CONTRASTS OF EFFECT LEVELS FOR POPs CHEMICALS FROM INTEGRATED TOXICOLOGY AND EPIDEMIOLOGY STUDIES - LESSONS FROM LOW-DOSE GENERATIONAL STUDIES

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Introduction

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 as well as 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. In general, it appears that the majority of toxicological studies overdose animals when compared to internal doses reported in human studies. In the context of endocrine disruptors, 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. This life-stage dependent risk, using low doses relevant to human exposure, needs to be accounted for in experimental toxicology and risk assessment. As a part of a larger effort[4,5] , 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). Reviewers questioned whether animal studies reporting internal doses are those where external doses were higher than those using low doses, such that the latter were possibly excluded in our selection. If so, the difference between epidemiology and *in vivo*, might be overestimated. Reviewers also asked why some experimental studies reporting internal doses similar to humans were not included. The aim of this paper is to present some results of revision to include additional statistical analysis of variance and contrasts of the reported significant effect concentrations of initially excluded studies. Included are both low-dose generational studies that examined the offspring of directly exposed dams, and a high dose study of older animals.

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). 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 review questions follow-up regarding studies not found by chance in our selection process, we reviewed three of these studies and compared them with the overall data base and results initially assembled.

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 2), 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)].

Table 2 Contrasts between Toxicological and Epidemiological Studies on mean Log_{10}(Dose or Body Burden in Molar units)

a) Wet weight [N, mean±SD)]

Effect	Toxicology in vivo	in vitro	All	Epidemiology	p-value	95% CI
DNT	35	66	101	24		
	-6.61±0.71	-5.26±0.79	-5.73±1	-8.87±1.15)		
	•			•	<0.001	(1.78, 2.74)
		•		•	<0.001	(3.18, 4.03)
			•	•	<0.001	(2.68, 3.6)
NTE	17	133	150	32		
	-6.88±0.74	-5.89±1.06	-6±1.07	-8.24±1.08)		
	•			•	<0.001	(0.78, 1.95)
		•		•	<0.001	(1.94, 2.76)
			•	•	<0.001	(1.83, 2.65)
Thyroid	12	83	95	85		
	-5.23±1.05	-6.76±1.33	-6.57±1.39	-9.03±1.18)		
	•			•	<0.001	(3.09, 4.52)
		•		•	<0.001	(1.89, 2.65)

Effect	Toxicology in vivo	in vitro	All	Epidemiology	p-value	95% CI
			•	•	<0.001	(2.08, 2.84)

Corresponding contrasts in lipid weight (Table 3) 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)]. These decreases in the magnitude and significance of the contrasts between lipid weight and the wet or whole weight, by effect category, mirror the analogous contrasts for all effects combined (data not shown).
However, please note that we suspect possible measurement error in lipids analysis of relatively leaner epidemiology matrices compared to in vivo.

Table 3 Contrasts between Toxicological and Epidemiological Studies on mean Log10(Dose or Body Burden in Molar units)

b) 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)

In follow-up, we examined a low-dose, prenatally exposed lambs study showing thyroid hormone declines, by Abdelouahab et al. (2009)[6] , or a low-dose study reporting growth alteration and body weight increase in perinatally exposed rat pups, by Suvorov et al. 2009[7] . Both studies tested for effects of PBDE-47 on the offspring of exposed pregnant sheep or rat dams. In this follow-up, it was found that a related, similar low-dose rat offspring study, by the same author, was included in the selection (Suvarov et al)[8] , and that a PBDE-47 high-dose, 7-week old rat study by Darnerud et al. (2007)[9] , was not found and therefore, not included.

In Abdelouahab et al. [6], 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). Study [9] also found thyroid effects measured as significant reductions in FT4, however, the external dosing (18 mg/kg) was 900 to 90000 times that of [6], was a different regimen, and the rats used 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 in [6]. These results for both studies compare with the lipid weight thyroid effect category overall mean dose of -6.61 for in vivo, and -7.18 for epidemiology (Table 3). The low-dose study (6) significant internal doses were among the lowest in the lipid weight thyroid range, and the high dose study (9) was the very highest dose of any we found.

Using data on plasma lipid percent (0.25 to 0.32) provided by [9], the wet weight significant doses were -5.64 to -5.55 (2.3 uM to 2.8 uM). These doses are slightly less than the overall wet weight thyroid mean dose of -5.23, but still far from the epidemiology mean at -9.03 (Table 3a). There are no comparable wet weight doses for the other study [6].

The other low-dose study considered [7], reported growth alteration and body weight increase in perinatally 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. For these same endpoint counts, the dams adipose tissue concentrations, measured at PND 27, were -7.59 to -6.32 (26 nM to 482 nM). The dams were dosed to PND 20. These doses are comparable with the lipid weight NTE effect category overall mean dose of 5.56 for in vivo, and -6.79 for epidemiology (Table 3b). The three lowest internal doses are the lowest in the range of all the in vivo lipid weight NTE results. The administered and internal doses in this study are the same as two of the three used in study [8], and were included as 6 observations out of 11 in the lipid weight DNT result calculations of the present paper.

At least three observations are apparent from this examination. First, the low-dose studies considered above [6, 7] not only used low doses, but are also generational studies that examined the offspring of directly exposed dams. The significant effect concentrations were generally below the overall lipid weight thyroid, and NTE, means reported in the larger study, and were closer to, or in some cases (NTE) equal to, the epidemiology overall means. Also, they were at the lowest end of the range of all results for NTE, thyroid, and DNT.

Second, 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. It appears that these life stages are able to discern effects at the low doses used, which are reported to be the lowest doses ever used, and comparable to human exposure and body burdens. Importantly, these low-dose study results are more consistent with the low effect association concentration results being seen in the epidemiology.

On the other hand, the high-dose study [133], presented the highest significant effect internal dose, in lipid weight, of any study examined in the larger paper, and used 7-week old animals. However, such studies are also used in assessing risk, and calculating Margins Of Exposure. Moreover, this study, when expressed in plasma wet weight, showed a lower effect concentration than the overall wet weight thyroid mean of the paper, but was still far away from the epidemiology mean. This is another indicator of the thyroid result inconsistencies found (data not shown), and also, the lipid weight, wet weight inconsistencies as well.

While beyond the present scope, these considerations support our recommendation that possible biases due to the study selection methodology, and the study purpose of quantifying variability and uncertainty across a range of study types, that are used to comment on hazard, risk, and "safety", might be addressed by expanding the database to include more studies. In particular, we suggest the inclusion of studies involving low doses (comparable to human exposures and body burdens) and generational models.

References

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