Dose response relationships for EROD induction in liver, lung and skin for dioxin and dibenzofurans.

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The relative potency of halogenated dioxins and dibenzofurans are a function of their binding affinity to the Ah receptor[1]. 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[1]. Pharmacokinetic properties such as absorption, distribution, metabolism, and excretion also contribute to the potency of a chemical. TCDD, the most potent congener of this class of chemicals, binds to the Ah receptor with greater affinity than any other ligand tested. It also has a relatively long half-life in animals (12-15 days in mice[2,3]). Other congeners have different binding affinities and pharmacokinetic properties that will influence their potency. In determining the potency of these congeners, experimental systems which are sensitive to differences in binding affinities and pharmacokinetic properties between chemicals should be employed. Acute *in vivo* or *in vitro* assays are sensitive to differences in binding affinity but are insensitive to the pharmacokinetic differences between congeners. *In vivo* studies using repeated low-dose exposures in which the chemicals approach steady-state conditions are sensitive to both the binding affinity of a chemical and its pharmacokinetic properties. The present study has compared the relative potency for induction of ethoxyresorufin-O-deethylase (EROD) of several dioxins and dibenzofurans in mice following sub-chronic exposures in which the chemicals are approaching steady-state conditions. Relative induction potencies estimated from these studies are then compared to the published Toxic Equivalency Factors (TEFs)[4].

METHODS:

2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD); 1,2,3,7,8-pentachlorodibenzo-p-dioxin (PeCDD); 2,3,7,8-tetrabromodibenzo-p-dioxin (TBDD); 2,3,7,8-tetrachlorodibenzofuran (TCDF); 1,2,4,7,8-pentachlorodibenzofuran (1-PeCDF); 2,3,4,7,8-pentachlorodibenzofuran (4-PeCDF); and octachlorodibenzofuran

(OCDF) were obtained from Ultra Scientific (purity>98%). All other chemicals were obtained from Sigma Chemical Co. (St Louis MO.). Female B6C3F1 mice were obtained from Charles River Laboratories (Raleigh, NC). Mice were treated with test chemicals 5 days/week for 13 weeks starting at 60 days old. Chemicals were administered in corn oil by gavage (10ml/kg). The doses are listed in Table 1. Three days after the last treatment animals were sacrificed. Liver, lung and skin were removed, prepared and EROD activity was determined as previously described[5].

RESULTS:

All compounds tested produced significant induction of EROD activity in all three tissues studied. The relative potency of each of these chemicals is presented on a molar basis (Table 1). EROD activity demonstrated a biphasic response that did not lead to a maximum effect at any of the doses tested for any of the chemicals or tissues examined. For all three tissues there is a steep rise in the dose response curve at low doses; at higher doses the response is less steep and never attains a maximum. Liver EROD activity was approximately 30 and 150 times greater than lung or skin EROD activity, respectively. For all chemicals except TBDD and OCDF, lung EROD activity was induced to a greater extent than was liver or skin EROD activity when compared as percent of controls and was also induced at doses that did not increase either liver or skin EROD activity. The order of relative liver EROD induction potency is TCDD>PeCDD>TBDD> 4-PeCDF>TCDF>OCDF. This order is changed in lung and skin to TCDD>PeCDD>4-PeCDF>TBDD>TCDF>1-PeCDF>OCDF. The relative induction potency is independent of the tissue examined except for TBDD and OCDF. TBDD and OCDF were 5 times more potent at inducing EROD activity in liver than in skin or lung.

Differences between the relative induction potency estimated from the present study and the published TEFs were observed for all of the compounds except PeCDD (Table 1). The largest difference was found for OCDF, which has been assigned a TEF of 0.001, while in the present study, the relative potency is between 0.00002 - 0.0001 or 10-50 times less than the present TEF. The relative induction potency and the published TEFs did not vary by more than an order of magnitude for the PeCDFs. The relative induction potency for TCDF was 10 times less than the TEF. For TBDD, the relative induction potency in liver was 5 times less than the TEF while the relative induction potency in lung and skin was 25 times less than the TEF.

DISCUSSION:

TEFs are estimates of the potency of a chemical based on all the available experimental data using both *in vivo* and *in vitro* assays. This results in a range of values for the relative potency of each chemical. The range is usually one to two orders of magnitude. A chemical is then assigned a conservative TEF value based on studies which demonstrate the greatest relative potency. The relative potencies

determined from the present study deviate less than an order of magnitude from the published TEFs[4] for PeCDD, 1-PeCDF and 4-PeCDF, suggesting that the TEFs may accurately represent the relative potency of these chemicals. The existing TEF for TCDF is approximately 10 times higher than its relative potency in the present study. A 10 fold reduction in the TEF for TCDF would decrease the contribution of TCDF to the estimated toxicity of an environmental sample. The relative potency of TBDD ranges from 0.75 - 5.3 in rats[1]. The relative induction potency of TBDD in mice ranges from 0.04 - 0.2. Differences in these estimates could be due to species differences. The TEF for OCDF is based on studies of octachlorodibenzo-p-dioxin (OCDD) in rats[6]. The present study indicates that the potency of this chemical is at least 10 times lower than its assigned TEF. Differences between the TEF for OCDF could be due to differences in the relative potency of OCDD compared to OCDF or due to species differences between rat and mouse. The relative potency of TBDD and OCDF varied between tissues. This may suggest that there the distribution of TBDD and OCDF are different than TCDD.

The relative induction potencies estimated from this study are consistently lower than the published ranges of relative potency for these chemicals[1]. One possible explanation for this is that the majority of studies comparing these compounds are *in vitro* or acute *in vivo* studies which are insensitive to pharmacokinetic differences between chemicals. Comparing the relative potencies of these compounds when they are approaching steady state concentrations may provide more a accurate estimate of a chemicals potency. Since all of the chemicals except PeCDD were less potent than the existing TEF, these data suggest that the current TEFs may be overly conservative.

This abstract does not represent USEPA policy.

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TABLE 1

THE RELATIVE POTENCY OF DIOXINS AND DIBENZOFURANS

CHEMICAL	DOSE (NG/KG/DAY)	LIVER EROD	LUNG EROD	SKIN EROD	PUBLISHED TEF[1]
TCDD	1.5, 4.5, 15, 45, or 150	1	1	1	1
PeCDD	90, 300, 900, 3,000, or 9,000	0.5	0.5	0.5	0.5
TBDD	30, 90, 300, 900, or 3,000	0.2	0.04	0.04	1
TCDF	15, 45, 150, 450, or 1,500	0.01	0.01	0.01	0.1
1-PeCDF	90, 300, 900, 3,000, or 9,000		0.009	0.009	0.05
4-PeCDF	9, 30, 90, 300, or 900	0.14	0.09	0.09	0.5
OCDF	1,500, 4,500, 15,000, 45,000, or 150,000	0.0001	0.00002	0.00002	0.001

[1] - TEFs are from EPA, 1989.