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Neurotoxicity of fragrance compounds: A review

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A B S T R A C T

Fragrance compounds are chemicals belonging to one of several families, which are used frequently and globally in cosmetics, household products, foods and beverages. A complete list of such compounds is rarely found on the ingredients-list of such products, as "fragrance mixtures" are defined as "trade secrets" and thus protected by law. While some information regarding the general toxicity of some of these compounds is available, their neurotoxicity is known to a lesser extent. Here, we discuss the prevalence and neurotoxicity of fragrance compounds belonging to the three most common groups: phthalates, synthetic musks and chemical sensitizers.

1. Introduction

The term "fragrance", frequently mentioned in the ingredients list of numerous cosmetic and household products, refers to a mixture of compounds which produces a variety of subjectively pleasant odors. The limited information currently available regarding the safety of fragrance compounds and rates of human exposure is due to ambiguity, as manufacturers are not required to elaborate on the ingredients of fragrance mixtures, as they are classified a "trade secret" (The Federal Fair Packaging and Labeling Act, 1973). In recent years, the accumulation of fragrance compounds in the environment, wildlife and humans, as well as related health issues have been studied, due to the ubiquitous presence of such compounds in cosmetics, cleaning products, air fresheners and many other products of daily use (Bridges, 2002).

Most fragrance compounds belong to one of three families: phthalates, synthetic musks and "sensitizers" – a group where some phthalates and synthetic musks might also be found (Bridges, 2002; Llompart et al., 2013; Siti Zulaikha et al., 2015). These compounds accumulate in the environment and wildlife, thus serving as a source for secondary exposure in humans (in addition to direct exposure following application). Seven fragrance allergens have been detected in wastewater from two water treatment plants in Spain, four of which in effluent water following two treatments – a finding pointing to the questionable degradability of such compounds (Godayol et al., 2015). Galaxolide and Tonalide, both synthetic musks, are two of the most common fragrance ingredients found in fish from rivers and lakes in the US (Chicago, Illinois; Dallas, Texas; Orlando, Florida; Phoenix, Arizona; and West Chester, Pennsylvania) and in Germany (Mottaleb et al., 2009; Ramirez et al., 2009; Rudel et al., 2006). These synthetic musks and other fragrance ingredients were also found in earthworms from agricultural soil (Chu and Metcalfe, 2007), in marine mammals and sharks (adults and fetuses) from Japanese coastal waters (Nakata, 2005), Danish farmed trout (Duedahl-Olesen et al., 2005) and, to a lesser extent, in remote Swiss alpine lakes (Schmid et al., 2007).

Unfortunately, evaluating direct exposure to such compounds is challenging: when two methods called "Matrix Solid-phase Dispersion" and "Gas Chromatography–Mass Spectrometry" were used to evaluate the quantity and variety of fragrance-related ingredients, 25 out of 26 different cosmetic products tested positively for phthalates and synthetic musks, 10 of which are on a prohibited list (Llompart et al., 2013) – a finding which further emphasizes the weak regulatory status of fragrance compounds. By applying analogous methods, 12 synthetic musks were found in 140 different personal care products: the highest concentration of these compounds was found in perfumes and shampoos for adults (5245.05 and 487.67 μg/g, mean, respectively) with Galaxolide, Exaltolide and Cashmeran as the most detected compounds (Homem et al., 2015a). These concentrations were used for calculating daily dermal exposure rates, which are 75.69 μg/kg/day for adults and 15.54 μg/kg/day for babies/children, with perfumes and lotion serving as the main contributors for exposures. Interestingly, while dermal exposure in adults is mainly to Exaltolide, Galaxolide and Tonalide (40%, 30% and 15% respectively), in children and babies 96% of dermal exposure is to Exaltolide, emphasizing the importance of the age variable in studying and regulating fragrance compounds.

Several health concerns are associated with exposure to fragrance compounds: skin, respiratory, neurological and systemic pathology are a few examples (Bridges, 2002). Fragrance compounds are consistently presented as either the first or second most common contributors to allergic contact dermatitis (ACD) and fragrance products, when

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compared to over 200 other commercial products, contain the highest number and concentration of endocrine disruptors and asthma-related compounds (Adams and Maibach, 1985; Biebl and Warshaw, 2006; Davis et al., 2008; de Groot and Frosch, 1997; Dodson et al., 2012; Kohl et al., 2002; Penchalaiah et al., 2000; Tomar et al., 2005; Wetter et al., 2010). In the following sections, neurotoxicity of the three fragrance-compound families will be discussed.

2. Phthalates

Phthalates are esters of phthalic acid and are found in a great variety of products, such as adhesives, detergents, pharmaceuticals, cosmetics and fragrances, to name a few (Crinnion, 2010). In perfumes, several phthalates are used for enabling slow evaporation of the fragrance in order for the scent to linger longer, such as dimethyl phthalate (DMP) and diethyl phthalate (DEP) (Orecchio et al., 2015). Phthalates are released into the environment, not only during their production and disposal, but also during normal use of plastic products, due to their weak bond to the plastic (Huang et al., 2013). The annual global rate of phthalates production is estimated at 8 million tons, with phthalate concentrations in the air, water and soil continuously rising (Zhang et al., 2015); furthermore, phthalates are among the most abundant endocrine disrupting compounds found in indoor air and dust (Larsson et al., 2017; Rudel et al., 2006). Various studies have addressed the detrimental effects of phthalate exposure, emphasizing endocrine disruption (Chen and Chien, 2014; Chen et al., 2014), bone mineral density (Min and Min, 2014), sperm function and morphology (Duty et al., 2005; Hauser et al., 2007) and obesity (Hatch et al., 2010).

Phthalate-related neurotoxicity in humans, animals and in vitro models has been addressed. The effects of phthalate exposure have been studied in children of different ages: Regarding children 2–5, higher concentrations of DEP, dibutyl phthalate (DBP), di-(2-ethylhexyl) phthalate (DEHP) and benzylbutylphthalate (BBzP) in house dust samples have been shown to be associated with the following: a) DEHP and BBzP were associated with developmental delay (DD), b) DEP and DBP were associated with poorer adaptive function (e.g. socialization, communication, motor skills) in typically developing children and c) DEP and DBP were also associated with greater hyperactivity and impulsivity in children with ASD or DD (Philippat et al., 2015). In children aged 4–9, prenatal exposure to specific phthalates (evaluated by measuring concentrations of phthalate metabolites in third-trimester maternal urine samples) was associated with poorer behavioral and executive functioning, including attention, problem-externalization, emotional control, aggression and depression, as indicated by scores on the Behavior Rating Inventory of Executive Function (BRIEF) and the Behavior Assessment System for Children Parent Rating Scales (BASC-PRS) (Engel et al., 2010). In children aged 8–11, higher urine concentrations of phthalates were associated with symptoms of attention-deficit/hyperactivity disorder (ADHD) and lower IQ scores (Cho et al., 2010; Kim et al., 2009). In children aged 6–15, phthalate urine concentrations were associated with the diagnosis of ADHD, the severity of its symptoms and impaired regional cortical maturation in diagnosed children (Park et al., 2015).

A considerable amount data is available regarding the effect of phthalate exposure in different developmental stages on hippocampal structure, function and gene expression. In a rodent model (rats and mice), both biochemical and behavioral evaluations were conducted following prenatal, perinatal and postnatal exposure to phthalates (Holahan and Smith, 2015). When tested on the Morris Water Maze for the evaluation of hippocampus-related spatial navigation, prenatally exposed 21 month old rats exhibited impaired spatial acquisition and retention, swimming a longer distance to the hidden platform while spending less time on average in the quadrant where the hidden platform was located (Sun et al., 2014); a month later, a reduction in insulin gene expression and an increase in Tau phosphorylation in the brains of the same rats was associated with the spatial deficits. These neurochemical findings not only point to structural and functional deficits in the hippocampus, but also suggest a relation to Alzheimer's disease (AD), corroborated by high levels of phosphorylated Tau proteins (Fiandaca et al., 2015).

Perinatal exposure to phthalates was also associated with neurobehavioral impairments: following the administration of DBP between gestation day 6 to postnatal day 21, neuronal apoptosis was observed in the hippocampus of 5 and 21 day old rats, as indicated by TUNEL, caspase-3 and Annexin V-propidium iodide (Li et al., 2013). Structural and functional alteration were also found in the hippocampus of exposed rats, as attested by synaptophysin expression and field excitatory postsynaptic potential respectively, along with behavioral deficits (spatial learning and memory in the Morris Water Maze); the suggested underlying mechanisms for the detrimental effects of perinatal exposure to DBP were dysregulation of aromatase, estrogen receptor β (ERβ), brain-derived neurotrophic factor (BDNF) and c-AMP-responsive element binding protein (CREB) (Li et al., 2014). Perinatal exposure to DEHP was also followed by anxiety and depressive-like behaviors (e.g. elevated plus maze, mirrored chamber), along with downregulation of androgen receptor in males, ERβ in females, and inhibited phosphorylation of extracellular signal-regulated protein kinases 1 and 2 (ERK1/2) in 6-week-old mice (Xu et al., 2015).

Additional studies addressed phthalate exposure at a later life stage: analysis of brains of 26 day-old rats following exposure to DEHP in postnatal days 16–22 showed reduced density of axonal innervation (indicated by synaptophysin) and cells (mature and immature, indicated by cresyl violet and doublecortin respectively) in the CA3 and DG areas in the hippocampus of males, suggesting a sex-specific effect of phthalate exposure on the development of the nervous system during a specific sensitive timeframe (Smith et al., 2011). Further evidence for the detrimental effects of DEHP exposure during this period of development on functional plasticity in the nervous system is derived from a study showing reduced spine density on basal and apical neurons in the CA3 hippocampal area in males only, consistent with downregulation of BDNF expression (Smith and Holahan, 2014).

Finally, neurobehavioral toxicity of phthalates in adults has also been documented: following an 8 week exposure to different doses of diisobutyl phthalate (DiBP) (50–1000 mg/kg), mice in the highest dose group exhibited learning impairments in the passive avoidance response test and increased rate of hippocampal cell apoptosis, while mice in all dose groups showed hippocampal ultrastructural damage (Ma et al., 2013a). Dysregulation of cAMP/PKA-CREB signaling pathway was invoked as an underlying mechanism for these findings (Ma et al., 2013b). A 14-day exposure to benzyl butyl phthalate (BBP) in adult mice, with doses ranging between 250–1250 mg/kg, was associated with impaired learning and memory abilities (Morris Water Maze), along with reduced locomotion in the forced swim test and depression (evaluated with the tail suspension test) (Min et al., 2014). Lower levels of phosphorylated CREB, secondary to altered serotonergic system, and oxidative stress were invoked to mediate these effects. In rats, neonatal exposure to phthalates led to motor hyperactivity and altered gene expression of *N*-methyl-D-aspartate receptor (NMDA) receptors, γ-aminobutyric acid (GABA) transporter, dopamine transporter 1, to name a few, in the striatum and midbrain (Masuo et al., 2004).

The effects of phthalate exposure on cortical neurotoxicity has also been studied in both in vitro and developmental models. Following exposure to DBP, primary cultures of mouse neocortical neurons show increased levels of apoptosis and decreased cell viability, related to reactive oxygen species (ROS) formation and caspase-3 and lactate dehydrogenase (LDH) activity stimulation (Wojtowicz et al., 2017). DBP exposure was associated with enhanced expression of aryl hydrocarbon receptor (AhR), and decreased expression of ESα, ESβ and peroxisome proliferator-activated receptor Gamma (PPARγ). When studied in vivo, prenatal exposure to DEHP in the mouse model increased cell death and reduced proliferation and neurogenesis in the dorsal telencephalon of fetal mice; in the neocortex of newborn mice,