in animals in chronic bioassays at relatively high doses are predictive for fatty liver changes in humans exposed to lower chemical concentrations environmentally.	**Unkown.** The two methods used here apply both an assumption that high dose responses for this endpoint have a clear threshold (Method 1) and the assumption that these risks translate linearly to much lower exposures (Method 2).
Risk of fatty liver degeneration assessed quantally in laboratory animals (0 or 1) can be extrapolated on a population basis to human risk of NAFLD.	**Unkown.** The quantal characterization of laboratory animal livers as demonstrating fatty liver degeneration or not is more simplistic than the human health condition in which a minor increase in fat levels in the liver may not translate to a frank diagnosis of NAFLD.
Use of the lower bound on the estimated benchmark dose.	**Conservative.** For the THMs, the BMDL values were approximately a factor of 3 lower than the corresponding BMDs.
Human liver tissue is 3-fold more sensitive than rodent or dog liver tissue to fatty changes induced by the same daily AUC exposure to THMs.	**Unknown.**
PBPK models for THMs provide accurate estimation of hepatic AUCs under the bioassay conditions	**Unknown.** The PBPK models used in the derivation of the BE values have varying levels of validation.
In the derivation of the BE values, all exposure to THMs occurs via the oral route.	**Conservative**. Assumption of mixed routes of exposure (likely in the general population due to volatilization and dermal exposure to water) would result in higher BE_{POD} and BE values associated with target hepatic AUCs (up to 6-fold higher) and therefore lower numbers of people affected and lower overall risk estimates.
Concentrations of THMs in spot blood samples are appropriate surrogates for long-term 24 hour average blood concentrations in individuals.	**Conservative** at the upper tails of the distributions observed in NHANES **Non-conservative** at the lower tails of the distribution of measured blood concentrations

[a] Conservative: not likely to underestimate risk but may overestimate risk; non-conservative: not likely to overestimate risk but may underestimate risk.

Figure 1: Schematic presentation of the derivation of BE values for the THM compounds.
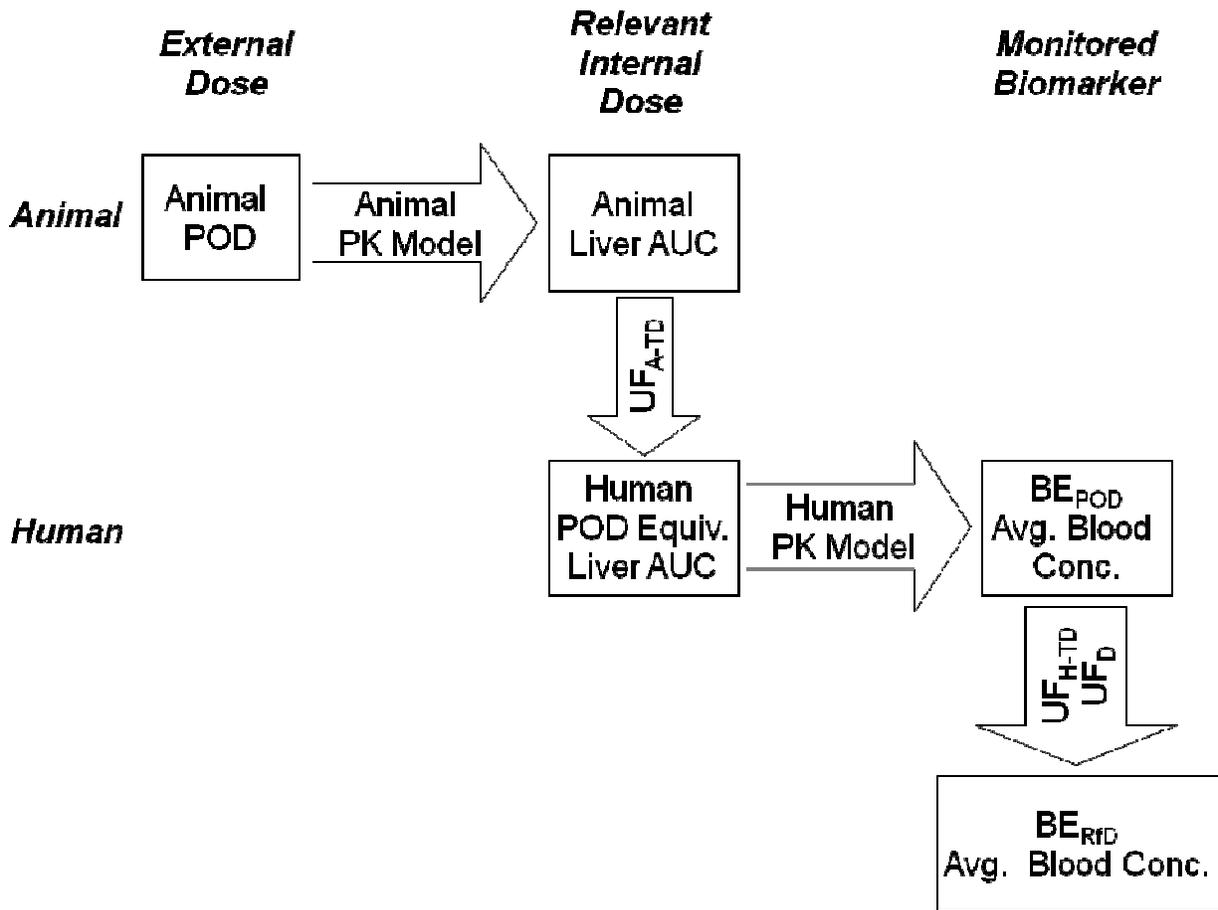
Figure 2: Illustration of methods for low-dose extrapolation from BE_{POD} evaluated in this case study (shown for chloroform). Method 1 assumes linear extrapolation between the BE_{POD} (assumed to correspond to a 10% risk of fatty liver degeneration, see text) and an assumed zero risk at the $BE_{RfD.}$ Method 2 assumes the risk is linearly extrapolatable from the BE_{POD} to zero risk at zero concentration of chloroform in blood.
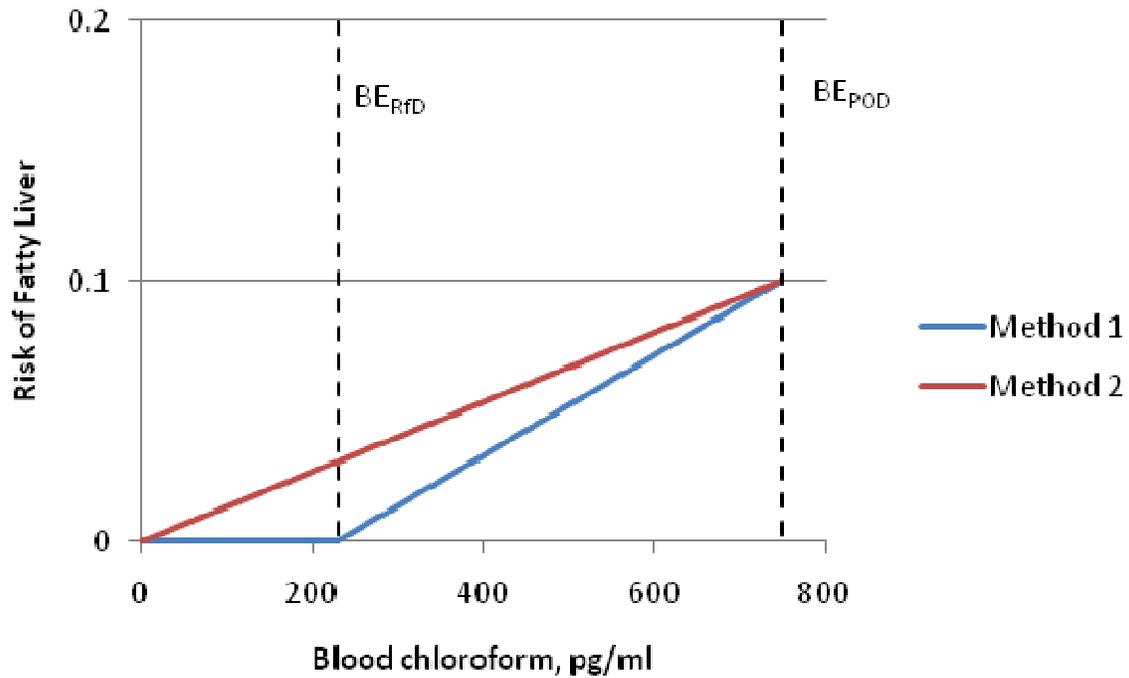
Figure 3: Box plots of hazard quotients for individual THM compounds and the hazard index for the combined THM hazard quotients calculated per equation 1. Box plots omit extreme values. The boxes represent the interquartile range and upper whiskers extend to the 75th %ile plus1.5 times the interquartile range.