Short Original Communications

Perfluorooctane Sulfonate Increases the Genotoxicity of Cyclophosphamide in the Micronucleus Assay with V79 Cells

Further Proof of Alterations in Cell Membrane Properties Caused by PFOS

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Abstract

Perfluorooctane sulfonate (PFOS; $C_8F_{17}SO_3^-$) is a fully fluorinated organic compound which has been manufactured for decades and was used widely in industrial and commercial products. The recent toxicological knowledge of PFOS mainly concerns mono-substance exposures of PFOS to biological systems, leaving the potential interactive effects of PFOS with other compounds as an area where understanding is significantly lacking. However, a recent study, reported the potential of PFOS to enhance the toxicity of two compounds by increasing cell membrane permeability. This is of particular concern since PFOS has been reported to be widely distributed in the environment where contaminants are known to occur in complex mixtures. In this study, PFOS was evaluated alone and in combination with cyclophosphamide (CPP) to investigate whether a presence of PFOS leads to an increased genotoxic potential of CPP towards hamster lung V79 cells. Genotoxicity was investigated using the micronucleus (MN) assay according to the recent draft ISO/DIS 21427-2 method. PFOS alone demonstrated no genotoxicity up to a concentration of 12.5 µg/ml. However, PFOS combined with two different concentrations of CPP, with metabolic activation, caused a significant increase in the number of micronucleated cells compared to treatments with CPP alone. These results provide a first indication that PFOS has the potential to enhance the genotoxic action of CPP towards V79 cells, suggesting, together with the alterations in cell membrane properties shown previously, that genotoxicity of complex mixtures may be increased significantly by changes in chemical uptake. Together with an earlier study performed by the own working group, it can be concluded that PFOS alone is not genotoxic in this bioassay using V79 cells up to 12.5 µg/ml, but that further investigations are needed to assess the potential interaction between PFOS and other substances, in particular regarding the impact of membrane alterations on the uptake of toxic substances.

Keywords: Alterations in cell membrane properties; genotoxicity; micronucleus assay; perfluorooctane sulfonate; PFOS

Introduction

Perfluorooctane sulfonate (PFOS, $C_8F_{17}SO_3^-$) belongs to a group of fully or 'perfluorinated' compounds which has recently received increasing attention based on its occurrence in the environment and toxicological effects. PFOS has been synthetically produced for more than 50 years and, due to its unique surface-active properties, is widespread in industrial and commercial products, e.g. fire fighting foams and coatings for textiles and paper products approved for food contact. The chemical structure of PFOS is characterized by an alkyl chain with fluorine substitutions forming strong carbon-fluorine bonds (C-F). Due to these high-energy bonds, PFOS demonstrates great resistance to hydrolysis, photolysis, microbial degradation and metabolism by vertebrates. PFOS is therefore considered to be persistent in the environment [1]. PFOS is among the most commonly detected perfluorinated chemical in the environment [2,3] and has been identified in arctic mammals [4,5,6] as well as in human blood and serum samples [7,8]. Recently, another perfluorinated chemical, perfluorooctanoic acid (PFOA), was detected at concentrations of 3,640 ng/L and 519 ng/L in German surface water and drinking water, respectively [9,10]. These findings are of concern and demonstrate the need for a comprehensive understanding of the toxic potential of these chemicals. Another issue that needs to be addressed in the context of the environmental toxicology of PFOS is the fact that contaminants in the environment almost always occur in complex mixtures. This leads to exposure situations where contaminants can interact and cause synergistic or antagonistic effects and, thus, gives rise to unexpected consequences compared to the individual toxicity of the contaminants. PFOS has been reported to be non-genotoxic in a number of microbial and mammalian assays [11]. The aim of the present study was to investigate the genotoxicity of PFOS as well as to evaluate whether PFOS possess the potential to increase the toxic action of a standard genotoxic substance, cyclophosphamide (CPP), towards hamster lung V79 cells. Genotoxicity was investigated using the micronucleus (MN) assay.

1 Method

The MN assay was performed according to the ISO Draft International Standard (ISO/DIS 21427-2, [12,13]). V79 cells were seeded at a density of 5 x 10^4 cells/ml onto slides in culture dishes and incubated at 37°C for 6 h. V79 cells with metabolic activation (rat liver S9 mix) were treated with 12.5 µg/ml PFOS both alone and in combination with 1.25 µg/ml or 2.5 µg/ml CPP for 4 h. Control cells were treated both with and without DMSO (1%). After fixation and air-drying preparations, slides were stained with Giemsa for 20 min. Two independent experiments were performed with two replicates for each treatment. Per treatment replicate, a total of 1000 cells were scored for the evaluation of the frequency of MN. In addition, the concentration of PFOS was determined using LC-MS/MS [10]. This was performed in order to assess differences in nominal and real concentrations. The chemical analysis indicated a good recovery of PFOS in the bioassay (100–110% of the nominal concentration).

2 Results

PFOS alone exhibited no increase in the frequency of micronucleated cells relative to the control (**Fig. 1**).

These results are in agreement with an earlier MN assay performed by our working group where PFOS (12.5 µg/ml) was evaluated towards V79 cells both with and without metabolic activation (data not shown). Co-exposure of cells to PFOS and CPP caused a significantly greater incidence of MN when compared to both controls. PFOS combined with CPP (2.5 µg/ml) resulted in a clear increase in micronucleated cells compared with the same concentrations of CPP and PFOS alone. A similar tendency was observed for PFOS combined with CPP (1.25 µg/ml), although cautious interpretation is required due to high variations in the treatment with CPP (1.25 µg/ml). Addition of PFOS to CPP (1.25 µg/ml) caused a greater frequency of micronucleated cells than the greater concentration of CPP (2.5 µg/ml) alone.

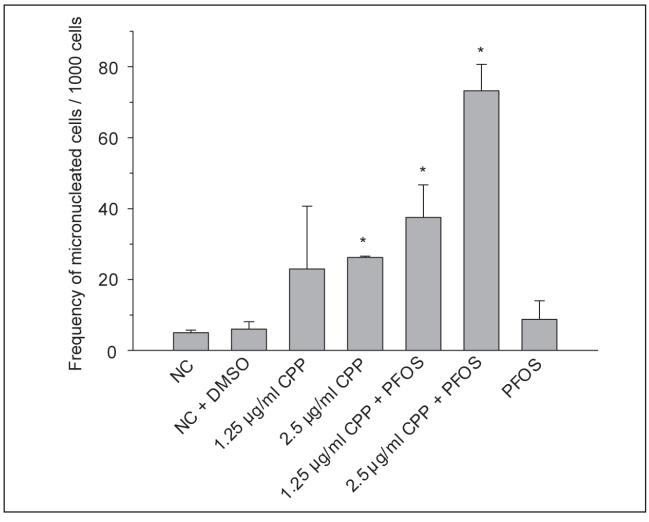

Fig. 1: Frequency of micronucleated V79 cells exposed to PFOS (12.5 µg/ml) combined with CPP (1.25 µg/ml and 2.5 µg/ml) with metabolic activation. The frequency of micronucleated cells shows a clear increase in treatments with both substances, compared with the single-substance treatments. Minimum Essential Medium (MEM) with and without DMSO (1%) served as a solvent and negative control (NC), respectively. Data are means ± SD of two independent experiments with 2 replicates each. 1000 cells were assessed for each replicate. * Significant genotoxicity (t-test with $p < 0.05$) compared to the controls

3 Discussion and Conclusions

The amphiphilic properties of PFOS suggest that cell membranes could be affected, potentially leading to an increase of the cellular 'accessibility' of other substances and a loss in homeostasis. Considering the global distribution of PFOS in wildlife and humans, the interactive effects of PFOS with other compounds could represent a cause for potential human and environmental health risks. This study offers the first indication of the potential of PFOS to increase the genotoxic action of CPP towards V79 cells. These results demonstrate that the frequency of micronucleated cells is greater in the combined treatments with metabolic activation compared to treatments with CPP and PFOS alone. PFOS along with metabolic activation showed no genotoxic potential towards V79 cells. Co-exposure of cells to PFOS and CPP (2.5 µg/ml) induced approximately three and eight times the amount of micronucleated cells compared to treatments with either CPP (2.5 µg/ml) or PFOS, respectively. Due to the high variation in treatments with 1.25 µg CPP/ml, no clear conclusions can be drawn concerning the same concentration combined with PFOS. PFOS combined with 1.25 µg CPP/ml, however, did reveal a slightly greater genotoxicity than 2.5 µg CPP/ml alone, i.e. the twofold increased concentration. It therefore seems reasonable to suggest that, while PFOS itself is inactive at micronuclei induction, the combination with CPP increases the mutagenic action of CPP by some undefined mechanism. Co-exposure of the two chemicals could suggest an additive effect with respect to the significant increase of micronucleated cells. The combination of PFOS and the higher concentration of CPP caused a larger increase of MN than would have been expected based on an additive toxicity assumption. Therefore, it appears that the greater genotoxicity of CPP in the presence of PFOS is caused by a potentiation. A possible explanation for this observation might be due to increased permeability of cell membranes. PFOS was found to affect membrane fluidity and mitochondrial membrane potential in fish leukocytes [14] and to inhibit gap junctional, intercellular communication in both rat liver cells and dolphin kidney cells [15]. These reports provide strong indications of the membrane alteration abilities of PFOS. Furthermore, PFOS was reported to increase the permeability of cell membranes to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and estradiol [14]. However, while PFOS (0.1 µg/ml) significantly increased responses to TCDD and E2, the increases observed were only approximately 40%. In the current study the potentiation of genotoxicity was greater. This observation may indicate that PFOS differentially increases the membrane permeability of structurally different compounds. The results suggest that the permeability of polar compounds, resulting from metabolic activation, may be more greatly enhanced than that of the previously studied, relatively non-polar compounds. From these observations, along with the present study, it seems possible that alterations in cellular membrane properties caused by PFOS could have considerable impact

on the availability of CPP to V79 cells, leading to the enhanced genotoxic action of CPP. We conclude that these results indicate a need for further and continued research activity to comprehensively assess the interactive effects of PFOS with other compounds in complex environmental mixtures, in particular with respect to the impact of membrane alterations on the uptake and effects of toxic substances. Because PFOS binds tightly to proteins [16] and the in vitro system studied here can not completely mimic the pharmacokinetics in an in vivo system, the combination studies need to be repeated in vivo with exposures that are environmentally relevant in both dose and dose rate.

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Perfluorinated Surfactants in Surface and Drinking Waters

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Abstract

Goal, Scope and Background. Perfluorinated surfactants (e.g. PFOS and PFOA) have shown different potentials for reproductory interference and carcinogenity in animal experiments as well as partly long half-lives in humans. They possess compound-dependent extreme recalcitrance against microbiological and chemical degradation and, in addition, they show variable potentials for bioaccumulation in animals and humans.

Methods. Surface and drinking water samples were collected from the rivers Rhine, Ruhr, Moehne and some of their tributaries. Further samples were taken from the Rhine-Herne-Canal and the Wesel-Datteln-Canal.

Conclusions. The concentrations found in drinking waters decreased with the concentrations of the corresponding raw water samples along the flow direction of the Ruhr river (from east to west) and were not significantly different from surface water concentrations. This indicates that perfluorinated surfactants have not been successfully removed by water treatment steps.

Recommendations and Perspectives. Because of their different problematic properties (persistence, mobility, toxicity, bioaccumulation), the concentrations of specific perfluorinated surfactants and their precursors in drinking waters and food have to be minimised. Therefore, it is of utmost importance to establish suitable legal regulations (limitations/ban) concerning the production and use of these surfactants and their precursors. Furthermore, it is indispensable to protect water resources from these compounds. A discussion on appropriate limit values in drinking water and foodstuffs is urgently needed.

Keywords: Drinking water; HPLC-MS/MS; organic waste; perfluorinated chemicals; perfluorinated surfactants; PFOA; PFOS; soil; surface water