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MiniReview

Polyfluorinated Alkyl Phosphate Ester Surfactants – Current Knowledge and Knowledge Gaps

Camilla Taxvig, Anna Kjerstine Rosenmai and Anne Marie Vinggaard

Division of Toxicology and Risk Assessment, National Food Institute, Technical University of Denmark, Søborg, Denmark

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Abstract: Fluorochemicals are a diverse group of synthetically produced compounds with the unique ability to repel water as well as oil. This property makes them ideal for multiple purposes in a variety of consumer and industrial products. Fluorochemicals have been detected in the environment, as well as in human blood, urine and milk. Due to their long half-life in human beings, there is an increased risk that exposure to these compounds can cause adverse effects. However, except for perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS), there is a large data gap regarding toxicological information on fluorochemicals. Polyfluorinated alkyl phosphate ester surfactants (PAPs) belong to the group of polyfluorinated alkyl surfactants. They have been detected in indoor dust and are widely used in food-contact materials, from which they have the ability to migrate into food. Toxicological data on PAPs are very limited, but current studies indicate that some PAPs have the potential to interfere with sex hormone synthesis *in vitro*. Disturbance of the sex hormone balance in foetal life has been suggested to be an important mechanism involved in adverse effects on, for example, male reproductive health and development. The current lack of toxicological data on PAPs impairs the risk assessment of this group of compounds. However, until more toxicological data on PAPs are available, the limited data currently accessible give reason to believe that these compounds might have the ability to cause potentially adverse effects, as seen for other perfluorinated chemicals, including some metabolic products of PAPs.

Perfluorinated alkyl carboxylic acid (PFCA) and perfluorosulphonic acids (PFSAs), such as perfluorooctanoic acid (PFOA) and perfluorooctane sulphonate (PFOS), respectively, have been detected in human blood at ng/mL levels from populations throughout the world [1]. Because of their global occurrence and known persistence (elimination half-lives ranging from hours in rodents up to 8 years in human beings, depending on the congener) [2, 3], in addition to their reported toxicity in animal and cell models [3–8], an increased focus has arisen regarding the potential health risk that continued exposure to these compounds may pose to human beings. This concern has further been supported by the increase in human epidemiological studies showing associations between various health effects in human beings, like breast cancer development and attention deficit hyperactivity disorder (ADHD), and exposure to specific fluorochemicals, for example PFOA and PFOS [9–12].

Federal environmental departments in the United States and Canada have already restricted the use of PFCAs and some PFCA precursors [13,14]. Within Europe, the use and regulations differ between countries, but in the EU, the use of PFOS has been regulated in the way that it may be present in finished or semi-finished products at a maximum content of 0.001% by weight (10 mg/kg) only, although some industrial applications have been exempted [15]. However, many other fluorinated compounds – including newly identified precursors to PFCAs (e.g. perfluorophosphonates, perfluorophosphinates) – remain in use [16]. Comparison of known PFCAs and PFSAs to total organic fluorine analysis in human blood has suggested that a large fraction of the organofluorine content in human beings has not been well characterized [17]. This highlights the need to identify and characterize unknown or lesser studied fluorochemicals, such as the polyfluorinated alkyl phosphate ester surfactants (PAPs), and evaluate both sources relevant to human exposure, as well as their toxicology, in order to better understand their potential to cause adverse effects in human beings.

The aim of this MiniReview is to give an overview of the current knowledge on the PAPs and to present the important knowledge gaps. The hope is that such an overview can be used as a tool in prioritization of future experimental studies.

Properties and Nomenclature

Fluorochemicals are a group of industrially produced compounds, with the unique ability to repel both water and oil, a property that makes them ideal for multiple purposes in a variety of consumer and industrial products.

The fluorochemicals can roughly be divided into two groups, the *per*fluorinated, containing only fluorinated carbon atoms, except for the carbon atom in the functional head

Author for correspondence: Camilla Taxvig, Division of Toxicology and Risk Assessment, National Food Institute, Technical University of Denmark, Mørkhøj Bygade 19, DK-2860 Søborg, Denmark (fax + 45 35887001, e-mail camta@food.dtu.dk).

group, and *poly*fluorinated compounds, containing at least one fully fluorinated carbon atom [18].

Phosphate ester surfactants belong to the group of polyfluorinated alkyl surfactants. Polyfluorinated alkyl phosphate ester surfactants are found in three forms depending on the levels of phosphate ester substitutions: monoesters (monoPAPs), diesters (diPAPs) and triesters (triPAPs). The PAPs exist as structural isomers with variations in alkyl chain lengths as they are synthesized from a mixture of fluorotelomer alcohols (FTOHs). Industrial PAPs mixtures consist primarily of diPAPs, with monoPAPs and triPAPs being produced as by-products [19–21]. The nomenclature for PAPs is based on the number of carbons in the perfluoroalkyl moiety *versus* the hydrocarbon linkage. For example, 4:2 diPAP corresponds to the disubstituted phosphoric acid containing two F $(CF_2)_4CH_2CH_2$ groups attached to the central phosphate group. For diPAPS with two different perfluorocarbon chain lengths x and y, the nomenclature follows x:2/y:2 diPAP (fig. 1). For example, polyfluoroalkyl groups in 6:2/8:2 diPAP are $F(CF_2)_6CH_2CH_2$ and $F(CF_2)_8CH_2CH_2$ substitutions [16].

Usage, Exposure and Biotransformation

Polyfluorinated alkyl phosphate ester surfactants are used as coating agents for food-contact materials of paper and board and have a documented ability to migrate from packaging into foods or food simulants [20]. diPAPs were found in 57% (42 of 74) of selected samples of food packaging taken from Danish, Swedish and Canadian retailers [22], and PAPs therefore constitute a class of compounds to which human beings are exposed. In a recent study, diPAPs were reported to represent more than 91% of the total measured fluorinated compounds in indoor dust samples (mean ∑diPAPs concentration above 7 μg/g dust), indicating that indoor dust may as well be a potential source of human exposure [16]. However, the authors also pointed out that diPAPs in the dust may also serve as a surrogate measure for PAPs-containing products being used in the households, meaning that direct exposure to these commercial products might be a more important source of human diPAPs exposure compared to dust [16].

Recent research confirms that human beings are exposed to PAPs, as several diPAPs congeners have been detected in human sera at μg/L levels, indicating that absorption occurs [23]. These findings are supported by animal studies showing that diPAPs can be absorbed across the gastrointestinal tract, both when administered as single diPAPs [24] or a mixture of homologues of diPAPs, but the absorption seems to decrease with increasing chain lengths [25]. Finally, the presence of PAPs in human milk (at ng/mL levels) was recently reported [26] suggesting that the newborn child may be exposed through breastfeeding. Fig. 2 illustrates some of the known sources of exposure to PAPs, such as food packaging material and indoor dust. However, high concentrations of diPAPs have also been detected in sludge from wastewater treatment plants [23], and several PAPs have been measured in drinking water [27]. Thus, besides being a proxy for human use, these matrices could also be considered potential sources for human exposure.

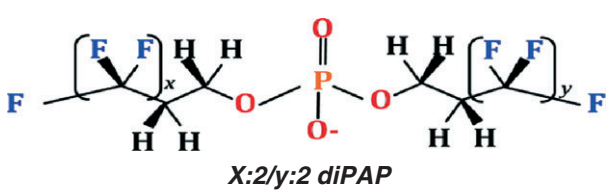

Fig. 1. Structural backbone of diPAPs (modified from [16]).

The elimination half-life of 8:2/8:2 diPAPs and 8:2 monoPAPs has been investigated in male rats after oral dosing, based on the disappearance of the chemicals after peak concentrations in whole blood. The whole blood half-life was approximately 3 days for monoPAPs and 1.3 days for diPAPs [24]. Biotransformation of diPAPs, in the rat [24] and in microbial systems [9,28], occurs by dephosphorylation leading to the formation of monoPAPs that can undergo further biotransformation to PFCA such as PFOA (fig. 2). Biotransformation of PAPs to PFCA is thought to be a contributing source to PFCA exposure [25]. The excretion of mixtures of diPAPs homologues or monoPAPs homologues in male rats showed that renal excretion is not the major direct elimination pathway [24]. However, more detailed studies on excretion of PAPs are needed to better understand the key elimination pathways. See fig. 2 for the metabolic pathway of PAPs.

Toxicological Data

The toxicological effects of PFCAs, such as PFOA, as well as PFOS, and to a lesser extent FTOHs, have been widely investigated and reviewed over the last years [4–6,9,29]. Polyfluorinated alkyl phosphate ester surfactants have been found in human blood and breast milk [23,26], indicating that the general population, foetuses, as well as infants might be exposed. As PAPs have been identified as potential precursors to PFCAs, meaning that PAPs are metabolized to PFCA in for instance the mammalian body, this raises concern that the toxicological effects reported for some PFCAs are also effects relevant for PAPs. A search through the available literature revealed only very limited data regarding the toxicology, mechanisms of action and potential adverse effects of PAPs. To our knowledge, only a single *in vitro* study has been published regarding the toxicology of PAPs. In this study, the potential of four PAPs (8:2 monoPAP, 8:2/8:2diPAPs, 10:2/10:2 diPAP and 8:2/8:2/8:2 triPAP) to interfere with steroid hormone synthesis and androgen receptor (AR) activation using two *in vitro* assays (the H295R steroidogenesis assay and a AR reporter gene assay) was examined [30]. All four PAPs caused decreased levels of progesterone and 17α-OH-progesterone in the H295R assay and for two of the four PAPs, 8:2/8:2 diPAP and 8:2 monoPAP, a decrease in androgens levels was also observed. Concerning effects on oestrogens levels, an increase was seen in the levels of estrone for all tested PAPs, but no significant effect was found on the level of 17-β-estradiol. In addition to this, a significant

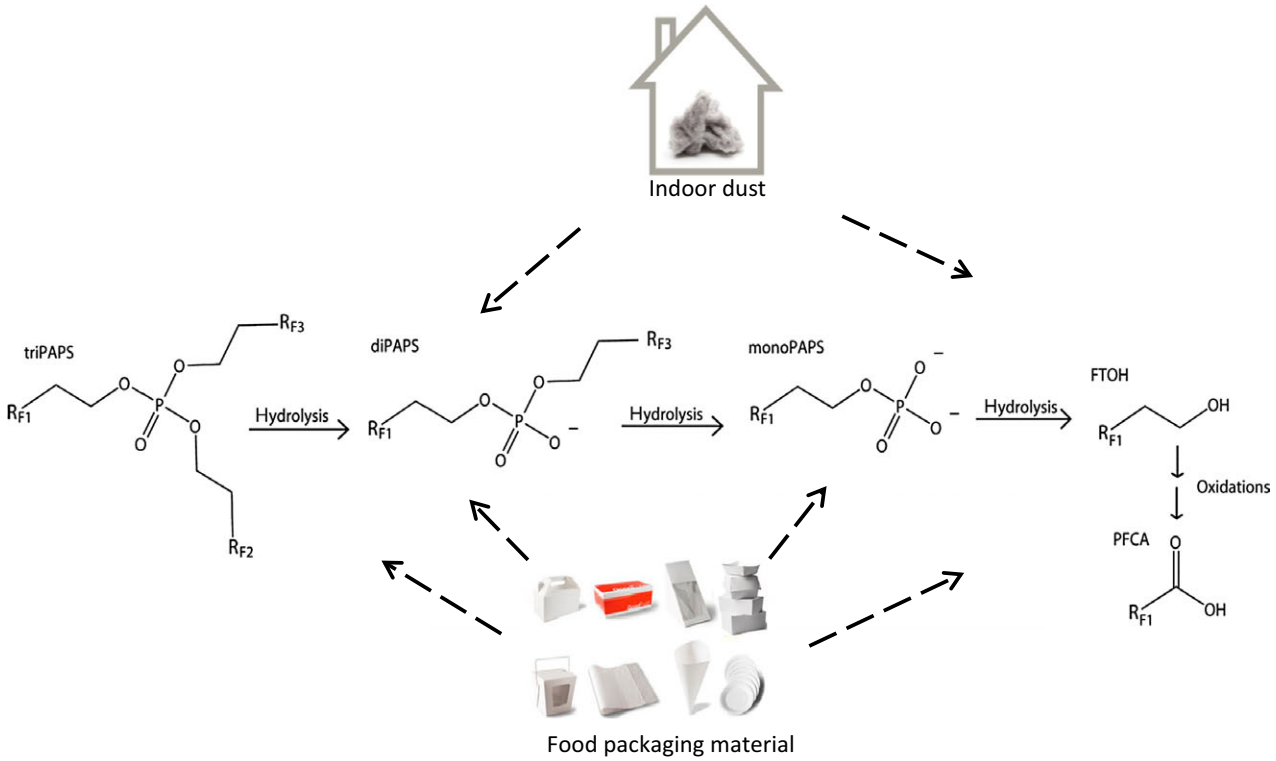

Fig. 2. Illustration of sources of polyfluorinated alkyl phosphate ester surfactants (PAPs) exposure (food packaging material and indoor dust) as well as the biotransformation of the PAPs. R_{F1}, R_{F2} and R_{F3} represent fully fluorinated carbon chains of varying lengths. Modified from [30].

increase in cortisol levels was observed for 8:2/8:2 diPAP. The study also included gene expression analysis, showing down-regulation of the peripheral benzodiazepine receptor (Bzrp) mRNA levels for 8:2 monoPAP. This finding indicated interference of 8:2 monoPAP with cholesterol transport over the inner mitochondrial membrane. Furthermore, increased aromatase mRNA expression was found for 8:2/8:2 diPAP and 8:2 monoPAP, which could be an explanation of the decreased androgen and the increased estrone levels [30]. No effects were found for the tested PAPs on human AR activation.

Overall, the toxicological data on the PAPs show that some of these compounds have the ability to affect steroid hormone production *in vitro*, primarily inhibiting androgen production, potentially through down-regulating Bzrp and/or up-regulating aromatase (CYP19) expression [30].

As mentioned previously, PAPs have been found *in vivo* to be biotransformed to PFCA such as PFOA. The toxicological effects of PFOA include among others developmental and reproductive effects, as well as effects on the thyroid and immune system. So far, activation of peroxisome proliferator-activated receptor α (PPARα) has been described as a prominent target of PFOA and a likely mechanism behind some of its reported effects *in vivo* [3]. It is likely that some of the known toxic effects of PFOA are also relevant for PAPs, and the PAPs effect on PPARs is one of the many toxicological end-points that would be relevant to address in further studies.

Conclusion and Perspectives

The very limited toxicological data so far available on PAPs suggest that these compounds have endocrine-disrupting abilities. This could be an issue in relation to potential adverse effects in wildlife and human beings, as many effects including impaired development of the reproductive system and development of neurological disorders can result from disturbance of the hormone system, for example inadequate androgen or thyroid hormone levels.

The effects of PAPs on steroid hormone production such as decreased testosterone levels are in accordance with what is seen for other fluorochemicals such as PFOS and some FTOHs [30, 31]. Previous data have shown that among the nuclear receptors studied, PPARα seems to be an important target of PFOS and PFOA and that disturbance of the thyroid hormone system as well as the oestrogen- and androgen receptors may also be targets for some fluorochemicals [7,29]. Whether the PAPs are also affecting these targets *in vivo* at real exposure levels is one of many questions needing to be addressed. It is evident that scientific and regulatory communities are only beginning to understand and effectively manage polyfluorinated compounds, including PAPs. As this class of compounds is persistent and accumulates in human beings with very long half-lives, and as the long-term human health consequences are poorly understood, it is strongly recommended that further research on the toxicology and exposure of the PAPs is prioritized and conducted. Until we know

more, we should exhibit precaution and consider the safety of PAPs as being cause of concern.

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