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CONTRASTS OF APPLIED DOSE EXPOSURE AND EFFECT LEVELS FROM INTEGRATED TOXICOLOGY AND EPIDEMIOLOGY STUDIES: ASSOCIATIONS OF DOSING AMOUNTS, INTERNAL DOSES, LIFE STAGE, AND SEX FOR THE THYROID EFFECT CATEGORY

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Introduction

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 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. 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. In the present effort, we assembled further data covering: (1) the detailed applied dose exposure protocols of the in vivo studies; (2) life stage of exposure for the in vivo and epidemiology studies; (3) sex identification; and (4) other relevant information describing the specific timing and number of doses applied. We can stratify this expanded data base to statistically explore the quantitative associations and contrasts between: (a) the applied exposure doses, internal toxicology doses, and internal epidemiological doses by each effect category; (b) the associations and contrasts by sex and exposure life stage; and (c) the associations of the range of dose amounts and life stage of exposure.

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. In this paper, we added two low-dose generational studies that examined the offspring of directly exposed dams – adding a sheep species -, and a high dose study of older rat animals, making the in vivo study n= 31. Further, we added data on the applied dose protocols for each in vivo study, with information on life stage of exposure, dose timing, and dose number. For the in vivo and epidemiology internal effect dose data we added information on sex and life stage of exposure. 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 and applied doses 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). In this paper, we further added the applied doses, sex, life stage of exposure and dose timing and number to this analysis. We contrasted with regard to the mean $\log\neg_{10}$ (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 aims of this paper we quantitatively analysed the entire database as noted and additionally with regard to dosing protocols, life stage of exposure, sex, and other criteria in the study designs. The effect category considered in this paper will be restricted to thyroid.

Results and Discussion

Table 1 shows the sample sizes by basis, study design, and effect category for the all toxin chemical category. We summarized 680 internal dose measurements in all studies (Lipid weight: Epidemiology 136, in vivo toxicology 41, in vitro toxicology 0, Wet weight: Epidemiology 152, in vivo toxicology 69, in vitro toxicology 282). Additionally, we summarized 234 applied dose measurements from all in vivo studies. Some internal dose sample sizes in Table 1 have increased a little from previous reports.[3] The n=234 is the count of all the exposures or ADs used regardless of the effect or basis of the reported internal significant effect doses. This count is reflected in Tables 2 and 3, which show the mean contrasts between the various dose categories considered in the data base and analysed.

Tables 2 and 3 show the wet weight and lipid weight contrasts, respectively, across the dose metrics. The consistent increase in the mean dose from epidemiology relative to in vivo and in vitro (not shown: $p<0.001$ for all contrasts except ww in vitro:in vivo, $p=0.04$; lw, 0.08), observed previously is maintained. The addition of the AD adds to this increasing trend. Table 4 shows the significance of inherent variation from the mean in exposure protocols and ADs that may be important, and are averaged over in a pooling of them. This is based on whether individual studies reported lipid or whole weight metrics. When the studies that reported lipid weight results are stratified together, the mean and summary statistics of the ADs decreases from -5.12 (2.2) to -6.63 (2.38). In this Table the ADs are not significantly different from the epidemiology or in vivo internal dose, and the median/mean relationship shows a skew towards the lower doses, and relative to the lipid/wet pooled sample. Table 5 shows the strictly wet weight strata and that this sample moves the mean AD in the higher direction, and this mean is significantly higher than all the other dose metrics ($p<0.001$). Tables 6 and 7 show the stratification of the wet wt and lipid wt pooled in vivo and epidemiology internal doses by selected categories of life stage at exposure. The variation in exposures and effects by life stage by wet wt and lipid wt reported studies is again apparent. It is evident that the internal doses in the in vivo reflect the ADs, however even at the lowest doses have effects, but small sample sizes can be problem. The sensitive life stages reflect in a dose-response manner the pattern inherent in the ADs used. In epidemiology, the maternal exposures are the lowest of any, including adult. In lipid wt, adult (-6.96) is equal to perinatal (-6.96), and maternal (-7.69) is close. Again, this reflects the ADs used. Tables 8 and 9 analyse life stage at exposure to the ADs. Again, the studies reporting lipid wt results used the lowest ADs, but these were the smallest samples. The comparative perinatal ADs show this, with lipid result at -8.39 (n=6) and wet at -4.04 (n=45). Regarding stratification by Sex (not shown), small samples are a problem for the in vivo. Epidemiology lipid wt results showed lower Female thyroid internal effect doses than males (-7.39 compared to -6.72; $p<0.02$). Interestingly, the M/F category also had a lower internal effect dose than Males (-7.32 compared to -6.72; $p<0.009$). A question is the details of the M/F group.

Reference

1. Diamanti-Kandarakis E, Bourguignon J-P, Giudice L, Hauser R, Prins G, Soto A et al. 2009. Endocrine Reviews 30(4): 293-342.
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3. Muir T and Michalek J. 2014. Organohalogen Compounds - Proceedings of Dioxin 2014

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

	Lipid Weight			Wet Weight		
		Toxicology			Toxicology	
Effect Category	Epidemiological	in vivo	applied	Epidemiological	in vivo	in vitro
DNT	21	11	75	24	35	66
NTE	42	12	77	32	17	133
Thyroid	73	18	82	96	17	83
Total	136	41	234	152	69	282

Table 2. Contrast between in vivo applied (Wet and Lipid) dose, in vivo internal, in vitro internal, and Epidemiology internal doses with regard to mean wet weight $Log_{10}(Dose)^1$ by Effect (27 May 2016)

Effect		*In vivo applied dose*	*In vivo internal dose*	*In vitro internal dose*	*Epidemiology internal dose*	*p-value*[2]	*p-value*[2,3]	*95% CI*[4]
Thyroid	**N**	**82**	**17**	**83**	**96**			
	Mean (SD)	**-5.12 (2.2)**	**-5.63 (1.54)**	**-6.76 (1.33)**	**-9.05 (1.12)**		**<0.001**	
	Median	**-4.74**	**-5.57**	**-7**	**-9.18**			
	Range	**-10.59, -2.22**	**-10.51, -3.66**	**-11.3, -4**	**-11, -4**			

Table 3 Contrast between in vivo applied dose (Lipid and Wet), and in vivo internal, in vitro internal, and Epidemiology internal doses with regard to mean Lipid weight $Log_{10}(Dose)^1$ by Effect (27 May 2016)

Effect		*In vivo applied dose*	*In vivo internal dose*	*In vitro internal dose*	*Epidemiology internal dose*	*p-value*[2]	*p-value*[2,3]	*95% CI*[4]
Thyroid	**N**	**82**	**18**		**73**			
	Mean (SD)	**-5.12 (2.2)**	**-6.35 (1.42)**		**-7.18 (0.75)**		**<0.001**	
	Median	**-4.74**	**-6.74**		**-7.17**			
	Range	**-10.59, -2.22**	**-8, -3.06**		**-9, -5.74**			

Table 4. Contrast between in vivo applied dose, in vivo internal dose, and Epidemiology internal dose
with regard to mean Lipid weight $Log_{10}(Dose)^1$ by Effect (17 May 2016)

Effect		**In vivo applied dose**	**In vivo internal dose**	**Epidemiology internal dose**	**p-value**[2]	**p-value**[2,3]	**95% CI**[4]
Thyroid	N	21	18	73			
	Mean (SD)	-6.63 (2.38)	-6.35 (1.42)	-7.18 (0.75)		0.03	
	Median	-5.8	-6.74	-7.17			
	Range	-10.45, -3.51	-8, -3.06	-9, -5.74			

Table 5. Contrast between in vivo applied dose, in vivo internal dose,in vitro internal dose and Epidemiology internal dose with regard to mean wet weight $Log_{10}(Dose)^{1}$ by Effect (7 May 2016)

		Dose						
Effect		*In vivo applied dose*	*In vivo internal dose*	*In vitro internal dose*	*Epidemiology internal dose*	*p-value*[2]	*p-value*[2,3]	*95% CI*[4]
Thyroid	N	61	17	83	96			
	Mean (SD)	-4.6 (1.89)	-5.63 (1.54)	-6.76 (1.33)	-9.05 (1.12)		<0.001	
	Median	-4.22	-5.57	-7	-9.18			
	Range	-10.59, -2.22	-10.51, -3.66	-11.3, -4	-11, -4			

Table 6. Contrast between Life Stage of Exposure and Internal Dose with regard to mean wet weight $Log_{10}(Dose)^{1}$ by Effect (28 May 2016)

Effect		*Perinatal*	*late postnatal*	*Childhood*	*Adult*	*Maternal*	*p-value*[2]	*p-value*[2,3]	*95% CI*[4]
Thyroid	N	8	4	1	56	40			
	Mean (SD)	-6.22 (1.84)	-5.61 (0.06)	-6.98 (.)	-8.52 (1.72)	-9.28 (0.95)		<0.001	
	Median	-5.72	-5.6	-6.98	-8.83	-9.28			
	Range	-10.51, -4.73	-5.68, -5.55	-6.98, -6.98	-10.96, -3.66	-11, -6.88			

Table 7. Contrast between Life Stage of Exposure and Internal Dose with regard to Lipid Weight $Log_{10}(Dose)^{1}$ by Effect (28 May 2016)

Effect		*Perinatal*	*late postnatal*	*childhood*	*adult*	*Maternal*	*p-value*[2]	*p-value*[2,3]	*95% CI*[4]
Thyroid	N	6	3	3	51	22			
	Mean (SD)	-6.99 (0.26)	-3.44 (0.64)	-6.38 (0.22)	-6.96 (0.63)	-7.69 (0.79)		0.001	
	Median	-6.95	-3.08	-6.4	-7.11	-7.8			
	Range	-7.41, -6.74	-4.17, -3.06	-6.6, -6.15	-8.05, -5.74	-9, -6.2			

Table 8. Contrast between Life Stage Exposure, Applied Dose with regard to mean lipid weight $Log_{10}(Dose)^{1}$ by Effect (29 May 2016)

Effect		*perinatal*	*late postnatal*	*childhood*	*adult*	*maternal*	*p-value*[2]	*p-value*[2,3]	*95% CI*[4]
Thyroid	N	6	12	3					
	Mean (SD)	-8.39 (0.89)	-4.8 (0.85)	-10.41 (0.06)				<0.001	
	Median	-8.39	-4.64	-10.45					
	Range	-9.39, -7.39	-6.33, -3.51	-10.45, -10.35					

Table 9. Contrast between Life Stage Exposure, Applied dose with regard to mean wet weight Log_{10}(Dose)[1] by Effect (29 May 2016)

Effect		*perinatal*	*late postnatal*	*childhood*	*adult*	*maternal*	*p-value*[2]	*p-value*[2,3]	*95% CI*[4]
Thyroid	N	45	5	7	4				
	Mean (SD)	-4.04 (1.54)	-4.74 (0.59)	-7.59 (2.16)	-5.55 (0.41)			<0.001	
	Median	-3.74	-4.48	-7.59	-5.55				
	Range	-9.39, -2.22	-5.68, -4.18	-10.59, -4.59	5.91, -5.2				