INTEGRATED EXPOSURE, TOXICOLOGY AND EPIDEMIOLOGY STUDIES: ASSOCIATIONS OF POPS DOSING AMOUNTS, INTERNAL DOSES, LIFE STAGE, AND SEX FOR NEUROTOXIC AND ENDOCRINE EFFECT CATEGORIES

Tom Muir[1*] Joel E Michalek[2] and Ray Palmer[3]

[1.] Environment Canada – Retired. 70 Townsend Ave, Burlington, Ontario, Canada. [2.] Department of Epidemiology and Biostatistics, University of Texas Health Science Center at San Antonio, 314.9 Admin Building, 7703 Floyd Curl Drive, San Antonio TX. [3] Dept. of Family and Community Medicine, University of Texas Health Science Center at San Antonio, 314.9 Admin Building,7703 Floyd Curl Drive San Antonio

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. 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[2, 3]. We further integrated data covering: (1) detailed *in vivo* applied dose exposure protocols; (2) life stage of exposure for the *in vivo* and epidemiology studies; and, (3) sex identification. We stratify this data 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) exposure life stage; and (c) sex.

Methods and Materials

In real time from 2000 to 2010, we have selected 68 relevant POPs *in vitro* (n= 40) and *in vivo* (n= 31) studies, and 53 epidemiological studies. We made the selection to include studies of BFRs, FRs and POPs with published applied dose amounts and protocols for *in vivo* studies, internal dose potencies and specification of the effect. Animal species included mouse, rat, monkey, sheep/lambs, kestrel, rainbow trout, flounder, and fathead minnow. Further, we added 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 added the applied doses, sex, life stage of exposure and dose timing and number to the analysis. 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 aims of this paper we report the results of our analysis for the NTE and DNT effect categories. Thyroid results were reported in 2016[4].

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.

Table 1. Toxicological sample sizes by basis, study type, and effect category

	Wet Weight	Lipid Weight				Wet Weight			
Effect Category	Applied Dose	Epidemiology	*in vivo*	*in vitro*	All	Epidemiology	*in vivo*	*in vitro*	All
DNT	75	21	11	0	11	24	35	66	101
NTE	77	42	12	0	12	32	17	133	150
Thyroid	82	73	18	0	18	96	17	83	100
Total	234	136	41	0	41	152	69	282	351

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

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]*
DNT	N	75	35	66	24			
	Mean (SD)	-5.56 (1.09)	-6.63 (0.71)	-5.26 (0.79)	-8.87 (1.15)		<0.001	
	Median	-5.81	-6.88	-5.19	-8.52			
	Range	-8.4, -3.81	-8.3, -5.69	-8, -3.71	-11.52, -7.35			
NTE	N	77	17	133	32			
	Mean (SD)	-5.47 (1.56)	-6.86 (0.76)	-5.89 (1.06)	-8.24 (1.08)		<0.001	
	Median	-5.33	-6.8	-5.6	-7.95			
	Range	-10.59, -2.22	-8.28, -5.68	-12, -4	-10.55, -6.8			

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

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]*
DNT	N	75	11		21			
	Mean (SD)	-5.56 (1.09)	-5.89 (1.12)		-6.46 (1.13)		0.005	
	Median	-5.81	-5.66		-6.18			
	Range	-8.4, -3.81	-7.59, -4.51		-9, -4.98			
NTE	N	77	12		42			
	Mean (SD)	-5.47 (1.56)	-5.93 (0.79)		-6.79 (1.13)		<0.001	
	Median	-5.33	-5.76		-7.13			
	Range	-10.59, -2.22	-7.59, -4.96		-8.52, -4.73			

Tables 2 and 3 show the wet weight and lipid weight contrasts, respectively, across the dose metrics. There is a consistent increase in the mean dose from epidemiology relative to *in vivo* and *in vitro* (not shown: $p<0.001$). The addition of the AD adds to this increasing trend. Among studies reporting DNT or NTE and

with regard to wet weight applied and internal doses (Table 2), the wet weight mean dose was significantly decreased in epidemiology relative to both *in vivo* and *in vitro* toxicology for all three effect categories. In wet weight, the *in vivo* ADs were not significantly different from the *in vitro* internal dose (DNT p=0.26; 95% CI -0.12 to 0.71: NTE p=0.8; 95% CI -0.87 to 0.03). Corresponding contrasts between toxicology and epidemiology in lipid weight internal doses (Table 3) were in the same direction as the wet weight contrasts, were generally smaller, and did not reach significance for studies expressing any effect category (DNT p=0.34; 95% CI -1.55 to 0.4: NTE p=0.14; 95%CI -1.93 to 0.21). In both effect categories, lipid weight *in vivo* internal dose is not significantly different from the AD, which is administered in whole weight (DNT p=0.62; 95% CI -0.51 to 1.17: NTE p=0.54; 95% CI -0.56 to 1.47). This combined result suggests that the ADs include doses that are environmentally relevant to humans, and that accumulation in lipids presents this. Integrating all lipid wt. results (data not shown), without regard to effect category, the epidemiology dose was significantly less than the *in vivo* dose (95% CI -1.45 to -0.24; p=0.003). This suggests that pooling data may average down significant effects otherwise seen in relevant stratifications. Table 4 show the results for life stage of exposure for wet wt. epi. There is no significant difference between any stage for DNT, however, for NTE, maternal exposure is more sensitive than both childhood (p=0.03), and adult (p<0.001). Table 5 shows results for lipid wt. DNT are only maternal exposure results. For NTE, adult and maternal exposure are almost the same (p=0.95). Maternal and adult compared to childhood are both marginally insignificant (p=0.09), but childhood is a small sample.

Table 4. Contrast between Life Stage of Exposure and Epi Internal Dose with regard to mean wet weight Log$_{10}$(Dose)[1] by Effect

Effect		*Perinatal*	*Childhood*	*Adult*	*Maternal*	*p-value*[2]	*p-value*[2,3]	*95% CI*[4]
DNT	N		6	1	17			
	Mean (SD)		-8.28 (0.64)	-7.4 (.)	-9.17 (1.19)		0.11	
	Median		-8.17	-7.4	-8.82			
	Range		-9, -7.35	-7.4, -7.4	-11.52, -7.59			
	NTE N		3	14	15			
	Mean (SD)		-7.48 (0.14)	-7.64 (0.75)	-8.95 (1.01)		0.001	
	Median		-7.4	-7.63	-9.07			
	Range		-7.64, -7.4	-9.65, -6.8	-10.55, -7.6			

Table 5. Contrast between Life Stage of Exposure and Epi Internal Dose with regard to Lipid Weight Log10(Dose)1 by Effect

Effect		*Perinatal*		*childhood*	*adult*	*Maternal*	*p-value*[2]	*p-value*[2,3]	*95% CI*[4]
DNT	N					21			
	Mean (SD)					-6.46 (1.13)		0.001	
	Median					-6.18			
	Range					-9, -4.98			
NTE	N			3	28	11			
	Mean (SD)			-5.43 (0.13)	-6.86 (1.08)	-6.98 (1.2)		0.09	
	Median			-5.36	-7.23	-7.32			

	Range			-5.59, -5.36	-8.52, -4.73	-8.4, -5.43			

Table 6. Contrast between Life Stage Exposure, In Vivo Internal Dose with regard to mean lipid weight Log_{10}(Dose)[1] by Effect

Effect		*perinatal*	*late postnatal*	*childhood*	*adult*	*maternal*	*p-value*[2]	*p-value*[2,3]	*95% CI*[4]
DNT	N	11							
	Mean (SD)	-5.89 (1.12)							
	Median	-5.66							
	Range	-7.59, -4.51							
NTE	N	12							
	Mean (SD)	-5.93 (0.79)							
	Median	-5.76							
	Range	-7.59, -4.96							

Table 7. Contrast between Life Stage Exposure, In Vivo Internal dose with regard to mean wet weight Log_{10}(Dose)[1] by Effect

Effect		*perinatal*	*late postnatal*	*childhood*	*adult*	*maternal*	*p-value*[2]	*p-value*[2,3]	*95% CI*[4]
DNT	N	35							
	Mean (SD)	-6.63 (0.71)						<0.001	
	Median	-6.88							
	Range	-8.3, -5.69							
NTE	N	10		1	6				
	Mean (SD)	-6.97 (0.57)		-7.09 (.)	-6.63 (1.07)			0.68	
	Median	-6.9		-7.09	-6.27				
	Range	-8.15, -6.28		-7.09, -7.09	-8.28, -5.68				

Note that Tables 6 and 7 internal doses directly reflect the ADs (Tables 2 and 3) used in the *in vivo* experiments, and therefore the life stages at exposure, and the dosing amounts and protocols, chosen and used. The majority of the 234 dose exposure samples were administered at the perinatal life stage (perinatal, 204; childhood, 17; adult 8). Therefore, early life stages are considered in our sample. However, the ADs used are relatively high at the mean (all > 1uM) although there are very high and very low doses used (10 pM to 100 pM low; to 6000 uM high), but even the lowest doses have effects, indicating that the data base contains early life, and low, environmentally relevant doses[4]. There are no significant differences between any life stage for DNT and NTE. Regarding sex (data not reported here), small samples are a problem, especially for *in vivo*, and the M/F category has the highest sample size - this raises questions of the details. For both wet wt. and lipid wt. *in vivo* studies there are no significant differences for any effect category but small samples confound lipid totals. Wet wt. *in vivo* totals show lower internal doses for M/F and M than Females, and M/F and M are the same. For epi, effect categories show no significant difference, but for totals M/F is lower than F ($p=0.02$) and almost lower than M ($p=0.07$).

References

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