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Brief communication

Application of a multiple endpoint bacterial reporter assay to evaluate toxicological relevant endpoints of perfluorinated compounds with different functional groups and varying chain length

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ABSTRACT

Perfluorinated compounds are widely distributed in the environment; good knowledge about the toxic mode of action of these compounds can contribute to improved molecular design and risk assessment.

The studied compounds were evaluated with a bacterial multiple endpoint reporter assay for responses in four different mode of action classes (oxidative damage, DNA damage, general cell lesions and membrane damage). The results of our study clearly demonstrate that inductions of stress responsive genes occur for the different compounds and confirm some of the known mechanisms of work for well studied compounds like PFOA and PFOS, and in addition provide new information for less studied compounds.

Few inductions were observed after exposure to the low carbon number carboxylic acids, PFBtA ($CF_3(CF_2)_2C(O)O^-$), PFPtA ($CF_3(CF_2)_3C(O)O^-$), PFHxA ($CF_3(CF_2)_4C(O)O^-$) and PFHpA ($CF_3(CF_2)_5C(O)O^-$) at equimolar concentrations (0.0156–1 mM). The induction of membrane damage markers (*MicF* and *OsmY*) is prominently present after exposure to PFOS ($CF_3(CF_2)_7SO_3^-$) and even more after exposure to PFNA ($CF_3(CF_2)_7C(O)O^-$).

This is the first report describing the mode of action of carboxylic acids with 11 and 12 carbon atoms; they are equally potent inducers relative to PFOS and PFNA. Overall, the effects seen at the level of gene expression were higher for the sulfonic acids than for the carboxylic acids, but the effect of the chain length is more important than the effect of the functional group.

1. Introduction

Perfluorinated compounds (PFCs) are "multi purpose" chemicals; used in many commonly used products such as textile, leather, paper, carpets, curtains, insecticides and floor polishes. PFCs such as the carboxylic acids and sulfonic acids are aliphatic compounds and consist of a hydrophobic carbon chain in which all the hydrogen atoms are replaced by fluorines. Because of the strong covalent bond between the fluor – and carbon ions PFCs are useful for industrial purposes.

It was incorrectly assumed for a long time that their inert character made them metabolically inactive and non toxic. About ten years ago, the first studies were published illustrating the presence of PFCs in wildlife. The results indicated that especially PFOS (perfluoroctane sulfonate, $CF_3(CF_2)_7SO_3^-$) accumulates in blood and liver of top predators, implicating biomagnification and addressing the liver as the major target organ (Giesy and Kannan, 2001). A recent Canadian study on mammals, fish and birds from the Canadian Arctic illustrated that besides PFOS many other perfluorinated compounds like perfluorononanoic acid (PFNA, $CF_3(CF_2)_7C(O)O^-$), perfluorodecanoic acid (PFDA, $CF_3(CF_2)_8C(O)O^-$), perfluoroundecanoid acid (PFUnDA, $CF_3(CF_2)_9C(O)O^-$) and perfluorododecanoic acid (PFDoDa, $CF_3(CF_2)_{10}C(O)O^-$) are also found in higher vertebrates though at much lower concentrations (Martin et al., 2004).

Recent studies have documented adverse effects at different levels of biological organisation after exposure to PFCs. Serious damage of the liver and lung were observed after rat exposure to PFOS and PFOA (Cui et al., 2009). Other studies illustrated that PFOS as well as PFOA (perfluorooctanoic acid, $CF_3(CF_2)_6C(O)O^-$) caused hepatic peroxisome proliferation in rats (Berthiaume and Wallace, 2002; Lau et al., 2007). PFOS exposure can have effects on cell membranes and lipid metabolism (Hu et al., 2003). Oxidative DNA damage was observed after exposure of HepG2 cells to PFOA, due to intracellular reactive oxygen species formation (Yao and Zhong, 2005).

Most studies focused on the mode of action of PFOS, PFOA and PFBS ($CF_3(CF_2)_3SO_3$), nevertheless many different perfluorinated compounds are found in the environment therefore more mechanistic information for this whole group of compounds is needed for identification of the potential risk(s). Several papers already pointed out the importance and relevance of toxicity studies with

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different types of PFCs. A study on perfluorinated carboxylic acids by Kleszczynski and colleagues revealed a structure activity relationship (SAR) between the number of carbon atoms and cytotoxicity (Kleszczynski et al., 2007). Upham and colleagues looked at structure activity relationships between gap junctional intercellular communication and the number of carbon atoms (Combes, 2000; Kleszczynski et al., 2007; Upham et al., 1998).

The importance of (semi) high throughput *in vitro* screening assays to elucidate the mode of action of compounds was illustrated by a recent study performed by Hickey et al. (2009). The effects of 18 perfluoroalkyl compounds were evaluated with *in* vitro chicken embryo hepatocyte assay and revealed that long chain PFCs (PFDA and PFUnDA) can affect genes associated with the cholesterol metabolism pathway, while after exposure to low chain PFCs cytochrome P450 genes were upregulated (Hickey et al., 2009).

In this study we used a bacterial gene profiling assay to gain further insight into the mode of action of the most commonly used PFCs (Dardenne et al., 2007a; Orser et al., 1995). Bacterial biosensors are frequently used in (eco)toxicological studies; they are particularly suitable for compound screening and classification according to mode of action (Sorensen et al., 2006). The use of semi-specific endpoints like DNA-, oxidative- and membrane damage combined in one assay provides a good tool to gain more insight in the mode of action of different PFCs. Effects at the level of gene expression were compared between sulfonic acids and carboxylic acids and the effects of chain length were analysed. To analyse the effects of the carbon chain length, gene expression profiles were compared between carboxylic acids with 4, 6, 7, 8, 9, 10, 11 and 12 carbon atoms. The same procedure was followed for the sulfonic acids with 4, 6 and 8 carbon atoms. Two telomeric alcohols (6:2 FTOH and 8:2 FTOH) were included in the study since they are widely distributed and human and environmental exposure can be expected.(Stock et al., 2004).

2. Materials and methods

2.1. Bacterial strains

All bacterial strains used, except *PQ37* were based on an *Escherichia coli* K-12 derivative SF1 containing the mutations *lac4169* deleting the entire *lac* operon, and *rpsL*. All the different *lacZ* fusions were present as single chromosomal inserts (Orser et al., 1995). A selected list of strains from the publication by Orser was used responding to different types of stress like DNA damage, oxidative stress, protein denaturation, membrane damage, osmotic stress, general cellular stress and heavy metal presence (Table 1). The PQ37 strain, called *SfiA*, is part of the SOS chromotest derived from *E. coli* GC4436 with a deletion in the *lac* operon carrying a sfiA:lacZ fusion so that responses to DNA damaging agents could be measured (Quillardet and Hofnung, 1985).

2.2. Chemicals

Stock solutions of all perfluorinated compounds were made in 100% dimethylsulfoxide (DMSO), 5% of DMSO as a final solvent concentration was used in the assay. All chemicals used were of at least analytical quality. The different compounds with purity, supplier and the used abbreviations that were tested with the prokaryotic gene profiling assay are listed in Table 2. The highest concentration tested for all compounds except perfluorododecanoic acid and the fluorotelomeric alcohols was 1 mM PFC dissolved in 5% DMSO, the range that was tested included seven different concentrations; 1 mM, 0.5 mM, 0.25 mM, 0.125 mM, 0.0625 mM, 0.03125 mM and 0.0156 mM. The longest chains tested for the carboxylic acids and the fluorotelomeric alcohols had lower test concentrations because solubility of the compound was limited. The concentration range that was tested for perfluorododecanoic acid was 0.25 mM, 0.125 mM, 0.0625 mM, 0.0313 mM and 0.0156 mM, 0.0078 mM and 0.0039 mM dissolved in 5% DMSO. Both fluorotelomeric alcohols (6:2 FTOH and 8:2 FTOH) were tested at 100 μM, 50 μM, 25 μM, 12.5 μM, 6.25 μM, 3.125 μM and 1.56 μM dissolved in 5% DMSO.

2.3. Prokaryotic gene profiling assay

A summarized description of the standard protocol of the prokaryotic gene profiling assay that was performed is given, for detailed specifications we refer to (Dardenne et al., 2007a). Cultures of the 14 different strains were grown overnight in 50 ml tubes (250 rpm and 37 °C). The assay was performed in triplicate in 96 well plates; column 2 till 11 received a uniform amount of the different cultures diluted in Luria Bertani (LB) medium, column one was used as a blank and only received LB. Optical density was measured at 600 nm to check uniformity. After 90 min of resuscitation (37 °C and 200 rpm) the plates received the compound to be tested at different concentrations, optical density (600 nm) was measured before and after dosing. Columns 5–11 received an increasing concentration of the compound in a ½ serial dilution, columns 2–4 (negative controls) were only dosed with the solvent (5% DMSO). After 90 min of exposure (37 °C and 200 rpm) optical density (600 nm) was measured again and the cells were lysed to allow measurement of β-galactosidase. The reduction of ONPG (*O*-nitrophenyl-β-D-galactopyranoside) (colorless) to ONP (*O*-nitrophenol)

Table 1
Type of stress responses, the main groups and the stress gene promoters fused to the *lacZ* gene and their major inducers as originally published by Orser et al. (1995).

Type stress response			
Main group	Promoter	Gene product/function	Responsive to
Oxidative stress	KatG	Hydrogen peroxidase I	Oxidative stress
	Zwf	Glucose-6-phosphate dehydrogenase	Oxidative stress
	Soi28	Superoxide inducible gene	Superoxide radical generating agents
	Nfo	Endonuclease IV	Ss and dsDNA breaks, oxidative DNA damage
Membrane damage	MicF	Antisense RNA to 5′ OmpF	Membrane integrity, osmotic stress
	OsmY	Periplasmic Protein	Osmotic stress
General cell lesions	UspA	Universal stress protein	Growth arrest
	ClpB	Proteolytic activation of ClpP	Protein perturbation
Heavy metal stress	MerR	Regulation of the mercury resistance operon (mer)	Heavy metals
DNA Damage	Nfo	Endonuclease IV	Ss and dsDNA breaks, oxidative DNA damage
	RecA	General recombination and DNA repair	SOS response
	UmuDC	DNA repair	Radiation and/or chemically induced DNA damage
	Ada	Adaptive response to alkylation	DNA damage, mainly methyl adducts
	SfiA	Inhibitor of cell division	SOS response
	DinD	Unknown function within the DNA damage inducible response	DNA damage