CONTRASTS OF EFFECT LEVELS FOR POPs CHEMICALS FROM INTEGRATED TOXICOLOGY AND EPIDEMIOLOGY STUDIES ARE HIGHLY ASSOCIATED WITH LIFE STAGE AND DOSING AMOUNTS

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In the context of endocrine disruptors, many chemicals have been identified as having similar effects on either thyroid and sex steroid homeostasis, or neurotoxicity, particularly showing a differential risk in fetuses and neonates, and may interfere with the operation of endocrine systems at concentrations far below those traditionally used in regulatory toxicology and screening[1]. This life-stage dependent risk, using low doses relevant to human exposure, needs to be accounted for in experimental toxicology and risk assessment. In general, it appears that the majority of toxicological studies overdose animals when compared to internal doses reported in human studies. As a part of a larger effort[2, 3], we assembled 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). We can stratify a relevant selection of this data base to explore the questions; (a) the extent to which experimental studies reporting internal doses similar to humans were included or not, and (b) the extent to which both low-dose and generational studies are included in our study selection. The aim of this paper is to examine how answers to these questions affect the difference between the epidemiology and *in vivo* results found in the larger integrated data base.

Methods and Materials

In real time from 2000 to 2010, 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). 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). We contrasted 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. In respect to the questions and aim of this paper we examined the stratified studies selected with regard to dosing protocols and life stage of exposure in the study design. We also included three studies not found by chance in our selection process. Included are two low-dose generational studies that examined the offspring of directly exposed dams, and a high dose study of older animals. These were all compared with the overall data base and results initially assembled, with a focus on the upper and lower bounds of the 95% confidence interval of the mean differences between the epidemiology and the *in vivo*.

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		
		Toxicology			Toxicology	
Effect 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

Among studies reporting DNT, NTE or Thyroid effects in wet weight (Table not shown), the mean dose was significantly decreased ($p<0.001$ for all contrasts) in epidemiology relative to wet weight *in vivo* and wet weight *in vitro* toxicology [DNT: in vivo toxicology -6.61±0.71, in vitro -5.26±0.79, epidemiology -8.87±1.15, CI (1.78, 2.74; 3.18, 4.03, respectively), NTE: in vivo toxicology -6.88±0.74, in vitro -5.89±1.06, epidemiology -8.24±1.08, CI (0.78, 1.95; 1.94, 2.76, respectively), Thyroid: in vivo toxicology -5.23±1.05, in vitro -6.76±1.33, epidemiology -9.03±1.18, CI (3.09, 4.52; 1.89, 2.65, respectively)].

Contrasts in lipid weight (Table 2) were in the same direction, but were generally smaller, and did not reach significance for studies expressing DNT effects. [DNT: in vivo -5.89±1.12, epidemiology -6.46±1.13, p=0.18, CI (-0.28, 1.43), NTE: in vivo -5.56±0.45, epidemiology -6.79±1.13, p=0.004, CI (0.41, 2.04), Thyroid: in vivo -6.61±1.02, epidemiology -7.18±0.75, p=0.04, CI (0.04, 1.09)].

Table 2 Contrasts between Toxicological and Epidemiological Studies on mean Log10(Dose or Body Burden in Molar units)

a) Lipid weight [N, mean±SD)]

Effect	in vivo Toxicology	Epidemiology	p-value	95% CI
DNT	11	21		
	-5.89±1.12	-6.46±1.13	0.18	(-0.28, 1.43)
NTE	8	42		
	-5.56±0.45	-6.79±1.13	0.004	(0.41, 2.04)
Thyroid	10	73		
	-6.61±1.02	-7.18±0.75	0.04	(0.04, 1.09)

Our focus is on examining what studies comprise the lower and upper bounds of the 95% confidence intervals. The lower bound is where the differences between the in vivo toxicology and the epidemiology are the least, or overlap, and may not be significant, and indicate what life stage and dosing the studies found are in contrast. Comparing the upper bound studies shows something about which kind of studies comprise that area of the distribution.

The range of the lipid weight in vivo toxicology studies for DNT is -7.59 to -4.51. For DNT the lowest effect concentration (-7.59 lw) is from Suvarov et al (2009)[4] which is a prenatal Wister rat model where the dam was dosed from GD15 to PND20 every 5 days to 0.002, 0.02, 0.2 mkd of PBDE- 47 by intravenous injection. These doses are some of the lowest ever used, and in a one-generation model. The highest effect concentration (-4.51 lw) is from Lilienthal et al (2009)[5], a benchmark, one-generation Wistar rat model using doses of 0.1, 0.3, 1.0, 3.0, 10.0, 30, 100 mkd of HBCD to the dam. Both of these studies are in utero

exposures, however, the higher doses of the Lilienthal et al study are 5 to 50 times the low three doses, and then from 15 to 500 times higher than the highest dose used by Suvarov et al (4).

In the epidemiology the lowest lipid weight effect concentration for DNT is -9.0 which was observed in Herbstman et al (2010)[6] (in a range of -8.0 to -9.0) for PBDEs in cord blood. This is longitudinal birth cohort from lower Manhattan assessed at ages 1 through 6 (all except at 5). The highest concentration of -4.98 is from Jacobson et al (1996)[7] for the highest total PCBs in cord blood, (range -5.24 to -4.98) lipid corrected (0.0024), associated with IQ loss at age 11. This was the offspring of a Lake Michigan fish-eating cohort.

The range of the lipid weight in vivo studies for NTE is -6.27 to -4.96. For NTE the lowest effect concentration is from Kuriyama et al (2006)[8], which is prenatal exposure, single dose of PBDE99 to Wistar rat dam by gavage on GD6 to either 60 or 300 ug/kg/bw (0.060 or 0.300 mkd). At the time of publication this was one of the lowest doses ever used. The highest effect concentration is from van der Ven et al (2009)[9], one generation Wistar rat model, with same dosing as Lilienthal et al noted above to HBCD. In this case, while both are prenatal exposures, and with different protocols, the dosing differences range from even (for 0.300 mkd) to 1.76 to 333.

The lowest effect concentrations in epidemiology lipid weight lie between -8.0 and - 8.5, as the lowest. In a birth cohort of newborn boys by Main et al (2007)[10], breast milk PBDEs (sum7), in this range, were associated with cryptorchidism. At the high end of -4.73 for T-DDT, Perry et al (2006)[11] found disruption of estrogen homeostasis in adult females.

The range of the lipid weight in vivo studies for Thyroid is -8.0 to -4.17. For Thyroid the lowest effect concentration (for thyroid gland hypertrophy) is from Palace et al (2007)[12] and Law et al (2006)[13], which are juvenile rainbow trout exposed to three HBCD stereoisomers (alpha, beta, gamma), separately, for 56 days to 10 to 30 ug/kg/body weight, (0.01 to 0.03 mkd) 3 times a week, then followed for another 112 days of depuration. Significant effects were seen to involve certain particular isomers fed, but also metabolites from bio-isomerization. The effect concentration of -8.0 is for the beta-HBCD metabolite of fed HBCD-gamma. The overall mean for all the effect concentrations -6.88. The highest effect concentration is from van der Ven et al, (2006)[14], which is a benchmark 28-day exposure study of 11 week old Wistar rats to technical HBCD. The dosing used was by gavage to 0.3, 1.0, 3.0, 10, 30, 100, 200 mkd, which are 30 to 6666 times higher than in Palace/Law et al. The effect of 10% increase in thyroid weight, the overall BMDL was 1.6 mkd with an internal dose calculated at 43 ug/g liver lw. This BMDL is 53 to 160 times higher than the doses in the Rainbow Trout study. These two studies are post-natal exposures, however, the dosing regimens are substantially different, as indicated.

In epidemiology (and toxicology), the thyroid system is a main target of POPs, with a lipid weight effect range of -9.0 to -5.74. At the lowest concentrations of between -9.0 and -8.0, Chevrier et al (2010)[15] found inverse associations between PBDEs and TSH in pregnant women (27 weeks), and elevated odds of subclinical hyperthyroidism (low TSH but normal T4). Also at the low end of -9.0 to -8.0, Herbstman et al (2008)[16] found associations between certain PCB and PBDE congeners in cord blood and neonatal blood spots in newborns, and increased odds of lower TT4 and fT4, and lower odds of high TSH. It is interesting to note the complementary results of this study showing thyroid effects, and the Herbstman et al (2010) on a different longitudinal birth cohort finding associations with DNT. At the high end of the thyroid epidemiology range (-5.74) is a study by Dallaire et al (2009)[17] finding a significant inverse association between sum20 PCBs and TSH in Inuit adults. However, in this study various thyroid metrics showed associations with individual PCBs at lower concentrations of -8.0 to -7.5.

We subsequently found other studies not in our data base. One is a low-dose, prenatally exposed lambs study showing thyroid hormone declines, by Abdelouahab et al. (2009)[18]. The doses of BDE-47 used were 0.2, 2.0, 20.0 ug/kg bw (0.0002, 0.002, and 0.02 mkd), applied from 5th to 15th week of gestation by IV injection weekly. Significant down regulation of TT4 was seen at lamb fat tissue concentrations of -7.41 (39 nM), and of both TT4 and TT3 at doses -7.14 (72 nM) and -6.74 (180 nM). A second is a PBDE-47 high-dose, 7-week old SD rat study by Darnerud et al. (2007)[19], was also found. This study also found thyroid effects measured as significant reductions in FT4, however, the external dosing (18 mg/kg) was 900

to 90000 times that of [18], was a different regimen, and the rats were 7 weeks old. The significant internal dose in rat plasma, lipid weight, was -3.06 (866 uM), or about 4 orders of magnitude higher than (18). A third study found is a low-dose study in perinatally exposed Wistar rat pups, by Suvorov et al. (2009b)[20] . In this study rat dams were exposed to doses of 2 or 200 ug/kg body weight (0.002 or 0.2 mkd) every 5th day from GD 15 to PND20 by IV injection. This study reported growth alteration and body weight increase in exposed rat pups. Internal doses in adipose tissue, lipid weight, were -7.1 to - 5.66 (80 nM to 2.2 uM) in the pups. The statistical analysis by the authors showed significant effects at -5.66 for six endpoints, and at -7.1 for three endpoints. The dams' adipose tissue concentrations (PND 27) were -7.59 to -6.32.

Specific summary conclusions are as follows.

- Very low applied dose (low ug/kg/bw) in vivo studies with generational exposures (prenatal/perinatal) yield the comparably lowest internal dose, significant effects responses that are most often the closest fit with the comparable epidemiology studies.
- Generational studies administering higher doses, in the low to high mg/kg/bw, yield significant responses at higher internal doses and are farther removed from the epidemiology.
- Juvenile, adolescent, or adult exposure models, at low to high mg/kg/bw applied dosing yield the highest internal dose effects levels, and are the farthest removed from the significant effects levels in the epidemiology.

In general conclusion, these studies reflect the emerging idea that toxicology must test for effects at the most sensitive life stages, including prenatal and perinatal stages of development, and at administered and internal doses that are comparable to human (or wildlife) exposure.

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