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Relative toxicity for indoor semi volatile organic compounds based on neuronal death

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Highlights

- Many indoor SVOCs are suspected to be neurotoxic, raising the question of cumulative risk assessment
- Benchmark concentrations (BMC) have been derived for 13 / 28 neurotoxic indoor SVOCs
- Experimental designs had a significant influence on BMC calculation
- More standardized protocols in terms of cell lines, species and exposure duration should be developed with a view to cumulative risk assessment

Abstract

Background: Semi Volatile Organic Compounds (SVOCs) are contaminants commonly found in dwellings as a result of their use as plasticizers, flame retardants, or pesticides in building materials and consumer products. Many SVOCs are suspected of being neurotoxic, based on mammal experimentation (impairment of locomotor activity, spatial learning/memory or behavioral changes), raising the question of cumulative risk assessment.

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The aim of this work is to estimate the relative toxicity of such SVOCs, based on neuronal death.

Method: SVOCs fulfilling the following conditions were included: detection frequency >10% in dwellings, availability of data on effects or mechanism of action for neurotoxicity, and availability of dose-response relationships based on cell viability assays as a proxy of neuronal death. Benchmark concentration values (BMC) were estimated using a Hill model, and compared to assess relative toxicity.

Results: Of the 58 SVOCs selected, 28 were suspected of being neurotoxic in mammals, and 21 have been documented as inducing a decrease in cell viability *in vitro*. 13 have at least one dose-response relationship that can be used to derive a BMC based on a 10% fall in neuronal viability. Based on this *in vitro* endpoint, PCB-153 appeared to be the most toxic compound, having the lowest BMC_{10} (0.072 μM) and diazinon the least toxic compound, having the highest BMC_{10} (94.35 μM). We showed that experimental designs (in particular choice of cell lines) had a significant influence on BMC calculation.

Conclusion: For the first time, the relative *in vitro* toxicity of 13 indoor contaminants belonging to different chemical families has been assessed on the basis of neuronal cell viability. Lack of comparable toxicity datasets limits the number of SVOCs that can be included. More standardized protocols in terms of cell lines, species and exposure duration should be developed with a view to cumulative risk assessment.

Abbreviations

AIF	Apoptosis-inducing factor
B(a)P	Benzo(a)pyrene
Bax	Bcl2 associated X protein
BBP	Benzylbutylphtalate

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BDE-X	Bromodiphenylether – congener X
BMC	Benchmark concentration
BMCL	Benchmark concentration lower bound
BMCU	Benchmark concentration upper bound
BMR	Benchmark response
BPA	Bisphenol A
Ca2+	Calcium
CCK-8	Cell counting kit-8
Cyto c	Cytochrome c
DBP	Di-n-butylphtalate
DDE or 4,4'-DDE	Di-chlorodiphenyldichloroethylene
DDT or 4,4'-DDT	Di-chlorodiphenyltrichloroethane
DEHP	Di-ethylhexylphtalate
DEP	Di-ethylphtalate
DiBP	Di-isobutylphtalate
DiNP	Di-isononylphtalate
DMEP	Di(2-methoxyethyl)phthalate
DMP	Di-methylphtalate
EFSA	European Food Safety Authority
EPA	Environmental Protection Agency
GD	Gestational day
HI	Hazard index
MTS	3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium

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MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NMDA	N-methyl-D-aspartate
PAH	Polycyclic aromatic hydrocarbon
PBDE	Polybromodiphenyl ethers
PCB	Polychlorobiphenyls
PND	Postnatal day
PODI	Point of departure index
ROS	Reactive oxygen species
RPF	Relative potency factor
SD	Standard deviation
SEM	Standard errors of the means
SVOC	Semi volatile organic compound

Keywords: Semi volatile organic compounds, benchmark concentrations, mixture, relative toxicity.

1. Background

For 50 years now, the emergence of new building materials coupled with increased use of consumer products and electrical and electronic equipment has led to the emission of semi volatile organic compounds (SVOCs) indoors. In this paper we consider SVOCs as defined by Weschler and Nazaroff: "organic compounds with vapor pressures between 10^{-14} and 10^{-4}atm (10^{-9} to 10Pa)" (Weschler and Nazaroff 2008), and which have a boiling point between 240° and 400°C according to French standards (NF ISO 16000-6, 2006). They include, for example, phthalates, polybromodiphenyl ethers (PBDEs), polychlorobiphenyls (PCBs),

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polycyclic aromatic hydrocarbons (PAHs) and various families of pesticides. The widespread use of products containing SVOCs, in association with their physicochemical characteristics, leads to their omnipresence (and sometimes persistent presence) in both air and settled dust (Mercier et al., 2011). Numerous SVOCs are known to be neurotoxic in experimental mammals, and questions arise as to their potential effects in humans. Most studied SVOCs are organochlorine, organophosphorus and pyrethroid pesticides, since these are produced precisely for their neurotoxic properties on insects. High to moderate levels of occupational exposure to these pesticides have been linked with neurobehavioral performance deficits and abnormalities in human nerve function (Kamel and Hoppin 2004), though whether chronic exposure to low (environmental) levels is also neurotoxic remains a matter of some controversy. Several recent epidemiological studies have shown an association between prenatal exposure to pesticides and neurological disorders (decreased IQ scores, cognitive development changes, abnormal reflexes, visual acuity, fine motor skills, attention deficit, hyperactivity) and in particular for organophosphorus (Munoz-Quezada et al. 2013; González-alzaga et al., 2014), pyrethroids (Viel et al. 2015), and organochlorines (Cartier et al., 2014). Prenatal exposure to polychlorobiphenylethers (PCBs) or bisphenol A (BPA) also appears to be related to cognitive impairments, as recently reviewed (Boucher et al. 2009; Polańska et al. 2013; Quinete et al. 2014; Mustieles et al. 2015). Neurodevelopmental deficits were also associated with prenatal exposure to polybromodiphenylethers (PBDEs) (Roze et al. 2009; Herbstman et al. 2010; Eskenazi et al. 2013; Chen et al 2014; Chevrier et al. 2016). The same observations were made of pre- and postnatal exposure to phthalates: prenatal exposure to DEHP and DBP and impaired behavior at 2 and 8 years of age (Polańska et al. 2014; Lien et al. 2015), postnatal DEP and DBP exposure associated with hyperactivity and impairment in adaptive functions (Philippat et al. 2015), and postnatal exposure to DEHP and attention / learning disorders (Chopra et al. 2014). Fewer epidemiological data are available

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for PAHs, but these show similar trends relating to cognitive function impairment (Edwards et al. 2010; Perera et al. 2011, Jedrychowski et al. 2015).

Experimental data confirm the neurotoxic potency of these SVOCs, especially on the developing brain, and support the biological plausibility of associating SVOC exposure with neurobehavioral disorders. Pre and/or postnatal exposures in rodents lead to: delayed spatial learning for BDE-99 (Blanco et al. 2013), decreased learning ability and memory for BDE-153 (Zhang et al., 2013) and benzo(a)pyrene (BaP) (Cheng et al. 2013; Chepelev et al. 2015), decreased motor activity for BDE-47 (Ta et al. 2011), and behavioral disturbance for a mixture of 6 PCBs (Elnar et al. 2012), BPA (Viberg and Lee, 2011) and fluorene (Peiffer et al. 2013), and attention deficit and hyperactivity for PCB-153 (Johansen et al. 2014) or phthalates (Miodovnik et al. 2014). Numerous critical reviews have already been published on the neurotoxicity of SVOCs in recent years, such as persistent SVOCs (PCB or PBDE) (Berghuis et al. 2015), pesticides (Burns et al. 2013; Mostafalou and Abdollahi 2017), phthalates (Miodovnik et al. 2014), PAHs (Wormley et al. 2004) or more broadly, endocrine disrupting compounds (Masuo et al. 2011). The main conclusions show neurodevelopmental outcomes for most SVOCs studied, with a large body of evidence for PCBs, PBDEs or pesticides (which are especially organophosphorus). While few or no epidemiological studies are available for PAHs, phthalates or some BPAs, supporting animal data provide potential evidence of their neurotoxicity.

These findings raise the question of the public health impact of SVOCs in general, which may be simultaneously present in indoor environments, thus exposing people to a complex mixture. Cumulative risk assessment could be used to assess this public health issue. A variety of methodologies have already been applied, and these have recently been reviewed (Fournier et al. 2014b): in practice, ‘Hazard Index’ (HI), ‘Point Of Departure Index’ (PODI) and ‘Relative Potency Factor’ (RPF) approaches are the most commonly used. These

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methodologies assume dose-additivity, based on the principle that synergism is less likely to occur at environmentally relevant low doses (ATSDR 2004; Kortenkamp et al. 2009). HI is the summation of individual hazard quotient for each chemical. The main benefit of the HI approach is its simplicity - which is why it is so broadly used in CRA. Drawbacks are the use of non-comparable toxicity indicators (different critical effects) leading to unsatisfactory results, and an extremely conservative approach. PODI avoids these drawbacks by directly comparing exposure to a toxicity indicator (POD) retrieved from the literature (a NOAEL, a LOAEL or a BMDL). However, because there is no guarantee that the POD for a given effect will necessarily be available in the literature, a reduced number of compounds can be studied. The RPF approach converts exposure into an index chemical equivalent by scaling the dose of each SVOC by its toxicity relative to the index chemical. This relies on both the existence of high quality dose-response data and a common mechanism of action for the SVOCs in question. The RPF approach demands more data on each chemical than do the other approaches. PODI and RPF have already been proposed for certain SVOCs such as pyrethroids, organophosphorus, phthalates, PAHs, fungicides and perfluorinated compounds (Borg et al. 2013; Audebert et al. 2012; Kortenkamp and Faust 2010; US EPA 2006; Wolansky et al. 2006). The approaches cannot however be used together in a single cumulative risk assessment, because they are not based on the same endpoints (reproductive or hepatic endpoints, anti-androgenic or genotoxic mechanisms, decrease in acetylcholinesterase or motor activity, chronic or acute exposure, etc...).

The cumulative risk assessment issue is challenging, and a consensus has been reached that hierarchical approaches should be adopted, each tier being more refined than the previous one (Meek et al. 2011). Instead of using a traditional reference dose based on a common target (as may be extracted from the US EPA IRIS database for some organs/systems, including the nervous system), we propose to complete the “tier 0 approach” recommended by Meek et al.

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(2011) using a less conservative approach, based on relative toxicity. The choice of a common outcome with sufficient comparable toxicity data was based on a previous work identifying neuronal death (Fournier et al. 2014) as a consequence of exposure to numerous SVOCs. Impairment of cognitive function may be linked, in part, to neuronal death, as has been suggested by several authors (Sharma et al. 2009; Lahouel et al. 2016). Neuronal death may be due to different mechanisms such as those involving oxidative stress (Grova et al. 2007; Dominico et al. 2002; Bouayed et al. 2009; Rammal et al. 2010), disruption of calcium signaling or modification of the expression of proteins required for brain development (Yuan et al. 2003). Neuronal death may be a consequence of various signaling pathways, one of which is apoptosis (Davis and Williams 2012; Green and Kroemer 2004; Niizuma et al. 2009) as shown in Figure 1. It induces activation of ~~the~~ different proteins, such as the cell surface death receptor, Bcl2, BH3 (a proapoptotic Bcl2 family member), Bax (proapoptotic Bcl2 associated X protein), caspases, AIF (apoptosis-inducing factor) or cytochrome C (Cyt C) (Davis and Williams 2012). Some SVOCs (PBDEs, organochlorines, organophosphorus pesticides, or PAHs in particular) may induce activation of such proteins (Karami-Mohajeri and Abdollahi 2011; Kodavanti et al. 2015; Mariussen and Fonnum 2006; Zhang et al. 2015; Costa et al. 2016; He et al. 2016).

The aim of this work is to estimate the relative toxicity of the indoor SVOCs that were found simultaneously in French dwellings, using comparable benchmark concentrations (BMCs) based on neuronal death.

2. Material and Method

2.1.Selection and grouping of indoor SVOCs

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The selection of chemicals was based on recent measurement campaigns in French dwellings in a range of environmental media (air, gas phase or particle matter and settled dust) (Mandin et al., 2013, 2016; Blanchard et al., 2014). 66 compounds were selected from a previous ranking on the basis of contamination data and reference doses (Bonvallot et al., 2010). SVOCs were included in the present work when they were detected in more than 10% of the dwellings investigated (from 30 to 285, according to the campaign). SVOC grouping was based on an approach previously described (Fournier et al. 2014a). For each chemical, an initial hazard identification was performed by means of a literature review covering its neurotoxic effects in humans and other mammals. As well as being of high enough quality to be taken into consideration, studies should describe at least one adverse effect on the neurological system, regardless of the experimental system used. SVOCs were then grouped by common key events, when known (Fournier et al. 2014a). An update of this work enabled selection of a group of SVOCs based on neuronal death as a common endpoint, taking into consideration the potential relationship between neuronal death and disturbance to neurobehavioral abilities (Sharma et al. 2009; Lahouel et al. 2016; Grova et al. 2007; Dominico et al. 2002; Bouayed et al. 2009; Rammal et al. 2010). In compliance with European Food Safety Authority (EFSA) recommendations, the present work takes into account contaminants from different chemical families, having a variety of mechanisms of action, provided they produce a common impact on the same target cell (EFSA 2013). EFSA recommends this approach for the assessment of the cumulative risks of pesticide residues in food.

2.2.Benchmark Concentration (BMC) derivation

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BMCs were derived using the following steps: i) selection of endpoint; ii) selection of suitable dose-response datasets; iii) choice of level of response associated with the BMC; iv) fitting models to experimental data and v) selection of the best fitting model.

Selection of endpoint: Several types of cell death exist, including apoptosis and necrosis. In this work, depending on data availability, cell viability assays may represent an advanced state of cell degeneration. Many methods for the assessment of neuronal cell viability have been developed (Aras et al. 2008). Most are based on measurement of metabolic (mitochondrial) activity, or on membrane integrity as a proxy of cell viability. The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was preferred because it is the one most frequently used as a proxy for cell viability measurement.

Selection of suitable dose-response datasets: For each SVOC, dose-response datasets were collected and selected on the basis of a compromise between the amount of available data and comparability of the data between compounds. Preferences were:

- *In vitro* test system: cell line, neurons, human
- Experimental design: 3 dose-levels and 1 control (4 groups)
- Exposure duration: 24 to 48 hours
- Result expression: raw data availability and standard deviation.

When not provided by the authors, raw data and Standard Deviations (SDs) were obtained using a graphical estimation, as described by the US EPA (US EPA 2005). Where only Standard Errors of the Means (SEMs) were given, these were converted to SDs using the following equation: $SD = SEM \times \sqrt{n}$, where n is the sample size.

Choice of level of response associated with the BMC: The benchmark responses (or BMRs) selected are the default values recommended by the US EPA (that is, one SD in relation to the control for continuous responses, or a 10% response rate for dichotomous responses).

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Selection of the best fitting model: BMCs were computed using PROAST software (www.rivm.nl/proast v38.9). As suggested by Levasseur, the Hill family models were selected for modeling of the dose-response relationships(Levasseur et al. 1998). Cells are considered mutually dependent - therefore responses measured from *in vitro* data could be considered continuous. The Hill models family is presented below in Equations 1 to 4 (2 to 4 parameters):

$$y = a\left(1 - \frac{x}{b + x}\right) \quad (1)$$

$$y = a\left(1 - \frac{x^d}{b^d + x^d}\right) \quad (2)$$

$$y = a\left(1 + \frac{(c-1)x}{b + x}\right) \quad (3)$$

$$y = a\left(1 + \frac{(c-1)x^d}{b^d + x^d}\right) \quad (4)$$

Where x is toxic compound concentration, a is the background response, b is the concentration of the compound inducing 50% of the effect, d is the slope, and $a*c$ is the response to an infinite dose.

Selection of the best fitting model: The best fitting model from the Hill family was selected according to the procedure described by Slob (Slob 2002), using the likelihood ratio test. To compare contaminant toxicity, the central estimation (BMC) was used in preference over the BMCL (Benchmark Concentration Lower Bound = lower limit of a one-sided confidence interval on the BMC) which integrates uncertainty of experimental data, as suggested by the US EPA in 2010 (US EPA 2010).

3. Results

3.1.Grouping of indoor SVOCs

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58 SVOCs were selected, since these were detected in at least 10% of dwellings (Table I). Of these, 28 were documented as having different neurotoxic properties, inducing neurobehavioral effects in mammals or humans. In 21 cases, neuronal death was hypothesized on the basis of viability assays. This outcome was caused by different upstream key events leading to membrane alteration (for 11 SVOCs), mitochondrial impairment (for 17 SVOCs) or neurotransmitter modulation (for 7 SVOCs), as shown in Figure 2. Neuronal death is the endpoint that includes the highest number of SVOCs (Fournier et al. 2014a) with exploitable dose/ concentration-response data, and is thus retained for the derivation of BMCs. For example, only 11 chemicals have at least 3 dose groups based on *in vivo* neurobehavioral changes with different responses measured (i.e. spatial learning and memory, anxiety or motor coordination).

3.2.Benchmark concentrations derivation

Concentration-response relationships fulfilling our criteria were available for 15 of the 21 SVOCs selected. Six contaminants (triclosan, 4,4'-DDT, DBP, DiBP, BDE-153 and permethrin) were excluded because there was no available concentration-response relationship in cell viability assays. Table II briefly summarizes the concentration-response data for each compound. Each response and standard deviation was converted into percentage of control. Concentration-response data were obtained from different species: rats (6/15), mice (4/15) or humans (5/15), usually on cell lines (12/15), and exposed for 24 to 48 hours (14/15). Only 3 experiments used primary culture in neonatal or prenatal rats.

The response measured in the toxicological studies was a decrease in cell viability estimated using 5 different tests corresponding to 2 measurements: membrane integrity (Trypan Blue dye assay), and mitochondrial activity (resazurin (CellTiter-Blue kit), MTT, MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) and

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CCK-8 (Cell Counting Kit-8) assays). Mitochondrial activity was thus measured in more than 90% of cases (14/15 SVOCs).

BMC_{10}, corresponding to a 10% decrease in neuronal viability, were presented in Table III. The number of parameters used for Hill equations ranged from 2 to 4. Resulting $BMCs_{10}$ range from 0.072 to 94.35 µM, corresponding to approximatively 3 orders of magnitude. Because no dose-response models fit their dataset, it was not possible to model the BPA and PCB-118 BMC_{10}. Indeed, BPA had a non-monotonic curve and PCB-118 had a response decreasing by more than 30% from the first tested dose.

Figure 3 shows the relative toxicity of the 13 different SVOCs based on the central estimation (BMC) and the lower and upper 90%-confidence bounds (BMCL and BMCU respectively). According to these BMC central estimations, we can distinguish three groups of compounds: 1) BMC below 1 µM with PCB-153 alone which appears the most toxic for the endpoint considered (cell viability); 2) BMCs between 1 and 10 µM: deltamethrin, B(a)P, BDE-47, BDE-209, BDE-99 and PCB-77; 3) BMCs between 10 to 100 µM: DEHP, dieldrin, chlorpyrifos, lindane, PCB-52 and diazinon.

4. Discussion and conclusion

Carrying out a CRA is currently a challenging matter. Some authors suggested the adoption of hierarchical approaches, with each tier being more refined than the last (Meek et al. 2011). In addition to the tier-0 HI approach (based on existing reference doses) we present elements for a first-tier approach based on relative toxicities estimated from data that is as comparable as possible. One way to do so is through the use of *in vitro* assays, which are standardized and widely available. In this way, we derived BMCs for 13 SVOCs pertaining to various chemical families to which people are exposed in indoor environments. We were able to rank 3 PCBs,

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5 pesticides, 3 PBDEs, 1 phthalate, and 1 PAH, according to their ability to decrease neuronal cell viability as observed *in vitro*.

BMCs were modeled from published dose-response relationships. The raw data differ in various aspects: 8 different cell lines, 3 different species strains, 3 distinct windows of exposure, 5 neuronal viability assays and 4 different durations of exposure.

In our work, studies based on human cells were preferred, but choice was limited by the availability of exploitable and reliable studies in the literature. Different human, rat and mouse cell lines were therefore used (Table II). However, the species tested do not seem to affect the BMC calculation, as shown by Goldoni et al. who tested the same toxicants on different cell lines from both rats and humans (Goldoni et al. 2003). They showed that different cell lines from the same species are capable of reacting differently to the same compound, using the same neuronal viability test (here the MTT test). For rats, they showed that C6 cells were more resistant to styrene oxide than PC12 cells. Similarly, in humans, D384 cells were more resistant to styrene oxide than SK-N-MC cells. In our work, the cells came from different strains (mice, rats and humans) and were from different cell lines for mice (hypothalamic and neuroblastoma cells, named GT1-7 and Neuro-2a respectively), rats (dopaminergic and pheochromocytoma cell, named N27 and PC-12 respectively or hippocampal and cortical neurons taken directly from the exposed animals), or humans (neuroblastoma cell from SK-N-MC and SH-SY5Y cell lines). Potency ranking could therefore be affected by the different cell lines used in these experiments.

As with the cell lines and species, selected studies also vary in their use of different neuronal viability assays. The MTT assay estimates cellular viability through measurement of mitochondrial dehydrogenase activity. This is the most widely used assay (more than 50% of our study SVOCs). We chose to also include studies using other kinds of neuronal viability assays: MTS (also known as CCK-8) and rezasurin assays (known as CellTiter-Blue assays)

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which also assess metabolic activity and are sensitive to the function of mitochondrial enzymes, which lose activity early in progression towards death (Aras et al. 2008), and the Trypan Blue assay, which assesses neuronal membrane integrity. The Trypan Blue method is based on the principle that live (viable) cells do not take up certain dyes, whereas dead (non-viable) cells do (Strober 2001). This is the least sensitive of the assays. The MTS assay appears easier to use, faster and slightly more sensitive than MTT. The resazurin assay appears to be more sensitive than the tetrazolium assays (MTS and MTT) (Riss et al. 2013). We can consider the following order of sensitivity for the various assays: Trypan Blue < MTT ≈ MTS (CCK-8) < resazurin (CellTiter-Blue). This difference in sensitivity might also affect the rank of potency of the tested SVOCs. The most toxic SVOC of the group was PCB-153, which was tested using the resazurin test. PCB-52, the second least toxic compound in this work, was tested using Trypan Blue. Without testing cell viability with other tests (MTT or MTS), it is difficult to assess whether their toxicity, relative to the others, is due to the sensitivity of the assays used or to their real ability to decrease cell viability. In addition, it is important to note that there is no guarantee that the decrease in metabolic activity is necessarily irreversible in MTT/MTS or resazurin assays. It is therefore not possible to demonstrate a direct link between metabolic activity, cell viability and neuronal death. Nevertheless, this parameter would only change the order of potencies for this group of contaminants.

As underlined by Deshpande et al., duration of exposure could also influence the calculated BMCs. These show a linear and time-dependent increase in neuronal cell death linked to extracellular calcium entering cells through the N-methyl-D-aspartate (NMDA) glutamate receptor channel (Deshpande et al. 2008). The studies retained in our work are also based on various exposure durations (5 hours to 48 hours). According to Deshpande's conclusion, one might expect lengthier exposure of cells to be more lethal - yet this was not confirmed in our

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data. In fact, dieldrin, lindane and diazinon appeared to be the least toxic compounds in spite of longer cell exposure. On the other hand, PCB-77 appeared to be 10 times more potent than diazinon after 5 hours of exposure (as against 48 hours for diazinon).

These relative toxicities could be used for cumulative risk assessment of real indoor mixtures of contaminants. Because they are based on *in vitro* data, these results should however be interpreted with caution. An important limitation is the absence of bioaccumulation data in short-term assays, while 9 SVOCs are persistent in the human body (PCB-153, B(a)P, BDE-47, BDE-99, BDE-209, PCB-77, dieldrin, lindane and PCB-52). Metabolic transformation has not been taken into account, since most *in vitro* studies test parent compounds. In our work, only B(a)P was tested using the S9 fraction in order to be metabolized.

Another potential confounder is that SVOCs are ubiquitous compounds; they can contaminate the labs in which the experiments are conducted. Control groups may thus differ across experiments, leading to a lack of comparability for the effect of treatment and statistical significance across SVOCs. This is particularly true of phthalates or bisphenols.

In addition, the use of such indicators should not prevent implementation of chemical-by-chemical risk assessment and "tier-0" cumulative risk assessment (i.e. HI) based on a larger biological scale (for example *in vivo* neurobehavioral changes), since neurotoxicity may be due to different mechanisms not necessarily leading to neuronal death. It is difficult to compare *in vivo* and *in vitro* data, because such studies do not share the same objectives. Two *in vivo* studies with BMD_{10} were found in the literature, for B(a)P (Chepelev et al. 2015) and BDE-47 (Yan et al. 2012). In these cases, the authors measured spatial learning and memory in orally exposed Sprague Dawley rats, using the Morris Water Maze test. B(a)P was tested postnatally of a period of 7 days (doses from 0.2 to 2 mg/kg/d) and BDE-47 was tested at the adult age over a period of 30 days (doses from 0.1 to 1 mg/kg/d). *In vivo* $BMDs_{10}$ were respectively 0.049 and 0.039 [CI90: 0.029 – 0.054] mg/kg/d for B(a)P and BDE-47. As a

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result, the *in vivo* relative toxicity of BDE-47 was found to be the same as B(a)P when studying spatial learning and memory, while it was 2 times less potent when studying *in vitro* cell viability. This difference might be related to the existence (beside neuronal death) of other toxicity pathways leading to neurobehavioral impact, such as interaction with the synaptic transmission (through potentiation of the GABAergic signaling pathway and inhibition of acetylcholine signaling, (Hendriks et al. 2010)) or interaction with the thyroid metabolism (Abdelouahab et al. 2009). It may also be related to the differences in *in vivo* experimental protocols, which can lead to inconsistencies in potency estimation - as demonstrated by Wolansky and Tornero-Velez (2013) for pyrethroids.

Lastly, the most important limitation of this work is the lack of available and comparable data in the scientific literature: of the initial 58 SVOCs detected in more than 10% of the French dwellings that had been investigated, 50% are documented as neurotoxic (Figure 2). Of these, 28% were excluded because no study focused on neuronal death, 20% because there was no dose-response relationship, and 7% because there was no suitable dose-response model. This was also recently underlined by Wignall et al. in their standardization of BMD calculations (Wignall et al. 2014). This team sought to apply a standardized process to the conduct of BMD modeling, to reduce inconsistencies in model fitting and selection. They identified 1,260 chemicals having at least one toxicity value, then applied a curation of chemicals and data in order to remove mixtures, chemicals missing structural information, and inorganic, organometallic, and duplicate structures. In the process of this curation, 374 chemicals were dropped. Of the 886 remaining chemicals, dose-response data was available for just 352. This corresponds to 28% of the initial chemicals.

The originality of this work lies in its grouping of compounds from the different chemical families people really are exposed to. Based on the methodological limitations mentioned, we particularly recommend the standardization of experimental protocols and the publication of

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results according to a format adapted for dose-response relationship modeling - both for *in vitro* and *in vivo* studies, given the pros and cons of such experiments. This will be needed to reduce uncertainty when using this type of indicators in a risk assessment context.

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Author's contributions

KF developed the first study outline which was extended in collaboration with EB. The results were discussed and interpreted by KF and NB. PG and DZ reviewed and contributed to the final version of the manuscript. All authors have approved the final version.

Competing interests

The authors declare that they have no competing interests.

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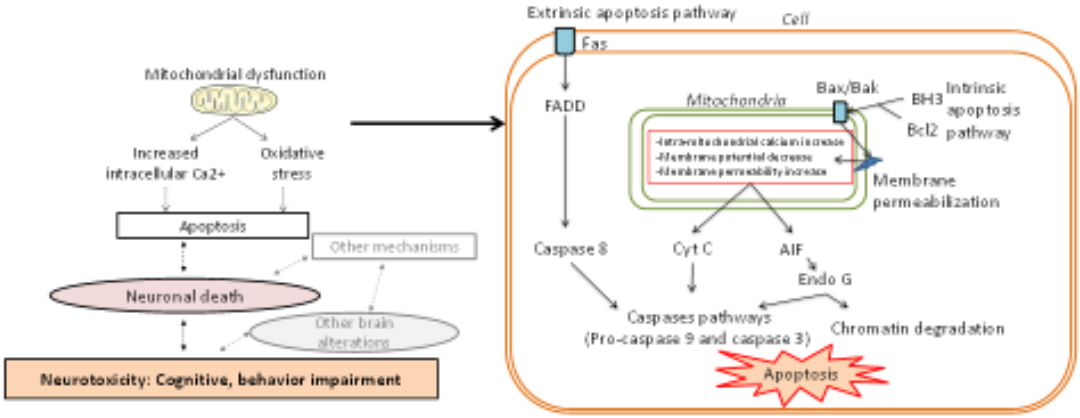
Zhang H, Li X, Nie J, Niu Q. 2013. Lactation exposure to BDE-153 damages learning and memory, disrupts spontaneous behavior and induces hippocampus neuron death in adult rats. Brain research;1517:44–56.

Zhang S, Chen Y, Wu X, Gao H, Ma R, Jiang C, Kuang G, Zhao G, Xia T, Zhang X, Lei R, Zhang C, Li P, Xu C, Wang A. 2015. The pivotal role of Ca(2+) homeostasis in PBDE-47-induced neuronal apoptosis. Molecular Neurobiology:1-11. doi:10.1007/s12035-015-9573-8.

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\Figure 1: The main mitochondrial pathways for cell death (inspired from Davis and Williams 2012)

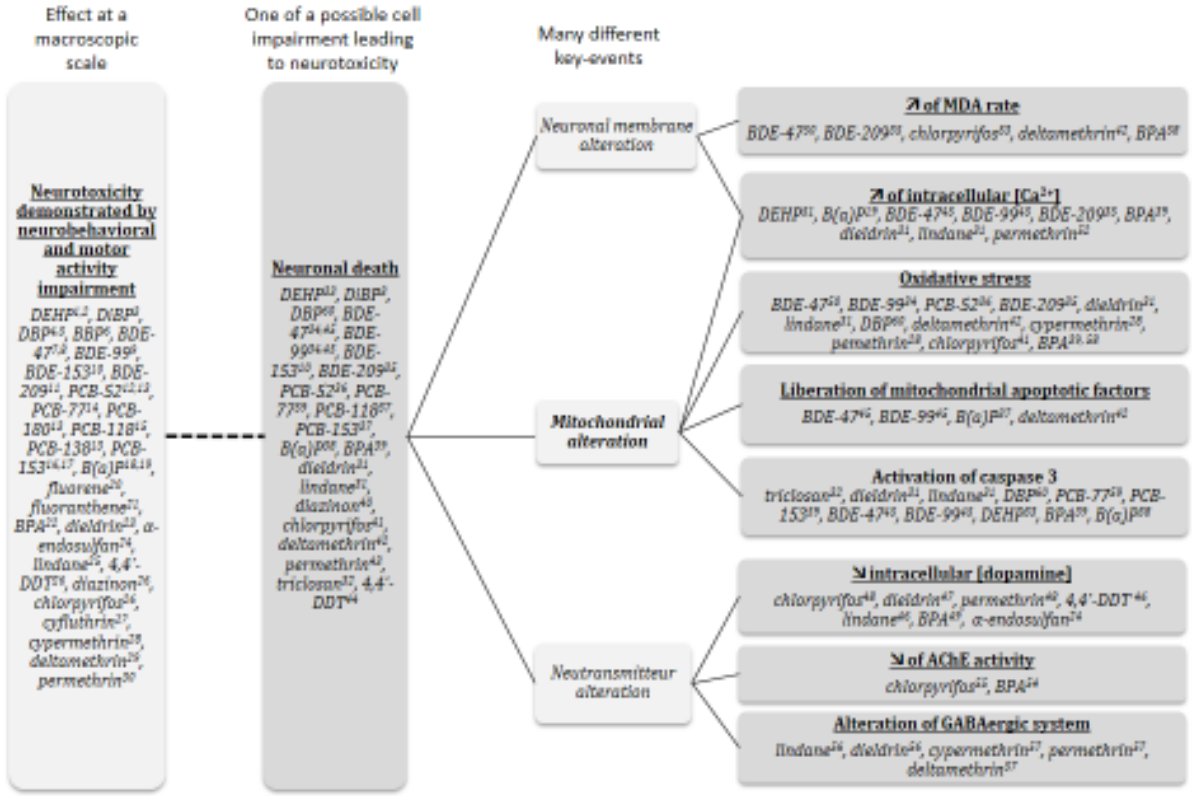
Bax: proapoptotic Bcl-2-associated X protein; Bak: Bcl-2 homologous antagonist killer; BH3: proapopotoic Bcl-2 family members; Endo G: endonuclease G; FADD: FAD domain protein; Cyt C: cytochrome C; Fas: transmembrane receptor



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Figure 2: Grouping of 29 indoor Semi Volatile Organic Compounds (SVOCs) based on neuronal death, adapted and updated from (Fournier et al. 2014a).

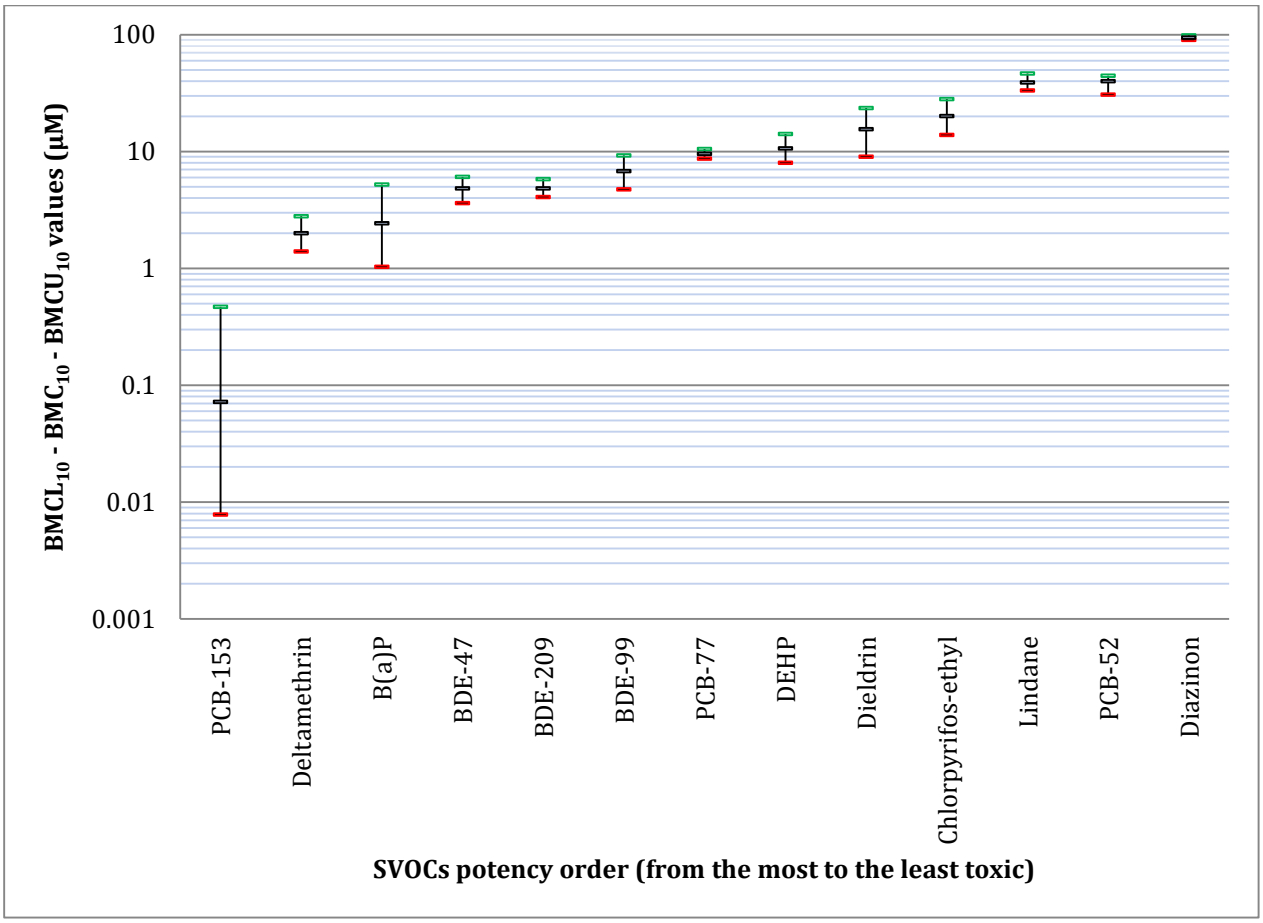
[1](Chopra et al. 2014); [2](Xu et al. 2015); [3](Ma et al. 2013); [4](Li et al. 2013); [5](Farzanehfar et al. 2016); [6](Min et al. 2014); [7](Ta et al. 2011); [8](Chen et al. 2014); [9](Blanco et al. 2013); [10](Zhang et al. 2013); [11](Johansson et al. 2008); [12](Eriksson et al. 2006); [13](Boix et al. 2010); [14](Simmons et al. 2005); [15](Kuriyama and Chahoud 2004); [16](Lynch et al. 2012); [17](Cheng et al. 2013); [18](Johansen et al. 2014); [19](Chepelev et al. 2015); [20](Peiffer et al. 2013); [21](Saunders et al. 2003); [22](Viberg et al. 2011); [23](Beard et al. 2014); [24](Lakshmana and Raju 1994); [25](Tussel et al. 1987); [26](Burns et al. 2013); [27](Soni et al. 2011); [28](Nasuti et al. 2007); [29](Chen et al. 2012); [30](Nasuti et al. 2012); [31](Sharma et al. 2010); [32](Szychowski et al. 2015); [33](Lin et al. 2011); [34](Tagliaferri et al. 2010); [35](Chen et al. 2010); [36](Lee et al. 2005); [37](Dickerson et al. 2009); [38](Nie et al. 2014); [39](Lee et al. 2008); [40](Bahrami et al. 2013); [41](Ki et al. 2013); [42](Romero et al. 2012b); [43](Elwan et al. 2006); [44](Kajta et al. 2014); [45](Yu et al. 2008); [46](Faro et al. 2009); [47](Kitazawa et al. 2001); [48](Karen et al. 2001); [49](Yoneda et al. 2003); [50](He et al. 2008); [51](Tully et al. 2000); [52](Meyer and Shafer 2006); [53](Jin et al. 2015); [54](Fan et al. 2013); [55](Farag et al. 2010); [56](Vale et al. 2003); [57](Eshleman and Murray 1991); [58](Agarwal et al. 2016); [59](Sanchez-Alonso et al. 2003) ; [60](Wójtowicz et al. 2016).



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Figure 3: Comparable BMC_{10} for 13 indoor Semi Volatile Organic Compounds (SVOCs)

Each BMC_{10} in represented by a black line, BMCL is below (red line) BMC, and BMCU is above (green line).



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Table I - Indoor Semi Volatile Organic Compounds (SVOCs) candidates for grouping (detected in more than 10% of the French dwellings investigated).

Chemical Family	**Name**
Phthalates	DEHP, DEP, DBP, BBP, DiBP, DiNP, DMP
Polybromodiphenyl ethers (PBDEs)	BDE-28, BDE-47, BDE-85, BDE-99, BDE-100, BDE-153, BDE-154, BDE-209
Polychlorobiphenyls (PCBs)	PCB-28, PCB-31, PCB-52, PCB-77, PCB-101, PCB-105, PCB-118, PCB-138, PCB-153, PCB-180
Polycyclic aromatic hydrocarbons (HAPs)	acenaphtene, anthracene, benzo(a)pyrene, fluorene, phenanthrene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(k)fluoranthene, benzo(b)fluoranthene, indeno(1,2,3-cd)pyrene, benzo(g,h,i)perylene, dibenzo(a,h)anthracene
Pesticides	4',4-DDE, 4',4-DDT, dieldrine, alpha-endosulfan, lindane, chlorpyrifos-ethyl, diazinon, cyfluthrine, cypermethrine, deltamethrine, permethrine, oxadiazon, tributylphosphate
Others	4-tert-butylphenol, 4-tert-octylphenol, bisphenol A, galaxolide, tonalide, triclosan

Based on Mandin et al., 2014a; Mandin et al., 2014b; Blanchard et al., 2014

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Table II - Available data on in vitro neurotoxic effect for the 15 Semi Volatile Organic Compounds (SVOCs) retained for BMC calculations

SVOC	Cell line	Duration of exposure (hours)	Test	Tested doses (µM)	Response: cell viability (standard deviation) (%)	Reference
DEHP[#]	Neuro-2a	24	MTT	0 0.1 1 10 40 80	100 (0) 101.54 (2.67) 103.08 (8) 92.31 (5.33) 56.15* (21.3) 42.31* (5.33)	(Lin et al. 2011)
BDE-47[#]	SK-N-MC	24	MTT	0 1 2.5 5 10 15 25 50	100 (6.25) 95 (3.75) 100 (5) 78.75* (5) 53.75* (8.125) 35.25* (7.5) 12.5* (3.125) 5* (1.25)	(Tagliaferri et al. 2010)
BDE-99[#]	SK-N-MC	24	MTT	0 1 5 10 20 30 40 50 100	100 (9.375) 97.5 (6.25) 95 (8.75) 85* (6.25) 72.5* (10) 52.5* (12.5) 45* (7.5) 30* (6.25) 17.5* (4.375)	(Tagliaferri et al. 2010)
BDE-209[#]	Neonatal hippocampal neurons of SD rats (PND1)	24	MTT	0 10.43 31.28 52.13	100 (13.78) 81.03* (11.72) 58.62* (9.31) 45.17* (3,45)	(Chen et al. 2010)

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PCB-52[#]	SK-N-MC	24	Trypan Blue	0 17.12 34.25 51.37 68.49 85.62	100 (0) 93.15 (1.61) 68.55* (3.63) 54.03* (4.03) 18.15* (2.02) 12.10* (0.46)	(Lee et al. 2005)
PCB-77[#]	Primary cortical cells of Wistar rats (GD 17-18)	5	MTT	0 30 50 100	100 (5.61) 72.95* (9.80) 60.38* (8.87) 46.86* (7.94)	(Sánchez-Alonso et al. 2003)
PCB-118[#]	GT1-7	24	CellTiter -Blue	0 0.1 1 10 100	100 (2,31) 67.47* (1.03) 69.27* (0.77) 69.88* (0.26) 63.26* (1.03)	(Dickerson et al. 2009)
PCB-153[#]	GT1-7	24	CellTiter -Blue	0 0.1 1 10 100	100 (1.54) 74.84* (1.03) 84.54* (0.77) 61.20* (0.51) 43.03* (2.05)	(Dickerson et al. 2009)
B(a)P[&]	Primary cortical cells of SD rats (PND 0-3)	40	CCK-8	0 0.5 1 2 5 10 20 40 80	100 (2.43) 94.77 (4.05) 94.25 (3.51) 90.29* (2.70) 86.46* (3.24) 80.48* (2.70) 74.56* (6.49) 67.37* (5.41) 59.58* (6.76)	(Nie et al. 2014)
BPA[#]	HT-22	24	MTT	0 1 10 50 100 500	100 (12.59) 154.55 (48.81) 210 (124.40) 308.18* (70.86) 217.27* (29.91) 27.27* (15.74)	(Lee et al. 2008)

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Dieldrin[#]	N27	48	MTT	0 10 25 50 75 100	100 (5.33) 82.75 (5.77) 65.26* (9.77) 37.30* (8.00) 23.55* (7.10) 12.12* (10.22)	(Sharma et al. 2010)
Lindane[#]	N27	48	MTT	0 10 25 50 75 100	100 (1.33) 99.49 (2.67) 92.05 (4.00) 87.95 (7.10) 81.03* (3.55) 79.49* (8.44)	(Sharma et al. 2010)
Diazinon[#]	PC12	48	MTS	0 100 200 300 400	100 (1.84) 91.32* (2.77) 79.62* (2.77) 75.47* (0.47) 67.92* (0.93)	(Bahrami et al. 2013)
Chlorpyrifos-ethyl[#]	SH-SY5Y	24	MTS	0 25 50 100 200	100 (0) 91.25* (7.27) 72.50* (10.62) 54.50* (10.62) 32.75* (10.06)	(Ki et al. 2013)
Deltamethrine[#]	SH-SY5Y	24	MTT	0 0.01 0.1 1 10 100 1000	100 (9.26) 105.10 (1.99) 99.38 (7.94) 93.13 (11.25) 76.57* (9.93) 62.19* (7.27) 43.13* (5.95)	(Romero et al. 2012a)

** Results differ significantly from the control*

[#] Data was obtained by manual measures conducted on the graphics directly

[&] Treated with S9 in order to metabolize the parent compound

MTT: Mitochondrial functionality test based on formazan product formation insoluble in water; MTS: Mitochondrial functionality test based on formazan product formation soluble in water; CCK-8: Commercial kit of the MTS test; CellTiter-Blue: Commercial kit of the resazurin test; Trypan Blue: Membrane integrity test; SD: Sprague Dawley; GD: Gestational day; PND: Postnatal day; GT1-7: Hypothalamic cells of mice; HT-22: Hippocampal cells of mice; N27:Dopaminergic cells of rats; Neuro-2a: Neuroblastoma cells of mice; PC12: Pheochromocytoma cells of rats; SH-SY5Y: Human neuroblastoma cells; SK-N-MC: Human neuroblastoma cell

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Table III - BMC_{10} of the 13 Semi Volatile Organic Compounds (SVOCs) ultimately selected, on the basis of in vitro neuronal death

SVOC	Number of Hill parameters	Log likelihood	BMC_{10} (μM)	$BMCL_{10}$ (μM)*	$BMCU_{10}$ (μM)*
PCB-153	3	21.82	0.072	0.00781	0.46987
Deltamethrin	3	50.12	1.996	1.3991	2.7856
B(a)P	4	66.52	2.43	1.0346	5.2125
BDE-47	4	41.4	4.83	3.6287	6.0664
BDE-209	2	21.4	4.84	4.0828	5.812
BDE-99	3	16.46	6.80	4.7495	9.2436
PCB-77	2	35.77	9.54	8.6715	10.533
DEHP	4	32.13	10.64	8.0276	14.096
Dieldrin	3	4.11	15.54	9.032	23.585
Chlorpyrifos	3	18.97	20.09	13.848	28.168
Lindane	2	38.9	38.93	33.302	46.513
PCB-52	4	12.71	39.89	30.724	44.39
Diazinon	2	80.21	94.35	90.24	98.788

* BMCL and BMCU are respectively the lower and upper 90%-confidence bounds.