Calculated Safety Factors from Integrated Toxicology and Epidemiology POPs Studies Based on Internal Dose

Tom Muir[1], Joel E Michalek[2*], and Raymond Palmer[2]

[1] Environment Canada – Retired, 70 Townsend Ave, Burlington, Ontario, L7T 1Y7, Canada

[2] University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, Texas 78229, USA, michalekj@uthscsa.edu

1 Introduction

Risk assessment (RA) and management (RM) are tools of policy and regulation. RA gives scientific advice on possible threats and RM uses this to propose actions. Exposures in humans are deemed "safe" if doses are below a no effect threshold, or margin of exposure (MOE), compared to doses in experimental animal models, and/or mechanistic or cell based modes of action. The ratio of toxicology over human concentrations is the MOE. Another issue is how to use the growing body of *in vitro* data, and how to account for human susceptibility inadequately/incompletely measured in surrogate systems. It is suggested that toxicokinetic and dynamic factors (e.g., "biomonitoring equivalents" [1, 2]) be estimated and used to scale the animal to human extrapolation of dose-response data used in RA. Our integrative work used in this paper[3] yielded empirical estimates of toxicokinetics and dynamic "scaling" or Calculated Safety Factors (CSF), and their 95% confidence intervals, derived from the multiple species, endpoints, and chemicals data assembled. The purpose of this study is to test our hypothesis that the Margin of Exposure (MOE) method to determine "acceptable dose", as a measure of "safety", is inadequate and potentially harmful because the simple MOE may underestimate human risk especially if the RA is conducted solely using *in vitro* data.

2 Methods and Materials

We conducted a quantitative review of the dose associated with harmful effects *in vivo*, *in vitro*, and in epidemiology by reviewing multiple published reports and articles pertaining to POPs. We arrived at empirical estimates of the relative internal dose in the three study types (*in vivo, in vitro*, and epidemiology), and effect categories [thyroid, non-thyroid endocrine (NTE), developmental and neurological (DNT)], and used these to compute a Calculated Safety Factor (CSF) addressing multiple factors. We selected 74 relevant POPs *in vitro* (n= 42) and *in vivo* (n= 32) studies, and 74 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, sheep/lamb, 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 26 categories), and effect (in multiple categories or markers (n=167), aggregated to DNT (n=44), thyroid (n=53), and NTE (n=70) 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 toxicology with epidemiology and in vivo toxicology with in vitro toxicology with regard to the mean $\log_{10}$ (Molar) using analyses of variance and, for each contrast, a 95% confidence interval for the mean difference (toxicology mean dose minus the epidemiology mean body burden). We provide 95% confidence intervals for the Calculated Safety Factors (CSF), defined as the toxicology effect dose distribution over the epidemiology effect dose distribution, by category of effect and basis of measurement (lipid weight, wet weight).

3 Results and Discussion

Table 1 shows the sample sizes by basis (wet weight versus lipid-adjusted weight dose), study design and effect. We summarized 954 internal dose measurements in all studies (Lipid weight: Epidemiology 273, *in vivo* toxicology 41, *in vitro* toxicology 0, Wet weight: Epidemiology 283, *in vivo* toxicology 71, *in vitro* toxicology 286).

Table 1: Sample Sizes by Basis, Effect, and Dose[1]

Basis	Effect	Epidemiology	Toxicology In vivo	Toxicology in vitro	Total
Wet	DNT	48	35	66	101
	NTE	120	19	134	153
	Thyroid	115	17	86	103
	Total	283	71	286	357
Lipid	DNT	70	11	0	11
	NTE	81	12	0	12
	Thyroid	122	18	0	18
	Total	273	41	0	41

Table 2 shows the CSF for the PBDE epidemiology compared to in vitro studies samples in wet weight, for each effect category, were high, ranging from 3 to 5 orders of magnitude at the CSF mean, and for the 95% CI from 2 to 6 orders, indicating that in vitro studies underestimate the effects of PBDEs indicated in human Epidemiology studies. The CSF for the individual chemical PBDE for DNT in Epidemiology compared to in vitro studies reporting wet weight results varied from 4 to 5 orders of magnitude; mean 156880 (95% CI 37163, 662251), the highest CSF we found in any comparison. For the PCB samples the CSF ranged from 2 to 3 orders and the 95% CI from 1 to 3 orders. For DDE, DDT, and OH-PCB samples the CSFs ranged from over 1 to 2 orders and the 95% CI 1 to almost 4 orders. For PFAS the CSF for thyroid is 14.8 and not significantly different from 1. The 95% CI at the lower bound is less than 1 and the upper bound is 2 orders of magnitude, with p=0.18. All other contrasts shown in Table 2a were significant (p<0.001).

Table 2: Calculated Safety Factor[1] (CSF) and 95% Confidence Interval by Basis and Effect

			Epidemiology		In Vitro Toxicology			
Toxin	*Basis*	*Effect*	*n*	Mean±SD	n	Mean± SD	CSF (95% CI)	p-value
PBDE	Wet	DNT	17	-10.7 ± 0.6	9	-5.5 ± 0.9	156880.8 (37163.5, 662251.9)	<0.001
		NTE	7	-9.9 ± 0.4	41	-5.6 ± 0.8	20617.7 (5131.2, 82844.4)	<0.001
		Thyroid	27	-9.2 ± 0.9	9	-5.8 ± 0.9	2424 (504.9, 11636.5)	<0.001
		All[2]	51	-9.8 ± 1	59	-5.6 ± 0.8	14320.7 (6535.8, 31378.1)	<0.001
PCB	Wet	DNT	10	-8.4 ± 0.6	37	-5.3 ± 0.9	1268.7 (318.9, 5046.4)	<0.001
		NTE	22	-8.6 ± 0.6	3	-5.6 ± 0.5	943.2 (163.5, 5442.7)	<0.001
		Thyroid	44	-8.9 ± 1.1	9	-6.9 ± 1.2	101.1 (15.4, 665)	<0.001
		All[2]	76	-8.7 ± 0.9	49	-5.6 ± 1.1	1460.2 (628, 3395.1)	<0.001
			Epidemiology		In Vitro Toxicology			
Toxin	Basis	Effect	n	Mean±SD	n	Mean± SD	CSF (95% CI)	p-value
DDE	Wet	NTE	6	-7.8 ± 0.5	4	-5.6 ± 0.7	157.6 (20.8, 1195.5)	<0.001
DDT	Wet	NTE	6	-7.4 ± 0.2	3	-5.4 ± 1	102.1 (11.7, 893.1)	
OH-PCB	Wet	Thyroid	13	-9.9 ± 0.8	15	-7.1 ± 2	587 (36.5, 9446.3)	<0.001
PFAS	Wet	DNT	16	-7.9 ± 0.4	0			
		NTE	60	-8.3 ± 0.7	1	-3.8 ± .		
		Thyroid	6	-7.5 ± 0.2	4	-6.3 ± 2	14.8 (0.2, 993.1)	0.18
		All[2]	82	-8.2 ± 0.7	5	-5.8 ± 2	236.8 (43.2, 1297.6)	<0.001

CSFs comparing PBDE Epidemiology with in vivo for DNT, NTE, Thyroid and combined effects (labelled as "All") in lipid weight (Table 3) were low, with all CSFs, except Thyroid at 139, below 100 as an example default value, ranging from 15 to 45. The 95% CI values at the lower bound ranged from 2.6 to 30.9, raising concerns for the highest exposures and the most sensitive.

Table 3: Lipid Weight Calculated Safety Factor[1] (CSF) and 95% Confidence Interval by Effect for In vivo Toxicology versus Epidemiology

			Epidemiology		In Vivo Toxicology			
Toxin	Basis	Effect	n	Mean±SD	n	Mean± SD	CSF (95% CI)	p-value
PBDE	Lipid	DNT	54	-7.9 ± 0.9	6	-6.7 ± 0.7	15.4 (2.6, 90.4)	0.003
		NTE	33	-7.5 ± 0.6	9	-6.2 ± 0.7	20.9 (7.1, 61)	<0.001
		Thyroid	58	-8.2 ± 0.7	8	-6 ± 1.8	139 (30.9, 624.4)	<0.001
		All[2]	145	-7.9 ± 0.8	23	-6.3 ± 1.2	45.1 (18.9, 107.6)	<0.001
	Wet	DNT	17	-10.7 ± 0.6	20	-6.3 ± 0.6	22324.7 (8674.9, 57452.5)	<0.001
		NTE	7	-9.9 ± 0.4	10	-6.8 ± 0.8	1492.4 (288.3, 7723.8)	<0.001
		Thyroid	27	-9.2 ± 0.9	10	-5.6 ± 2	3520.4 (406.5, 30487.1)	<0.001
		All[2]	51	-9.8 ± 1	40	-6.3 ± 1.2	3325.3 (1168.3, 9464.7)	<0.001
	All	All[2]	196	-8.4 ± 1.2	63	-6.3 ± 1.2	137 (63.1, 297.2)	<0.001
PCB	Lipid	DNT	11	-6 ± 0.6	2	-5.2 ± 0.2	6.3 (0.6, 65.5)	0.11
		NTE	28	-6.8 ± 1				
		Thyroid	47	-7.1 ± 0.7				
		All[2]	86	-6.9 ± 0.8	2	-5.2 ± 0.2	50.4 (3.2, 783.6)	0.006
	Wet	DNT	10	-8.4 ± 0.6	8	-7.3 ± 0.5	10.9 (2.9, 41.5)	0.002
		All[2]	96	-7 ± 0.9	10	-6.9 ± 1	1.3 (0.3, 5.6)	0.69

Table 4 summarizes the CSF for chemicals in Epidemiology compared to combined in vitro and in vivo Toxicology. Given the lack of in vitro lipid weight data, results for PBDE lipid weight are the same as those in Table 2b. CSFs for PBDE wet weight were 3 to 4 orders of magnitude for all effect categories; the 95% CI ranged from 2 to 5 orders when individual effect categories considered and 3 to 4 orders when all effects were combined. PCB wet weight CSFs were 2 orders of magnitude at the mean, and the 95% CI ranged from 1 to 3 orders. Thyroid effect category exhibited the lowest CSF of approximately 100 and a 95% CI ranging from 15 to 665. For all effect categories combined the CSF was 2 orders of magnitude and the 95% CI ranged from 2 to 3 orders. The wet weight CSF for DDE, and OH-PCB were the same as in Table 2a, as expected. The CSF for DDT in NTE was 15.2 (95% CI 0.7 to 310), p=0.07. All other contrasts in Table 3a were significant (p<0.001).

Table 4: Calculated Safety Factor[1] (CSF) and 95% Confidence Interval (95% CI) by Toxin Category, Basis and Effect in Toxicology versus Epidemiology for PBDE and PCB Internal Dose

			Epidemiology		Toxicology			
Toxin	Basis	Effect	n	Mean±SD	n	Mean± SD	CSF (95% CI)	p-value
PBDE	Lipid	DNT	54	-7.9 ± 0.9	6	-6.7 ± 0.7	15.4 (2.6, 90.4)	0.003
		NTE	33	-7.5 ± 0.6	9	-6.2 ± 0.7	20.9 (7.1, 61)	<0.001
		Thyroid	58	-8.2 ± 0.7	8	-6 ± 1.8	139 (30.9, 624.4)	<0.001
		All[2]	145	-7.9 ± 0.8	23	-6.3 ± 1.2	45.1 (18.9, 107.6)	<0.001

			Epidemiology		Toxicology			
Toxin	Basis	Effect	n	Mean±SD	n	Mean± SD	CSF (95% CI)	p-value
	Wet	DNT	17	-10.7 ± 0.6	29	-6.1 ± 0.8	40886.6 (14265.6, 117185)	<0.001
		NTE	7	-9.9 ± 0.4	51	-5.9 ± 0.9	12320.8 (2513.3, 60399)	<0.001
		Thyroid	27	-9.2 ± 0.9	19	-5.7 ± 1.5	2950 (573.8, 15167.2)	<0.001
		All[2]	51	-9.8 ± 1	99	-5.9 ± 1	7938.6 (3594.3, 17533.8)	<0.001
	All	All[2]	196	-8.4 ± 1.2	122	-6 ± 1.1	276.5 (152.8, 500.2)	<0.001
PCB	Wet	DNT	10	-8.4 ± 0.6	45	-5.6 ± 1.2	544.8 (95.6, 3104)	<0.001
		NTE	22	-8.6 ± 0.6	3	-5.6 ± 0.5	943.2 (163.5, 5442.7)	<0.001
		Thyroid	44	-8.9 ± 1.1	9	-6.9 ± 1.2	101.1 (15.4, 665)	<0.001
		All[2]	76	-8.7 ± 0.9	57	-5.8 ± 1.2	832.1 (355.7, 1946.4)	<0.001
DDE	Wet	NTE	6	-7.8 ± 0.5	4	-5.6 ± 0.7	157.6 (20.8, 1195.5)	
DDT	Wet	NTE	6	-7.4 ± 0.2	5	-6.3 ± 1.4	15.2 (0.7, 309.8)	0.07
OH-PCB	Wet	Thyroid	13	-9.9 ± 0.8	15	-7.1 ± 2	587 (36.5, 9446.3)	<0.001

4 Conclusions

Overall, these results data suggest a systematic toxicology versus epidemiology difference to the detriment of regulatory agency efforts to establish standards for safety in people.

5 References

1. Hayes S, Becker R, Leung A, Aylward L, Pyatt D. 2007. *Regulatory Toxicology and Pharmacology* 47 (2007) 96–109
2. Rhomberg L, and Lewandowski T. 2004. Gradient Corporation, Prepared for Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, DC. Contract 3W-0477-NASX
3. Muir, Tom, Michalek, Joel E and Palmer, Raymond F. "Determination of safe levels of persistent organic pollutants in toxicology and epidemiology" *Reviews on Environmental Health*, vol., no., 2022. https://doi.org/10.1515/reveh-2021-0105