THE ORAL BIOAVAILABILITY OF POLYCHLORINATED DIBENZO-*P*-DIOXINS/DIBENZOFURANS IN SOIL: REVIEW OF THE STATE OF THE SCIENCE

Morinello EJ, Warmerdam JM, Finley BL

ChemRisk, Inc., 25 Jessie St., Suite 1800, San Francisco, CA 94105, United States

Introduction

Estimates of the relative oral bioavailability (RBA) of 2,3,7,8-tetrachloro-*p*-dioxin (TCDD) and other polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) in contaminated soils have been reported in numerous studies.[1-6] In many environmental settings, RBA estimates are considered critical to accurate human health risk assessments. The RBA of PCDD/Fs in soil has typically been calculated by comparing the liver concentration of the congener(s) of interest in animals orally dosed with contaminated soil vs. an oral reference formulation containing PCDD/Fs dissolved in corn oil or some other vehicle. Our review of these studies indicates some study design and results interpretation issues that merit consideration. First, the RBA estimates based on PCDD/F-spiked soils have been consistently higher than those derived from studies which evaluated soils collected from contaminated sites. Second, a number of study design-related issues have the potential to introduce substantial bias into RBA determinations. Confounding factors that may result in under- or over-estimated RBA values include congener- or dose-dependent differences in the distribution, metabolism, excretion, or toxicity of PCDD/Fs. In this review, data from animal studies which reported the oral bioavailability of PCDD/Fs in spiked or contaminated soils are evaluated, and potential sources of systematic error are discussed.

Methods and Materials

Studies of the oral bioavailability of PCDD/Fs in soil which included tissue concentration data were reviewed. RBA was calculated by comparing the reported PCDD/F concentrations or the percentage of the administered dose in the liver of animals treated with reference oral formulations vs. that found in animals treated with contaminated soil *per os*. When data from multiple oral reference groups were reported, RBA values were calculated based on comparisons between the reference and soil-treated groups that were found to have the most similar liver concentrations of PCDD/Fs. Studies which did not provide sufficient tissue concentration data for the reference or treatment groups and data from animals that died prematurely were excluded. RBA estimates reported by the authors were also presented when available.

Results

Table 1. Relative Oral Bioavailability of PCDD/Fs in Spiked Soils

Ref	Analyte	Study Day(s) Dosing	Study Day(s) Sacrifice	Species	Route	Calculated Relative Bioavailability
1	TCDD	1	2	rat	gavage	44%[a] – 66%[b]
2	TCDD	1-7	8	rabbit	gavage	56 – 100%

[a] Soil spiked 8 days prior to dosing
[b] Soil spiked 10-15 hours prior to dosing

The results from studies in which clean soils were spiked with PCDD/Fs[1-2] are summarized in Table 1. These studies examined TCDD only. The TCDD liver concentration data indicated that the RBA of TCDD in spiked soils ranged from approximately 44% to 100%. The upper bound values indicate that the RBA of TCDD added to soil in

experimental settings may in some cases be comparable to the absorption of TCDD from corn oil or other vehicles commonly used to prepare reference oral formulations. Data from one study which compared soil spiked with TCDD 8 days vs. 10-15 hours prior to dosing indicated that the leachability of PCDD/Fs from soils may be inversely related to contact time.[1]

The results from studies which evaluated the RBA of PCDD/Fs in soils collected from contaminated sites are summarized in Table 2. In contrast to the spiked soil studies, these data suggest that RBA values of PCDD/Fs in soil from contaminated sites are likely to be less than 50%.

Table 2. Relative Bioavailability of PCDD/Fs in Soils from Contaminated Sites

				Study Day(s)			Relative Bioavailability	
Ref	Analyte	Species	Route	Dosing	Sacrifice	Source of Soil	Reported	Calculated
2	TCDD	rabbit	gavage	1-7	8	Seveso	< 50%	33%[a] – 41%[b]
3	TCDD	guinea pig	gavage	1	31	Times Beach	NR	16%[c,d]
						Minker Stout	NR	27%[c,e]
3	TCDD	rat	gavage	1	7	Minker Stout	NR	45%
4	TCDD	rat	gavage	1	7	Minker Stout	NR	22 – 45%[f]
5	TCDD	rat	gavage	1	2	Times Beach	37 – 49%	NA
6	PCDD/Fs[h]	rat	diet	1-30	31	Midland	36%[g,j]	NA
		swine					44%[j,k]	NA
6	PCCD/Fs[i]	rat	diet	1-30	31	Tittabawassee	63%[g,j]	NA
		swine					40%[j,k]	NA

NR = not reported
NA = not applicable (additional comparisons were not performed due to study design or reporting limitations)
[a] Low dose (80 ng/day) soil treatment group compared to 80 ng/day reference group
[b] High dose (160 ng/day) soil treatment group compared to 80 ng/day reference group
[c] Animals that died prior to scheduled sacrifice were excluded from the RBA estimates due to likely differences in excretion.
[d] Group 5 compared to low-dose reference Group 2
[e] Group 8 compared to low-dose reference Group 2
[f] Calculations were based on soil and reference groups that were matched by administered dose of TCDD because no substantial differences in hepatic enzyme activities between the reference and treatment groups were evident.
[g] The authors reported that the RBA estimates may have been erroneously high because significant differences in hepatic EROD activity between the reference and soil-treated groups were evident in rats.
[h] The following congeners which made substantial contributions to the soil TEQ were included in the reported data: 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, 1,2,3,6,7,8-HxCDD, 1,2,3,4,6,7,8-HpCDD, and 2,3,4,7,8-PeCDF.
[i] The following congeners which made substantial contributions to the soil TEQ were included in the reported data: 2,3,7,8-TCDF, 1,2,3,7,8-PeCDF, 2,3,4,7,8-PeCDF, 1,2,3,4,7,8-HxCDF, and 1,2,3,6,7,8-HxCDF.
[j] Mean non-weighted RBA of the five congeners that were measured; see report for congener-specific results
[k] Values below the lower limit of quantitation were considered to be equal to 50% of the detection limit for the purpose of these calculations.

Data from McConnell et al. (1984) indicated that RBA estimates may be highly dependent on between-group differences in the distribution or excretion of PCDD/Fs. Because a substantial fraction of the administered dose of TCDD is retained in adipose tissue, and because TCDD is excreted by rodents at an appreciable rate, any toxicity that results in body weight changes or premature death would be expected to affect RBA determinations that are based on tissue concentrations. When data from animals that died prematurely were excluded from the calculations, the calculated RBA was 16% for Times Beach soil and 27% for Minker Stout soil (Table 2). This RBA estimate for the Times Beach soil is substantially lower than the 85% value that was previously reported based on the data from

this study,[7] and the lower estimates are more consistent with the RBA values calculated from other studies of the Times Beach and Minker Stout soils (Table 2).[4,5] These results suggested that chemical toxicity resulting from the proportionately greater absorption of TCDD from the vehicle administered to the reference groups may skew RBA estimates to erroneously high values, especially when highly sensitive species are utilized.

A recent study[6] of contaminated soils from the Midland and Tittabawassee River flood plain areas in Michigan provided evidence for another mechanism by which RBA estimates may be influenced by the dose administered to the reference group. This study was unique because 1) it provided RBA estimates for non-TCDD congeners; 2) the animals were exposed to the soil via feed rather than gavage; and 3) the confounding effects of hepatic enzyme induction were considered in the RBA estimates. Although the calculated mean RBA of PCDD/Fs in rats was significantly higher than in swine, this was apparently due to differences in hepatic enzyme induction which were only evident in the smaller species. The authors hypothesized that the greater enzyme induction in the rat groups treated with the reference formulation may have accelerated the metabolism and excretion of PCDD/Fs and yielded an over-estimated RBA in rats. In addition, significant differences in the apparent RBA of individual PCDD/F congeners were observed. In swine treated with the Midland soil, the RBA of individual congeners ranged from 38% (TCDD) to 64% (1,2,3,4,6,7,8-HpCDD). There was even more (approximately 4-fold) variation in the RBA of individual congeners when swine were treated with the Tittabawassee soil. The apparent RBA of individual congeners ranged from 30% to 44% in rats treated with the Midland soil and from 56 to 90% in rats treated with the Tittabawassee soil; however, the authors noted that much of the data from the rat study may be unreliable due to the likelihood of differences in the excretion rates of the various congeners in the reference and soil-treated groups.

Discussion

RBA estimates used for risk assessment purposes should be based on evaluations of site-specific soils

Factors which are likely to influence the RBA of PCDD/Fs in soil collected from a contaminated site include, but are not limited to: 1) the form of the material which contaminated the site; 2) the rate at which the soil was contaminated; 3) the time that elapsed since the soil was contaminated; 4) the extent of weathering; 5) the specific PCDD/F congeners that were involved; and 6) the particle size, total organic content, and other physical or chemical properties of the soil. Because detailed analyses of the soils were not provided, the influence of total organic content and other soil characteristics on RBA could not be evaluated with the data from these studies. However, it is clear that data from studies which utilize soils collected from distant, unrelated sites or soils which were contaminated in an experimental setting are likely to be of limited value when considering the RBA of PCDD/Fs in soil from a particular site of interest.

RBA estimates should be based on comparisons between reference and treatment groups which have similar concentrations of PCDD/Fs in the liver

It is now clear that differences in hepatic enzyme induction may cause the RBA of PCDD/Fs to be overestimated if the amount absorbed from the soil is substantially less than the amount absorbed from the reference formulation. This potentially confounding effect may be minimized by administering a lower dose to the reference groups to help ensure that the concentrations of individual PCDD/Fs in the liver of animals in the control and reference groups, and therefore the extent of enzyme induction, are similar. Hepatic ethoxyresorufin-*O*-deethylase activity or other appropriate indicators of enzyme activity which may influence the metabolism or excretion of PCDD/Fs should be included in study designs to help determine if such differences were likely to have affected RBA determinations.

It is useful to characterize both the absolute and relative bioavailability of the individual PCDD/Fs that make significant contributions to the contamination at a specific site

Although only TCDD has been considered in most bioavailability studies, other congeners are likely to make appreciable contributions to the TEQ concentration of PCDD/Fs in soils from contaminated sites. However, relatively little is known about the absorption of PCDD/F congeners other than TCDD from the vehicles that are commonly used to prepare reference formulations. Because only relative measures of bioavailability can be characterized by comparing groups treated with different oral formulations, it would be useful for future bioavailability studies to also include intravenously treated reference groups. Such data could play an important role in site-specific quantitative risk assessments because accurate estimates of absorbed dose can only be made when the absolute bioavailability values of individual congeners from soil or reference oral formulations have been well characterized.

Short-term studies in species that are relatively insensitive to the toxic effects of PCDD/Fs provide appropriate estimates of RBA in contaminated soils

Although long-term, multiple-dose studies may be more appropriate for studying the pharmacokinetics or toxicity of PCDD/Fs, the short-term study designs that have been used for many soil RBA studies may provide more accurate oral bioavailability estimates because they minimize the potential for confounding effects due to hepatic enzyme induction. Because hepatic enzyme induction does not peak until approximately 72 hours after administration of a single dose of PCDD/Fs,[8] the group- and congener-specific differences in the metabolism and excretion of PCDD/Fs may be minimized when an early tissue collection time point is utilized. In contrast, between-group differences in hepatic enzyme activity appeared to substantially bias the RBA values calculated from the 30-day rat feeding study.[6] When analytical detection limits are not a concern, multiple-dose regimens do not appear to offer any clear advantages over single-dose designs for the purpose of PCDD/F soil bioavailability determinations.

Although differences in the test materials and study designs preclude statistical comparisons, no substantial species-specific differences in RBA were apparent in the data from these studies. However, PCDD/F-induced toxicity may confound RBA determinations by causing between-group differences in the fraction of the absorbed dose that is excreted (*i.e.*, due to tissue damage or premature death) or differences in tissue distribution (*i.e.*, due to wasting and other body weight-related effects). The results of a guinea pig study[3] indicated that the use of a highly sensitive species or high doses which result in overt toxicity should be avoided. As was demonstrated in the recent feeding study[6], the use of large, rapidly-growing animals such as swine may complicate RBA determinations because resulting tissue concentrations of PCDD/Fs may be near or below the analytical detection limits. Therefore, in most cases rats or rabbits are more likely to be suitable for use in PCCD/F soil bioavailability studies than highly sensitive species such as guinea pigs or large animals such as swine.

References

1. Poiger H, Schlatter C. *Fd Cosmet Toxicol* 1980; 18:477.
2. Bonaccorsi A, di Domenico A, Fanelli R, Merli F, Motta R, Vanzati R, Zapponi GA . *Arch Toxicol* 1984; S7:431.
3. McConnell EE, Lucier GW, Rumbaugh RC, Albro PW, Harvan DJ, Hass JR, Harris MW. *Science* 1984; 223:1077.
4. Lucier GW, Rumbaugh RC, McCoy Z, Hass R, Harvan D, Albro P. *Fund Appl Toxicol* 1986;6:364.
5. Shu H, Paustenbach D, Murray FJ, Marple L, Brunck B, Dei Rossi D, Teitelbaum P. *Fund Appl Toxicol* 1988;10:648.
6. Exponent. 2005; study report available online at: http://www.deq.state.mi.us/documents/deq-whm-dioxin-PilotStudyReportFINALFeb24.pdf
7. Umbreit TH, Hesse EJ, Gallo MA. *Science* 1986;232:497.
8. Santostefano M, Wang X, Richardson V, Ross D, DeVito M, Birnbaum L. *Toxicol Appl Pharmacol* 1998:294.