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Short Communication

Negative Results of *umu* Genotoxicity Test of Fluorotelomer Alcohols and Perfluorinated Alkyl Acids

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Abstract

Objectives: Recently, perfluorooctanoate (PFOA) has been ubiquitously detected in the environment as well as in human serum. Fluorotelomer alcohols (FTOHs), a precursor of PFOA, undergo biodegradation via several metabolic routes which leads to formation of various biodegradation products. The degradation of FTOHs produces an α,β-unsaturated aldehyde that seems possibly to be electrophilic and may react with cellular macromolecules including DNA.

Methods: We investigated the genotoxicity of three FTOHs (6:2 FTOH, 8:2 FTOH and 10:2 FTOH), PFOA and perfluorooctane sulfonate (PFOS) using the *umu* test.

Results: The FTOHs, PFOA and PFOS showed no significant increases in β-galactosidase activity at 0–1000 μM in the absence of S9 mix. The results were unchanged by the metabolic activation with S9 mix.

Conclusion: The genotoxicities of FTOHs, PFOA or PFOS are not detectable using the present method, suggesting that they are unlikely mutagens.

Key words: perfluorooctane sulfonate, perfluorooctanoic acid, fluorotelomer alcohols, genotoxicity, *umu* test

Introduction

Perfluorochemicals such as perfluorooctanoate (PFOA) are environmental contaminants that may pose their health risks (1). The sources of PFOA in the environment remain unclear, however, the degradation of fluorotelomers, particularly fluorotelomer alcohols (FTOHs), might be an indirect source of perfluorinated carboxylic acids (PFCAs) (2). FTOHs are currently produced and used as intermediates for the synthesis of coatings, polymers, and surfactants among others (3).

FTOHs have been detected in ambient air in several countries (4, 5). Human exposure to FTOHs has not been established but it has been shown that FTOHs are metabolized to PFCAs *in vivo*. Higher chain length PFCAs which have relatively minor application compared with PFOA, were detected in human serum (6).

PFOA is a carcinogen for rodents (7), but is considered to be nonmutagenic because of its chemical stability. 8:2 FTOH undergoes biodegradation, and various metabolic products have been identified (8). One of the metabolites, an α,β-unsaturated aldehyde seems to be possibly electrophilic and may react with cellular macromolecules including DNA.

Here, we investigated the genotoxicity of FTOHs using the *umu* test.

Materials and Methods

umu test procedure

The assay for *umuC* gene expression was carried out according to the procedure described previously (9, 10). Briefly, bacterial cells were grown overnight at 37°C in Luria-Bertani broth containing ampicillin (50 μg/mL). The culture was diluted 100-fold with tryptone-glucose-agar medium consisting of 1% Bactotryptone (w/v), 0.5% NaCl (w/v), 0.2% glucose (w/v), and 20 μg/mL ampicillin and further incubated at 37°C until the bacterial OD600 reached about 0.3. The cultures were subdivided into 1 mL aliquots in test tubes, and 10 μL of a test compound in dimethylsulfoxide was added to each tube. These mixtures were incubated at 37°C for 5 hr with vigorous shaking. For metabolic activation of a chemical with S9 mixture, the cultures were subdivided into 0.85 mL aliquots in test tubes, to which the S9 mixture (0.15 mL) and a test

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chemical (10 μL) were added. These mixtures were incubated at 37°C for 3 hr with vigorous shaking, and then the bacterial density and β-galactosidase activity were measured by the method of Miller with slight modifications as described by Oda et al. (11, 12). The effect of the chemicals on bacterial growth was determined in the reaction mixture by measuring the absorbance at 600 nm.

The results are presented as means of results from two tubes from two to three independent experiments.

Bacterial strain

The bacterial strain used in this work was *Salmonella typhimurium* TA1535/pSK1002 (*hisG46*, *rfa*, *uvrB*).

Chemicals

The test chemicals used were obtained from the following sources: pentadecafluorooctanoic acid ammonium salt (PFOA) and heptadecafluorooctane sulfonic acid potassium salt (PFOS) from Fluka, Milwaukee, WI; 1H,1H,2H,2H-perfluorooctanol (6:2 FTOH), 1H,1H,2H,2H-perfluorodecanol (8:2 FTOH) and 1H,1H,2H,2H-perfluoro-1-dodecanol (10:2 FTOH) from Alfa Aesar, Ward Hill, MA; 2-aminoanthracene (2-AA) from Katayama Chemical Co., Ltd., Tokyo; 4-nitroquinoline 1-oxide (4-NQO) from Wako Pure Chemical, Osaka. Rat liver S9 fraction and cofactors were obtained from Oriental Yeast Co., Tokyo.

Table 1 Effects on cell growth and induction of *umuC* gene expression by 6:2 FTOH, 8:2 FTOH, 10:2 FTOH, PFOS, and PFOA in *S. typhimurium* TA1535/pSK1002 strain with or without S9 mixture

Chemical	S9	Concentration (μM)	Cell growth (OD_{600})	β-galactosidase activity (units)	
6:2 FTOH	–	0	2.094	65	±2
	–	250	2.089	65	±8
	–	500	2.086	66	±3
	–	1000	2.077	73	±6
	+	0	1.946	80	±5
	+	250	1.925	87	±4
	+	500	1.829	87	±5
	+	1000	1.777	92	±5
8:2 FTOH	–	0	2.094	65	±2
	–	250	2.070	67	±7
	–	500	2.071	68	±8
	–	1000	2.086	63	±2
	+	0	1.946	80	±5
	+	250	1.932	76	±1
	+	500	1.988	88	±1
	+	1000	2.025	86	±1
10:2 FTOH	–	0	2.094	65	±2
	–	250	2.105	74	±11
	–	500	2.091	65	±1
	–	1000	2.096	70	±0
	+	0	1.946	80	±5
	+	250	1.880	84	±4
	+	500	1.956	81	±11
	+	1000	1.955	79	±6
PFOS	–	0	2.249	55	±2
	–	30	2.271	41	±1
	–	100	2.300	38	±1
	–	300	2.370	46	±2
	–	1000	2.487	44	±9
	+	0	2.145	89	±7
	+	30	2.130	91	±6
	+	100	2.089	92	±16
	+	300	2.045	92	±8
	+	1000	1.916	93	±4
PFOA	–	0	2.190	62	±5
	–	30	2.222	58	±6
	–	100	2.163	67	±8
	–	300	2.227	63	±11
	–	1000	2.080	66	±5
	+	0	2.145	89	±7
	+	30	2.208	95	±1
	+	100	2.145	94	±0
	+	300	2.123	94	±1
	+	1000	2.032	86	±0
4-NQO	–	1.6	2.119	344	±78
2-AA	+	5.2	1.940	466	±5

4-NQO, 4-nitroquinoline 1-oxide; 2-AA, 2-aminoanthracene.

Data represent the means and standard deviations of two to three independent experiments.

Results and Discussion

As shown in Table 1, all of the tested FTOHs, PFOA and PFOS showed no significant increases in β-galactosidase activity at 0–1000 μM in the absence of S9 mixture. The results were unchanged by the metabolic activation with S9 mixture (Table 1).

The biotic and abiotic degradations of FTOHs lead to the accumulation of various products including PFCAs and cause secondary pollution. The toxicities of FTOHs and their metabolites have not been well understood. In this study, three FTOHs did not show detectable genotoxicity in the *umu* test. The S9 mixture activation of FTOHs also showed the same results although one of the metabolites of FTOHs, an α,β-unsaturated aldehyde, was suggested to be electrophilic. PFCAs, particularly PFOA, have been reported to cause liver cancer, Leydig cell tumor and pancreatic acinar cell tumor, the combination of which is known as the "tumor triad" (13). PFOA is classified as a peroxisomal proliferator (14) which is well known to induce the tumor triad (15). FTOHs might indirectly induce tumors via PFCAs. However, a direct action of FTOHs cannot be disregarded: FTOHs have been reported to exhibit estrogen-like properties in MCF-7 breast cancer cells (16). Leydig cell tumors are also induced by estradiol administration in mice (17). Additional studies on the nonmutagenic carcinogenicity of FTOHs are required.

In conclusion, the genotoxicities of FTOHs, PFOA or PFOS are not detectable using the present method, suggesting that they are unlikely mutagens.

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