Disposition of PCDD/PCDF in Mice

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1. Introduction

Polychlorinated dibenzo-p-dioxins and dibenzofurans are environmental contaminates that produce a variety of toxicities in experimental animals[1]. The actions of these chemicals are mediated through the Ah receptor and their relative potencies are a function of their binding affinity to the Ah receptor[2,3]. There is a rank order structure activity relationship for binding to the Ah receptor and effects, such as enzyme induction, thymic atrophy and weight loss[3]. Pharmacokinetic properties such as absorption, distribution, metabolism and excretion also contribute to the potency of a chemical[4].

The distribution of TCDD and its congeners is dose- and time-dependent [5]. Hepatic retention increases with dose while the percentage of the dose retained in extrahepatic tissue decreases [6]. The retention of these congeners in liver has been explained by a proposed inducible hepatic binding site [5]. One possible binding protein is CYP1A2. This protein is induced through an Ah receptor dependent mechanism and appears to be inducible only in liver. In addition, there are several studies indicating that TCDD and its congeners bind to this cytochrome. In determining the potency of these congeners, experimental systems which are responsive to differences in binding affinity as well as differences in the pharmacokinetic properties of a chemical should be employed[5]. The present study compares the distribution of these chemicals in liver and fat in mice following sub-chronic exposures in which the chemicals are approaching steady-state conditions.

2. Methods

Chemicals: 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), 1,2,3,7,8-pentachlorodibenzo-p-dioxin (PeCDD), 2,3,7,8-tetrachlorodibenzofuran (TCDF), 1,2,3,7,8-pentachlorodibenzofuran (1-PeCDF), 2,3,4,7,8-pentachlorodibenzofuran (4-PeCDF), and octachlorodibenzofuran (OCDF) were obtained from Ultra Scientific (purity > 98%). These chemicals were initially dissolved in acetone and these solutions were diluted with corn oil. The acetone was removed from the corn oil solution by evaporation. Dosing solutions were prepared by diluting the remaining corn oil solutions with additional corn oil. All other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO) and were of the highest grade available.

Animals and Treatment. Female B6C3F1 mice were obtained from Charles River Laboratory (Raleigh NC) and allowed 7 days to acclimate. Mice were randomly assigned to treatment groups (five mice/group), group housed in plastic cages with hardwood shavings and allowed free access to food (Purina rodent chow) and water. The room was maintained on a 12-hr light:dark cycle at 72 $\pm$ 2°F and 50 $\pm$ 5% humidity. Mice were treated with test chemicals 5 days/week for 13 weeks starting at 60 days old. Chemicals were administered in corn oil solutions by gavage at a dosing volume of 10 ml/kg. Doses administered for each chemical are shown in Table 1. The doses were derived from the relative potency of each chemical, based on the USEPA interim TEFs as well as pilot studies[17]. Three days after the last treatment animals were sacrificed. A 200 mg sample of liver and fat were obtained from each animal. These samples were pooled according to treatment and the pooled sample was used to determine dioxin and dibenzofuran levels.

Tissue analysis for PCDDs and PCDFs. Tissue samples were ground with sodium sulfate and extracted with a diethyl ether:hexane (30:70%) solution. The diethyl ether:hexane mix was then passed through a silica gel column and a carbon column chromatography. Once the chemicals were extracted, PCDDs and PCDFs were quantitated using HRGC/HRMS. Calibration and quantitation of all samples and standards associated with this study used the equations presented in EPA method 1613: Tetra-through Octa-chlorinated dioxins and furans by isotope dilution HRGS/HRMS.

3. Results

Effects on body and organ weights. The administration of PCDDs and PCDFs for 13 weeks did not produce any treatment related mortality for any of the chemicals examined. Body weight changes and liver, lung, thymus, spleen and uteri weight were examined for all chemicals tested. PeCDD was the only chemical administered at high enough levels to produce any signs of toxicity as evidenced by alterations in body and organ weights. Liver weights and liver/body weight ratios were increased by PeCDD at doses of 3,000 and 9,000 ng/kg/day. Body weights at the time of sacrifice and thymus/body weight ratios were decreased at the two highest doses of PeCDD tested. In addition, a dose-dependent increase in absolute lung weights and lung/body weight ratios were seen in animals treated with PCDD (9,000 ng/kg/day). No other chemical treatment resulted in changes in body weights or organ/body weight ratios for liver, uterus, spleen, thymus, and lung (data not shown).

Distribution of PCDDs and PCDFs in liver and fat. The distribution of these chemicals was dose-dependent (Table 1). The % dose retained in the liver increased with dose for TCDD, PeCDD, 4-PeCDF, and OCDF. The % dose retained in the liver in animals treated with TCDF was not altered by dose, but was decreased with dose in animals treated with 1-PeCDF. The % dose retained in the fat decreased with dose in animals treated with TCDD, PeCDD, 1-PeCDF, and 4-PeCDF. There was no dose related trend for the % dose retained in the fat in animal treated with TCDF and OCDF. For all chemicals examined, liver/fat ratios were increased in a dose-dependent manner. The increases in liver/fat ratios were consistent with the induction of an hepatic dioxin binding species. The liver/fat ratios were similar for most chemicals with the exception of PeCDD and 4-PeCDF, which were greater than those of the other chemicals studied.

Compared to TCDD, the % dose retained in either liver or fat was lower in animals treated with TCDF, 1-PeCDF, and OCDF. This is consistent with the observations that the apparent half-lives of TCDF and 1-PeCDF are less than TCDD[5]. The small amount of OCDF retained in the liver and fat compared to TCDD is most likely a function of its limited uptake compared to TCDD rather than differences in half-lives. Approximately 10-42% of the administered dose of 4-PeCDF was retained in the liver indicating that the hepatic sequestration of this chemical is much greater than that of TCDD or any of the other PCDDs or PCDFs examined.

4. Discussion.

The relative potency of dioxins and dibenzofurans is dependent upon their binding affinity to the Ah receptor and the pharmacokinetic properties of the individual congener. The present study compared the relative disposition of these chemicals following 13 weeks of treatment. Significant differences were observed in the distribution of these chemicals. For example, approximately 1% of the dose of TCDF compared to 10-42% of the dose of 4-PeCDF was retained in the liver. The differences in the disposition of these chemicals can contribute to the differences in relative potency between congeners. Factors affecting the different dispositions of these chemicals are their relative half-lives, absoprtion, and hepatic sequestration. Some of these differences may not be related to the binding affinity of these chemicals to the Ah receptor.

In addition, this study provides indirect evidence that there is an inducible hepatic dioxin binding species and that the relationship between structure and Ah receptor binding and hepatic sequestration may be different. The affinity of these chemicals for hepatic tissue will be dependent upon the binding affinity of the chemicals to the hepatic binding sites and the relative potency to induce these binding sites. Initial analysis of this data indicates that the relative affinity for hepatic tissue based on liver/fat ratios is 4-PeCDF > PeCDD > OCDF > TCDF = TCDD > 1-PeCDF. This is in contrast to the relative binding affinity to the Ah receptor in which TCDD is the most potent[3]. However, in the present study CYP1A2 concentrations, the putative hepatic binding site, have not been determined and the amount of CYP1A2 may be a crucial determinant of how much sequestration occurs. Efforts to determine the amount of CYP1A2 in these tissues is ongoing.

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TABLE 1
DISPOSITION OF DIOXINS AND DIBENZOFURANS IN LIVER AND FAT

CHEMICAL/DOSE (ng/kg/d)	% DOSE/g LIVER	% DOSE/g FAT	LIVER/FAT
TCDD/ 1.5	2.89	7.87	0.37
TCDD/ 4.5	2.56	5.17	0.49
TCDD/ 15	5.20	3.97	1.3
TCDD/ 45	5.52	2.44	2.26
TCDD/ 150	6.62	2.62	2.5
PeCDD/ 90	8.45	1.8	4.66
PeCDD/ 300	10.3	1.7	6.04
PeCDD/ 900	11.6	1.3	8.85
PeCDD/ 3,000	10.00	1.2	8.3
PeCDD/ 9,000	7.60	1.10	6.88
TCDF/ 15	1.3	1.1	1.14
TCDF/ 45	0.75	0.20	3.66
TCDF/ 150	0.89	0.42	2.11
TCDF/ 450	0.84	0.47	1.77
TCDF/ 1,500	1.18	0.22	5.39
1-PeCDF/ 30	3.15	3.73	0.84
1-PeCDF/ 90	2.29	2.40	0.95
1-PeCDF/ 300	1.87	1.67	1.12
1-PeCDF/ 900	1.85	1.65	1.12
1-PeCDF/ 3,000	1.05	0.82	1.29
4-PeCDF/ 9	6.1	0.9	6.5
4-PeCDF/ 30	10.4	0.87	12.1
4-PeCDF/ 90	3.8	0.37	10.29
4-PeCDF/ 300	42	0.94	44
4-PeCDF/ 900	22.7	0.48	47
OCDF/ 1,500	0.18	0.11	1.69
OCDF/ 4,500	0.21	0.091	2.27
OCDF/ 15,000	0.12	0.06	1.88
OCDF/ 45,000	0.28	0.076	3.76
OCDF/ 150,000	1.17	0.19	6.2