INTEGRATING TOXICOLOGY AND EPIDEMIOLOGY FOR RISK ASSESSMENT

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Abstract

Risk assessment (RA) is a tool of policy and regulation. Presently, RA policy is to assess individual chemicals in isolation. Use is allowed if exposures are below some estimated "no effect" concentration, or "margin of exposure" (MOE). The MOE does not usually account for measurement uncertainty and "safety", or modulating factors such as: *in utero* and infant exposure; complex mixtures additivity and synergism; and polymorphisms. "Default uncertainty factors" exist, however, these are subjective, not empirical, and appear to be used arbitrarily, or only in legally enforceable Reference Dose (RfDs), or related standards, e.g., in the U.S. The MOEs are often reported as large, but the ignored uncertainty and safety factors may be even larger. Exploring this uncertainty quantitatively, by assessing potency variance within and between toxicological and epidemiological studies, found toxicology-epidemiology discordance, and *in vitro – in vivo* toxicology discordance. There is an apparent trend of increasing potency, with *in vitro* < *in vivo* < epidemiological. Contrasts in log units yielded one to four order of magnitude differences in means between these study types. *In vitro* data contrasts with epidemiology for DNT by 3 to 4 orders of magnitude and *in vivo* data contrasts with epidemiology for DNT by more than two orders of magnitude. The relation between log dose and study type changes with lipid weight versus wet weight basis.

Introduction

The single chemical in isolation risk assessment (RA) process can lead to an unlimited number of chemicals, in high volume use and subsequent environmental and human exposure. One example is an observed proliferation of Brominated Flame Retardants (BFRs), other Flame Retardants (FRs) and degradation products widely detected in the environment, for example, in Great Lakes Herring Gulls, humans, and globally – referred to as Pandora's Box. This process includes no rigorous attempt at hypothesis testing, leading to inappropriately large Type II Error and repetitive acceptance of the zero risk null over the alternative harm hypothesis. The assumptions are that a) toxicological models predict the observed outcome in epidemiological studies and b) protecting the adult animal protects the fetus and neonate. Evidence of additive or greater effects from the real world mixtures exposures is ignored. It is also implicitly assumed that RA describes a pure risk proposition, a proposition that we believe is false. We do not know all the outcomes or endpoints, all the mechanisms, or the outcome probabilities, and there may in fact be novelties. This makes RA inherently uncertain. The aim of this paper is to explore RA uncertainty quantitatively by assessing potency variance within and between toxicological and epidemiological studies to address the possible integration of toxicological and epidemiological data in the RA process[1]. In this context we discuss implications for risk assessment and management.

Materials and Methods

We integrate detailed micro information to get a macro description of relations between exposures and endpoints and assess the statistical significance of variation in the results with study type (toxicology, epidemiology), basis (wet, lipid), and endpoint (endocrine, developmental neurotoxicity (DNT), thyroid). We selected 45 relevant BFR and related POPs *in vitro* (n=26) and *in vivo* (n=19) toxicology studies, and 19 epidemiological studies (Muir and Michalek, manuscript in preparation) [2 to 36]. We made the selection to include studies of BFRs and POPs with published potencies and specification of the effect. We expressed the potencies in all studies in a common molar metric of internal dose expressed in log base 10. Studies that did not provide internal dose estimates, but only applied doses, were not used. Often the epidemiological effects data were reported as ranges, or as cut-off points, or as certain percentiles, or as point estimates; we used the maximum, minimum and mean when available. With

one exception the epidemiological data were for legacy POPs, because we found no suitable BFR epidemiological studies during our data collection phase. The in vivo toxicology data were also reported effects ranges, or point estimates, or Bench Mark Dose Lower confidence limits (BMDLs); we used all of these. Species included mouse, rat, monkey, kestrel, frog, zebra fish, rainbow trout, flounder, and fathead minnow. Life stage of exposure varied. The in vitro toxicology studies reported significant effect ranges, point estimates, EC50, IC50, LOEC, EC2X, Kd, Ki, RIC20, REC20; we used all of these, however, we did not use reported EC01-10 for xenoestrogens. Our intent was to select studies that examined a wide range of BFRs and legacy POPs.

We stratified the data by basis (lipid weight, wet weight), study (in vivo toxicology, in vitro toxicology, epidemiology), chemical (in multiple categories), and effect (in multiple categories, reduced to DNT, thyroid, and endocrine due to sample size constraints). Most of the epidemiological studies used wet weight (described as "whole weight") although some used lipid weight or both lipid and wet weight measurements. If the lipid weight measure was not given and the lipid percent for the matrix was reported, we used the lipid percent to estimate the lipid weight result. Few of the in vivo studies provided lipid weight estimates. We contrasted toxicology with epidemiology and in vivo toxicology with in vitro toxicology with regard to the mean $\log_{10}$ (dose) using one-way analyses of variance and, for each contrast, displayed the Mahalanobis distance, defined as the mean difference divided by the square root of the mean squared error. All statistical testing was two-sided with a significance level of 5%. We used SAS Version 9.1.3 for Windows (SAS Institute, Cary, NC) for all statistical analyses and graphics.

Results and Discussion

Table 1 shows the sample sizes by basis, study design, and effect category for the all toxin chemical category.

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
Acute developmental toxicity	0	0	0	0	3	0
Acute toxicity	0	0	0	0	2	0
DNT	13	6	0	17	27	24
Endocrine	5	5	0	8	6	53
Thyroid	0	2	0	21	7	42
Total	18	13	0	46	45	119

Tables 2, and 3, show contrasts between toxicological and epidemiological studies with regard to mean Log base 10 molar internal doses or body burdens. Table 2 shows that wet weight in vivo studies report effect concentrations that are significantly more than an order of magnitude higher (15X to 21X) in mean or median measure than the epidemiological results. Table 2 also shows that the lipid weight contrast reverses this difference, and shows that the lipid metric observed in epidemiological studies is higher than the in vivo toxicological results, although the difference is insignificant by conventional measures. This result is interesting because it raises the question, on empirical grounds, of what is the appropriate and toxicologically relevant dose metric, wet or whole weight, or lipid corrected weight. At the least, target tissue hydrophobic binding site abundance influences chemical accumulation to concentrations not reflected in the wet or whole weight averaging that loses detailed variance information and adds uncertainty. As well, the epidemiological studies usually reflect hydrophobic POPs mixtures exposures, several life stages, and susceptibilities.

Table 2. Contrasts between in vivo Toxicological and Epidemiological Studies with regard to mean Log_{10} (Dose or Body Burden)

Basis		Study Design In vivo Toxicological	Epidemiological	p-value[2]	Distance[3]
Lipid Weight	N	13	18	0.16	-0.52
	Mean (SD)	-6.31 (1.13)	-5.85 (0.64)		
	Median	-6.27	-5.89		
	Range	-8.55, -4.17	-6.85, -4.73		
Wet Weight	N	45	46	<0.001	1.02
	Mean (SD)	-7.25 (1.24)	-8.43 (1.07)		
	Median	-7.2	-8.52		
	Range	-10, -3.66	-10.15, -4		

Table 3. Contrasts between in vitro Toxicological and Epidemiological Studies with regard to mean Wet Weight Log_{10} (Dose or Body Burden)

Basis		Study Design in vitro Toxicological	Epidemiological	p-value[2]	Distance[3]
Wet Weight	N	119	46	<0.001	1.65
	Mean (SD)	-6.14 (1.49)	-8.43 (1.07)		
	Median	-6	-8.52		
	Range	-12, -3.71	-10.15, -4		

Table 3 shows a significantly increased potency in epidemiological studies of more than two orders of magnitude (195X to 331X) greater than the effects concentrations of in vitro.

Figure 1 shows the lipid weight and wet weight contrast in Table 2 graphically, with the lipid weight epidemiology generally plotted to the right of the lipid weight in vivo toxicology and the wet weight epidemiology clearly to the left of in vivo toxicology. This reversal in relative location is statistically significant ($p<0.001$). In other words, the relation between log dose and study changes with basis ($p<0.001$). Also interesting is the intersection or overlap between the two at about 1 uM, below which about 40 to 60% of the results fall.

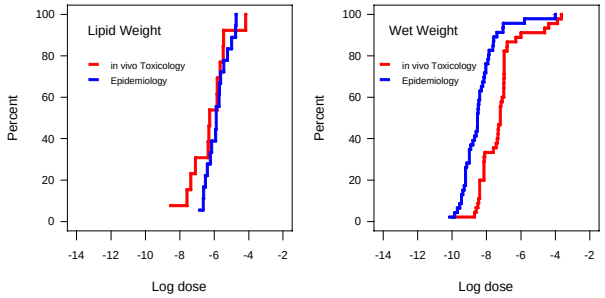

Figure 1: Lipid and wet weight distribution functions (in vivo toxicology versus epidemiology); the percent of values less than or equal to the abscissa is given on the vertical axis.

It is important to consider some results that reflect the endpoint or effect reported. Table 4 shows that the in vitro sample for the DNT comparison is 3 to 4 orders of magnitude higher in effect concentration than the epidemiological reports (mean at 3,890X; median 3,548X). The distance M is 3.94 or 8,710X for the mean.

Table 4 Contrasts between in vitro Toxicological and Epidemiological Studies on mean wet weight Log_{10} (Dose or Body Burden) for Studies Exhibiting DNT, Endocrine, and Thyroid Effects

		Study Design			
Effect		in vitro Toxicological	Epidemiological	p-value[2]	Distance[3]
DNT	N	24	17	<0.001	3.94
	Mean (SD)	-5.16 (1.03)	-8.75 (0.7)		
	Median	-5.15	-8.7		
	Range	-8, -3.71	-10.15, -7.59		
Endocrine	N	53	8	0.001	1.29
	Mean (SD)	-6.07 (1.36)	-7.74 (0.63)		
	Median	-6	-7.62		
	Range	-12, -4	-8.8, -7.03		
Thyroid	N	42	21	<0.001	1.11
	Mean (SD)	-6.77 (1.58)	-8.44 (1.32)		
	Median	-7	-8.54		
	Range	-11.3, -4	-9.85, -4		

The data in Table 4 are graphically displayed in Figure 2. Separation between in vitro toxicology and epidemiology is evident in all three-effect categories.

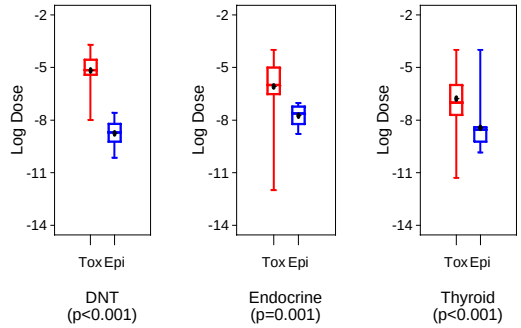

Figure 2. Contrasts between in vitro Toxicological and Epidemiological Studies on mean wet weight Log_{10} (Dose or Body Burden) for Studies Exhibiting DNT, Endocrine, and Thyroid Effects. Whiskers extend to the maximum and minimum, rectangles are determined by quartiles, and the mean is indicated with a dot.

The results presented here are new data that reflect an empirical analysis of published toxicological and epidemiological data to quantitatively describe measurement uncertainty in assessments of risk and safety, particularly based on margin of exposure concepts. To our knowledge, this is the first time that collections of toxicological and epidemiological data have been organized and analyzed this way, in the same molar metric and as internal dose, and the first empirical display of measurement uncertainty in potency. Such data can supplant the "default uncertainty factors" that exist, but are, however, subjective, not empirical, and appear to be used arbitrarily, or only in legally enforceable Reference Dose (RfDs), or related standards, e.g., in the U.S.

The results support the hypotheses of toxicology-epidemiology discordance, and *in vitro – in vivo* toxicology discordance; there is an apparent trend of increasing potency, with in vitro studies exhibiting the lowest, in vivo toxicology studies exhibiting intermediate, and epidemiological studies exhibiting the highest potency. Various contrasts yielded one to almost four order of magnitude differences in means between these study types in the direction indicated. In particular, *In vitro* data contrasts with epidemiology for DNT by 3 to 4 orders of magnitude and *In vivo* data contrasts with epidemiology for DNT by more than two orders of magnitude.

The epidemiological results of observed effects at generally lower internal doses reflect several modulating factors, including *in utero and infant* exposure, complex mixtures, polymorphisms, induced susceptibility, and metabolism (such as pharmacokinetics in infants). Variation in these factors will contribute to variance in the measured potencies. Previous studies have shown, for example, that mixtures additivity and synergism can add from 10 to 1000-fold uncertainty[30, 37]. Accounting for the dose per unit weight to the fetus as compared to newborn through 12 months of life, adds another 100 to 1000- fold factor[37]. Uptake and metabolism by newborns can add a 4 to 10 factor to internal dose.[38,39] Polymorphisms might account for another 2 to 10 factor. RA has many sources of uncertainty to account for, and an apparent inherent limit of at least 5 or 6 orders of magnitude in the precision with which estimates of safety and risk can be made. At present, basically none of these factors and uncertainties is explicitly or visibly accounted for in general RA practice.

To the extent that RA continues as single chemical exercises, and does not account for measurement uncertainty in estimates of risk and safety, the evidence and analysis presented here supports the idea that any MOE estimate derived using present practices must be divided by at least 5 to 6 orders of magnitude to determine whether or not

the chemical use and presence in the environment, human and wildlife exposure, and body burdens are acceptable. This may provide a reasonable possibility that all the stages and susceptibilities of human and environmental health and life can be protected.

References

1. Ritter L, Arbuckle T. 2007 *Tox Sci.*, Advance Access, January 18.
2. Kelce W, Stone C, Laws S, Gray E, Kemppainen J, Wilson E. 1995. *Nature* 375, 581-585.
3. Hamers T, Kamstra J, Sonneveld E, Murk A, Kester A, Andersson P, Legler J, Brouwer A. 2006. *Toxicol Sci* 92(1), 157-173.
4. Pacyniak E, Cheng X, Cunningham M, Crofton K, Klaassen C, Guo G. 2007. *Toxicol Sci* 97(1) 94-102.
5. Kodavanti P. 2005; *Dose-Response*, 3, 273-305.
6. Birnbaum SG, Yuan P.X, Wang M, Vijayraghavan S, Bloom A.K, Davis DJ., Gobeske K.T, Sweatt JD, Manji HK, and Arnsten AFT. 2004. *Science* 306, Issue 5697, 882-884.
7. Canton R, Sanderson T, Letcher R, Bergman A, van den Berg M. 2005. *Toxicol Sci* 88, 447-55.
8. Eriksson P, Fischer C, Fredriksson A. 2003. *Organohalogen Compounds*, Vol.61, 81-83.
9. Schantz S, Widholm J, Roegge C, Powers B. 2003. *Organohalogen Compounds*, Vol. 65, pp. 16-19.
10. Bemis, J.C., and Seegal, R.F. 1999. *Environ. Health Perspec.* Vol. 107, Number 11, 879-885.
11. Fischer C, Fredriksson A, Eriksson P. 2004. *Organohalogen Compounds*, Volume 66, 3160-3162.
12. Eriksson P, Fischer C, Wallin M, Jakobsson E, Fredriksson A. 2006. *Env. Tox Pharmac*., 21, 317-322.
13. van der Ven L, Verhoef A, van de Kuil T, Slob W, Leonards P, Visser P, Hamers T, Herlin M, Ha°kansson H, Olausson H, Piersma A, and Vos J. 2006. *Toxicol Sci* 94(2), 281-292.
14. Kuriyama, S., Talsness, C., Grote, K., Ibrahim, C. 2005. *Environ Health Perspect*. 113: 149-154.
15 Silva, E., Scholze, M., Kortenkamp, A., 2007. *Environ Health Perspect*. Vol. 115, S-1, December.
16. Jacobson, J.J., and Jacobson, S.W. 1996. *The New England Journal of Medicine*, 335, Number 11, September 12, 783-789.
17. Weiss, J. 2006. *PhD Thesis*, Dept. of Environmental Chemistry, Stockholm University, Sweden.
18. Stewart, P., Reihman, J., Lonky, E., Darvill, T., Pagano, J. 2000. Neurotox and Terat, 22; 21-29.
19. Ribos-Fita N, Torrent M, Carrizo D, Munoz-Ortiz L, Julvez J, Grimalt J, Sunyer J. 2006. *Am. J. Epi. A*dv. Access. September 12.
20. Ribos-Fita N, Torrent M, Carrizo D, Julvez J, Grimalt JO, Sunyer J. 2007. *Env. Health Perspec*t. 115(3) 447-450.
21. Cohn, B., Wolff, M., Cirillo, P., Sholtz, R. 2007. *Environ. Health Perspect. 10260*, On-line 24 July, 2007.
22. Perry M, Ouyang F, Korrick S, Venners S, Chen C, Xu X, Lasley B, Wang X. 2006. *Am. J. of Epidemiology*, Adv Access, Oct. 5.
23. Persky, V., Turyk, M., Anderson, H., Hanrahan, L., Falk, C., Steenport, D., Chatterton, Jr., R., Freels, S, and Great Lakes Consortium. 2001. *Environ. Health Perspect.*Vol. 109, No. 12).
24. Rice DC. 1999. *Environmental Research* Section A, 80, S113-S121.
25. Fernie K, Shutt J, Mayne G, Hoffman D, Letcher R, Drouillard K, Ritchie I. 2005. *Toxicol Sci* 88(2), 375-383.
26. Bahn, A, Bialik, Oler, J., Houton, L., Landau, E. 1980. *U.S. EPA* 560/6-80-001).
27. Wania F. 2004. *Proceedings of BFR 2004*. 221-224.
28. Gouin T, Harner T. 2003. *Environment International* 29, 717-724.
29. Seegal, R. 1999. *Environmental Research*, Volume 80, No. 2; S38-45.
30. Fischer, C. 2008. *PhD Dissertation.* Uppsala University, Sweden.
31. Eriksson P, Johansson N, Fredriksson A.2006. *Organohalogen Compound*s 68.
32. Bemis, J.C., and Seegal, R.F. 1999. *Environ. Health Perspec.* Vol. 107, Number 11, 879-885.
33. Rajapaske N, Silva E, Kortenkamp A. 2002. *Environmental Health Perspectives* Volume 110, Number 9, September, pp. 917-921.
34. Crofton K, Craft E, Hedge J, Gennings C, Simmons J, Carchman R, Carter Jr,W, DeVito M. 2005. *Environmental Health Perspectives*, on-line July, 2005.
35. Metzdorf I, Dalgaard M, Christiensen S, Axelstad M, Hass U, Kiersgard M, Scholze M, Kortenkamp A, Vinggaard AM. 2007. *Tox Sci*, Advance Access, April 19.
36. Schantz S, Widholm J, Roegge C, Powers B. 2003. *Organohalogen Compounds*, Vol. 65, pp. 16-19.
37. Muir T. 2008. *Organohalogen Compounds*, Vol. 70.
38. Edginton AN, and Ritter L. 2009. *Environmental Health Perspectives*, On-line, March 1.
39.Kodama H, and Ota H. 1980. *Archives of Environmental Health*, Vol. 35, No. 2, pp. 95-100.