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

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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. 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.

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}–10 Pa)" (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), 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

Abbreviations: AIF, apoptosis-inducing factor; B(a)P, benzo(a)pyrene; Bax, Bcl2 associated X protein; BBP, benzylbutylphtalate; 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; 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

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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 (Muñoz-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 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 and Ishido, 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 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. (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., 2014a) 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 Fig. 1. It induces activation of 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 (CytC) (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

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