Human Dietary Intake of Aryl Hydrocarbon (Ah) Receptor Agonists: Mass Balance Estimates of Exodioxins and Endodioxins and Implications for Health Assessment

Stephen Safe
Veterinary Physiology and Pharmacology, Texas A&M University, College Station, TX 77843-4466

1. Introduction

Several studies including the recent EPA Health Assessment Document for 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin and Related Compounds have suggested that the diet is the major route of human exposure to polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) [1-4]. Levels of these compounds are highly variable; however, concentrations of PCDDs/PCDFs tend to be higher in foods with a high fat content. Other factors which influence the concentration of these compounds in foodstuffs include contaminant levels in the immediate environment and, for fish and wildlife samples, their position in the food chain. There is evidence that the concentration of PCDDs/PCDFs in milk and other dairy products are higher in more industrialized regions or where there are point source emissions of these compounds [5]. Although the absolute levels and congener distribution patterns of PCDDs and PCDFs in foods and in human tissues are highly variable, octachlorodibenzo-*p*-dioxin (octaCDD) appears to be the dominant congener in most samples. OctaCDD is also the major by-product derived from pentachlorophenol and from combustion sources. Total adipose tissue levels of PCDDs/PCDFs in industrialized countries can vary from 150 to 1500 ppt on a fat weight basis; however, octaCDD is the dominant congener (~80%) in all samples [6]. Using the toxic equivalency factor (TEF) approach [7], it has been shown that the daily intake of TCDD or toxic equivalents (TEQs) from all food sources ranges from 80 to 120 pg/day in the U.S., Netherlands and Germany, and these values may be typical of dietary intakes for most industrialized countries [1-4]. It has been suggested in the recent E.P.A. reassessment of TCDD and related compounds that current background exposures to these compounds may be at or near levels which could elicit adverse health effects [1]. Based on the carcinogenic potency of TCDD in female Sprague-Dawley rats [8], E.P.A. has recommended an R_fd (0.01 pg /kg/day) which is approximately 100-fold lower than the current daily intakes of PCDDs/PCDFs (TEQ) (i.e. 1 to 2 pg/kg/day) suggesting that background levels of the compounds are already inducing cancer incidences > 1 in 10^6. In contrast, R_fd values from 1 to 10 pg/kg/day have been recommended by most other regulatory agencies using a safety factor approach and these values are within the range or lower than current daily intakes.

In order to assess the hazards of human dietary exposure to PCDDs/PCDFs, it is also important to evaluate current ongoing exposures to other compounds in the diet. This approach has previously been used by Ames and coworkers [9,10] in their studies on dietary exposures to endogenous and exogenous carcinogens/mutagens and their evaluation of the relative dietary impacts of these compounds.

2. Results and Discussion

There are several different structural classes of chemicals which bind to the aryl hydrocarbon (Ah) receptor and these include TCDD and related halogenated aromatic hydrocarbons HAHs [7,11,12] which are derived from industrial and combustion sources. Figure 1 illustrates the structures of some of the most toxic members of this class of chemicals which have been named "exodioxins". HAHs induce a diverse spectrum of Ah receptor-mediated toxic and biochemical responses in mammalian cells in culture, laboratory animals and humans [11,12]. The expression of these responses is dependent on the species, strain, sex and age of the animal and the reasons for these differences are not well understood.

Raw and cooked foods also contain relatively high levels of Ah receptor agonists which include polynuclear aromatic hydrocarbons (PAHs) and heterocyclic aromatic amines which are formed on cooking and indole-3-carbinol (I3C) and related compounds which are found in *Brassica* vegetables such as cauliflower, broccoli and Brussels sprouts [13-19]. These compounds can be termed "endodioxins" (see Figure 1) although it is clear that PAHs, could fit into the endo- or exodioxin category due to other human exposure pathways from combustion-derived processes. The natural endodioxins all exhibit a diverse spectrum of Ah receptor-independent responses which include the potent carcinogenic and mutagenic activities of the PAHs and aromatic amines [18-20]. However, exodioxins and endodioxins exhibit several common properties which include binding to the Ah receptor and induction of CYP1A1 gene expression in mammalian cells, laboratory animals and humans [11,12,21-29]. For example, exposure to relatively high levels of HAHs in the Yu Cheng poisoning in Taiwan resulted in induction of placental CYP1A1-dependent activity [26,27] and, using the caffeine breath test, it was shown that CYP1A2 levels were elevated in this population [28]. Exposure to PAHs also results in CYP1A2 induction in humans [26] and a recent study showed that CYP1A2 was also induced in individuals consuming pan-fried beef which contained relatively high levels of aromatic amines [23]. Human CYP1A2 levels were also elevated in individuals exposed to indole-3-carbinol in the diet [29]. Endo- and exodioxins also elicit many other common Ah receptor-mediated responses including antiestrogenic effects and immunotoxicity; however, a methodical comparison of Ah receptor-dependent responses by these compounds has not been carried out. Differences in their activities as Ah receptor agonists would be related to their structure-dependent pharmacokinetic and metabolic properties in which endodioxins such as I3C, PAHs and aromatic amines are rapidly metabolized and excreted. These factors would result in the assignment of low TEFs (i.e. potencies relative to that of TCDD) for endodioxins. However, despite the rapid metabolism of endodioxins in food, these

compounds elicit Ah receptor-mediated responses in humans and this is related, in part, to daily exposures to these compounds [23,26,29]. Table 1 summarizes the estimated daily human exposures to dietary endodioxins and exodioxins. The mass balance ratio of endodioxins/exodioxins is > 500,000/1 and clearly demonstrates that endodioxins are the major source of "dioxin-like" compounds in the diet. However, the endodioxin/exodioxin ratios in terms of TEQs are unknown since the TEF values for PAHs, I3C and related compounds, and aromatic amines are unknown. It is possible for some responses that exodioxins may contribute only a small fraction of the TEQs. For example, the antiestrogenic TEFs for PAHs in cell culture are approximately 0.001 [24] and using this value, the TEQs (PAHs) » TEQs (HAHs). The TEF values for PAHs would be less than 0.001 for *in vivo* responses due to their rapid metabolism in most species. Therefore, the ratio of TEQs for PAHs and PCDDs/PCDFs is unknown and is currently being investigated in this laboratory.

With the exception of the carcinogenic PAHs, the remaining endodioxins are weak Ah receptor agonists and it is possible that some of these compounds may exhibit Ah receptor antagonist activity. Research in my laboratory has shown that I3C inhibits several TCDD-induced responses including CYP1A1 induction in various cell lines and mouse liver, and immunosuppressive activity in B6C3F1 mice. Results of these studies are summarized in other Abstracts. Moreover, the ratio of I3C/TCDD required to observe these nonadditive (antagonistic) interactions in B6C3F1 mice are considerably lower than the corresponding ratios of these compounds in the diet.

Peterson and coworkers previously reported that *in utero* exposure to TCDD results in demasculinization of the offspring male rats [30]. This model has also been utilized to investigate the comparative effects of TCDD and I3C. The results of initial studies with TCDD (1 μg/kg) and I3C (1 mg/kg) showed that both compounds demasculinized male rats and the TEF for I3C would 0.001 to 0.0001 for this response. The surprising results of this study were the unusually high potency of I3C in the female rat model since I3C is generally regarded as a weak Ah receptor agonist [31]. Since the mass balance human dietary intake ratio of I3C/PCDDs+PCDFs is > 500,000/1, the relative contribution of the PCDDs/PCDFs compared to I3C for the demasculinization response (in rats) would be minimal. The relative effects and potencies of I3C and TCDD in humans for the demasculinization response are unknown. However, if the rat model is predictive for human responses, then I3C, a component of *Brassica* vegetables, would be the predominant toxin for this response in the human diet compared to TCDD and related HAHs.

In summary, the human diet contains relatively low levels of exodioxins and much higher levels of endodioxins. The relative contributions of these different classes of Ah receptor agonists to the total dietary TEQs is unknown and will require further research in this area. These studies should account for both additive and possible non-additive (antagonistic) interactions since recent results in animal studies suggest that I3C can exhibit response- and species-specific Ah receptor agonist and antagonist activities.

Table 1. Mass and potency balance for endodioxins and exodioxins in the human diet.

Compounds	Mass Intake (pg/day)	Potency Intake (TEQs) (pg/day)
PCDDs/PCDFs [1-4]	150 - 1500	80 - 120
PAHs [13-15]	1,200,000 - 5,000,000	?
I3C [a]	735,000,000 (73,500 ICZ)	?
Aromatic amines [23] (MeIQx, DiMeIQx, PhIP)	11,000,000	?

[a] It was assumed that the average daily intake of I3C-containing vegetables was 25 g and the I3C content was 5 μmol/25 g; the conversion of I3C into indolo[3,2-b]carbazole (ICZ) is approximately 0.01%.

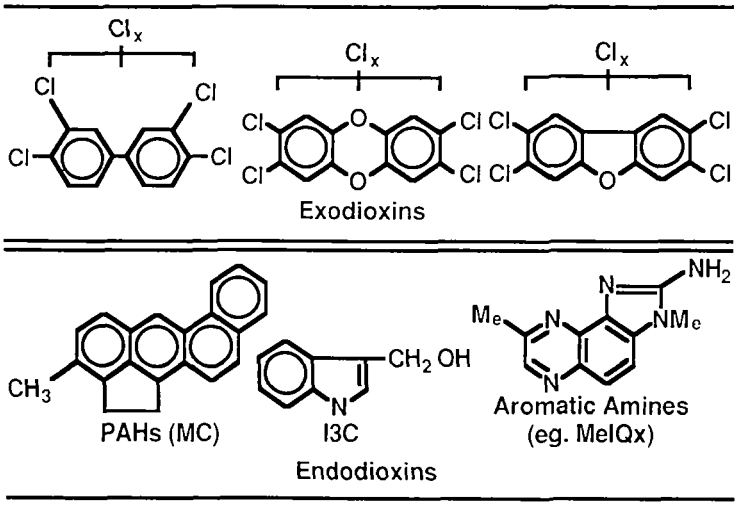

Figure 1. Structures of representative exodioxins and endodioxins in the human diet.

3. References

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