The use of body burdens vs daily dose in comparisons of endo- and exodioxins and in assessing human health risks.

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1.Introduction

The TEF methodology has been applied to the polyhalogenated aromatic hydrocarbons (PHAHs), such as the chlorinated dibenzo-p-dioxins, dibenzofurans and more recently PCBs, to estimate health risks associated with exposures to these chemicals [1]. One criticism of the use of TEFs is the exclusion of "endodioxins" and the lack of comparison of the contribution of dioxin equivalents from endodioxins vs exodioxins [2]. Endodioxins are "natural" or "endogenous" chemicals that bind to the Ah receptor and induce the same effects as TCDD in experimental systems. Polynuclear aromatic hydrocarbons (PAHs) and indole-3-carbinol (I3C) are considered endodioxins. The PAHs are produced when cooking meats primarily by grilling or broiling. I3C is a glucosinate that is present in cruciferous vegetables such as broccoli, brussel sprouts and cabbage. Exodioxins are synthetic or "man-made" chemicals that bind to the Ah receptor and also produce the same effects as TCDD. PHAHs such as the dioxins, dibenzofurans and PCBs are considered exodioxins.

Exposure to endodioxins are considerably higher on a mass basis compared to exodioxin exposure. PAH exposure is estimated at 1.6 - 16 ug/d [3]. Exodioxin exposure in the US is approximately 210 - 420 pg/d[4]. Exposure to I3C in the UK is approximately 100 ug/d[5] . Although the exposure to endodioxins is considerable, these chemicals are weak ligands of the Ah receptor and are much less potent than the prototype Ah receptor agonist, TCDD[6].

One of the major differences between endodioxins and exodioxins is pharmacokinetics. Exodioxins are resistant to metabolism and are highly persistent in both environmental and biological systems. For example, the half-life for elimination of TCDD in humans is approximately 8.7 years[7] while in rats the half-life is 23 days[8]. In contrast, the endodioxins have considerably shorter half-lives of elimination. Benzo[a]pyrene has a half-life in humans of approximately 4.4 hours[9]. The half-life of indole-3-carbinol is humans is unknown, however, its half-life in rats is approximately 24 hours[10], which is considerably less than that of TCDD. These differences in half-life could have significant effects on the relative potency of these

chemicals as well as the relative contribution to the total dioxin equivalents in humans.

The difference in half-life is not only important when comparing different chemicals but is equally important in species extrapolations. Estimation of human health risks associated with exposure to dioxins are based on extrapolating animal data to humans. The two-year bioassay by Kociba et al., (1978)[11] and the multigeneration study by Murray et al (1979)[12] are the most frequently used studies for these purposes. The risk assessments have used either a linear model or a safety factor method. The safety factor method uses a dose of 1 ng/kg/d as the NOEL for cancer and LOEL for reproductive toxicity and, depending on the regulatory body, divides this by a safety factor of 100 or 1,000. However, the use of daily dose as the appropriate dosimetric has been questoined[13]. There is over a 110 fold difference in the half-life of TCDD between rats and humans. Because of the large difference in half-life between humans and animals, the use of daily dose may not be the appropriate dosimetric in assessing human health risks.

The present exercise attempts to illustrate the relevance of pharmacokinetics in assessing the health risks associated with exposure to dioxins. Initially this exercise examines the contribution to dioxin equivalents of PHAHs, PAHs and indole-3-carbinol based on daily intake and body burdens under steady-state conditions. The present analysis indicates that while the endodioxins contribute significantly to the dioxin equivalents based on daily intake, under steady state conditions, the PHAHs are the predominate contributors to the total dioxin equivalents. Secondly, this exercise compares the margin of exposure from the cancer NOEL and the reproductive toxicity LOEL of 1 ng/kg/d in rats with human exposure in the general population based on either daily intake or body burden. These comparisons indicate that the choice of dosimetric can lead to widely divergent estimates of a margin of exposure and strongly indicate that the use of daily dose is inappropriate for risk assessment purposes for this class of chemicals.

2.Methods

A classical pharmacokinetic model was used to estimate human body burdens of PHAHs, PAHs and I3C following 1,2,5,10 and 20 years of daily exposures to these chemicals. Daily intake of the PHAHs was expressed on a TEQ basis and was assumed to be 3 pg/kg/d based on estimates of US intake of these chemicals[4]. Daily intake of PAHs was estimated at 229 ng/kg/d[2]. Daily intake of I3C was estimated assuming 100g of cruciferous vegetables each day with a concentration of 50 μmol/100g[6]. Using these assumptions, daily intake of I3C is approximately 105 μg/kg/d. The half-life of the PHAHs was 8.7 years[7]. All PAHs were assumed to have a half-life of 4.4 hr which is equivalent to benzo[a]pyrene[9]. I3C has a half-life of 24 hr in rodents[10] and the half-life of its active metabolites was assumed to be 24 hr in humans.

Once body burdens were estimated, these values were converted to TEQ by multiplying body burdens by the TEF values. Since the PHAHs were initially expressed on a TEQ basis, the body burdens were not further adjusted. The relative potency or TEF of I3C was estimated at 0.00001 based on hepatic EROD induction in

rats[14,15]. The relative potency of all PAHs was assumed to be equivalent to 3-methylcholanthrene which is approximately 0.001[16].

Margin of exposures were estimated by dividing the exposure in rodents that resulted in a NOEL (cancer) or LOEL (reproductive toxicity) by the present human exposure in the general population. Exposures were estimated based on either daily intake or body burdens. Body burdens in rats were estimated assuming a half-life of 23 days and a daily intake of 1 ng/kg/d. In humans body burdens were estimated based on the assumption of a daily intake of 3 pg TEQ/kg/d and a half-life of 8.7 years.

3.Results

The daily intake of TEQs from PHAHs, PAHs and I3C is 1282 pg TEQ/kg/d (Table 1). I3C accounts for 81% of the total TEQs while the PAHs account for 18%. The PHAHs account for less than 1% of the daily TEQ intake. However, of these three classes of chemicals only the PHAHs are persistent and bioaccumulative. After 20 years of exposure, the total TEQ body burden increases by almost 10 fold to 12003 pg/kg. Because of their persistence, there is an accumulation of the PHAHs which eventually attain concentrations to account for over 90% of the TEQ body burden (Table 1). The PAHs have the shortest half-life and account for less than 1% of the body burden of TEQs following 1 to 20 years of exposure. Daily intake of I3C for 20 years results in a body burden of 1050 pg TEQ/kg which is approximately 10% of the total TEQ.

Rats exposed to 1 ng TCDD/kg/d accumulate approximately 30 ng TCDD/kg/d under steady state conditions. The daily exposure of 3 pg/kg/d in the background population results in a steady state body burden of approximately 10 ng/kg (Table 2). The margin of exposure based on daily intake is 333 fold. However, the margin of exposure based on steady state body burdens is approximately 3 fold. The difference in the margin of exposure estimates is due to the much longer half-life of dioxins in humans compared to rats.

4.Discussion

Several assumptions were made in the comparisons of endo- and exodioxins. The theoretical population studied was assumed to ingest 1 serving of cruciferous vegetables daily. Similar assumptions were used to estimate ingestion of I3C in the UK resulting in equivalent ingestion estimates[5]. More important were the assumptions used in estimating the relative potency and pharmacokinetics. No studies have directly compared the relative potency of I3C with TCDD, hence estimates of the relative potency of I3C were based on induction of hepatic EROD activity in studies from different laboratories. Future studies comparing the relative potency of I3C and TCDD for a variety of dioxinlike effects are required to adequately examine the relative contribution to the TEQ body burdens and intakes. I3C does not bind to the Ah receptor. In the acidic environment of the gastrointestinal tract I3C is converted to a number of cyclic and acyclic oligomers, some of which bind to the Ah receptor[6]. The half-life of these compounds was assumed equal to the half-life of I3C in rats[10]. Clearly this assumption requires future research.

The assumptions used in comparing endo- and exodioxins allow only for imperfect estimates of the contribution to the total TEQs present in human populations. However, this exercise emphasizes that initial comparisons between endodioxins and exodioxins have ignored the importance of the pharmacokinetic differences between these chemicals. Dioxins are of concern not just because they are highly toxic but because they are extremely persistent and bioaccumulate. While the present study indicates that exodioxins are the predominate contributor to the total TEQ body burden, other investigations support the importance of exodioxin exposures. Recent studies demonstrate that in populations with no known high exposures to dioxins, TEQ concentrations in human milk correlate with lower levels of thyroid hormones, decreased psychomotor skills and immune alterations in infants[17,18,19]. These studies suggest that it is unlikely that endodioxins are significant contributors to the overall body burdens of dioxins. Future studies on the relative potency and pharmacokinetics of I3C metabolites are required to more accurately determine the importance of these chemicals.

Pharmacokinetic differences are also important when extrapolating between species. Several regulatory agencies throughout the world have applied the safety factor approach in order to derive acceptable or tolerable exposure levels of dioxins. Typically these approaches have divided the cancer NOEL or reproductive toxicity LOEL dose in rats expressed as ng/kg/d by 100 or 1000 which result in acceptable human exposures between 1 - 10 pg/kg/d. These "acceptable" exposures are equivalent to the present daily exposures in the US populations and result in a margin of exposure of approximately 333. If the dosimetric used is body burden, the margin of exposure drops to less than an order of magnitude. Due to the large difference in half-life of dioxins between animals and humans, deriving an acceptable or tolerable intake based on daily intake is clearly inappropriate. Human risk assessments must incorporate the differences in pharmacokinetics between species and between congeners in order to adequately assure protection of human health.

This abstract does not necessarily reflect USEPA policy

5.References

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Table 1.
Comparisons of body burdens of endo- and exodioxins over time.

	BODY BURDEN TEQ (pg/kg)				%TEQ		
YEARS	PHAH	PAH	I3C	TOTAL	PHAH	PAH	I3C
0	3	228	1050	1282	<1.0	17.8	81.9
1	1052	0.4	1050	2103	50.1	0.02	49.9
2	2024	0.4	1050	3075	65.8	0.01	34.2
5	4516	0.4	1050	5566	81.1	0.007	18.9
10	7548	0.4	1050	8599	87.8	0.005	12.2
20	10951	0.4	1050	12003	91.2	0.004	8.8

Table 2
Comparisons of Margin of Exposures based on Daily Dose or Body Burdens

DOSIMETRIC	RAT	HUMAN	MOE
DAILY DOSE	1 ng/kg/d	3 pg TEQ/kg/d	333
BODY BURDEN	30 ng/kg	10 ng/kg	3