NATURALLY OCCURRING AH-RECEPTOR AGONISTS IN THE DIET: WHY A VEGAN DIET WON'T REDUCE YOUR "DIOXIN DOSE"

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Introduction

It is known that many naturally occurring compounds in the diet are Ah receptor (AhR) agonists that exert effects at dietary levels. Given the ongoing concern regarding trace levels of anthropogenic dioxins and dioxin-like compounds in the food supply, it would be useful to compare the dietary dioxin "toxic equivalence" (TEQ) associated with chemicals vs. natural compounds. Preliminary comparative estimates have been developed by Finley et al.[1], Safe[2] and DeVito and Birnbaum[3], with conflicting results.

These earlier comparisons assumed that the "background" PCDD/F TEQ dose in the U.S. is approximately 120 pg/day, an assumption based on 1994 U.S. EPA estimates of PCDD/F levels in food and food consumption rates. More recent U.S. EPA estimates suggest that the PCDD/F TEQ dose in the U.S. is actually 3-fold lower, approximately 40 pg TEQ/day[4].

In this study, we update the chemical vs. natural agonist comparison with refined exposure and potency estimates and conduct a preliminary sensitivity analysis. We review the results of published studies from which relative estimates of potency (to TCDD) can be developed for indole-3-carbinol (I3C) and indolo(3,2-b)carbazole (ICZ) and derive a range and best relative estimate of potency (REP) for each compound. In addition, we compare the influence of different dose metrics (administered dose vs. accumulated body burden vs. area-under the curve (AUC)) using a consistent set of exposure values.

Materials and Methods

Table 1 summarizes those studies that can be used to derive REP values for I3C and ICZ. While the relative potency data for I3C are based on competitive AhR binding only; one study measured competitive binding in conjunction with AhR transformation . Jellinck et al.[5] measured binding of the AhR complex to a dioxin-responsive element (DRE) and reported that transformation potency paralleled binding potency. Therefore, the REP from this study (8.7×10^{-7}) was chosen to represent I3C potency in this analysis. Studies with ICZ have also examined competitive AhR binding in conjunction with receptor activation and have found ICZ to be nearly equipotent to TCDD[6]. In addition, Kleman et al.[6] measured the induction of a DRE-linked reporter gene product contained in human hepatoma cells and found the potencies of TCDD and ICZ to be within the same order of magnitude. The REPs from this study (0.5—1.0) are at the high end of the REP range for ICZ; however, the use of a human-derived cell line may make these data the most relevant (data from others used animal-derived cell lines). Several studies have also measured induction of CYP1A1-dependent EROD activity in mouse Hepa1c1c7 cells and the REPs from

these studies range from 0.0001 to 0.01. In this analysis, a value of 0.01 is chosen to represent the central tendency of REPs for ICZ. Average daily TEQ dose, accumulated TEQ body burden, and TEQ AUC were calculated for a 30-year adult exposure. Exposure and potency estimates are summarized in Table 2; values are presented as a baseline value followed (where appropriate) by a range of reported values. Accumulated body burden was estimated assuming steady-state conditions. AUC was calculated using the exact solution for a one-compartment pharmacokinetic mode.

Table 1. Comparison of Relative Potency Estimates for I3C and ICZ

Investigators	Relative Potency of I3C	Relative Potency of ICZ	Endpoint
Johannsson et al.[7]	3.8×10^{-4}	—	AhR binding affinity in rat intestinal mucosa
Gillner et al.[8]; d'Argy et al.[9]	—	0.4	AhR binding affinity in rat liver cytosol
d'Argy et al.[9]	—	1×10^{-5}	Lymphoid toxicity in murine fetal thymus cells
Bjeldanes et al.[10]	2.6×10^{-7} (AhR binding)	0.037, 1.4×10^{-4}	AhR binding affinity and EROD activity in Hepa1c1c7 (mouse hepatoma) cells
Jellinck et al.[6]	8.7×10^{-7}	—	Competitive AhR binding, AhR transformation to DRE-binding form in rat liver cytosol
Kleman et al.[7]	—	~0.5–1	DRE binding, reporter gene activity in HepG2 human hepatoma or Hepa1c1c7 cells
Liu et al.[11]	—	~0.001, 0.01	Maximum induced EROD activity, antiestrogenic effects in MCF-7 human breast cancer cells
Chen et al.[12]	—	0.01, 1×10^{-4}	AhR transformation and CYP1A1 mRNA transcription, EROD induction in Hepa1c1c7 cells
Park et al.[13]	—	~1.0×10^{-4}	Induction of EROD activity in Hepa1c1c7 cells

Note: Approximate values (~) indicate that relative potencies were not based on EC_{50}s, but were estimated with the results from a single dose level.

Three different measures of I3C/ICZ and PCDD/F TEQ AUC were calculated and compared. The "lower bound" comparison used estimates that gave the highest PCDD/F TEQ and the lowest I3C/ICZ TEQ; the "median" was based on the baseline values; and the "upper bound" used estimates that gave the lowest PCDD/F TEQ AUC and the highest 13C/ICZ TEQ AUC.

Results and Discussion

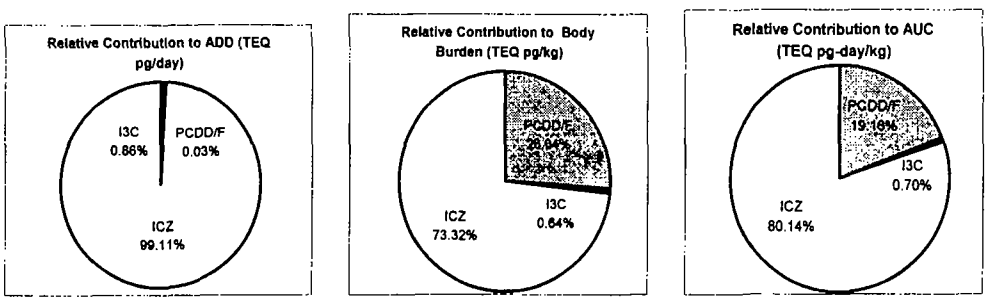
Figure 1 presents a comparison of alternative TEQ dose metrics: average daily dose (ADD) vs. accumulated body burden (BB) vs. AUC. Only baseline exposure and potency estimates are used

in this comparison. ICZ accounts for greater than 73% of the TEQ dose, irrespective of the dose metric. Figure 2 presents a comparison of lower bound vs. median vs. upper bound estimates of PCDD/F vs. I3C/ICZ AUC TEQ. I3C and ICZ contribute more than 74% of the AUC TEQ for

Table 2. Baseline and range of exposure and potency estimates

Compound	Daily Dietary Dose (pg/day)	REP	Bioavailability (%)	Half-life
PCDD/Fs	40	1	50 (50-100)	7.2 years (6-9 years)
I3C	7.35×10^{8}	8.7×10^{-7} (2.6×10^{-7} – 2.4×10^{-3})	100	48 hours (24-48 hours)
ICZ	7.35×10^{6}	0.01 (0.0001-0.4)	100	48 hours (24-48 hours)

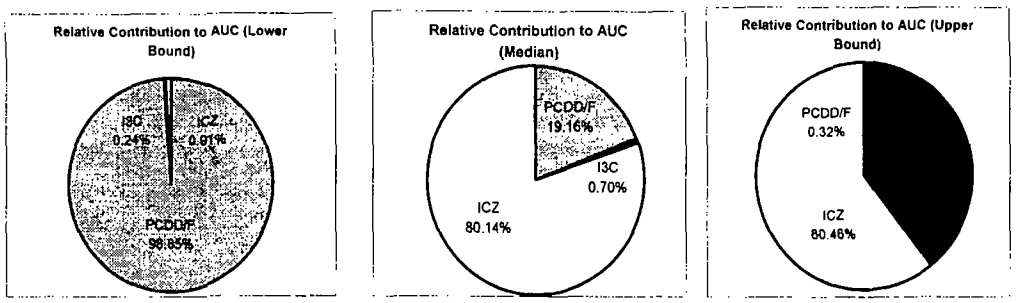
Figure 1. Comparison of relative steady-state TEQ contribution using three different dose metrics



the median and upper bound scenarios; PCDD/Fs contribute the majority of the AUC TEQ only when the most conservative estimates of PCDD/F dose (half-life of 9 years and 100% bioavailability) are used in conjunction with the least conservative estimates of I3C/ICZ potency and dose. The results indicate that, regardless of the dose metric or exposure and potency assumptions, the I3C/ICZ TEQ dose typically far exceeds that of the PCDD/Fs.

For many reasons, the percent contribution from naturally occurring compounds is actually far greater than estimated here. We examined the TEQ contribution of only one chemical class from one specific food type; in reality there are several known and possibly hundreds of naturally occurring compounds in the food supply that have AhR agonist or antagonistic activity at dietary doses. High TEQ doses of naturally occurring compounds (relative to PCDD/Fs) would suggest that the contribution by PCDD/Fs may be trivial and not a health concern. Similarly, high

Figure 2. Comparison of relative steady state TEQ AUC contribution for three different cases



doses of naturally occurring PCDD/F antagonists suggest that any potential effects exerted by dietary PCDD/Fs would be inhibited. For example, Hitoshi et al[14] recently asserted that dietary levels of flavones and flavonols, known AhR antagonists found in produce, are sufficient to inhibit the effects of dietary PCDD/Fs.

These findings bring into question recent claims concerning the (lack of) safety of the food supply, particularly suggestions that ingestion of beef, chicken, pork, and dairy products should be decreased to minimize PCDD/F intake. If the TEQ dose from naturally occurring agonists in fruits and vegetables is orders of magnitude greater than that associated with meat and dairy products, then even total elimination of meat and dairy from the diet would result in no decrease in TEQ dose. We recently showed that in fact a purely vegan diet (no meat or dairy) would result in less than a 0.001% reduction in TEQ dose[1]. We believe it would be useful to continue research into this area via a TEQ analysis of a typical diet to better understand the relative contribution of anthropogenic vs. naturally occurring compounds.

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