CONTRASTS OF ENDOCRINE DISRUPTING EFFECT LEVELS FOR SELECTED POPs CHEMICALS FROM INTEGRATED TOXICOLOGY AND EPIDEMIOLOGY STUDIES

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Introduction

In the United States, the Toxic Substances Control Act (TSCA) enactment in 1976 grandfathered about 62,000 chemicals as "existing" and not subject to testing or regulation unless proven to "present(s) an unreasonable risk of injury to health or the environment." The TSCA inventory now lists more than 84,000 substances, with new ones adding 1% of the TSCA production volume, and subject to the same regulatory standards and burdens. This burden of proof has been unworkably onerous and only 5 substances have been banned or restricted since 1976. As a result, many POPs chemicals are used in high volume such that environmental levels and body burdens are of health and scientific concern[1,2]. Important among these are a broad suite of endocrine-disrupting chemicals (EDCs) that include natural or synthetic hormones and compounds that mimic hormones and may interfere with the operation of endocrine systems at concentrations far below those traditionally used in regulatory toxicology and screening, such as under TSCA. While Congress in 1996 authorized EPA to create its Endocrine Disruptors Screening Program to assess potential endocrine risks, the program has been slow to get off the ground. As a result, risk assessment of persistent organic pollutants (POPs) is being challenged by diverse opinions in emerging science, on government regulatory reform, and from institutional and advisory group reports and recommendations. In the context of endocrine disruptors, this critical voice has been particularly noteworthy, since many chemicals have been identified as having similar effects on either thyroid and sex steroid homeostasis, or neurotoxicity,[3] particularly showing a differential risk in fetuses and neonates, so as to be deemed an unreasonable risk to health. As a part of a larger effort[4,5] , we used a weight of evidence approach to assemble a data-base of comparable internal dose and response effect concentration data, from a number of toxicological (*in vitro* and *in vivo*) and epidemiological studies reporting on a range of POPs chemicals, multiple species, and for multiple toxicological responses or endpoints, which we aggregated into 3 categories - thyroid, non-thyroid endocrine (NTE), and developmental neurotoxicity (DNT). The aim of this paper is to present some results of this effort in the form of statistical analysis of variance and contrasts of the reported significant effect mean concentrations of individual POPs chemicals stratified by the thyroid and NTE effect categories.

Methods and Materials

We selected 68 relevant POPs *in vitro* (n= 40) and *in vivo* (n= 28) studies, and 53 epidemiological studies. We made the selection to include studies of BFRs, FRs and POPs with published internal dose potencies and specification of the effect. Animal species included mouse, rat, monkey, kestrel, rainbow trout, flounder, and fathead minnow. We stratified by basis (lipid weight, wet weight), study (*in vivo* toxicology, *in vitro* toxicology, epidemiology), chemical (in 22 categories), and effect (in multiple categories or markers (n=102), aggregated to DNT (n=22), thyroid (n=35), and NTE (n=45) due to sample size constraints). The chemical category number (22) was not selected by us, but emerged from the studies used. We expressed the internal dose in a common Molar metric expressed in log base 10. We assessed the statistical significance of variation in reported or minimum internal dose observed to be associated with an effect with study type (in vitro (toxicology), in vivo (toxicology), epidemiology), basis (wet, lipid), and effect category (non-thyroid endocrine (NTE), developmental neurotoxicity (DNT), thyroid). Where sample size was sufficient, we assessed variation by individual chemical or group, with study type and endpoint category. We contrasted chemicals and study types with regard to the mean $\log_{10}$ (Molar) using analyses of variance and, for each contrast, a 95% confidence interval for the mean difference. We applied the Tukey method to correct multiple pairwise comparisons. All statistical testing was two-sided with a

nominal experimentwise significance level of 5%. We used SAS Version 9.2 for Windows (SAS Institute, Cary, NC) throughout[6].

Results and Discussion

Table 1 shows the sample sizes by basis, study design, and effect category for the all toxin chemical category. We summarized 652 dose measurements in all studies (Lipid weight: Epidemiology 136, in vivo toxicology 29, in vitro toxicology 0, Wet weight: Epidemiology 141, in vivo toxicology 64, in vitro toxicology 282).

Table 1. Sample sizes by basis, effect, and study

	Lipid Weight			Wet Weight		
Effect		**Toxicology**			**Toxicology**	
Category	**Epidemiological**	**in vivo**	**in vitro**	**Epidemiological**	**in vivo**	**in vitro**
DNT	21	11	0	24	35	66
NTE	42	8	0	32	17	133
Thyroid	73	10	0	85	12	83
Total	136	29	0	141	64	282

In vitro studies expressing NTE effects (Table 2) showed heterogeneity with regard to the mean log of the wet weight dose. The required dose of MeO-PBDE (-5.5±0.4, p=0.007), OH-PBDE (-5.8±0.8, p=0.01), and PBDE (-5.6±0.8, p<0.001) were significantly increased compared to "Others" [comprised of Chlordanes (n=1), Dieldrin (n=2), HCH (n=7), Other OHC (n=4) and TBECH (n=8)] (-6.7±1.7).

Table 2. Contrasts between 6 POPs and Other chemicals in in vitro Toxicology Studies exhibiting NTE Effects on mean wet weight $\log_{10}$(Dose in Molar units) [N, Mean±SD]

	HBCD	**MeO-PBDE**	**OH-PBDE**	**PBDE**	**PCB**	**TBBPA**	**Others**
N	5	15	37	41	3	7	22
Mean±SD	-5.6±0.6	-5.5±0.4	-5.8±0.8	-5.6±0.8	-5.6±0.6	-6.2±1.1	-6.7±1.7

In vitro expressing thyroid effects (Table 3) did not exhibit heterogeneity; all 7 pairwise contrasts were non-significant (p>0.05). These results for thyroid have sample size n=83, of which n=49 are transthyretin or thyroxine-binding prealbumin (TTR) competitive binding assays, and n=11 are thyroxine-binding globulin (TBG) competitive binding assays. For TTR, hydroxylated, and halogenated phenolic compounds (including TBBPA, TCBPA and triclosan) dominated the sample. For TBG, hydroxylated compounds (OH-PBDE, n=6; OH-PCB, n=1; OH-PFB, n=1; and triclosan, n=1) dominated, with PBDE, n=2, the remainder. Summary statistics for TTR were: mean -7.23; range -8.59 to -4.44 and for TBG: mean -6.09; range -7.0 to -4.18.

Table 3. Contrast between 6 POPs and Other Chemicals in in vitro Toxicology Studies with regard to mean wet weight Log_{10}(Dose in Molar units) for Thyroid effects

	HBCD	**OH-PBDE**	**OH-PCB**	**PBDE**	**PCB**	**TBBPA**	**Others**
N	2	23	15	7	9	10	13
Mean±SD	-5.5±0.71	-7.11±0.58	-7.14±1.99	-5.51±0.74	-6.9±1.23	-6.95±1.48	-6.85±1.19

For the non-TTR/TBG markers, (n=23), the OH-PCB (n=9), OH-PBDE (n=2), and TBBPA/TCBPA (n=4) dominate, with PCB (n=1), PBDE (n=4), and HBCD (n=3) sharing the remainder. Overall, these markers were predominantly associated with TH pathway down regulation, or inhibition, or antagonism, through several mechanisms (n=15). The balance show T3/TR inhibition combined with TH induction and agonism

(TBBPA/TCBPA, n=2; 4-OH-PCBs, n=4), or T3 potentiation (TBBPA/PBDE/HBCD, n=2). Summary statistics for these data were: mean -6.09; range -11.3 to -4.0.

Table 4 Contrasts between 4 POPs and Other chemicals in Epidemiology Studies exhibiting NTE Effects on mean wet weight Log_{10}(Dose of Body Burden in Molar units) [N, Mean±SD]

DDE-DDT	PBDE	PCB	PFC	Others[a]	95% CI
9	7	7	4	4	
-7.7±0.5	-10.0±0.5	-8.1±0.7	-7.7±0.5	-7.5±0.8	
•	•				(1.4, 3.1)**
	•	•			(-2.7, -0.9)**
	•		•		(-3.3, -1.2)**
	•			•	(-3.5, -1.4)**

a. HCB (n=2), PBB (n=1). Chlordanes (n=1)
**p<0.001

In epidemiological studies showing NTE effects (Table 4), of 4 chemicals, PBDE had the lowest mean (-10±0.5) and was significantly less than that of DDE-DDT (-7.7±0.5, p<0.001), PCB (-8.1±0.7, p<0.001), PFC (-7.7±0.5, p<0.001) and Others [HCB (n=2), Chlordanes (n=1), and PBB (n=1)] (-7.5±0.8, p<0.001). In epidemiology expressing thyroid effects (Table 5), of 6 chemicals, mean dose for OH-PCB was smallest (-10.0±0.8), and was significantly less than the means for DDE (-8.4±0.1, p=0.03), PCB (-8.8±1.2, p=0.01), and PFC (-7.5±0.2, p<0.001). PFC had the largest mean of these 6 chemicals, and was significantly greater than HCB (-9.5±0.3, p=0.03), PBDE (-9.6±1,1, p=0.003), PCB (-8.8±1.2, p=0.04), and Others [DDT (n=1), HCH (n=1), Me-SO2-PCB (n=4), PBB (n=2) and PCP (n=1)] (-9.2±1.4, p=0.03).

Table 5 Contrasts between 6 POPs and Other chemicals in Epidemiology Studies exhibiting Thyroid Effects on mean wet weight Log_{10}(Dose or Body Burden in Molar units) [N, Mean±SD]

DDE	HCB	OH-PCB	PBDE	PCB	PFC	Others[a]	95% CI
7	5	13	8	37	6	9	
-8.4±0.1	-9.5±0.3	-10.0±0.8	-9.6±1.1	-8.8±1.2	-7.5±0.2	-9.2±1.4	
•		•					(0.1, 3.0)*
	•				•		(-3.9, -0.2)*
		•		•			(-2.2, -0.2)*
		•			•		(-4.1, -1)**
			•		•		(-3.9, -0.5)*
				•	•		(-2.8, -0.02)*
					•	•	(0.1, 3.4)*

a. DDT (n=1), HCH (n=1), MeSO2-PCB (n=4), PBB (n=2), PCP (n=1)
*p<0.05
**p<0.001

Not shown here, the wet weight in vivo thyroid results (n=12) correspond to TBBPA (liver) in two one-generation rat studies (n=5, range -4.73 to -5.98; and n=2, -6.74 and -5.74), and PBDE-47 in one fathead minnow (muscle), and one flounder (carcass) study (n=4, range -3.66 to -4.62; and n=1, -6.98, respectively). Overall results statistics: mean -5.23; SD, 1.05. This compares to the lipid weight in vivo results from two studies - one 28-day rat OECD model (liver; n=1) testing HBCD as a technical mixture, and one rainbow trout model (muscle; n=9) testing the three main HBCD diastereoisomers (alpha, beta, and gamma) administered individually, with measurements of each of the three diastereoisomers for each test that showed significant effects. The rat liver effect concentration is -4.17, and the rainbow trout muscle mean is -6.88. The reported effect in each case was thyroid epithelial cell and gland hypertrophy.

The in vivo lipid weight results for NTE (n=8) consist of: PBDE in Kestrel corporal (-5.83); Deca-BDE in Rat plasma (-5.82; -5.48); HBCD in Rat liver (-5.7 to -4.96 in females; -4.96 males); PBDE-99 in Rat dam adipose (-6.27 to -5.96). Overall results statistics: mean, -5.56; SD, 0.45. In vivo wet weight results for NTE (n=17) consist of PBDE/DE71 in Kestrel corporal (-6.8), Kestrel whole egg (-6.35 to -5.74; -6.35 to 5.74); HBCD in Kestrel whole egg (unintended; -8.28 to 7.62); DE71/PBDE-47 in flounder muscle (-7.09); PBDE-99 in Rat dam liver (-8.15 to -7.44); Deca-BDE in Rat liver (-7.34 to -6.99); TBBPA in Rat liver (-6.63 and -6.76); HBCD in Rat female liver -7.05 to -6.29, and -6.28). Overall results statistics: mean, -6.88; SD 0.74.

To provide some context to these details, for the overall data analysis (not shown here) there was an apparent trend of increased significant effect concentrations means, with *in vitro* studies exhibiting the highest concentrations, *in vivo* exhibiting intermediate, and epidemiology the lowest. An exception to our general trend hypothesis is the in vitro result for thyroid showing a lower mean effect concentration than the wet weight in vivo (-6.76, SD 1.33 versus -5.23, SD 1.05, respectively). As well, the in vivo whole weight (-5.23) is higher than the lipid weight (-6.61, SD -1.02). In the overall contrasts for thyroid, in vivo contrasts with epidemiology by 3 to 4.5 orders of magnitude, and in vitro contrasts by almost 2 to almost 3 orders of magnitude. The overall ratio of in vivo dose distributions to the epidemiology dose distributions, an empirical measure of the relative toxicokinetics and dynamics (TK), is the highest in thyroid, ranging from mean 6357 (95th CI; 1229.9, 32856.5). The in vitro ratio was mean 185.5 (95th CI, 76.8, 448.4), but this is not TK. These results, at least, reflect the chemical, species, and model type, as data above show, however, they may also reflect the findings of Parham et al[6], where predicted rat dose-responses for thyroid effects of PCBs were generally orders of magnitude (1 to 6) lower than those for humans, based on integrated studies. This present paper, and Parham et al, suggests greater human sensitivity to the thyroid pathway effects is possible, but there are other influences. The results also support the importance of considering, together, mixtures of chemicals that can affect the same common adverse outcome or pathway.

The overall findings indicate that the multiple POPs, and metabolites, studied have multiple or common dose-response markers, and potencies, at several levels of upstream and downstream biological organization varying with study type. These suggest a combination effect as responses involve similar and multiple effects on either thyroid and sex steroid homeostasis and related pathways, (or neurotoxicity), further suggesting concentration addition metrics. Emerging chemicals appear to have lower effect concentrations (PBDEs and epidemiology), as do metabolites. However, we found no significant differences between the means of POPs and metabolites expressing thyroid effects *in vitro* (Table 3), contrary to other evidence that OHs are more potent in the thyroid markers, are retained in blood with thyroid hormone transport proteins, such as TTR and TBG, and passed to the placenta and fetus. Moreover, legacy compounds, such as PCBs are declining and PBDEs are increasing, and human exposure sources vary, possibly leading to a lack of correlation between these compounds in human matrices, possibly preventing the discovery of uncorrelated compounds as confounders or cofactors, even though they are part of the overall body burden background, and have reasonably similar dose-response relations that may act in concert. These results raise questions about the application of thresholds and low-dose hypotheses used in the regulatory RA and management paradigm of assessing single chemicals in isolation, particularly for the endocrine disrupting class.

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