Review

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# Determination of safe levels of persistent organic pollutants in toxicology and epidemiology

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**Abstract:** We reviewed published manuscripts from toxicology and epidemiology reporting harmful health effects and doses of persistent organic pollutants (POPs), published between 2000 and 2021. We found 42 *in vitro*, 32 *in vivo*, and 74 epidemiological studies and abstracted the dose associated with harm in a common Molar unit. We hypothesized that the dose associated with harm would vary between animal and human studies. To test this hypothesis, for each of several POPs, we assessed the significance of variation in the dose associated with a harmful effect [categorized as non-thyroid endocrine (NTE), developmental neurotoxicity (DNT), and Thyroid] with study type (*in vitro, in vivo*, and Epidemiology) using a linear model after adjustment for basis (lipid weight, wet weight). We created a Calculated Safety Factor (CSF) defined as the toxicology dose divided by epidemiology dose needed to exhibit significant harm.

Significant differences were found between study types ranging from <1 to 5.0 orders of magnitude in the dose associated with harm. Our CSFs in lipid weight varied from 12.4 (95% confidence interval (CI) 3.3, 47) for NTE effects in Epidemiology relative to *in vivo* studies to 6,244 (95% CI 2510, 15530) for DNT effects in Epidemiology relative to *in vitro* in wet weight representing 12.4 to 6.2 thousand-fold more sensitivity in people relative to animals, and mechanistic models, respectively. In lipid weight, all CSF 95% CI lower bounds across effect categories were less than 6.5. CIs for CSFs ranged from less than one to four orders of magnitude for *in vivo*, and two to five orders of magnitude for *in vitro* vs. Epidemiology. A global CSF for all Epidemiology vs. all Toxicology was 104.6 (95% CI 72 to 152), significant at $p<0.001$.

**Keywords:** endocrine disruptors; experimental, mechanistic and animal studies; flame retardants; human health studies – epidemiology; neuro-development disruption; persistent organic pollutants; risk assessment; sex steroid disruption; thyroid disruption.

## Background

Risk assessment (RA) and management are essential tools of regulatory policy. However, the scientific approach used follows assumptions and regulatory guidance that determine what scientific aspects are examined and which ones are not. RAs for persistent organic pollutants (POPs) are being challenged by diverse opinions in emerging science calling for reform by institutional and other advisory groups [1, 2]. The view that traditional chemical RA faces multiple challenges in predicting and preventing disease and is discordant with human environmental health monitoring is widely held [1–3]. In the United States, the Toxic Substances Control Act (TSCA), enacted in 1976, considered approximately 62,000 chemicals as "existing" and not subject to testing or regulation unless proven to "present(s) an unreasonable risk of injury to health or the environment [1, 2]." A more recent report puts the number of chemicals at 83,000 and states that the TSCA laws are outdated and need reform [4].

This study originated in response to the emerging issue of exponentially increasing trends in the environmental presence and human body burdens of brominated flame retardants (BFRs), and a particular sub-group, the polybrominated diphenyl ethers (PBDEs) [5]. These trends were emerging parallel to an expressed increase in prevalence and burden of thyroid and neurodevelopmental deficits [5].

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

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solely using *in vitro* data. To address this hypothesis, 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 [6–20].

# Methods

We examined variation in the dose associated with harm between Toxicology and Epidemiology and, if present, characterized its source and magnitude. We assembled a database of applied dose, internal dose, and response effect data from toxicological (*in vitro* and *in vivo*) and Epidemiological studies reporting significant effects pertaining to a range of POPs. We subsequently assessed the significance of variation in the dose required to produce harm within and between these studies. For brevity, we summarize only internal dose results in this paper. Applied dose results will be shown in a subsequent manuscript.

Using the internal dose distributions of each study type, we estimated the mean and 95% confidence intervals for our CSFs, by category of effect (NTE, DNT, Thyroid) and basis of measurement (lipid weight, wet weight). We converted all reported *in vivo* doses and Epidemiological body burdens to Molar units prior to computation. We report the mean and standard error of the dose in Molar units by study type, and basis (wet weight, lipid weight) and effect category. CSF was calculated as the ratio of the mean Toxicology dose to the mean Epidemiological body burden associated with a harmful effect. All data were $\log_{10}$ transformed prior to analysis; doses are reported in $\log_{10}$ and CSF is in original units.

The Epidemiology studies included birth cohorts, prospective, longitudinal, and adult cross-sectional designs, and covered all age groups. In the Thyroid category, of 35 effects reported, 30 were thyroid hormone complex disruption, and five presented as thyroid disease. In the NTE category, of 40 effect markers, 23 reflected sex steroid disruption and/or related morbidity, and 17 were frank disease. In the DNT effect category there were 29 effect markers and all could be considered clinically presented neurobehavioral development issues, such as ADHD, IQ loss, hyperactivity, and numerous verbal/memory/attention deficits [Supplement References 21–72, 137–160]. Life stage data will be summarized in a separate manuscript. All lipid weight calculations were considered adjusted due to known complexities of lipid composition.

*In vivo* studies were dominated by the BFRs, with only two studies covering PCBs, and two for DDT. Of 32 studies reviewed, 21 were published between 2005 and 2014, and two were published before 2001 [Supplement References 73–103, 150]. The studies were a mix of acute, intermediate, and chronic exposures. The *in vivo* toxicology doses were reported as effects ranges, or point estimates, or Benchmark Dose Lower confidence limits (BMDLs); we used all of these in the calculations and transforms. Species included: monkey [74]; mouse [75–81, 150]; rat [83–93]; kestrel [94–98]; rainbow trout [99–100]; flounder [101]; fathead minnow [102]; and sheep/lamb [103].

Life stage of exposure varied, including; *in ovo* (kestrel); *in utero* (rat, sheep); perinatal (mouse, rat, kestrel); juvenile (flounder, rainbow trout); 7 weeks (rat); 11 weeks (rat); adult (fathead minnow); birth to 20 weeks (monkey). Dose matrices included brain, liver, carcass, whole egg, adipose, muscle, plasma, whole blood, and corporal body burden.

The *in vitro* studies were largely from government agency funded research programs stimulated by steadily rising levels of BFRs in the North American, European, and Asian environments, including fish, wildlife, and humans, with accompanying concerns about their persistence, bioaccumulation, and toxicity (see www.bfr2013.com for study abstracts from 2001) and reported effect ranges, point estimates, and multiple indices of effective or inhibitory concentrations, and pharmacokinetic constants (EC50, IC50, EC1 to EC10, LOEC, EC2X, $K_d$, $K_i$, RIC20, REC20; see list of abbreviations); we used all of these. For the DNT markers, all 11 reflected mechanistic or major modes of action aspects of the lower rungs of the translation ladder. For example, genes, molecules, enzymes, receptors, cells, circuits and pathways for nervous system neurotransmitters, intracellular signaling, calcium homeostasis, and altered protein kinase C translocation and signaling [9–15; Supplement References 104–136].

The PCB data were examined to account for dioxin-like activity regarding ortho and non-ortho chemical differences by TEF. We document Epidemiology and *in vitro* studies of PCB exposure by congener, metabolites, groups, and mixtures, the latter two of which might have included mono-ortho and co-planar dioxin-like congeners. We limited *in vivo* studies of PCBs to two studies, one using pure congeners (PCB 28, 52, 153) in mice [Supplement References 75], and the other a custom mixture in monkeys [Supplement References 74] which included five mono-ortho congeners (PCB 105, 118, 156, 157, 189), constituting 24% of the mixture.

The data in these studies were reported as internal doses which we converted to Molar units by computing molecular weights (MW) where the congeners or homologues, or pure chemical, were given. Molarity (molar, M) is defined as moles/Liter (L), assuming a tissue density of water, moles/L = moles/kg. We report Internal dose effect concentrations in weight metric (expressed in terms of L or kg)/MW = molar metric M. Thus, based on transforms by physical-chemical constants, all the study concentration results are expressed in a common, uniform metric, M.

For studies reporting technical formulations, or other mixture chemicals, particularly total PCBs, we derived estimates of weighted MW based on relevant reported congener or homologue proportions in related studies. For Stewart et al. [Supplement References 22, 45], we used converging evidence [Supplement References 72] looking at the homologue proportions of the maternal cohort in studies of Lake Ontario salmon consumption, compared with maternal breast milk, and umbilical cord blood in newborns, to derive an estimated weighted MW of 350. For Jacobson et al. [Supplement References 21], Persky et al. [Supplement References 48], Schantz et al. [Supplement References 60], and Karmaus et al. [Supplement References 43], all Lake Michigan fish-eater cohorts, we used the Schantz and Widholm [Supplement References 73] reported serum PCB congener proportions data on Lake Michigan fish eaters, which yielded a weighted MW of 369. For Hertz-Picciotto et al. [Supplement References 35], reporting on maternal serum total PCBs, we used an MW of 353 from Schantz and Widholm [Supplement References 73], corresponding to an estimate of the actual congener distribution in human milk used by Rice [Supplement References 74] in a monkey study. For Brucker-Davis et al. [Supplement References 32], we used weighted MW 353 for total PCBs in maternal milk colostrum.

## Statistical methods

Significance was assessed with a fixed effects linear model of $\log_{10}$ dose in terms of study type and differences of least square means and their standard errors stratified by basis. Sample sizes, means, standard errors, and mean differences and their 95% confidence intervals and associated p-values are reported. The GLM procedure in SAS was used. The Tukey method was applied to correct for multiple pairwise comparisons within tables exhibiting multiple pairwise contrasts. All statistical testing was two-sided with a nominal experiment-wise significance level of 5%. SAS Version 9.4 for Windows (SAS Institute, Cary, NC) was used throughout.

## Results

Table 1 shows the sample sizes by basis (wet, lipid), study design (*in vitro*, *in vivo*, Epidemiology) and effect (DNT, NTE, Thyroid). 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). We found no *in vitro* studies reporting lipid weight dose measurements; *in vitro* wet weight studies of NTE effects reported the greatest number (n=134) measurements, and *in vivo* lipid weight studies of DNT and NTE reported the least (n=11 and 12, respectively).

Table 2a shows the CSF for the PBDE epidemiology compared to *in vitro* studies samples in wet weight, for each effect category, were high, ranging from three to five orders of magnitude at the CSF mean, and for the 95% CI from two to six orders, indicating that *in vitro* studies underestimate the effects of PBDEs indicated in Epidemiology studies. The CSF for the individual chemical PBDE for DNT in Epidemiology compared to *in vitro* studies reporting wet weight results varied from four to five orders of magnitude; mean 156,880 (95% CI 37,163, 662,251), the highest CSF we found in any comparison (Table 2a). For the PCB samples the CSF ranged from two to three orders and the 95% CI from one to three orders. For DDE, DDT, and OH-PCB samples the CSFs ranged from over one to two orders and the 95% CI one to almost four 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 one and the upper bound is two orders of magnitude, with p = 0.18. All other contrasts shown in Table 2a were significant (p<0.001).

Table 2b shows the CSF for PBDE and PCB Epidemiology compared to *in vivo*. Lipid weight studies for PBDE show the lowest CSF for all effect categories with means within one to two orders of magnitude and the 95% CI ranging at one to two orders, all significant. Wet weight CSF was higher than lipid weight. CSFs over effect categories ranged over three to four orders, and 95% CI range over two to four orders. PCB wet weight results for DNT show CSF of one order, at 10.9, and 95% CI ranges one to two orders.

CSFs comparing PBDE Epidemiology with *in vivo* for DNT, NTE, Thyroid and combined effects (labelled as "All") in lipid weight (Table 2b) 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. For PCB wet weight comparisons of DNT for epidemiology and *in vivo* (Table 2b), the CSF of 10.9 (95% CI (2.9, 41.5)) is of concern for the highest exposed and most sensitive, for which there is no practical protection and consistent with accumulated evidence on DNT effects from prenatal exposure to PCB.

Table 3a 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 three to four orders of magnitude for all effect categories; the 95% CI ranged from two to five orders when individual effect categories are considered and three to four orders when all effects were combined. PCB wet weight CSFs were two orders of magnitude at the mean, and the 95% CI ranged from one to three orders. The 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 two orders of magnitude and the 95% CI ranged from two to three orders. The wet weight CSF for DDE, and OH-PCB were the same as in Table 2a, as expected. The CSF

**Table 1:** Sample sizes by basis, study type, and effect category.

| | | | Toxicology | | |
|---|---|---|---|---|---|
| **Basis** | **Effect** | **Epidemiology** | ***In vivo*** | ***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 2a:** Calculated Safety Factor[a] (CSF) and 95% confidence interval by toxin category, basis and effect.

| | | | $\log_{10}$ Internal dose | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Epidemiology | | *In vitro* toxicology | | | |
| Toxin Category | 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 | 156,880.8 (37,163.5, 662,251.9) | <0.001 |
| | | NTE | 7 | −9.9 ± 0.4 | 41 | −5.6 ± 0.8 | 20,617.7 (5,131.2, 82,844.4) | <0.001 |
| | | Thyroid | 27 | −9.2 ± 0.9 | 9 | −5.8 ± 0.9 | 2,424 (504.9, 11,636.5) | <0.001 |
| | | All[b] | 51 | −9.8 ± 1 | 59 | −5.6 ± 0.8 | 14,320.7 (6,535.8, 31,378.1) | <0.001 |
| PCB | Wet | DNT | 10 | −8.4 ± 0.6 | 37 | −5.3 ± 0.9 | 1,268.7 (318.9, 5,046.4) | <0.001 |
| | | NTE | 22 | −8.6 ± 0.6 | 3 | −5.6 ± 0.5 | 943.2 (163.5, 5,442.7) | <0.001 |
| | | Thyroid | 44 | −8.9 ± 1.1 | 9 | −6.9 ± 1.2 | 101.1 (15.4, 665) | <0.001 |
| | | All[b] | 76 | −8.7 ± 0.9 | 49 | −5.6 ± 1.1 | 1,460.2 (628, 3,395.1) | <0.001 |
| DDE | Wet | NTE | 6 | −7.8 ± 0.5 | 4 | −5.6 ± 0.7 | 157.6 (20.8, 1,195.5) | <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, 9,446.3) | <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[b] | 82 | −8.2 ± 0.7 | 5 | −5.8 ± 2 | 236.8 (43.2, 1,297.6) | <0.001 |

[a]*In vitro* toxicology internal dose divided by Epidemiology internal dose in Molar units. [b]DNT, NTE, or Thyroid.

**Table 2b:** Calculated Safety Factor[a] (CSF) and 95% confidence interval by toxin category, basis and effect.

| | | | $\log_{10}$ internal dose | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Epidemiology | | *In vivo* toxicology | | | |
| Toxin Category | 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[b] | 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 | 22,324.7 (8,674.9, 57,452.5) | <0.001 |
| | | NTE | 7 | −9.9 ± 0.4 | 10 | −6.8 ± 0.8 | 1,492.4 (288.3, 7,723.8) | <0.001 |
| | | Thyroid | 27 | −9.2 ± 0.9 | 10 | −5.6 ± 2 | 3,520.4 (406.5, 30,487.1) | <0.001 |
| | | All[b] | 51 | −9.8 ± 1 | 40 | −6.3 ± 1.2 | 3,325.3 (1,168.3, 9,464.7) | <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[b] | 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[b] | 96 | −7 ± 0.9 | 10 | −6.9 ± 1 | 1.3 (0.3, 5.6) | 0.69 |

[a]*In vitro* toxicology internal dose divided by Epidemiology internal dose in Molar units. [b]DNT, NTE, or Thyroid.

**Table 3a:** Calculated Safety Factor[a] (CSF) and 95% confidence interval by toxin category, basis and effect for PBDE, PCB, DDE, DDT, and OH-PCB.

| | | | $\log_{10}$ internal dose | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Epidemiology | | Toxicology | | | |
| Toxin Category | 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[b] | 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 | 29 | −6.1 ± 0.8 | 40,886.6 (14,265.6, 117,185) | <0.001 |
| | | NTE | 7 | −9.9 ± 0.4 | 51 | −5.9 ± 0.9 | 12,320.8 (2,513.3, 60,399) | <0.001 |
| | | Thyroid | 27 | −9.2 ± 0.9 | 19 | −5.7 ± 1.5 | 2,950 (573.8, 15,167.2) | <0.001 |
| | | All[b] | 51 | −9.8 ± 1 | 99 | −5.9 ± 1 | 7,938.6 (3,594.3, 17,533.8) | <0.001 |
| | All | All[b] | 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, 3,104) | <0.001 |
| | | NTE | 22 | −8.6 ± 0.6 | 3 | −5.6 ± 0.5 | 943.2 (163.5, 5,442.7) | <0.001 |
| | | Thyroid | 44 | −8.9 ± 1.1 | 9 | −6.9 ± 1.2 | 101.1 (15.4, 665) | <0.001 |
| | | All[b] | 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, 1,195.5) | |
| 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, 9,446.3) | <0.001 |

[a]*In vitro* toxicology internal dose divided by Epidemiology internal dose in Molar units. [b]DNT, NTE, or Thyroid.

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).

Without regard to effect category or chemical, the CSF for lipid weight *in vivo* Toxicology compared to Epidemiology (Table 3b) was 18.9 (95% CI 8.6 to 41.1) and did not exhibit numerically large variation by effect category with CSFs less than 40. The largest CSF was for DNT at 38 (95% CI 6.5 to 216.9). The CSF for NTE was 12.4 (95% CI 3.3 to 4,736) and for Thyroid it was 16.4 (95% CI 5.5 to 49); all of these were significant (p<0.001).

**Table 3b:** Calculated Safety Factor[a] (CSF) and 95% confidence interval by toxin category, basis and effect for all chemicals.

| | | | | Toxicology | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Epidemiology | | *In vivo* | | *In vitro* | | All | | | |
| Basis | Effect | n | Mean ± SD | n | Mean ± SD | n | Mean ± SD | n | Mean ± SD | CSF (95% CI) | p-Value |
| Lipid | DNT | 70 | −7.5 ± 1.2 | 11 | −5.9 ± 1.1 | | | | | 37.7 (6.5, 216.9) | <0.001 |
| | NTE | 81 | −7 ± 1 | 12 | −5.9 ± 0.8 | | | | | 12.4 (3.3, 46.6) | <0.001 |
| | Thyroid | 122 | −7.6 ± 0.9 | 18 | −6.3 ± 1.4 | | | | | 16.4 (5.5, 48.7) | <0.001 |
| | All[b] | 273 | −7.4 ± 1 | 41 | −6.1 ± 1.2 | | | | | 18.9 (8.6, 41.1) | <0.001 |
| Wet | DNT | 48 | −9.1 ± 1.3 | 35 | −6.6 ± 0.7 | | | | | 269.5 (86.6, 838.8) | <0.001 |
| | NTE | 120 | −8.5 ± 1 | 19 | −6.9 ± 0.8 | | | | | 40.9 (13.6, 123.7) | <0.001 |
| | Thyroid | 115 | −9 ± 1.1 | 17 | −5.6 ± 1.5 | | | | | 2,580.3 (677.2, 9,831.8) | <0.001 |
| | All[b] | 283 | −8.8 ± 1.1 | 71 | −6.5 ± 1.1 | | | | | 542.6 (355.9, 827.2) | <0.001 |
| Wet | DNT | 48 | −9.1 ± 1.3 | | | 66 | −5.3 ± 0.8 | | | 6,243.6 (2,510.1, 15,530.3) | <0.001 |
| | NTE | 120 | −8.5 ± 1 | | | 134 | −5.9 ± 1.1 | | | 465.1 (256.9, 841.8) | <0.001 |
| | Thyroid | 115 | −9 ± 1.1 | | | 86 | −6.8 ± 1.3 | | | 182.4 (85.1, 390.7) | <0.001 |
| | All[b] | 283 | −8.8 ± 1.1 | | | 286 | −6 ± 1.2 | | | 542.6 (355.9, 827.2) | <0.001 |
| All | All | 556 | −8.1 ± 1.3 | | | | | 398 | −6.1 ± 1.2 | 104.6 (72.1, 151.8) | <0.001 |

[a]*In vitro* toxicology internal dose divided by Epidemiology internal dose in Molar units. [b]DNT, NTE, or Thyroid.

Without regard to effect category or chemical, the CSF for wet weight *in vivo* (Table 3b) was 542 (95% CI 356 to 827), but varied numerically by effect category ranging from 41 to 2,580 and 95% CI ranging from 14.0 to 9,831. The smallest CSF was for NTE at 41 (95% CI 14 to 124.0), and the largest was for Thyroid at 2,580 (95% CI 677 to 9,831). The CSF for DNT was 270 (95% CI 87 to 839); all were significant at p<0.001.

With restriction to *in vitro* toxicology studies (Table 3b) the combined CSF was 543 (95% CI 356 to 827), and the maximum, 6,245 (95% 2,510 to 15,530) occurred in studies expressing DNT effects. The CSF for Thyroid was the lowest at 182 (95% CI 85 to 391) and NTE was intermediary at 465 (95% CI 257 to 842); all of these were significant (p<0.001). The integrated all Epidemiology and all Toxicology CSF was 104.6 (95% CI 72 to 152).

## Discussion

Our results generally supported our study hypothesis, that the dose associated with harm would vary between animal and human studies. The results suggest that in total the experimental studies underestimate hazard and risk in people because epidemiology studies exhibit a lower threshold of harm than the *in vivo* and *in vitro* studies. In addition to the lower threshold for harm in epidemiology, frequently reflecting developmental exposure, the experimental applied doses are high and for the most part not environmentally relevant to people.

The CSF calculated for the individual chemical PBDE for DNT in epidemiology compared to *in vitro* studies reporting wet weight results showed the highest CSF we found in any comparison (Table 2a). The PBDE epidemiology internal dose mean (–10.7) was one of the lowest found in all the studies. One epidemiology study of prenatal PBDE exposure effects on neurodevelopment, represented by tests of mental and physical development in a longitudinal cohort initiated after 9/11/01 including 329 participants who delivered in one of three hospitals in lower Manhattan exhibited –11.5. The study examined prenatal PBDE exposure and neurodevelopment at ages 1–4 and 6 years. 210 cord blood specimens were analyzed for selected PBDE congeners. The compared *in vitro* mechanistic effects involved neurotransmitters, second messengers, fetal human brain cell disruption, and Cerebellar granule cell death, all of which are at the molecular and cellular levels of biological organization. They are far removed from the behavioral or clinical presentation level measured as the human effect of the prenatal exposure in childhood. PBDE exhibited very low dose effects on the thyroid in people, which has implications for possible thyroid mediated DNT effects.

The CSF calculated for the individual chemical PCB for Thyroid in epidemiology compared to *in vitro* studies reporting wet weight results varied (mean 101 (95% CI 15.4, 665)), partly spanning usual default uncertainty factors but not protective of the most exposed and sensitive (Table 3a). Wide CI reflects sample size and difference in mean doses of effect – Epidemiology n=44, mean dose –8.9 +/– 1.1; *in vitro*, n=9, mean dose 6.9+/– 1.2.

The epidemiology effects markers were a variety of thyroid hormone system disruption effects, and frank and sub-thyroid disease. The Thyroid *in vitro* effects markers for PCB are TTR inhibition, and TR inhibition and TR-DNA disruption. PCB also has DNT effects in this CSF comparison and this possible relation to the Thyroid should be noted. Thyroid effects in epidemiology and *in vitro* are found for OH-PCB and PFAS (Table 3a). Markers are similar to PCBs. These thyroid results are again suggestive of concerns for development induced effects in the fetus and offspring.

For PCB wet weight comparisons of DNT for epidemiology and *in vivo* (Table 2b), the CSF of 10.9 (95% CI (2.9, 41.5)) is of concern for the highest exposed and most sensitive, for which there is no practical protection. This is consistent with the accumulated evidence on DNT effects from prenatal exposure to PCB.

The thyroid CSF for PFAS is interesting (mean 14.8 95% CI 0.2 to 993.1) but is small sample and not significant at p = 0.18. For DNT (n=16) and NTE (n=60) PFAS epidemiology has larger samples but with insufficient toxicology data to estimate a CSF (Supplement References 137, 138, 142, 143, 146, 147, 148, 152, 154, 157, 159). For DNT the log10 internal dose is –7.9 +/– 0.4, and for NTE is –8.3 +/– 0.7 (Table 2a).

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

It may be relevant that in many cases, for *in vitro* data, there was little statistical difference in the multi-chemical contrasts except for the thyroid category, attributable to the predominance of hydroxylated PBDEs, PCBs, and phenolic chemicals. In epidemiology studies, the PBDEs [and OH-PBDEs and MeO-PBDEs] overall showed the most potency with effects at the lowest internal concentrations followed by OH-PCBs, PCBs, HCB, and sometimes a group of several chemicals of concern considered together (e.g., chlordane, DDE, DDT, dieldrin, HCB, HCH, MeSO2-PCB, PBB, PCP, and PFAS). Most often, except for PBDEs and sometimes others, the chemical groups contrasted were not statistically different in their mean potencies.

This raises the important question of why *in vitro* toxicology methods dominate regulatory agencies' analyses, and how they can be used to develop guidance on uncertainty factors using *in vitro data* alone. We are not aware of any standards being based primarily on *in vitro* toxicology. In this study, we interpreted results of the *in vitro* work as mechanistic effects, showing and clarifying likely modes of action upstream from the *in vivo* and epidemiology effects, observed in the three effect categories. In our experience, however, in scientific studies *in vitro* results are sometimes compared with human internal concentrations from epidemiology or biomonitoring studies. These studies may reflect the large differences we found in our analyses, and may conclude, based on usually applied default safety factors, that there is a more or less large margin of safety [9–16, 115].

Moving forward, we suggest that an integrated data base can be used to model the statistical relationships or associations between the observed variables and the network of relationships between them, and estimate an empirical component (for example, our CSF) in the determination of an appropriate uncertainty factor in RA, including an exploration of uncertainty factors for *in vitro* data.

Strengths of this study are that the results were compiled directly from articles published in peer-reviewed scientific journals or proceedings, and the data analytical methods were uniformly applied across all contrasts. The lognormal assumption was met in all analyses, as shown by the small SD relative to the mean, and similarity of the mean and median in the log-transformed results.

The relevance of these factors is inherent in their empirical basis, providing data-derived uncertainty or calculated safety factors. However, these data themselves contain possible limitations of accuracy due to uncertainties in unaccounted for differences in chemical properties (e.g., ortho vs. non-ortho), complex mixture exposures in epidemiology, life stage, and possible synergistic or antagonistic effects.

## Authors' information

TM worked for Environment Canada as an environmental research analyst from 1974 to 2004, when he retired. He worked on a wide range of environmental and health related issues, using integrative and trans-disciplinary approaches. Since then he has remained professionally active as an independent researcher writing papers and attending professional meetings. His interest is in seeing the manuscript published along with all the data and supporting documentation so that it will be freely available and can be used by others.

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