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Biomonitoring Equivalents (BE) dossier for 2,4-dichlorophenoxyacetic acid (2,4-D) (CAS No. 94-75-7)

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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 2,4-dichlorophenoxyacetic acid (2,4-D) and applies these data and models to existing health-based exposure guidance values from the US Environmental Protection Agency to estimate corresponding BE values for 2,4-D in plasma and urine. These values can be used as screening tools for evaluation of biomonitoring data for 2,4-D in the context of the existing USEPA risk assessment and for prioritization of the potential need for additional risk assessment efforts for 2,4-D.

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 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. 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 (POD, a dose corresponding to the low end of the dose–response relationship such as a No Observed Effect Level [NOEL] or Benchmark Dose [BMD]) 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 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.

2,4-Dichlorophenoxyacetic acid (2,4-D) (CAS number 94-75-7) is used as a herbicide to control broadleaf weeds in the cultivation of wheat, corn, barley, and sorghum and in rangeland and pastureland. It is the active ingredient in more than 1500 herbicide products. There are few data documenting specific exposure pathways or media for human exposure to 2,4-D in the general population (http://www.epa.gov/ttn/atw/hlthef/di-oxyac.html).

This BE dossier describes the scientific basis for and derivation of BE values for 2,4-D and discusses issues that are important for the interpretation of biomonitoring data using Biomonitoring Equivalents. This dossier is not designed to be a comprehensive compilation of the available hazard, dose–response or risk assessment information for 2,4-D.

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1.1. Current health-based exposure guidance values

Acute high-level oral or inhalation exposure to 2,4-D causes neurotoxicity, with symptoms including stiffness of arms and legs, incoordination, lethargy, anorexia, stupor, and coma in humans. 2,4-D is also an irritant to the gastrointestinal tract, causing nausea, vomiting, and diarrhea, and can cause a rash or dermal irritation after direct skin exposure. The most sensitive effects observed following chronic exposure in animals are hematologic, renal, and hepatic effects. At higher doses, decreased fetal weights, increased fetal mortality, and skeletal abnormalities have been observed in offspring of treated animals. Additional information and references regarding 2,4-D toxicity are available at the USEPA Air Toxics Web site (http://www.epa.gov/ttn/atw/hlthef/di-oxyac.html).

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 [RfD or RfC]), the Agency for Toxic Substances and Disease Registry (ATSDR) (Minimal Risk Levels or MRLs), and Health Canada and the World Health Organization (Tolerable Daily Intakes or TDIs). The chronic health-based exposure guideline values are designated with different names and have somewhat different definitions, but generally describe a rough estimate for a chemical that are expected to be without adverse or deleterious effects in the general population, including sensitive subpopulations.[1] For chemicals considered to be carcinogenic, the USEPA also establishes estimates of the cancer potency of the chemicals 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 2,4-D. The USEPA Integrated Risk Information System (IRIS) evaluated 2,4-D in 1988 and established a chronic RfD. However, more recently, the USEPA Office of Pesticide Programs conducted an updated review of 2,4-D and adopted a revised chronic RfD as well as acute RfDs (applicable to single-day exposures) for 2,4-D (USEPA, 2004). These revised values are described in Table 1, including information regarding the studies used as the basis for the derivation, the identified point of departure (POD) (no observed adverse effect level [NOAEL], lowest observed adverse effect level [LOAEL], or benchmark dose) and the uncertainty factors applied to the POD to obtain the RfD. USEPA has not classified 2,4-D with respect to carcinogenicity and has not established any UCR estimates for 2,4-D.

1.2. Pharmacokinetics

The pharmacokinetics of 2,4-D have been studied in two studies of human volunteers. Kohli et al. (1974) and Sauerhoff et al. (1977) studied the fate of 2,4-D after oral administration of single doses of 5 mg kg^{-1} in six and five human volunteers, respectively, reporting time courses for both plasma concentrations and urinary excretion (Sauerhoff et al. report plasma time courses for only three of the five individuals studied). 2,4-D was eliminated as the parent compound (~82%) or as a conjugate of the parent compound in urine (Sauerhoff et al., 1977). Different estimates of the elimination pharmacokinetics of 2,4-D were made by the two groups. Sauerhoff et al. (1977) estimated an average urinary elimination half-life of approximately 17 h and a plasma half-life of approximately 11 h. This elimination rate is rapid enough that pseudo-steady-state plasma levels would be reached within a few days of constant exposure. At that point, the total quantity of compound eliminated per day in urine would be equivalent to the amount absorbed into the body (from dietary or other exposure sources). Kohli et al. (1974) found slower overall elimination kinetics, with a plasma half-life of approximately 33 h. The differences between the two models stem primarily from significant differences in measured plasma concentrations at time points 5–7 days after administration, with Kohli et al. reporting significantly higher remaining plasma concentrations after this time period. Both groups fit their plasma concentration data to a one-compartment kinetic model. The parameters derived by these authors can be used to predict plasma concentrations of 2,4-D following repeated exposures. Under either set of kinetic assumptions, however, continuing exposure at the RfD or other daily exposure guideline for more than 1 week of exposure would result in a steady-state in which the amount excreted daily in urine would be approximately equivalent to the amount absorbed each day.

The pharmacokinetics of 2,4-D have been studied in numerous species including rats, the species used in the studies that serve as the basis for the RfD values (reviewed in Timchalk, 2004). As in humans, 2,4-D does not undergo oxidative metabolism but is excreted in urine as the parent compound or a conjugate in rats. Renal excretion occurs via active transport, and is saturable in rats at chronic dosing rates between 10 and 100 mg kg^{-1} d^{-1} (Timchalk, 2004; Saghir et al., 2006; van Ravenzwaay et al., 2003). In general, toxicity is not observed below doses required to saturate the active renal transport mechanisms. A pharmacokinetic model for 2,4-D in rats has been developed with a focus on examining distribution to the adult and developing rat brain (Kim et al., 1994, 1995, 1996, 2001). More recently, Saghir et al. (2006) duplicated the dietary exposure regimens in rats used in the studies underlying the RfD derivation for 2,4-D and other pesticides and collected blood and urine samples from dosed rats at various time points to evaluate the daily variation in plasma and urine levels. The measured plasma concentrations in rats exposed at the NOEL allow direct comparison with the human plasma data reported by Sauerhoff et al. (1977) from human volunteers ingesting a single dose of 2,4-D.

1.3. Biomarkers and dose metrics

The objective of using BEs is to provide a human health risk framework for evaluation of human biomonitoring data. The choice of the biomarker (analyte and medium) and dose metric (peak, daily average, creatinine corrected, etc.) should be optimized to facilitate this objective. The key criterion for the choice of a biomarker and dose metric then is that they be as closely related to the critical dose metric associated with toxicity as possible and feasibly obtained 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 directly related to the target tissue concentration as possible. Likewise, the dose metric should be chosen to reflect the mechanism of toxicity of the compound, where known.

Several mechanisms of action of toxicity for 2,4-D are known, but these are most clearly observed after high-exposure acute-tox-

[1] 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. It can be derived from a NOAEL, LOAEL, or benchmark dose, with uncertainty factors generally applied to reflect limitations of the data used. Generally used in EPA's noncancer health assessments. http://www.epa.gov/NCEA/iris/help_gloss.htm#r.

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 OPP reference doses (RfDs) for 2,4-D (USEPA, 2004)

Exposure guideline	Description of study used as the basis for the value	Effects observed at doses above the NOAEL	POD (mg kg^{-1} d^{-1})	UFs	RfD mg kg^{-1} d^{-1}
Chronic RfD (general population)	Rat chronic toxicity study, dietary administration	Decreased bodyweight gain and food consumption, alterations in hematology and clinical chemistry parameters, increased thyroid weights, and decreased testes and ovarian weights	NOAEL: 5 LOAEL: 75	1000 10—interspecies variation; 10—interindividual variation; 10—database	0.005
Acute dietary RfD (females age 13–50)	Rat developmental toxicity study via oral gavage, gestation days 6–15	Skeletal malformations and skeletal variations	NOAEL: 25 LOAEL: 75	1000 10—interspecies variation; 10—interindividual variation; 10—database	0.025
Acute dietary RfD (general population)	Rat acute neurotoxicity study via oral gavage, single dose	Gait abnormalities	NOAEL: 67 LOAEL: 227	1000 10—interspecies variation; 10—interindividual variation; 10—database	0.067

icity events. These include (but are not limited to) dose-dependent cell membrane damage, uncoupling of oxidative phosphorylation, and disruption of acetylcoenzyme A metabolism (Bradberry et al., 2000). The specific mechanisms of action underlying the neurotoxicity and other effects observed at lower exposures are not fully understood, and a full discussion of the mechanism of action of 2,4-D is outside the scope of this review. Researchers have developed models relating tissue exposure in the brain to plasma concentrations of 2,4-D, indicating that plasma concentration is a useful surrogate for brain concentrations (Kim et al., 1995). With respect to potential developmental effects, plasma concentrations probably are superior to administered dose measures as surrogates for target tissue dose, but no specific research on this point has been done. Since 2,4-D does not undergo oxidative metabolism in mammals (reviewed in Timchalk, 2004), it is likely that the toxicity moiety for most endpoints is the parent compound, 2,4-D. Based on this conclusion, the concentration of 2,4-D in plasma should be directly relevant to the critical dose metric(s) for a range of toxic endpoints, even if the specific mechanism of action and critical dose metrics are not fully understood.

Because 2,4-D is excreted as the parent compound in urine, most biomonitoring evaluations of exposure to 2,4-D have relied on urinary samples (Knopp and Glass, 1991; Knopp, 1994; CDC, 2005). A few kinetic studies have examined plasma concentrations of 2,4-D in humans and animals as well (Kohli et al., 1974; Saghir et al., 2006; van Ravenzwaay et al., 2003; Sauerhoff et al., 1977). The relative ease of collection of urine samples compared to blood samples contributes to this choice. From a toxicologic point of view, plasma concentrations of 2,4-D are probably more informative for predicting target tissue concentrations and responses (e.g., neurotoxic responses). This would be particularly true under conditions of episodic, higher level exposures. However, under conditions of chronic, low-level exposures, urinary excretion rates should be specific and quantitatively relevant in a framework of a mass-balance assessment. That is, under exposure conditions that approximate steady-state, daily urinary excretion should equal daily intake. Table 2 summarizes the advantages and disadvantages of available biomarkers.

2. BE derivation

This section presents the methods used to derive BE values for use in evaluation of biomonitoring data for 2,4-D and the resulting BE values. As discussed above, either plasma or urine can be used as the medium for biomonitoring.

The following BE values are derived and presented for assessment of urinary concentrations of 2,4-D:

- Estimated human creatinine-adjusted urine concentrations under conditions of exposure at the chronic RfD, or following single-day exposure at the acute RfD (see Table 1 for RfD values).
- Estimated concentration of 2,4-D in urine on a volume basis (μg L^{-1}) following exposure at the chronic RfD or following single-day exposure at the acute RfD.

For plasma, the following values are derived and presented:

- Measured laboratory rat plasma concentrations at the point of departure, 5 mg kg^{-1} d^{-1} (a NOAEL), used as the basis of the derivation of the chronic or acute RfD, as appropriate;

Table 2
Possible analytes and media for use as a biomarker of exposure in biomonitoring studies

Analyte	Medium	Advantages	Disadvantages
2,4-D	Plasma	Highly relevant as a surrogate for target tissue dose for CNS effects (Kim et al., 2001); relevance to other target organ effects likely given lack of significant metabolism	Invasive sampling required
	Urine	Sampling is non-invasive	Not directly relevant to target tissue concentrations; requires creatinine correction or other method of accounting for hydration status, and variations in creatinine excretion can impact interpretation
No relevant metabolite available as 2,4-D undergoes little metabolism			

- Measured peak human plasma concentrations following acute dosing with the same dose, 5 mg kg^{-1} d^{-1} as reported in two studies of volunteer exposure;
- Human plasma concentrations consistent with both the acute and chronic RfDs RfD were estimated in two ways:
  - Using the available human pharmacokinetic models to estimate the plasma concentration resulting from steady-state exposure at the chronic RfD or single-dose exposure at the acute RfD and
  - Estimated human plasma concentrations derived based on extrapolation of measured plasma concentrations in animals dosed at the PODs that underlie the acute and chronic RfDs.

2.1. Methods

The basic methods and approaches for deriving BE values are described in Hays et al. (2007), with specific guidelines for derivation presented in Hays et al. (2008). The key considerations to be assessed in selecting a method for derivation include:

- Identification of internal dose metrics that are likely to be relevant to the mechanism of action;
- Evaluation of the relationship between the biomarker and the relevant internal dose metric; and
- Consideration of the available pharmacokinetic data.

For 2,4-D, as discussed above, plasma concentrations are likely to be highly relevant to the critical internal dose(s) associated with toxic responses. Thus, plasma concentration as a biomarker is directly relevant to toxic responses. In contrast, urinary concentration is less directly related to critical internal dose metrics, but rather, serves most directly as a biomarker of exposure. Thus, the approaches for derivation of BE values for these two biomarkers differ.

For urinary 2,4-D concentration, a simple mass-balance approach is taken here, estimating the 24-h average concentration of 2,4-D in urine expected at steady-state exposure at the RfD. For plasma 2,4-D as the biomarker, an approach that takes into account plasma concentrations in the laboratory experiment at the POD along with appropriate uncertainty factors is used. These approaches are detailed below.

2.1.1. Urine

As discussed above, the straightforward elimination kinetics of 2,4-D (as parent compound in urine with essentially no metabolism) and the lack of direct relationship between urinary concentration and critical internal dose metrics suggests a simple mass-balance approach for derivation of BE values for urinary 2,4-D concentration, illustrated in Fig. 1. The following sections discuss the implementation of this approach for 2,4-D.

2.1.1.1. Chronic exposure conditions. An ideal biomonitoring regimen for 2,4-D and for other compounds excreted in urine would include collection of 24-h urine specimens so that daily excretion could be quantified directly. In practice, however, collection of such samples is difficult and impractical for large biomonitoring studies such as the NHANES/CDC effort. As a result, urinary concentrations are generally reported based on spot urine sample collection. The absolute concentration of compounds in such samples can vary substantially due simply to differences in hydration rates and to other factors (reviewed by Mage et al., 2004). Thus, in addition to reporting absolute urinary concentrations of such chemicals (e.g., in units of μg L^{-1}), CDC and other researchers generally also report levels adjusted to creatinine levels (e.g., μg chemical g^{-1} creatinine).

While hydration status introduces variability into interpretation of urinary concentrations on a volume basis, creatinine adjustment also introduces variability into the analysis (Garde et al., 2004). As reviewed by Mage et al. (2004) and Barr et al. (2005), creatinine excretion is a function of age, gender, and lean body mass (a function of height and weight), dietary patterns, and other factors including kidney function status. Daily creatinine excretion can vary substantially: typical estimates for adults vary by a factor of 4 or more, from approximately 0.5 to more than 2 g d^{-1} depending on those factors and other sources of individual variability (www.nlm.nih.gov/medlineplus/ency/article/003610.htm), and

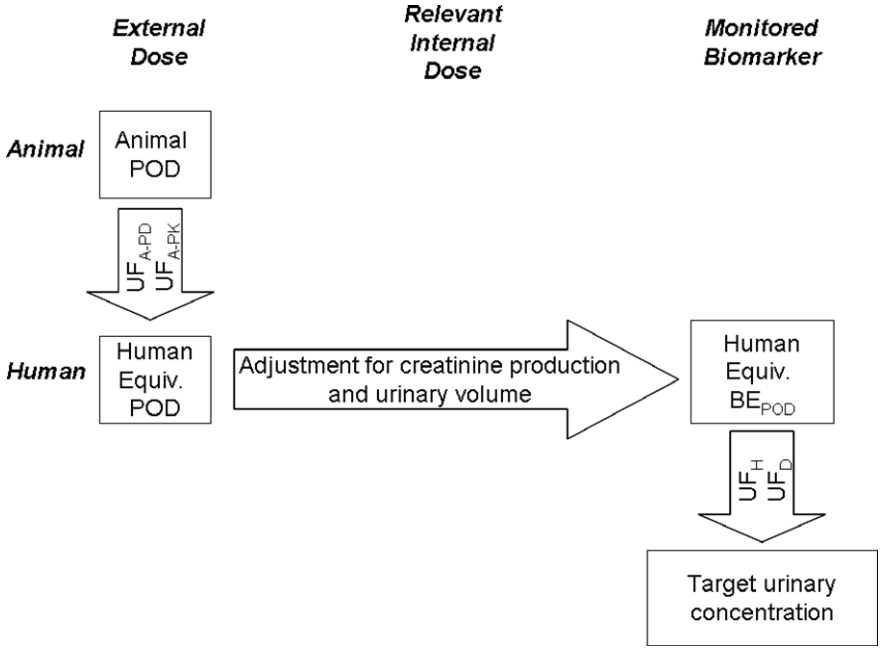

Fig. 1. Schematic for derivation of the urinary chronic BE_{RfD}.

variability in creatinine excretion rates in children may be greater (Kissel et al., 2005; O'Rourke et al., 2000). Because the total intake at any health-based exposure guideline such as the RfD is also a function of weight (these values are generally specified in terms of milligrams of intake per kilogram bodyweight per day), estimates of the creatinine-adjusted concentration in urine associated with exposure at the RfD can vary substantially among individuals.

Two main approaches to derivation of urinary BE values are taken here: creatinine adjustment (μg chemical g^{-1} creatinine) and urinary volume adjustment (μg chemical L^{-1} of urine).

2.1.1.1.1. Creatinine-adjusted bias. The modeling approach taken in this effort adopts the same basic assumptions used by Mage et al. (2004) in their back-extrapolation of estimated doses for pesticides and metabolites analyzed in urine based on NHANES III data:

- The values derived are based on the assumption that there is no pathology involved, such as kidney failure or a muscle wasting disease;
- Typical food intake patterns are assumed. That is, creatinine production will be assumed to be from a mixed diet rather than from a strict vegetarian diet, which could reduce creatinine production and excretion, or from a highly meat-intensive diet, which could increase creatinine excretion.
- The maximum rate of renal transport for 2,4-D is assumed not to be exceeded at the RfD, an assumption supported by the available kinetic data in both humans and laboratory animals (Timchalk, 2004).

Adults. Mage et al. (2004) derived predictive equations specific for men and women to estimate daily creatinine excretion as a function of height, weight, and age based on established formulas scaled to body surface area (Eqs. (3a) and (3b) in Mage et al., 2004):

$$\text{Male Cn} = 1.93(140 - A) * \text{BW}^{1.5} * h^{0.5} \quad (1)$$

and

$$\text{Female Cn} = 1.64(140 - A) * BW^{1.5} * h^{0.5} \quad (2)$$

where Cn is creatinine excretion in μg d^{-1}, A is age in years, BW is bodyweight in kg, and h is height in cm.

These formulas for adults were used in a probabilistic assessment incorporating gender-specific approximate distributions of height, weight, and age in the adult US population for two age groups: 20–60 years, and 60–80 years, corresponding to age categories used in the past by CDC to report results. The modeling was conducted using Crystal Ball® version 7.2.2. Table 3 presents the distributions used for each of the key parameters for men and women. For each selection of height, weight, and age from the distributions, a specific creatinine excretion rate in μg/d was calculated. For each iteration of the simulation, excretion of 2,4-D equal to a daily dose corresponding to the RfD times the specific bodyweight for that iteration was assumed. This daily excretion amount of 2,4-D was divided by the iteration-specific creatinine excretion estimate. The result is the predicted creatinine-adjusted concentration of 2,4-D in μg g^{-1} Cn for that iteration of the simulation:

$$[2,4\text{-D}]_{\text{Cn-adj}} = \frac{\text{RfD}_{\text{chronic}} * \text{BW}}{\text{Cn}(A, \text{BW}, h)} * \text{CF} \quad (3)$$

where the RfD is given in mg kg^{-1} d^{-1}, BW in kg, Cn in μg d^{-1} from Eq. (3) above, and CF is a conversion factor equal to 10^9.

Children. In children, height and weight are correlated with age in complex relationships, so a more direct approach based on empirical distributions of creatinine excretion from the literature was used. Daily (24-h) creatinine excretion per kilogram bodyweight was reported by age groups by Remer et al. (2002) based on measured creatinine in 24-h urine samples taken from 225 boys and 229 girls (Table 4). This is the most current and largest data set available regarding children's creatinine excretion rates, although a limitation of this data set is that it is composed entirely of Caucasian children. This distribution of normal creatinine excretion as a function of bodyweight for specific age groups can be used to estimate the range of concentrations of 2,4-D per gram creatinine that is consistent with exposure at the RfD for each age group. Values for the mean, median, 5th, and 95th percentile were calculated by dividing the RfD by those values as reported for each age group and gender.

2.1.1.2. Acute exposure conditions. The USEPA OPP has also identified permissible acute (single day) exposure levels for the general population and for females aged 13–50 (see Table 1). These values could apply in circumstances involving direct use of 2,4-D or other close contact to the compound during application. In human volunteers given a single dose of 2,4-D, Sauerhoff et al. (1977) found that approximately 50% of the total dose was excreted in urine within the first 24 h. Thus, the creatinine-adjusted concentration estimated to be associated with a single exposure at the acute RfD, $\text{BE}_{\text{RfD acute, urine}}$ can be estimated as follows:

$$\text{BE}_{\text{RfD acute, urine}} = \text{BE}_{\text{RfD chronic, urine}} \left[\frac{\text{RfD}_{\text{acute}}}{\text{RfD}_{\text{chronic}}}\right] * 0.5 \quad (4)$$

where the $\text{BE}_{\text{RfD chronic, urine}}$ is the BE value resulting from Eq. (3). This approach was used to scale the estimated chronic creatinine-adjusted values for the corresponding acute RfD values.

2.1.1.2.1. Urinary volume basis. Estimates of average 24-h urinary volume are available in the literature for both adults and children (Perucca et al., 2007; Remer et al., 2006). These values can be used to derive an estimate of average 24-h urinary concentration consistent with steady-state exposure at the RfD for use as a

Table 3
Distributions used for parameters in the Monte Carlo model of urinary creatinine excretion

Gender	Age (years) uniform	Variable and distribution description			
		Height (cm) normal		Weight (kg) lognormal	
		Mean	SD	Geometric mean	GSD
Men	20–60	178	7.6	82	1.16
	60–80	170	5	74	1.04
Women	20–60	162	6.6	74	1.1
	60–80	158	5	67	1.04

Table 4
Age-based reference ranges for 24-h urinary creatinine excretion in 225 boys and 229 girls[a]

	Creatinine excretion (mg kg^{-1} d^{-1})				
	3 years	4–5 years	6–8 years	9–13 years	14–18 years
Boys					
Mean	15.2	17.1	19.5	20.6	22.7
SD	2.6	2.9	2.9	4.1	4.9
Median	14.8	17.0	19.3	20.7	23.3
5th percentile	10.9	12.0	15.2	11.3	13.2
95th percentile	21.4	24.2	25.6	27.7	33.3
Girls					
Mean	14.4	16.1	18.1	19.3	20.6
SD	2.9	2.8	3.5	4.1	3.2
Median	14.5	15.7	17.9	18.9	20.9
5th percentile	8.9	12.3	12.4	13.2	14.6
95th percentile	20.6	21.2	26.6	27.6	26.9

[a] Values from Remer et al. (2002), Table 2, converted from mmol kg^{-1} d^{-1} to mg kg^{-1} d^{-1} using the molecular weight of creatinine, 113.12 g mol^{-1}.

screening value. For each group at steady-state exposure, a quantity of 2,4-D equal to the estimated daily intake at the RfD is assumed to be excreted in the 24-h urinary volume ($V_{24\text{-h}}$), so that the predicted concentration in urine ($\mu g\,L^{-1}$) following steady-state exposure at the chronic RfD can be calculated as follows:

$$[2,4\text{-D}] = \frac{\text{RfD}_{\text{chronic}} * \text{BW}}{V_{24\text{-h}}} \quad (5)$$

For adults, 24-h urinary volume estimates from the literature for healthy adult men and women are summarized by Perucca et al. (2007, Table 2). A weighted average for the four studies of healthy individuals tabulated there yields estimates of average urinary volumes for men and women of 1.7 and 1.6 L/24 h, respectively, with coefficients of variation of approximately 30%. Remer et al. (2006) reported that 24-h urinary volume for children aged 6–12 (average bodyweight for boys and girls of 28.4 and 28 kg, respectively) was not sex-dependent and averaged 0.66 L/24 h. No data were found for 24-h urinary volume for adolescents or children under 6 years of age. For adolescents, an assumption was made that urinary volume is equivalent to that of adults; for children under 6, an assumption was made that urinary volumes are similar to those for children aged 6–12. In both cases, these assumptions may overestimate actual urinary volumes, resulting in underestimates of urinary BE values for these age groups.

To derive urinary concentration values associated with exposure at the acute RfD, the assumption was again made that following a single-day exposure at the acute RfD, 50% of the acute ingested dose would be excreted in urine in the first 24 h following exposure, as for the creatinine-based values (Eq. (4)).

2.1.1.2.2. An alternative method: mass per time basis. Finally, because the assumption of steady-state implies that the amount excreted in urine is equal to the RfD, an estimate of 24-h excretion on a mass per time basis can be made for different standard bodyweights assuming steady-state exposure at the chronic RfD. If urinary spot collections are reported with sufficient information (volume of collection, time since last void, and concentration of analyte), estimates of urinary elimination in mass per time can be estimated and compared directly to a standard excretion rate equal to the chronic RfD on a mg kg^{-1} d^{-1} basis. For acute exposure scenarios, the excreted mass per time in the first 24 h following exposure can be compared to 50% of the acute RfD on a mg kg^{-1} d^{-1} basis. Specific values are not calculated here due to the lack of requisite information in current reports of biomonitoring data. However, if appropriate data were collected, the calculations can easily be made for a given bodyweight through use of the RfD.

2.1.2. Plasma

As discussed above, guidelines for derivation of BE values call for an assessment of relevant dose metrics, relationship between those metrics and the biomarker concentration, and evaluation of the available pharmacokinetic data. In addition, the availability of both human and animal pharmacokinetic data influences the approach used to derive BEs (Hays et al., 2008). The derivation of chronic and acute BE values for 2,4-D in plasma are described below.

2.1.2.1. Chronic BE value. Fig. 2 presents a schematic of the approach used to derive the chronic BE values for 2,4-D in plasma. The approach involves the following steps:

- Estimation of the relevant internal dose metric in the animal study at the POD. As discussed above, plasma concentration is likely to be directly related to the critical target tissue doses, and is also the biomarker of interest. Thus, the estimation of plasma concentration in the animal study at the POD is the BE_{POD_animal}.
- Extrapolation to a human equivalent internal dose metric through application of an interspecies uncertainty factor component for pharmacodynamic differences between the laboratory species and humans ($UF_{A\text{-}PD}$). No component for pharmacokinetic differences is applied because the pharmacokinetics are

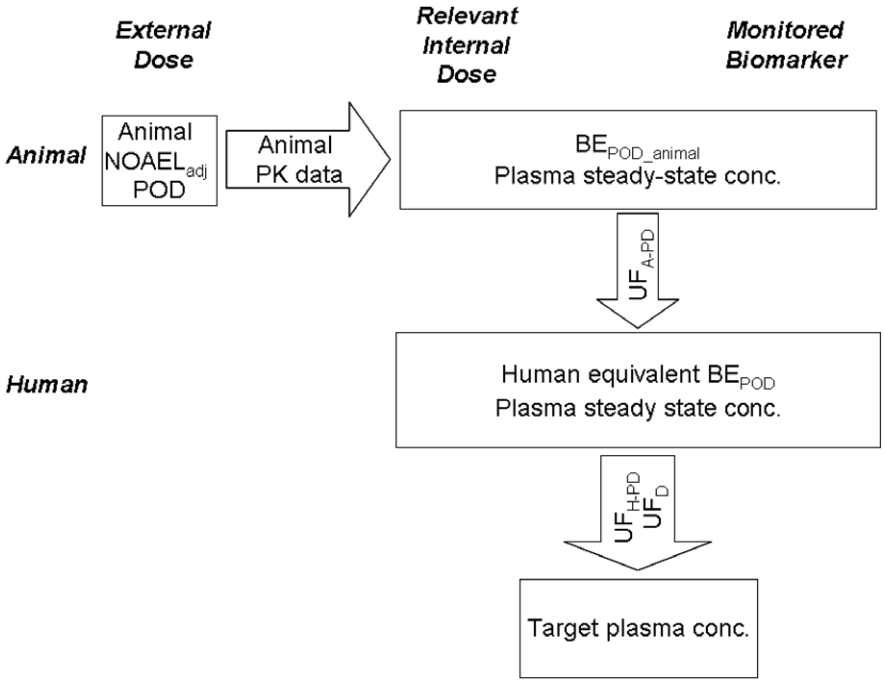

Fig. 2. Schematic of approach to derivation of the plasma chronic BE_{RfD}.

explicitly accounted for through the use of measured plasma concentrations in the animal study at the POD, and the interspecies extrapolation is being conducted on an internal dose basis.
- Assessment of the biomarker concentrations in humans associated with that internal dose metric. For 2,4-D, the relevant internal dose metric (plasma concentration) is the monitored biomarker, and the concentration in plasma is the human equivalent BE_{POD}; and
- Application of appropriate uncertainty factors. These factors include components for intraspecies pharmacodynamic and pharmacokinetic sensitivity ($UF_{H\text{-}PD}$ and $UF_{H\text{-}PK}$) and for database uncertainties (UF_D) consistent with the derivation of the chronic RfD.

In assessment of the application of uncertainty factors, the pharmacokinetic component of the intraspecies (within human) uncertainty factor that is normally applied in external dose-based risk assessments is replaced by the measurement of plasma concentration during biomonitoring. That is, persons who are pharmacokinetically sensitive will demonstrate higher plasma concentrations than those who are not from the same external dose. Since the risk assessment process used in the derivation of the BE is based on target internal dose metrics, no additional component of the intraspecies UF for pharmacokinetic sensitivity is necessary in the derivation of the BE value. This topic is discussed in more detail in the BE derivation guidelines (Hays et al., 2008).

2.1.2.2. Acute BE values. Two approaches were evaluated for the derivation of acute BE values for plasma. The acute RfD values are based upon studies in rats employing single-dose gavage administration of 2,4-D (see Table 1). Measured peak concentrations of 2,4-D in plasma in rats in the dose ranges used in these studies are available from pharmacokinetic studies (Kim et al., 1994; see Table 7). The bolus doses used in these studies are in the non-linear range for pharmacokinetics, resulting in substantial elevations in plasma concentrations above those that would be predicted on the basis of the dietary administration plasma data from Saghir et al. (2006). Because of the non-linearity in kinetics and the challenges presented by the single-dose administration (transient concentration profiles), the full complement of inter- and intraspecies uncertainty factors, including pharmacokinetics components (composite uncertainty factors of 1000 for both acute RfD values), were retained in the extrapolation from the peak plasma values in rats at the POD to human plasma BE values.

A second approach was available. As discussed above, both Kohli et al. (1974) and Sauerhoff et al. (1977) assessed the pharmacokinetics of 2,4-D in human volunteers following ingestion of 5 mg kg^{-1} of 2,4-D, measuring plasma concentrations and urinary excretion over a period of 6–7 days following ingestion. Based on these data, each group of authors estimated parameters for a one-compartment model of plasma clearance (Table 5). The clearance values estimated are generally consistent with allometric scaling from several other species (Timchalk, 2004) and produce estimates for short-term average blood concentration following a bolus dose that are somewhat consistent, but the models predict plasma concentrations that diverge over the course of chronic exposure. In order to provide context for the BE values derived using the approach described above and in Fig. 2, both of these one-compartment models were used to estimate the plasma concentrations of 2,4-D associated with exposures at the acute RfD values in a single-exposure event. The average of the estimates derived from the two models of 24-h average plasma concentration in humans exposed at the acute RfD values provide an alternative basis for the acute BE values.

Table 5
Parameters used in the one-compartment models of plasma clearance of 2,4-D from Sauerhoff et al. (1977) and Kohli et al. (1974)

Parameter	Sauerhoff et al. (1977) model	Kohli et al. (1974) model
Oral absorption rate (h^{-1})	0.3085	0.274
Volume of distribution (ml kg^{-1} BW)	288.5	101
Elimination rate (h^{-1})	0.0785	0.021

Table 6
Selected percentiles of the estimated creatinine-adjusted urinary concentration of 2,4-D consistent with exposure at the US EPA OPP chronic RfD for individuals by gender and age group

Gender/age group (years)	Percentiles of creatinine-adjusted 2,4-D concentration (μg g^{-1})		
	5th	50th	95th
Boys			
4–5	210	300	420
6–8	200	260	330
9–13	180	240	440
14–18	150	220	380
Girls			
4–5	240	320	410
6–8	190	280	400
9–13	180	270	380
14–18	190	240	340
Men			
20–60	180	220	280
60–80	190	230	300
Women			
20–60	220	280	350
60–80	350	430	510

Values for adults were generated through Monte Carlo modeling; values for children were estimated by dividing the RfD (in mg kg^{-1} d^{-1}) by the percentiles of total 24-h urinary creatinine excretion from Table 3 for each age group.

2.2. Results of modeling and identification of BE values

2.2.1. Urine

2.2.1.1. Creatinine-adjusted basis. The estimated ranges of creatinine excretion resulting from the prediction formulas correspond well to the typical "normal" range for creatinine excretion of approximately 0.5–2 g/day, although the upper end of the predicted distributions for men were somewhat higher, ranging up to approximately 2.8 g d^{-1}. Selected percentiles of the distributions of creatinine-adjusted 2,4-D concentrations consistent with the chronic RfD predicted by gender and age group are tabulated in Table 6 for each gender and age group ranging from children aged 3 through adults aged 80. The pattern of estimated concentrations of 2,4-D per gram creatinine reflect known patterns in creatinine excretion with age. Creatinine excretion in infants and very young children, as well as elderly persons and especially elderly women, is lower than in healthy older children and adults. As a result, the creatinine-adjusted concentration of 2,4-D in these young and elderly age groups consistent with exposure at the RfD is somewhat higher. Within each age group, the 5th and 95th percentiles of creatinine-adjusted 2,4-D concentration generally differed by a factor of two or less. The central tendency across age groups is relatively consistent. The average of the median values for adults (males and females) is approximately 290 μg g^{-1} creatinine; this value is also consistent with the range of median values identified for children of various ages. Because BEs are screening values, estimated BE values are generally presented with only one significant figure. Thus, the chronic BE_{RfD} for urinary 2,4-D concentrations on a creatinine-adjusted basis is 300 μg g^{-1} creatinine.

Table 7
Comparison of modeled and measured plasma concentrations after various exposure scenarios in humans and rats

Dose (mg kg^{-1} d^{-1})	Description	Measured plasma concentrations (μg L^{-1})		Modeled human plasma concentrations (μg L^{-1})		
		Rat	Human	Sauerhoff et al. model	Kohli et al. model	Average of model predictions
5	POD for chronic RfD, NOAEL in chronic rat dietary study	410–720[a]				
5	Rat gavage, peak concentration	9000–14,000[b]				
5	Human volunteers single dose, peak concentration		8000–50,000[c]	12,000	40,000	26,000
0.005	Chronic RfD, single dose per day			4–14[d]	80–110[d]	60
0.005	Chronic RfD, three divided doses per day			6–12[d]	90–100[d]	55
25	POD (NOAEL) for developmental effects study used as the basis of the acute RfD for females of reproductive age; rat gavage	80,000[e]				
0.025	Acute dietary RfD, females aged 13–50, one single dose in 1 day (non-steady-state)			32[f]	134[f]	83
67	POD (NOAEL) for neurological effects in rats following single oral gavage; used as the basis of the acute RfD for the general population	200,000[e]				
0.067	Acute dietary RfD, general population, one single dose in one day (non-steady-state)			85[f]	361[f]	223

[a] C_{min} and C_{max} measured over the course of 24 h in rats dosed for 28 days via diet (Saghir et al., 2006).
[b] Peak concentration following oral gavage administration to rats (male and female, respectively; van Ravenzwaay et al., 2003).
[c] Range of peak measured concentration measured by Kohli et al. (1974; six volunteers) and Sauerhoff et al. (1977; three volunteers) following ingestion of a single dose of 5 mg kg^{-1}.
[d] Modeled range of C_{min} to C_{max} consistent with chronic exposure at the RfD (0.005 mg kg^{-1} d^{-1}) under the specified exposure pattern using the specified one-compartment model (Sauerhoff et al., 1977 or Kohli et al., 1974).
[e] Peak concentration estimated by interpolation using Fig. 2, Kim et al. (1994).
[f] Average plasma concentration over 24 h as estimated for a single dose as described using the specified one-compartment model (Sauerhoff et al., 1977 or Kohli et al., 1974).

The age-specific estimates of creatinine-adjusted 2,4-D concentrations at the chronic RfD presented in Table 6 were adjusted using Eq. (4) to obtain the corresponding BE values associated with the acute RfD values (which vary by age and sex, see Table 1). For women of childbearing age (age 13–50), the BE value associated with the acute RfD of 0.025 mg kg^{-1} d^{-1}is 700 μg g^{-1} creatinine. For the remainder of the population, the acute RfD of 0.067 mg kg^{-1} d^{-1} corresponds to a BE of 2000 μg g^{-1} creatinine.

2.2.1.2. Urinary volume basis. The assumptions used in the calculation of urinary concentrations consistent with exposure at the chronic and acute RfDs and the resulting urinary concentrations are presented in Table 8. The variations in estimated concentrations reflect variations in estimated urine volume, bodyweight (which affects the daily dose consistent with the RfD), and the difference in the acute RfD for women of childbearing age compared to the rest of the general population. The results presented in Table 8 demonstrate that for chronic exposure, a BE value of approximately 200 μg L^{-1} is consistent with exposure at the RfD for all age groups evaluated.

BE values corresponding to the acute RfDs were again derived using Eq. (4). The age-specific urinary BE values on a volume basis consistent with exposure at the acute RfD presented in Table 8 vary due to factors discussed above (variations in bodyweight, urinary volume, and RfD value). Rounded to one significant figure, the acute BE value (appropriate for single-day episodic exposures) for women of childbearing age is 400 μg L^{-1}; the value for the remainder of the population is 1000 μg L^{-1}.

2.2.2. Plasma

Table 7 summarizes the available measured 2,4-D concentrations in animals and humans at various doses under both dietary and gavage dosing regimens and presents the modeled plasma concentrations in humans following various dosing regimens at the chronic RfD and for the acute RfD values using the two available models.

Table 9 presents the derivation of the plasma BE value associated with the chronic RfD using the approach outlined above and in Fig. 2. The derived BE value, 5 μg L^{-1}, is similar to the plasma concentrations associated with exposure at the chronic RfD using the Sauerhoff model, but is lower than the values obtained using the Kohli model.

BE values for assessing plasma 2,4-D concentrations following short-term (1 day) exposures are 80 and 200 μg L^{-1} for females (age 13–50) and the rest of the general population, respectively (Table 10). These values represent the average from the two available models of the estimates of 24-h average blood concentrations following a single oral dose at the acute RfD values (see Table 7). These values are similar to the values that would result from application of the full composite uncertainty factors used in the derivation of these acute RfD values (1000; see Table 1) to the estimated rodent plasma concentrations at the POD for each RfD value (see Table 7).

The urinary and plasma BE values corresponding to the chronic and acute RfD values are summarized in Table 10.

2.3. Sources of variability and uncertainty

This section presents a brief overview of sources of variability in the estimates of plasma and urine concentrations that are consistent with exposure to 2,4-D at the RfD values listed in Table 1.

2.3.1. Model uncertainty

In the case of the urinary BE values, model uncertainty is very low because 2,4-D is not metabolized and essentially 100% of intake is excreted in urine. Thus, under the conditions of chronic, steady-state exposure, by definition, the daily urinary excretion rate will be equal to the daily intake rate.

With respect to the plasma BE values, the chronic BE values are derived directly from measured plasma concentrations in rats exposed to 2,4-D under the conditions of interest in the key study underlying the derivation of the chronic RfD (Saghir et al., 2006). Thus, the estimate of relevant internal dose at the POD has low uncertainty, and extrapolation of this dose metric on an internal dose basis to humans also has low uncertainty.

However, with respect to the acute plasma BE values, uncertainty regarding model parameters has an impact. The acute BE values were derived using two approaches, which produced sim-

Table 8
Assumptions for bodyweight, 24-h urinary volume, and estimates of volume-based urinary concentration of 2,4-D consistent with exposure at the chronic or acute RfDs for different age and gender groups

Age/gender group (years)	Bodyweight[a] (kg)	24-h urinary volume (L)	RfD (see Table 1) ($mg\ kg^{-1}\ d^{-1}$)		Corresponding average urinary concentration of 2,4-D ($\mu g\ L^{-1}$)	
			Chronic	Acute	Steady-state exposure at the chronic RfD[b]	Single-day exposure at the acute RfD[c]
Children, 4–12	30	0.66[d]	0.005	0.067	230	1500
Adolescents, 13–18, male	55	1.7[e]	0.005	0.067	160	1100
Adolescents, 13–18, female	55	1.6[e]	0.005	0.025	170	430
Men, >19	70	1.7[e]	0.005	0.067	210	1400
Women, 19–50	55	1.6[e]	0.005	0.025	170	430
Women, >50	55	1.6[e]	0.005	0.067	170	1100

[a] Estimated from Table 11-6 of the USEPA (2001) Interim Child-Specific Exposure Factors Handbook.
[b] Assumed intake at the chronic RfD from Table 1; urinary concentration estimated using Eq. (5).
[c] Assumed intake at the age- and gender-specific acute RfD values presented in Table 1; urinary concentration estimated using Eq. (6), which assumes that 50% of a single dose will be eliminated in urine in the first 24 h.
[d] Remer et al. (2006).
[e] Weighted average from studies of healthy adults presented in Table 2 of Perucca et al. (2007). Values for adolescents were assumed to be the same as for adults.

Table 9
Derivation of the BE_{RfD} for plasma concentration of 2,4-D consistent with the USEPA chronic RfD

Exposure guidance value	Administered animal dose $NOAEL_{adj}$ POD[a] ($mg\ kg^{-1}\ d^{-1}$)	BE_{POD_animal}		Human equivalent BE_{POD}				BE_{RfD}
		Corresponding animal average plasma concentration ($\mu g\ L^{-1}$)	$UF_{A\text{-}PD}$	Human equivalent average plasma concentration ($\mu g\ L^{-1}$)	$UF_{H\text{-}PD}$	$UF_{H\text{-}PK}$	UF_D	Average plasma concentration ($\mu g\ L^{-1}$)
USEPA RfD (chronic)	5	540[a]	3.2	170	3.2	1	10	5

[a] Average estimated from measured 24 h AUC from Saghir et al. (2006).

Table 10
Summary of derived BE values for 2,4-D in plasma and urine

Exposure guidance value	Analyte	Biological matrix	Human equivalent BE_{POD}	Composite UFs[a]	BE_{RfD}	Confidence
USEPA RfD (chronic)	2,4-D	Plasma	170 $\mu g\ L^{-1}$	32	5 $\mu g\ L^{-1}$	High
	2,4-D	Urine (creatinine adjusted)	30,000 $\mu g\ g^{-1}$	100	300 $\mu g\ g^{-1}$	High
	2,4-D	Urine (volume basis)	20,000 $\mu g\ L^{-1}$	100	200 $\mu g\ L^{-1}$	High
USEPA RfD (acute, single-day exposure, females aged 13–50)	2,4-D	Plasma	8000 $\mu g\ L^{-1}$	100	80 $\mu g\ L^{-1}$	Medium
	2,4-D	Urine (creatinine adjusted)	70,000 $\mu g\ g^{-1}$	100	700 $\mu g\ g^{-1}$	Medium
	2,4-D	Urine (volume basis)	40,000 $\mu g\ L^{-1}$	100	400 $\mu g\ L^{-1}$	Medium
USEPA RfD (acute, single-day exposure, remainder of general population)	2,4-D	Plasma	20,000 $\mu g\ L^{-1}$	100	200 $\mu g\ L^{-1}$	Medium
	2,4-D	Urine (creatinine adjusted)	200,000 $\mu g\ g^{-1}$	100	2000 $\mu g\ g^{-1}$	Medium
	2,4-D	Urine (volume basis)	100,000 $\mu g\ L^{-1}$	100	1000 $\mu g\ L^{-1}$	Medium

These values represent an estimate of the central tendency for each of the BE values; however, normal variability in hydration status and creatinine excretion rates can result in approximately 2-fold variation from the central tendency of the urinary BE values. Similar variability around the plasma values might occur due to timing of recent exposures.
[a] Composite UFs for intraspecies extrapolation and database uncertainties from the human equivalent BE_{POD} to the target value, BE_{RfD}.

ilar results. In one approach, the two simple pharmacokinetic models for 2,4-D derived from human exposure studies were used to predict 24-h average plasma concentrations following a single exposure at the acute RfD values, and the results from the two models were averaged to estimate the BE values. In the other approach, measured plasma concentrations in rats following single-dose gavage administration were used to estimate peak plasma concentrations at the POD for each key study. These peak plasma concentrations are well into the range of non-linear kinetics where the renal transport maximum is exceeded. In light of this, the full composite uncertainty factor was applied to each of these values, resulting in an estimate of plasma concentrations consistent with the acute RfD values. The two approaches produced nearly identical results. Thus, although the human pharmacokinetic models produce somewhat disparate results, the overall estimate of the acute plasma BE values is consistent from two approaches, and the model uncertainty has limited impact on the derived BE values.

2.3.2. Exposure patterns

Exposure to 2,4-D at the RfD via the oral route of exposure could theoretically occur with a number of temporal patterns ranging from a single exposure once per day to a more distributed exposure across time. It is possible that higher creatinine-adjusted urinary levels than those predicted here could occur in a given spot urine sample depending upon when the sample was taken compared to when an exposure occurred, even though exposure did not exceed the RfD. However, data from Sauerhoff et al. (1977) indicate that approximately equal proportions of a single dose of 2,4-D are eliminated in the first 12 h after dosing compared to the 2nd

12 h after dosing. This would suggest that due to the kinetics of absorption, distribution in plasma, and renal transport into urine, under chronic exposure conditions variations in spot sample creatinine-adjusted concentrations associated with the pattern of within-day exposure would be relatively minor.

Estimated peak blood plasma levels vary depending upon whether chronic exposure is estimated following single daily doses or divided doses. The degree of variation associated with this factor is approximately 15%. The variation between peak following a single dose and the average steady-state concentration is approximately 10%.

2.3.3. Analytical issues

The sample preparation and analytical methodologies used by the Centers for Disease Control and Prevention (CDC) to assess urinary 2,4-D concentrations capture both the conjugated and unconjugated 2,4-D excreted in urine (L. Needham, personal communication). The BE values presented here assume that the conjugated compound is detected analytically. However, if the analytical method used in a given study does not capture the conjugated compound, the BE values here should be reduced by approximately 15% to reflect only the unconjugated parent compound expected to be excreted in urine before being used as a screening tool.

2.3.4. Pharmacokinetics

The available pharmacokinetic data for 2,4-D is extensive, with data available for several species including humans (Timchalk, 2004). However, within-human variability in the pharmacokinetics is less well-characterized. As discussed above, the two studies of pharmacokinetic behavior of 2,4-D in human volunteers resulted in different estimates of plasma clearance rates that result in substantially different estimates of chronic steady-state plasma concentrations following exposure at the RfD. This pharmacokinetic uncertainty impacts the estimation of peak or steady-state plasma concentration values associated with exposure at the chronic RfD, but would have little impact on the estimation of BE values for steady-state urinary elimination rates which would, by definition, be determined only by the RfD and the creatinine-adjustment factors. The human pharmacokinetic models were not used directly in the estimation of the chronic plasma BE values derived here (Table 9), so the substantial uncertainty associated with the prediction of steady-state plasma concentrations due to the observed variability in pharmacokinetics of 2,4-D in human volunteers does not directly impact the BE values estimated here.

2.3.5. Gender and age

Known effects of gender and age on creatinine excretion rates were explicitly taken into account in the estimation of urinary BE values in this effort. However, some variability can still occur, with variations of twofold (in either direction from the mean values given here) possible. Other effects of gender and age, for example on the efficiency of renal organic acid transport systems, could also affect the estimated BE values for plasma, but no data were found to address this issue.

2.3.6. Health status

Conditions that affect the body's glomerular filtration rate or creatinine excretion rate would affect the accuracy of the creatinine-adjusted BE values. Conditions that result in reduced creatinine excretion would result in higher creatinine-adjusted values being consistent with exposure at the given RfD values.

Conditions that affect the efficacy of the renal organic acid transport system could result in reduced clearance rates and therefore higher plasma values associated with exposure at the chronic RfD. However, no information on how these issues might specifically impact the kinetics of 2,4-D could be found.

2.3.7. Smoking, drugs, alcohol, or other co-exposures

No specific information was found regarding the potential effects of smoking, drugs, or alcohol consumption on the estimated 2,4-D BE values presented here. Drug or other exposures that impact or compete for renal organic acid transport mechanisms in the kidney could impact elimination of 2,4-D, particularly if the serum concentrations of these drugs or 2,4-D approach the renal transport maximum for the organic acid transporter systems involved. In the recent evaluation of chronic and acute RfDs by USEPA OPP (USEPA, 2004), explicit consideration was given to information regarding the renal clearance of these compounds, and the RfD values for 2,4-D were set at levels that were expected to be well below the renal transport maximum for 2,4-D.

2.3.8. Genetic variability

Recent studies have examined polymorphisms in the renal organic acid transport (OAT) system proteins (reviewed in Kerb, 2006). To date, few polymorphisms have been observed in the OAT system, and the overlapping functionality of different OAT transporter proteins suggest that modifications to individual proteins may have limited effects on overall clearance. However, information regarding such genetic variability is still quite limited.

2.3.9. Variation in hydration status affecting urinary excretion

The use of creatinine-adjustment addresses some of the variability associated with variations in hydration state; however, it introduces additional variability as discussed above. Factors impacting creatinine production or excretion could affect the accuracy of the BE values, as discussed above. For the BE values set on a volume basis, the variations in hydration status could have a severalfold effect on the observed concentrations in urine taken from spot samples (Scher et al., 2007), and a lower influence on the concentrations observed in 24-h samples.

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 Plasma BE values

The use of plasma concentrations as biomarker for 2,4-D exposure is attractive from the toxicological perspective because plasma concentrations should be directly relevant to critical target tissue concentrations and do not require corrections for hydration state as is needed for urinary excretion of 2,4-D. Furthermore, the study relied upon as the basis of the chronic RfD BE derivation, Saghir et al. (2006) reports careful and detailed measurements of plasma concentrations in rats exposed at the POD under the relevant experimental conditions. The chronic BE_{RfD} for plasma thus is based on a relevant biomarker and is derived based on robust pharmacokinetic information. For both aspects, confidence is high in the derived BE values.

For the acute plasma BE values, data on plasma concentrations in humans following acute exposures were relied upon. These data draw from two studies in human volunteers, and the two studies demonstrated variability among individuals. Thus, while the biomarker is considered to be relevant to the internal dose metric (high confidence), confidence in the pharmacokinetic data is med-

ium, and overall confidence in the estimated plasma concentrations consistent with the acute RfD values is medium.

Summary of confidence ratings for plasma BE values:

- Relevance of biomarker to relevant dose metrics: HIGH
- Robustness of pharmacokinetic data/models: HIGH for chronic BE, MEDIUM for the acute BE values.

2.4.2. Confidence in urinary BE values

The basic concept that daily urinary excretion equals daily intake under chronic, steady-state conditions lends support to use of BE values based on urinary excretion. Because most biomonitoring efforts use spot samples rather than 24-h collections, issues associated with temporary alterations in hydration status arise. Both a creatinine-adjusted and urinary volume-based analysis are presented here. Each method incorporates some variability in the resulting estimates (Garde et al., 2004). However, the variability inherent in each of the methods is acceptable in the context of use of these values as screening tools. Levels of 2,4-D in urine are less directly related to relevant target tissue concentrations than are plasma concentrations;, however, urinary excretion of 2,4-D is probably well correlated with plasma levels. Refinement of biomonitoring data collection practice and reporting to expand the information available about spot collection samples would also be helpful in interpretation of results. Specifically, when spot samples are collected, an estimate of the time of last void and reporting of the volume of the collected sample, as well as the concentration of analyte in the sample, would allow for an estimate of mass excreted per unit time, which can be interpreted directly in terms of the RfD values. Issues related to the variability in spot sample concentrations may impact interpretation of biomarker concentrations following acute exposure episodes more than under chronic exposure conditions.

Summary of confidence ratings for urinary BE values:

- Relevance of biomarker to relevant dose metrics: MEDIUM
- Robustness of pharmacokinetic data/models: HIGH for chronic BE values; MEDIUM for acute values.

3. Discussion and interpretation of BE values

The BE values presented here represent the concentrations of 2,4-D in plasma and urine that are consistent with exposure at the exposure guideline values that have been established by the USEPA (Table 1), based on the current understanding of the pharmacokinetic properties of this compound. These BE values should be regarded as interim screening 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.

The BE values presented here are screening values and can be used to provide a screening level assessment of measured plasma or urine levels of 2,4-D 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 (see Fig. 3, for example). 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. Further discussion of interpretation and communications aspects of the BE values is presented in LaKind et al. (2008).

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. Measured values in excess

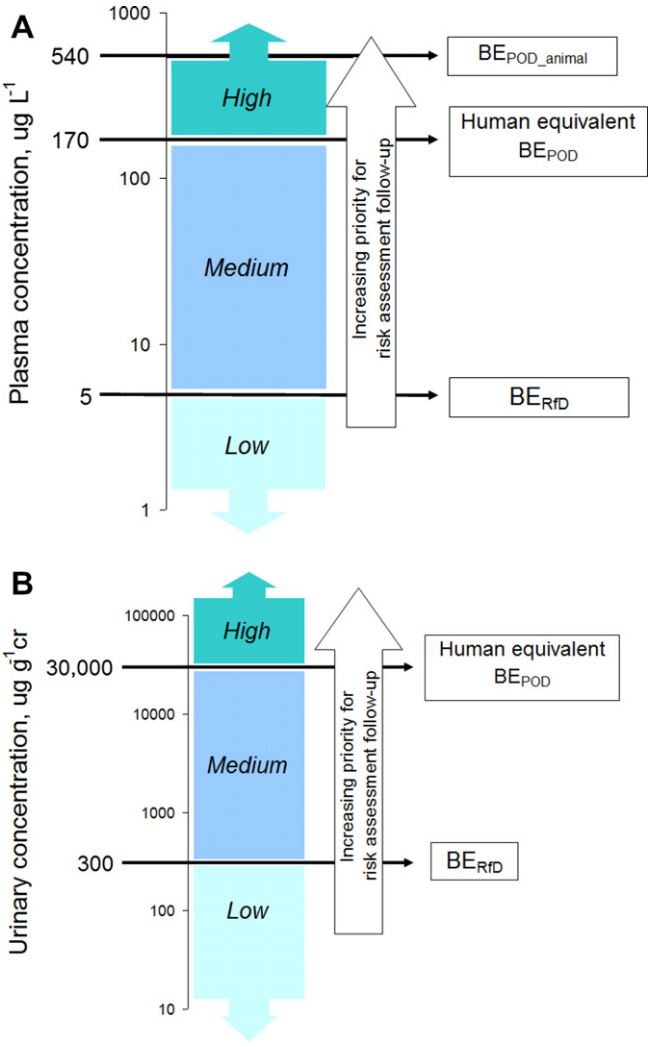

Fig. 3. Guide for interpretation of measured concentrations of 2,4-D in urine or plasma in the context of the chronic BE_{RfD}. Measured concentrations below the BE_{RfD} present a low priority for risk assessment follow-up, while those exceeding that value but below the human equivalent BE_{POD} indicate medium priority, and those in excess of that value suggest high priority. (A) BE values for 2,4-D in plasma; (B) BE values for 2,4-D in urine (creatinine adjusted).

of the identified BEs may indicate exposures at or above the current health-based exposure value that serves as the basis for the BE. However, because of various uncertainties, including those associated with correction for hydration status, an exceedance (particularly in an individual) may be an artifact of temporary unusually low hydration or creatinine excretion, particularly if the exceedance is relatively small.

In addition, such health-based exposure guidelines are generally derived with a substantial margin of safety built in, and these values are not "bright lines" distinguishing safe from unsafe exposures. In the case of 2,4-D, the RfD values derived by USEPA OPP are 1000-fold lower than the exposure levels resulting in no observed adverse effects in rat studies. Chronic RfD values are set at levels that are designed to be health-protective for daily exposure for a full lifetime of exposure. For short-lived compounds, an exceedance of the corresponding BE value in a single sample of blood or urine may or may not reflect continuing elevated exposure. Thus, occasional exceedance of the RfD or the BE_{RfD} in an individual in a cross-sectional study does not imply that adverse health effects are likely to occur, but can serve as an indicator of relative priority for further risk assessment follow-up. Further discussion of

interpretation and communications aspects of the 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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