Negative results of mutagenecity of fluorotelomer alcohols and perfluorinated alkyl acids

Yoshimitsu Oda [1], Shoji Nakayama [2], Kouji H. Harada [2] and Akio Koizumi [2]

[1] Osaka Prefectural Institute of Public Health, Osaka 537-0025, Japan

[2] Department of Health and Environmental Sciences, Kyoto University Graduate School of Medicine, Kyoto 606-8501, Japan

*Address correspondence to Akio Koizumi M.D., Ph.D., Department of Health and Environmental Sciences, Kyoto University Graduate School of Medicine, Yoshida Konoe-cho, Sakyo-ku, Kyoto, 606-8501, Japan. Tel: 81-75-753-4456. Fax: 81-75-753-4458. E-mail: koizumi@pbh.med.kyoto-u.ac.jp

Running Title: *umu* test for fluorotelomer alcohols

Abstract

Objectives: Recently, perfluorooctanoate (PFOA) was reported to be ubiquitously detected in the environment, as well as in human serum. A precursor of PFOA, fluorotelomer alcohols (FTOHs) undergo biodegradation via several metabolic routes and leads to formation of various biodegradation products. Degradation of FTOHs produces α,ß-unsaturated aldehyde which seemed to be electrophilic and may react with cellular macromolecules including DNA.

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

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

Conclusion: Mutagenicities of FTOHs or PFOA or PFOS are not discernible by the present method suggesting that they are unlikely mutagens.

Keywords: Perfluorooctane sulfonate, Perfluorooctanoic acid, Fluorotelomer alcohols, mutagenicity, *umu* test

Introduction

Perfluorochemicals such as perfluorooctanoate (PFOA) are environmental contaminants raising concerns regarding its health risks (1). The sources of PFOA in the environment remain unclear, however degradation of fluorotelomers, particularly fluorotelomer alcohols (FTOHs), might be indirect sources of perfluorinated carboxylic acids (PFCAs) (2). FTOHs are currently produced and used as intermediates for the synthesis of coatings, polymers, surfactant and so on (3).

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

PFOA is carcinogens for rodents (7), but are thought to have no mutagenicity due to their chemical stability. 8:2 FTOH undergoes biodegradation and various metabolic products were identified (8). One of the metabolites, α,β-unsaturated aldehyde seemed to be electrophilic and may react with cellular macromolecules including DNA.

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

Materials and Methods

umu test procedure

Assay for *umuC* gene expression was carried out according to the procedure described previously (9,10). Briefly, bacterial cells were grown for overnight at 37°C in Luria-Bertani broth including ampicilline (50 μg/mL). The culture was diluted 100-fold with TGA medium consisting of 1% Bactotryptone (w/v), 0.5% NaCl (w/v), 0.2% glucose (w/v), and ampicillin (20 μg/mL)and further incubated at 37°C until the bacterial OD600 reached about 0.3. The cultures were subdivided into 1 mL portion in test tube, and 10 μL of a test compound in dimethyl sulfoxide was added to each tubes. These

mixtures were incubated at 37°C for 5 hr with vigorous shaking. For metabolic activation of a chemical with S9 mixture, the cultures subdivided into 0.85 mL aliquots in the test tubes, to which S9 mixture (0.15 mL) and a test chemical (10 μL) added. These mixtures were incubated at 37°C for 3 hr with vigorous shaking, and then the bacterial density and the β-galactosidase activity were measured by the method of Miller with slight modification as described by Oda et al. (11,12). The cell growth effect of chemicals on bacterial cell was determined in reaction mixture by measuring the absorbance at 600 nm.

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

Bacterial strain

The bacterial strain used in this work was *S. 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 the cofactors were obtained from Oriental Yeast Co., Tokyo.

Result and Discussion

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

Biotic and abiotic degradation of FTOHs leads to the accumulation of various products including PFCAs and to cause secondary pollution. Toxicity of FTOHs and their metabolites has not been well understood. In this study, three FTOHs did not show detectable mutagenicity in *umu* test. S9 mix activation of FTOHs also showed same results although one of metabolites of FTOHs, α,ß-unsaturated aldehyde was suggested to be electrophilic. PFCAs in particular to 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 tumor triad (15). FTOHs might indirectly induce tumors via PFCAs. However, a direct action of FTOHs cannot be discarded: FTOHs have been reported to exhibit estrogen-like properties for MCF-7 breast cancer cells (16). Leydig cell tumors are also induced by estradiol administration in mice (17). Further investigation for non-mutagenic carcinogenecity for FTOHs is needed.In conclusion, mutagenicities of FTOHs or PFOA or PFOS are not discernible by the present method suggesting that they are unlikely mutagens.

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TABLE 1. The effects of 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})	ß-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	± 1 6
	+	300	2.045	92	± 8
	+	1000	1.916	93	± 4
PFOA	-	0	2.190	62	± 5
	-	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
	-	30	2.222	58	± 6
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 are the mean of two or three independent experiments.