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Biomonitoring Equivalents (BE) dossier for toluene (CAS No. 108-88-3)

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ABSTRACT

Recent efforts by the US Centers for Disease Control and Prevention and other researchers have resulted in a growing database of measured concentrations of chemical substances in blood or urine samples taken from the general population. However, few tools exist to assist in the interpretation of the measured values in a health risk context. Biomonitoring Equivalents (BEs) are defined as the concentration or range of concentrations of a chemical or its metabolite in a biological medium (blood, urine, or other medium) that is consistent with an existing health-based exposure guideline. This document reviews available pharmacokinetic data and models for toluene and applies these data and models to existing health-based exposure guidance values from the US Environmental Protection Agency, the Agency for Toxic Substances and Disease Registry, Health Canada, and the World Health Organization, to estimate corresponding BE values for toluene in blood. These values can be used as screening tools for evaluation of biomonitoring data for toluene in the context of existing risk assessments for toluene and for prioritization of the potential need for additional risk assessment efforts for toluene.

1. Introduction

Measurements of environmental chemicals in air, water, or other media can be compared to health-based exposure guidelines to identify chemical exposures that may be of concern, or to identify chemicals for which a wide margin of safety appears to be present. Interpretation of human biomonitoring data for environmental compounds is hampered by a lack of similar screening criteria applicable to measurements of chemicals in biological media such as blood or urine. Such screening criteria 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, and appropriate data for such values may never be available for many compounds. As an interim effort, the development of Biomonitoring Equivalents (BEs) has been proposed (Hays et al., 2007).

A Biomonitoring Equivalent (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. Existing 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]) used as the basis for the exposure guidance value and to estimate biomarker concentrations that are consistent with the guidance value. BEs can be estimated using available human or animal pharmacokinetic data. Guidelines for the derivation and communication of BEs are available (Hays et al., 2008; LaKind 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, and are designed to provide a basis for prioritization of chemicals for risk assessment follow-up. BEs are only as robust as the underlying health-based exposure guidelines that they are based upon and the underlying animal and/or human pharmacokinetic data used to derive the BEs. BEs are not designed to be diagnostic for potential health effects in humans, either individually or among a population.

Toluene is used as a solvent in numerous products including industrial paints, adhesives, coatings, inks, and cleaning products. Toluene is also added to aviation fuel to improve octane ratings and as a raw material for the manufacture of polymers used to make nylon, plastic soda bottles, and polyurethanes. It is also used in processes for manufacture of pharmaceuticals, dyes, cosmetic nail products, and in the synthesis of organic chemicals including benzene. According to the US Environmental Protection Agency (USEPA), the primary pathway for exposure to toluene is inhalation from ambient and indoor air, although ingestion may also occur through trace amounts of toluene that may occur in food or water.

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Intentional inhalant abuse can result in high exposure to toluene vapors. Additional general information regarding toluene can be found at http://www.epa.gov/ttn/atw/hlthef/toluene.html.

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

1.1. Current health-based exposure guidance values

The primary effects of toluene in both humans and animals after either acute or chronic exposure are effects on the central nervous system (CNS). Acute exposure to high concentrations of toluene causes symptoms including fatigue, sleepiness, headaches, and nausea. Chronic exposure to toluene at levels above current occupational exposure guidelines has been associated with subtle changes in sensory function including reduced color vision (reviewed in USEPA, 2005). High level exposures through intentional inhalant abuse or accidental or intentional ingestion of toluene have also been reported to cause effects on the liver, kidneys, and other organ systems. With respect to carcinogenicity, both the International Agency for Research on Cancer (IARC) and the US Environmental Protection Agency (USEPA) consider toluene as "not classifiable" as to human carcinogenicity (Groups 3 and D, respectively) (IARC, 1999; USEPA, 2005).

Health-based exposure guidelines and toxicity values have been established for many chemicals for the general population by the USEPA (Reference Doses or Reference Concentrations [RfDs or RfCs]), the Agency for Toxic Substances and Disease Registry (ATSDR) (Minimal Risk Levels or MRLs), and Health Canada and the World Health Organization (WHO) (Tolerable Daily Intakes or TDIs). Although these health-based exposure guidance values have different labels and slightly different definitions, they all generally describe an approximation of daily intake rates (or air concentrations) for a chemical expected to be without adverse effects in the general population, including sensitive subpopulations.[1]. For chemicals considered to be carcinogenic, the USEPA also establishes estimates of cancer potency by assigning a quantitative estimate of the upper bound of potential increased cancer risk associated with a unit of intake or air concentration (unit cancer risks, or UCRs). Finally, several organizations set chemical-specific air concentrations that are considered to be safe for workers in the occupational environment (for example, Threshold Limit Values [TLVs], Permissible Exposure Limits [PELs], and Maximum Air Concentrations [MAKs]). These values are generally not appropriate for application to the general population on a chronic basis, but can provide perspective for evaluating non-workplace environmental exposures.

Several health-based exposure guidelines and toxicity values are available for toluene including guidelines for both inhalation and oral exposures. These values are summarized in Table 1. As discussed above, toluene is generally not considered to be carcinogenic so no cancer potency estimates for toluene are available. In addition, biological monitoring values for toluene in blood or toluene metabolites in urine in occupationally exposed individuals (Biological Exposure Indices (BEIs) and Biological Tolerance Values (BATs)) have also been established (ACGIH, 2001; Angerer et al., 1998). As discussed above, these are not appropriate for application to the general population.

[1] See the definition of RfD at http://www.epa.gov/NCEA/iris/help_gloss.htm#r; 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.ox.ac.uk/MSDS/glossary/tolerable_daily_intake.html.

1.2. Pharmacokinetics

The pharmacokinetics of toluene have been studied extensively in human volunteers and persons occupationally exposed as well as in laboratory animals. Toluene is well absorbed following inhalation and oral exposure. Toluene undergoes metabolism, principally via CYP2E1, and metabolites are excreted in urine. Toluene is also eliminated as parent compound in urine and exhaled air. The recent USEPA IRIS review of toluene includes a detailed description of the metabolic pathways for toluene (USEPA, 2005). Detailed physiologically based pharmacokinetic (PBPK) models for toluene in humans and laboratory rats have been developed by several groups of researchers and can accurately predict blood levels associated with a variety of inhalation exposure regimens (human and rat models by Tardif et al., 1993, 1995; human models by Jang, 1996; Pierce et al., 1998).

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 criterion for the choice of a biomarker is 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.

Several potential biomarkers are available for assessing internal exposure to toluene (Table 2). Toluene is excreted unchanged in exhaled air. However, as a quantitative biomarker, toluene in exhaled breath is relatively insensitive and it is difficult to obtain reliable, reproducible measurements. In the occupational setting, exposure to toluene has been monitored through measurement of metabolites in urine and parent compound in blood (ACGIH, 2001). However, use of urinary metabolites of toluene as markers for assessing exposure in persons in the general population is of limited utility because neither marker measured, hippuric acid or *ortho*-cresol, is specific to toluene exposure. Instead, each can be observed as metabolites of numerous parent compounds (Dossing et al., 1983). Hippuric acid levels in urine are relatively poorly correlated with exposure even under occupational exposure conditions (Truchon et al., 1999). Under conditions of higher occupational exposure levels, elevated *ortho*-cresol levels are closely correlated with inhalation exposures, but at environmental exposure concentrations *ortho*-cresol levels are non-specific. For instance, *ortho*-cresol is present in cigarette smoke. Thus, it cannot serve as a specific marker for toluene exposure at low environmental levels (Dossing et al., 1983). Two other urinary metabolites, *S*-*p*-toluylmercapturic acid and *S*-benzylmercapturic acid, could potentially serve as specific markers for toluene exposure (Angerer et al., 1998; Inoue et al., 2004). However, current analytical techniques are probably not sensitive enough to quantitate concentrations following environmental exposures, and insufficient data on quantitative relationships between these metabolites and toluene exposure or blood levels are currently available. Finally, unchanged toluene in urine has also been proposed as a biological marker for exposure to toluene in the occupational setting (Fustinoni et al., 2007; Kawai et al., 2008). There is relatively little literature relating toluene in urine to external exposures, and none of the current models for toluene pharmacokinetics explicitly include this pathway to allow quantitative prediction of elimination in urine under differing exposure conditions. Thus, although

Table 1
Health-based exposure guidance values for toluene from various agencies

Organization, criterion, and year of evaluation	Study description	Critical endpoint and dose	Uncertainty factors	Value
Inhalation exposure guidelines				
USEPA RfC (USEPA, 2005)	Multiple studies of human occupationally exposed populations	Transient and persistent neurological effects NOAEL (average): 34 ppm (128 mg/m^3) NOAEL (adjusted for 24 h, 7 day/week exposure): 46 mg/m^3	10—total 10—interindividual	5 mg/m^3 (1.3 ppm)
Health Canada TDI—inhalation (Health Canada, 1996)	Study of human occupationally exposed populations	Nervous system effects in humans NOAEL: 150 mg/m^3 NOAEL (adjusted by a factor of 6/24 for 24 h, 7 day/week exposure): 38 mg/m^3	10—total 10—interindividual	3.8 mg/m^3 (1 ppm)
World Health Organization Air Quality Guideline (WHO, 2005)	Occupationally exposed workers with mean exposure at 332 mg/m^3	Impacts on neurological performance tests LOAEL of 332 mg/m^3, duration adjusted to 80 mg/m^3	300—total 10—interindividual 10—use of a LOAEL 3—potential sensitivity of developing CNS	0.26 mg/m^3 (0.07 ppm)
ATSDR acute inhalation MRL (1–14 days exposure) (ATSDR, 2000)	Human volunteers, exposure to 10, 40, or 100 ppm, 6 h per day for 4 days	Trend of decreased neurological performance, with NOAEL at 40 ppm. Duration adjusted	10—total 10—interindividual	3 mg/m^3 (0.8 ppm)
ATSDR chronic inhalation MRL (>1 year) (ATSDR, 2000)	Occupationally exposed workers with mean exposure at 35 ppm	Decreased color vision after adjustment for age and alcohol use, with LOAEL at 35 ppm. Duration adjusted	100—total 10—minimal LOAEL to NOAEL 10—interindividual	0.3 mg/m^3 (0.08 ppm)
Oral exposure guidelines				
USEPA RfD (USEPA, 2005)	Rat gavage, 13 weeks (NTP, 1990)	Kidney weight changes as a precursor to kidney toxicity at higher doses NOAEL: 223 mg/kg day LOAEL: 446 mg/kg day (duration adjustment applied) BMDL: 228 mg/kg day	3000—total 10—interspecies 10—interindividual 10—subchronic to chronic 3—database uncertainties	0.08 mg/kg day
Health Canada TDI—oral (Health Canada, 1996)	Rat gavage, 13 weeks (NTP, 1990)	Liver and kidney weight changes NOAEL: 223 mg/kg day LOAEL: 446 mg/kg day (duration adjustment applied)	1000—total 10—interspecies 10—interindividual 10—subchronic to chronic	0.22 mg/kg day
World Health Organization TDI (WHO, 2004)	Mouse gavage, 13 weeks (NTP, 1990)	Marginal hepatotoxic effects at the lowest dose	1000—total 10—interspecies 10—interindividual 10—subchronic to chronic and use of a LOAEL	0.2 mg/kg day
ATSDR acute oral MRL (1–14 days exposure) (ATSDR, 2000)	Rats exposed by single dose corn oil gavage to 0, 250, 500, or 1000 mg/kg day	Decreased flash-evoked potential at all dose levels (no dose–response trend)	300—total 3—minimal LOAEL to NOAEL 10—interspecies 10—interindividual	0.8 mg/kg day
ATSDR intermediate oral MRL (up to 1 year) (ATSDR, 2000)	Mice exposed by drinking water for 28 days to 5, 22, or 105 mg/kg day	Changes in neurotransmitter levels	300—total 3—minimal LOAEL to NOAEL 10—interspecies 10—interindividual	0.02 mg/kg day

toluene in urine may prove useful as a specific biomarker for environmental exposure to toluene, the current data are not sufficient to rely on toluene in urine for the Biomonitoring Equivalent process. Thus, no urinary biomarker for exposure to toluene is currently useful for assessing general environmental exposures.

Toluene has also been measured in blood and correlated with inhalation exposure levels in persons exposed occupationally (see, for example, Neubert et al., 2001), in volunteers under conditions of controlled exposure (see, for example, Pierce et al., 1998), and in the general population (see, for example, Sexton et al., 2005). Toluene in blood has also been identified as a useful biomarker in the occupational setting (ACGIH, 2001).

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. The USEPA oral RfD is based on subtle kidney toxicity following oral gavage dosing in rats, while the ATSDR acute and intermediate MRL values are based on changes in neurological endpoints in rats and mice. Inhalation criteria from all agencies are based on subtle neurological effects observed in humans after acute and chronic exposure to toluene.

The mechanisms of the renal toxicity observed in rats following subchronic oral gavage in the National Toxicology Program study are unknown, but recent *in vitro* studies by Al-Ghamdi et al. (2003a,b) in proximal tubule cell cultures suggest that the toxicity may be attributable to benzyl alcohol, a toluene metabolite produced via CYP2E1. Al-Ghamdi et al. (2003a,b) showed that inhibiting CYP2E1 activity prevented toxicity in cell culture following toluene exposure. Renal toxicity has also been observed in humans following intentional or accidental ingestion of large amounts of toluene and following chronic inhalation abuse (Stengel et al., 1998). However, such toxicity has not been reported in occupational populations exposed to more moderate air concentrations. For example, Stengel et al. (1998) reported a no-observed-adverse-effect-level (NOAEL) at the TLV of 50 ppm (188 mg/m^3) for renal function changes in a chronically exposed occupational cohort. Renal toxicity is most likely a phenomenon associated with

Table 2
Potential biomarkers of exposure to toluene

Analyte	Medium	Advantages	Disadvantages
Toluene	Blood	Sensitive and specific; highly relevant to target tissue concentrations	Requires blood draw
	Urine	Sample easily obtained; specific biomarker	Lack of robust data set or model to quantify relationship between exposure and observed levels; not directly relevant to target tissue concentrations
	Exhaled air	Sample easily obtained; specific biomarker	Insensitive, difficult to obtain reproducible results
Hippuric acid	Urine	Sample easily obtained	Non-specific metabolite; not directly relevant to target tissue concentrations
ortho-Cresol	Urine	Sample easily obtained	Non-specific metabolite at environmental exposure levels; not directly relevant to target tissue concentrations
S-*p*-Toluylmercapturic acid	Urine	Sample easily obtained; specific biomarker	Lack of robust data set or model to quantify relationship between exposure and observed levels; not directly relevant to target tissue concentrations; analytical sensitivity may not be sufficient
S-Benzylmercapturic acid	Urine	Sample easily obtained; specific biomarker	Lack of robust data set or model to quantify relationship between exposure and observed levels; not directly relevant to target tissue concentrations; analytical sensitivity may not be sufficient

high peak toluene blood levels resulting in high rates of metabolism and subsequent activity of metabolites in the kidney (Al-Ghamdi et al., 2003a,b).

Neurological responses following inhalation exposure to toluene in humans or oral exposure in rats and mice are likely to be related directly to brain concentrations of toluene, which in turn are directly related to blood concentrations (Benignus et al., 2007; Bushnell et al., 2007). The RfC for toluene derived by USEPA is based on evaluation of potential neurotoxicity, which appears to be the most sensitive endpoint identified in numerous studies of long-term occupationally exposed populations (see USEPA, 2005, for a complete description of these studies and populations). These studies are characterized by long-term exposure with monitored air exposure concentrations. The mechanisms underlying the observed neurotoxicity are not fully understood, but appear to be related to concentrations of the parent compound (rather than metabolites) reaching the brain (van Asperen et al., 2003; Benignus et al., 2007; Bushnell et al., 2007). However, there are insufficient data to conclusively identify whether peak or average toluene concentration in blood is the most appropriate dose metric for various neurological responses. The direct correlation between toluene blood concentration and neurological responses supports use of blood concentration of toluene as a biomarker, and under chronic exposure conditions, average blood concentration should be directly relevant.

2. BE derivation

2.1. Methods

2.1.1. Urine

As discussed above, data do not support the use of urinary markers for toluene exposure at environmental exposure levels at this time, although, as discussed above, selected specific metabolites or unchanged toluene in urine might serve as reliable biomarkers if more data can be developed and analytical techniques for those markers become sufficiently sensitive. No urinary BE values were derived for toluene exposure.

2.1.2. Blood

In order to estimate human blood levels associated with exposure to toluene at the various health-based inhalation and oral exposure guidelines detailed in Table 1, the human PBPK model for toluene developed by Tardif et al. (1993, 1995) was implemented. Models by Pierce et al. (1996, 1998) and Jang (1996) were also available. Each is similar to the model developed by Tardif et al. (1995), but of the three, the Tardif et al. (1995) model has been used the most extensively and was therefore chosen for use in this BE derivation. The rat PBPK model by Tardif et al. (1993) was also used to estimate blood concentrations in rats at the dose levels used as the point of departure for derivation of the oral RfD and oral MRL values. These blood concentrations at the point of departure for risk assessment can provide additional context for interpretation of measured blood concentrations in humans in the general population.

Both the human and the rat PBPK models required minor modifications to incorporate the oral route of exposure. These additions are described below.

2.1.2.1. Rat model. The PBPK model of Tardif et al. (1993) for toluene inhalation exposure in rats was implemented in MS Excel® with physiological and physicochemical parameters as described in Tables 1 and 2 of that publication. The model was modified to incorporate oral dosing by adding a virtual gastrointestinal tract compartment with a first-order absorption process to the liver. To parameterize the absorption rate from oral dosing, the gastrointestinal absorption rate was calibrated visually against graphical data from Sullivan and Conolly (1988) for the time course of blood toluene concentrations following oral gavage at four different dose levels in Sprague–Dawley rats. The absorption rate from the rat gastrointestinal tract was adjusted to result in peak blood concentrations between 2 and 2.5 h post-gavage, as reflected in the Sullivan and Conolly (1988) data set. All other parameters were retained as reported by Tardif et al. (1993). The parameters used in the rat oral and inhalation toluene PBPK model are presented in Table 3.

2.1.2.2. Adult human model. The human PBPK model of Tardif et al. (1995) with parameters as reported by Nong et al. (2006, Table 1) for toluene inhalation exposure was similarly implemented in MS Excel®. The model was able to accurately reproduce the central tendency of the measured blood and exhaled air concentrations in an independent data set for volunteers exposed to 50 ppm toluene for 2 h from Pierce et al. (1998; results not shown).

An oral dose route was also added to the human PBPK model. Addition of this dose route required parameterization of an oral absorption rate constant. An oral absorption rate was calibrated against the time course to peak exhaled air concentrations following administration of toluene at measured drip rates for specified time periods to human volunteers via nasal-gastric tube (Baelum et al., 1993). The exhaled air concentrations peaked approximately 15–30 min following cessation of exposure. The full set of model parameters for the adult human model is included in Table 3.

Table 3
Model parameters used in the rat and human PBPK models

Parameter	Adult[b]	Rat[a]
Physiological parameters		
Body weight (kg)	70	0.25
Tissue volumes (L)		
Liver	1.82	0.0123
Fat	13.3	0.0225
Richly perfused	3.5	0.0125
Poorly perfused	43.4	0.18
Cardiac output (L/h)	418	5.3
Alveolar ventilation (L/h)	418	5.3
GI Tract emptying rate[c] (h^{-1})	0.69	0.23
Tissue blood flow rates (L/h)		
Liver	109	1.33
Fat	21	0.48
Richly perfused	184	2.70
Poorly perfused	104	0.80
Partition coefficients		
Blood:air	15.6	18
Liver:blood	2.98	4.64
Fat:blood	65.8	56.7
Richly perfused:blood	2.66	4.64
Poorly perfused:blood	1.37	1.54
Metabolic constants		
V_{max} (mg/h)	116.2	1.7
K_m (mg/L)	0.55	0.55

[a] From Tardif et al. (1993).
[b] From Tardif et al. (1995) and Nong et al. (2006).
[c] Fit to data sets as described in text.

2.1.2.3. Evaluation of BEs for oral exposure guidelines. The general approach for the derivation of BE values for the oral exposure guidelines is presented in Figs. 1 and 2.

For those exposure guidance values derived based on rat toxicity study data, the rat and human PBPK models were used in combination to derive BE values. Briefly, the process (Fig. 1) is as follows:

- Step 1: *Calculate relevant animal internal dose at POD.* In this case, effects on liver and/or kidney following chronic gavage administration of toluene are the most sensitive effects and serve as the basis for the derivation of oral exposure guidelines. These effects are likely related to production of metabolites in these organs. Production of these metabolites is likely to be proportional to area under the curve of toluene in these organs. Thus, toluene area under the curve for kidney (modeled as richly perfused tissue) or liver (modeled explicitly) was selected as the relevant internal dose metric, and an estimate of the target organ AUC at the duration- and LOAEL-to-NOAEL adjusted POD was made for each of the oral exposure guidelines. The average blood concentration in the animals at the POD (BE_{POD_animal}) was also estimated using the PBPK model.
- Step 2: *Interspecies extrapolation.* Interspecies extrapolation of this relevant internal dose metric to a corresponding human target organ AUC by application of an interspecies uncertainty factor for pharmacodynamic differences. An interspecies factor for pharmacokinetic differences was also applied. This factor accounts for unknown differences between humans and the experimental animals of interest in the pharmacokinetics of the metabolites believed to be responsible for the organ-specific toxicity.
- Step 3: *Calculate BE_{POD}.* Application of the human pharmacokinetic model to identify an average blood concentration corresponding to the relevant target organ internal dose measure identified above (human equivalent BE_{POD}).
- Step 4: *Calculate BE.* Application of relevant intraspecies uncertainty factor(s) and any additional applicable uncertainty factors identified by the organizations that derived the oral exposure guidelines initially (for example, database uncertainty factors sometimes applied by USEPA). Because the measured biomarker is directly related to the internal dose metric of interest, direct measurement of this biomarker concentration replaces application of the pharmacokinetic component of the intraspecies uncertainty factor in derivation of the BE values (Hays et al., 2008); only the pharmacodynamic factor is appropriate on an internal dose basis in this case.

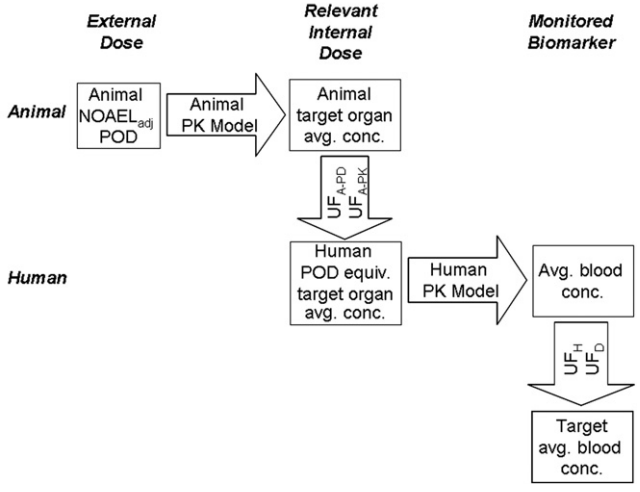

Fig. 1. Schematic of approach used to estimate BE values for toluene in humans corresponding to oral exposure guidance values based on rat toxicity data. $NOAEL_{adj}$ POD: Point of departure, adjusted for duration and LOAEL to NOAEL, as appropriate; UF_{A-PD}: component of interspecies uncertainty factor for pharmacodynamic sensitivity; UF_{A-PK}: component of interspecies uncertainty factor for pharmacokinetic sensitivity; UF_H: intraspecies uncertainty factor; UF_D: uncertainty factor component for database uncertainties, where applicable. See text for discussion.

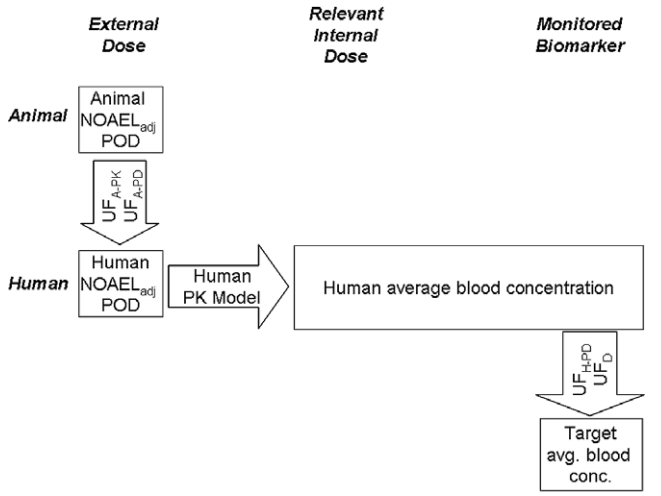

Fig. 2. Schematic of approach used to estimate BE values for toluene in humans corresponding to oral exposure guidance values based on mouse toxicity data. UF_{H-PD}: component of intraspecies uncertainty factor for pharmacodynamic sensitivity.

For those oral exposure guidance values derived based on mouse toxicity data (the WHO TDI and the ATSDR intermediate MRL), a modified process outlined in Fig. 2 was used because of the lack of a mouse PBPK model. In this approach, the interspecies extrapolation is conducted on an external dose basis to obtain the human equivalent external dose POD. The human average blood concentrations associated with this POD were then estimated using the human PBPK model to obtain the human equivalent BE_{POD}.

2.1.2.4. Evaluation of BEs for inhalation exposure guidelines. The general approach for the derivation of BE values for the inhalation exposure guidelines is presented in Fig. 3. Each of the applicable guidelines is derived based on human data. Thus, the derivation process does not involve an interspecies extrapolation. Briefly, the process is as follows:

- Step 1: *Calculate the BE_{POD}.* The steady-state blood concentrations in humans exposed at the duration- and LOAEL-to-NOAEL adjusted PODs (based on human study data) were modeled using the PBPK model described above. Because blood concentration has been identified as a directly relevant dose metric for neurological effects, the relevant internal dose metric and the monitored biomarker concentration are the same. Thus, these modeled blood concentrations are the BE_{POD} values used in the derivation of the BEs for the inhalation exposure guidelines.
- Step 2: *Calculate the BE.* Application of relevant intraspecies uncertainty factor(s) and any additional applicable uncertainty factors identified by the organizations that derived the oral exposure guidelines initially (for example, database uncertainty factors sometimes applied by USEPA). As above, because the measured biomarker is directly related to the internal dose metric of interest (they are the same for this set of exposure guidelines, blood toluene concentration) direct measurement of this biomarker concentration replaces application of the pharmacokinetic component of the intraspecies uncertainty factor in derivation of the BE values (Hays et al., 2008); only the pharmacodynamic factor is appropriate on an internal dose basis in this case.

2.2. Results of modeling and identification of BE values

2.2.1. Urine

As discussed above, no specific and useful urinary markers for toluene exposure at environmental exposure levels currently exist. No urinary BE values were derived for toluene exposure.

2.2.2. Blood—oral exposure

All of the available chronic oral exposure guidelines are based upon extrapolation from the same study of subchronic (13 weeks) administration of toluene by gavage to rats or mice at duration-adjusted doses of 223, 446, 893, 1786, or 3571 mg/kg day (NTP, 1990). Two organizations, the USEPA and Health Canada, based their guidance values on liver or kidney toxicity observed in the rat gavage study, while the WHO based its oral guidance value on results from the mouse study. The ATSDR also derived exposure guidance values for acute (1 to 14 day) and intermediate (up to 1 year) exposures. The acute MRL was derived based on a single dose rat gavage study, while the intermediate MRL was derived based on a 28-day mouse drinking water study.

Table 4 presents the modeling results and BE derivation for the oral exposure guidance values based on rat toxicity data using the general approach outlined in Fig. 1. Table 5 presents the corresponding results and BE derivation for those oral exposure guidance values derived from mouse toxicity data according to the approach in Fig. 2.

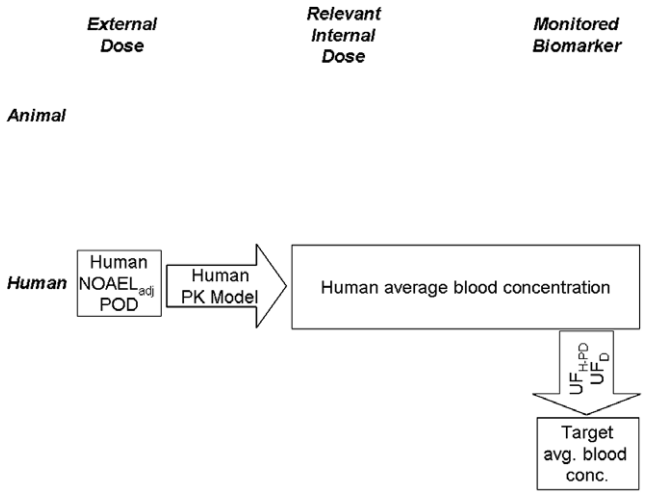

Fig. 3. Schematic of approach used to estimate BE values for toluene corresponding to inhalation exposure guidance values. See text for discussion.

2.2.3. Blood—inhalation exposure

All of the available inhalation exposure guidance values are based on studies of human occupationally exposed populations with a focus on a range of potential neurological effects. Several studies in human occupational cohorts provide LOAEL or NOAEL exposure estimates for all studied neurological endpoints including both transient and persistent effects, as well as for a wide range of other biochemical and health effect endpoints. Different agencies have made slightly different choices in their selection of points of departure for derivation of exposure guidance values, as summarized in Table 1. Selected occupational exposure levels were adjusted to an equivalent continuous exposure concentration from intermittent exposures experienced in the workplace. This adjustment is applied to account for the presumption that the general public could be continuously exposed in air. Note that this adjustment implicitly assumes that the average concentration (or area under the curve) is the critical dose metric. However, it is possible that peak concentrations or time above a threshold level is as—or more—important than average concentration in producing neurotoxic effects. Estimated peak blood concentrations following exposure under actual occupational exposure concentrations are approximately 3-fold higher than the duration-adjusted average blood concentrations (modeling not shown). The ATSDR has also derived an acute duration MRL (1–14 day exposure) based on dose–response for neurological effects observed in a volunteer study.

The results of PBPK modeling and BE derivation for the inhalation exposure guidance values are presented in Table 6. The BE values from different agencies differ substantially due to different judgments regarding whether selected occupational exposure levels represent NOAELs or LOAELs. The BE values corresponding to the USEPA RfC and the Health Canada inhalation TDI are higher than the human equivalent BE_{POD} values derived from the WHO and ATSDR exposure guidance values.

2.3. Discussion of sources of variability and uncertainty

2.3.1. Model uncertainty

The PBPK model used here has been used extensively to evaluate data sets for human and rat inhalation exposure and can reproduce the observed blood concentration vs. time behavior from independent data sets. The model incorporates understanding of the physiological, physicochemical, and metabolic determinants of toluene pharmacokinetics. However, as discussed above, its application to oral exposures introduces some additional uncertainty due to the behavior of rapidly eliminated volatile compounds and the uncertainties associated with estimation of peak blood concentrations associated with bolus oral dosing. At very high oral exposures, saturation of metabolism may become an issue resulting in non-linear relationships between external doses and resulting blood concentrations. However, at environmentally relevant exposures, such saturation is

Table 4
Estimated internal dose metrics and average human blood concentrations consistent with the derivation of oral exposure guidance values for toluene based on rat toxicity data (see Fig. 1)

BE derivation step	USEPA chronic RfD	Health Canada chronic oral TDI	ATSDR acute MRL
Target organ	Kidney	Kidney, liver	Brain
Administered dose regimen	321 mg kg^{-1} day^{-1}, rat gavage, 5 day/week, 13 weeks (NOAEL)	321 mg kg^{-1} day^{-1}, rat gavage, 5 day/week, 13 weeks (NOAEL)	250 mg kg^{-1} rat single dose gavage (LOAEL)
LOAEL-to-NOAEL adjustment	None	None	3
Duration adjustment and/or benchmark dose modeling	Adjust for 5/7 day/week; benchmark dose modeling	Adjust for 5/7 day/week	None
Subchronic to chronic adjustment	10	10	NA
POD, mg kg^{-1} day^{-1}	23	22	83
BE_{POD_animal}, μg L^{-1} (Corresponding animal avg. blood conc. from PBPK model)	90	90	830
Animal avg. target organ conc. from PBPK model, μg L^{-1}	390[a]	390[a]–450[b]	3650[a]
Interspecies uncertainty factors			
Pharmacodynamic	$10^{0.5}$	$10^{0.5}$	$10^{0.5}$
Pharmacokinetic	$10^{0.5}$	$10^{0.5}$	$10^{0.5}$
Human equivalent target organ avg. conc., μg L^{-1}	39	39–45	365
Human equivalent BE_{POD}, μg L^{-1} (corresponding human avg. blood conc. from PBPK model)	16	12–16	150
Intraspecies uncertainty factors			
Pharmacodynamic	$10^{0.5}$	$10^{0.5}$	$10^{0.5}$
Pharmacokinetic	1[c]	1[c]	1[c]
Other uncertainty factors	3—database uncertainties	NA	NA
BE value, μg L^{-1}	2	3–5	50
Confidence rating[d]	Medium	Medium	Medium

[a] Average daily toluene concentration in kidney resulting from once daily bolus dosing at the $NOAEL_{adj}$ POD as estimated from the richly perfused compartment of the PBPK model.
[b] Average daily toluene concentration in liver resulting from once daily bolus dosing at the $NOAEL_{adj}$ POD as estimated from the liver compartment of the PBPK model.
[c] Measurement of a biomarker that is directly relevant to the internal dose metric of interest replaces the default uncertainty factor for pharmacokinetic sensitivity. See text for discussion.
[d] See text for discussion.

Table 5
Derivation of BEs for oral exposure guidance values for toluene based on mouse toxicity data (see Fig. 2)

BE derivation step	ATSDR intermediate MRL	WHO chronic TDI
Target organ	Brain	Liver
Administered dose regimen	5 mg kg^{-1} day^{-1} mouse drinking water, 28 day (LOAEL)	321 mg kg^{-1} day^{-1}, mouse gavage, 5 day/week, 13 weeks (LOAEL)
LOAEL-to-NOAEL adjustment	3	$10^{0.5}$
Duration adjustment and/or benchmark dose modeling	NA	Adjust for 5/7 day/week
Subchronic to chronic adjustment	NA	$10^{0.5}$
POD, mg kg^{-1} day^{-1}		22
Interspecies uncertainty factors		
Pharmacodynamic	$10^{0.5}$	$10^{0.5}$
Pharmacokinetic	$10^{0.5}$	$10^{0.5}$
Human equivalent POD, mg kg^{-1} day^{-1}	0.17	2.2
Human equivalent BE_{POD}, μg L^{-1} (corresponding human avg. blood conc. from PBPK model)	1.7	23
Intraspecies uncertainty factors		
Pharmacodynamic	$10^{0.5}$	$10^{0.5}$
Pharmacokinetic	1[a]	1[a]
Other uncertainty factors	NA	NA
BE value, μg L^{-1}	0.5	7
Confidence rating[b]	Medium	Medium

[a] Measurement of a biomarker that is directly relevant to the internal dose metric of interest replaces the default uncertainty factor for pharmacokinetic sensitivity. See text for discussion.
[b] See text for discussion.

unlikely to occur. Thus, as a tool for predicting the central tendency of blood concentrations associated with inhalation exposure to toluene, the model uncertainty is low, while uncertainty is somewhat higher for estimating peak concentrations following oral exposures. Another potential uncertainty relates to the modeling of kidney concentrations. The existing published PBPK models used here do not include an explicit kidney compartment. The use of the "richly perfused" compartment for simulation of kidney concentrations is appropriate, but inclusion of an explicit kidney compartment could be considered if future data support this refinement of the model.

2.3.2. Analytical

The analytical methods for measuring toluene in blood are well established (ACGIH, 2001). The variability due to analytical issues should be minor in the context of the BE values.

2.3.3. Interindividual variations in pharmacokinetics

Differences in body composition (body fat levels, etc.), level of physical activity, metabolic capability, and other factors can lead to variations in blood concentrations of toluene associated with a given exposure level. Data sets from controlled exposure experiments show variations in blood levels in individuals exposed to

Table 6
Estimated internal dose metrics and average human blood concentrations consistent with the derivation of inhalation exposure guidance values for toluene based on human toxicity data for central nervous system (CNS) effects (see Fig. 3)

BE derivation step	USEPA chronic RfC	Health Canada chronic inhalation TDI	WHO air quality guideline	ATSDR chronic inhalation MRL	ATSDR acute MRL
Target organ	CNS effects	CNS effects	CNS effects	CNS effects	CNS effects
Administered dose regimen	34 ppm (128 mg m^{-3}) NOAEL, occupational exposure	40 ppm (150 mg m^{-3}) NOAEL, occupational exposure	88 ppm (332 mg m^{-3}) LOAEL, occupational exposure	35 ppm (132 mg m^{-3}) LOAEL, occupational exposure	40 ppm (150 mg m^{-3}) NOAEL, volunteers exposed 6 h/day, 4 day
LOAEL-to-NOAEL adj.	NA	NA	10	10	NA
Duration adjustment	Adjust to continuous exposure	Adjust to continuous exposure	Adjust to continuous exposure	Adjust to continuous exposure	Adjust to continuous exposure
Subchronic to chronic adjustment	None	None	None	NA	NA
POD, mg m^{-3} continuous	46	38	8	3	30
Human equivalent BE_{POD}, μg L^{-1} (corresponding human avg. blood conc. from PBPK model)	170	135	30	10	100
Intraspecies uncertainty factors					
Pharmacodynamic	$10^{0.5}$	$10^{0.5}$	$10^{0.5}$	$10^{0.5}$	$10^{0.5}$
Pharmacokinetic	1[a]	1[a]	1[a]	1[a]	1[a]
Other uncertainty factors	NA	NA	3—potential sensitivity of developing CNS	NA	NA
BE value, μg L^{-1}	50	40	3	3	30
Confidence rating[b]	High	High	High	High	High

[a] Measurement of a biomarker that is directly relevant to the internal dose metric of interest replaces the default uncertainty factor for pharmokinetic sensitivity. See text for discussion.
[b] See text for discussion.

the same external air concentrations. Pierce et al. (1998) exposed individuals to controlled concentrations of toluene in air for 2 h and followed blood concentrations for approximately 100 h after exposure ceased. At each time point, variations of a factor of two to three from the mean were observed among the individuals, consistent with a default assumption that interindividual pharmacokinetic differences could account for 3-fold variations from the mean. These experimental results are consistent with the variations predicted when physiological variability is incorporated into PBPK modeling using the Tardif et al. models (Tardif et al., 2002).

2.3.4. Gender and age

Nong et al. (2006) conducted a modeling study to evaluate the impact of the development of CYP2E1 metabolic capability in infants and children on the predicted blood concentration of toluene following inhalation exposure. Nong et al. (2006) used data on the concentration of hepatic CYP2E1 protein (Johnsrud et al., 2003) and the change in liver tissue volume as a function of age to estimate total CYP2E1 metabolic capability as a fraction of adult capability. Using these data with age-specific physiologic parameters in the Tardif et al. (1995) PBPK model, Nong et al. predicted that blood levels in newborn infants could be as much as 3 times higher than blood levels predicted in adults at the same air exposure level. Predicted blood concentrations in older children and adolescents were more similar to those predicted in adults. Nong et al. (2006) noted that this degree of variability was consistent with the pharmacokinetic component of the interindividual uncertainty factor used in the derivation of the RfC. No data on the impact of gender on the pharmacokinetics of toluene were identified, other than those resulting from physiological variability, which can be accounted for using the PBPK model. Varying bodyweight and other physiological parameters in the model to account for female vs. male physiology does not result in marked changes in predicted blood concentrations (variations generally less than about 10 percent) (Pelekis et al., 2001).

2.3.5. Smoking, drugs, alcohol co-exposures

Ethanol can inhibit metabolism of toluene through competition for CYP2E1 (Baelum, 1991). Thus, co-exposure to these or other compounds that are substrates for or inhibitors of CYP2E1 may result in prolonged elevation of toluene blood concentrations compared to those resulting from exposure to toluene alone. Smoking is a source of toluene exposure and smokers consistently demonstrate higher blood concentrations of toluene than non-smokers (see, for example, Churchill et al., 2001). However, no information is available regarding the impact of smoking on elimination rates of toluene.

2.3.6. Polymorphisms in enzymes or other factors

Researchers are beginning to identify polymorphisms in genes coding for key metabolic enzymes and examine the impact of such polymorphisms on potential responses. Polymorphisms in several of the enzymes known to be involved in the metabolism of toluene, including CYP2E1, have been identified. However, researchers have focused on correlating the occurrence of such polymorphisms with susceptibility to various conditions (see, for example, Heuser et al., 2007; Kezic et al., 2006) rather than directly assessing the effects of these polymorphisms on metabolic capability. Some studies have indicated an impact of such polymorphisms on enzymatic activity, but data are limited to date (reviewed in Gemma et al., 2006). Thus, we cannot draw any conclusions regarding the impact of such polymorphisms on predicted blood concentrations in individuals exposed at an exposure guideline. Such polymorphisms may account for some of the variability in blood levels observed among individuals after controlled exposures (see above).

2.4. Confidence assessment

Guidelines for 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 relationship between the measured biomarker and the critical or relevant target tissue dose metric.

2.4.1. Confidence in BE values based on oral exposure guidelines

As discussed above, the oral exposure route introduces additional uncertainties in estimating blood BE values corresponding to the oral exposure guidelines. These uncertainties stem from uncertainty regarding the appropriate dose metric (for example,

area under the curve vs. peak target organ concentrations) and uncertainty in the active metabolite responsible for liver or kidney toxicity. In this assessment we have relied on area under the curve of the parent compound in the target organ of interest as the relevant dose metric. However, if peak concentration (or peak metabolite production) is more relevant, then uncertainty regarding the appropriate oral absorption rate value for the models (which impacts estimates of peak, but not average, blood concentrations), and uncertainty regarding the appropriate dosing regimen to assume for exposure at the health-based exposure guidelines (once per day bolus vs. divided dose; again, impacting peak but not average blood concentrations) affects confidence in the BE values. Blood concentration as a biomarker for toluene should be directly related to average target organ concentration, but may be less informative regarding peak target organ concentration. For this reason, confidence in the BE values associated with the oral dosing route is lower than that for the inhalation exposure route.

In summary, the confidence ratings for BE values for oral exposure guidance values are:

- Relevance of biomarker to relevant dose metrics: MEDIUM.
- Robustness of pharmacokinetic data/models: MEDIUM.

2.4.2. Confidence in BE values based on inhalation exposure guidelines

Blood toluene concentrations are directly related to target tissue concentrations in the brain. The available PBPK models are well-validated and have been extensively used in combination with occupational data sets in humans. The BE values for inhalation exposure guidance values for toluene based on blood concentration as the biomarker thus have HIGH confidence for both aspects.

In summary, the confidence ratings for BE values for inhalation exposure guidance values are:

- Relevance of biomarker to relevant dose metrics: HIGH.
- Robustness of pharmacokinetic data/models: HIGH.

The summary confidence ratings are presented in Tables 4–6.

3. Discussion and interpretation of BE values

The BE values presented here represent the concentrations of toluene in blood that are consistent with exposure at the exposure guideline values that have been established by various agencies (Table 1). These BE values should be regarded as interim values that can be updated or replaced if the exposure guideline values are updated or if the scientific and regulatory communities develop additional data on acceptable or tolerable concentrations in human biological media based directly on epidemiological data.

The BE values presented here are screening values and can be used to provide a screening-level assessment of measured blood concentrations of toluene in population- or cohort-based studies. Comparison of measured values to the values presented here can provide an initial evaluation of whether the measured values in a given study are of low, medium, or high priority for risk assessment follow-up. Fig. 4 illustrates the presentation of the BE value corresponding to the Health Canada inhalation TDI. Measured biomarker values in excess of the human equivalent BE_{POD} indicate a high priority for risk assessment follow-up. Values below the BE_{POD} but above the BE suggest a medium priority for risk assessment follow-up, while those below the BE values suggest low priority for risk assessment follow-up. Based on the results of such comparisons, an evaluation can be made of the need for additional studies on exposure pathways, potential health effects, other aspects affecting exposure or risk, or other risk management activities.

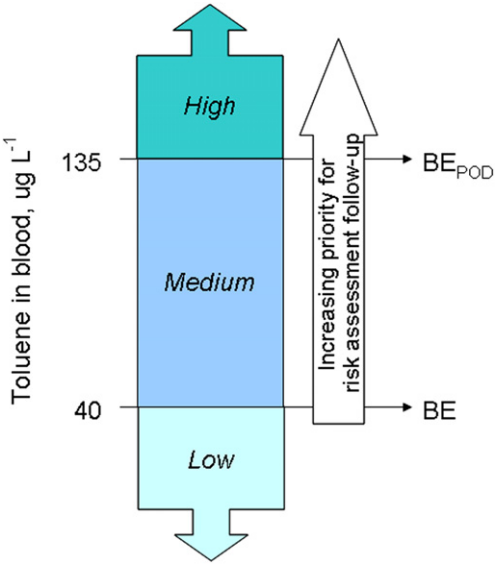

Fig. 4. Example presentation of the BE value corresponding to the Health Canada inhalation TDI. The BE_{POD} corresponds to the average blood concentrations estimated at the identified human no-observed-adverse-effect-level used as point of departure for the guideline derivation. The BE value presents the blood concentration consistent with the TDI (see Table 6 and the text for details on the derivation). Similar graphs could be prepared for the BE values derived for each of the available exposure guidance values.

Numerous exposure guideline values and thus BEs exist for interpreting human biomonitoring data for toluene. Selecting the most appropriate BE (and BE_{POD}) for interpreting biomonitoring data may depend on several factors including: the year the exposure guidance value was established (and thus potentially reflects advancement in understanding of toluene toxicity, mechanism of action, or available studies for deriving an exposure guideline value); whether the exposure guidance value was based upon animal or human toxicity data; the route of exposure from which the exposure guideline value was derived (and thus potentially reflects the more predominant pathway for exposure in the environment); the degree of uncertainty involved in the derivation of the exposure guidance value; and other judgments regarding the reliability of the underlying exposure guidance value.

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. In the case of toluene, BE values corresponding to exposure guidance values from different agencies differ widely, and interpretation of biomonitoring data results may depend upon which guidance value is regarded as most reliable and appropriate for a given situation. Further discussion of interpretation and communications aspects of the BE values is presented in LaKind et al. (2008).

Disclaimer

This work was reviewed by EPA and approved for publication, but does not necessarily reflect official Agency policy. Mention of trade names or commercial products does not constitute endorsement or recommendation by EPA for use.

Conflict of interest disclosure statement

The authors declare that they have no conflicts of interest.

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