ENVIRONMENTAL HEALTH & TOXICOLOGY
Vol. 25, No. 3, 181 ~ 186 (2010)

Toxicological Effects of PFOS and PFOA on Earthworm, *Eisenia fetida*

Ki-Eun Joung※, Eun-Hye Jo, Hyun-Mi Kim[1], Kyunghee Choi and Junheon Yoon

Risk Assessment Division, Environmental Health Risk Research Department, National Institute of Environmental Research
[1]Research Planning Division, Research Strategy and Planning Office, National Institute of Environmental Research

ABSTRACT

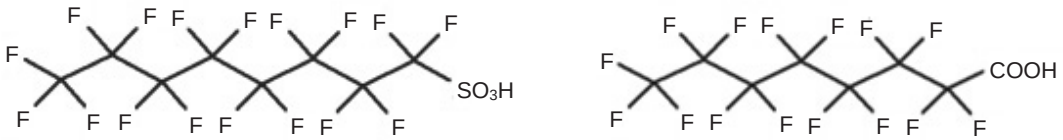
Perfluorinated Compounds (PFCs) are anthropogenic compounds found in trace amounts in many environmental compartments far from areas of production. Along with the highly persistent nature of PFCs, there are increasing concerns over the potential adverse effects of them on the ecosystems. Most of highly fluorinated compounds degrade into PFOS and PFOA that are very stable compounds hard to break down. So, in this study, we tried to determine the toxicity of PFOS and PFOA in the terrestrial invertebrate. Acute toxicity test using earthworm, *Eisenia fetida*, was performed according to the OECD test guideline 207 (Earthworm, Acute Toxicity Tests). In the 14 day acute toxicity tests, the highest concentration causing no mortality and the lowest concentration causing 100% mortality of PFOS were 160 and 655 mg/kg (dry weight), respectively. And the highest concentration causing no mortality and the lowest concentration causing 100% mortality were 500 and 1,690 mg/kg (dry weight), respectively in the PFOA-exposure group. 14 day-LC_{50} values were estimated at the level of 365 and 1,000 mg/kg (dry weight) in the PFOS and PFOA-exposed group. These results indicate that under laboratory conditions PFOS is about 3 times more toxic to earthworms than PFOA. Based on known environmental concentrations of PFOS in the soil of Korea, which occur in the 0.42 ~ 0.73 ng/L range, there is no apparent risk to terrestrial invertebrate, earthworms. However, further work is required to investigate long-term effects on these and other terrestrial organisms.

Key words : PFOS, PFOA, *Eisenia fetida*

INTRODUCTION

Perfluorinated Compounds (PFCs) such as PFOS and PFOA have been recognized as emerging environmental pollutants and proposed as a new class of Persistent Organic Pollutants (POPs). Recently, many studies repeatedly reported relatively high concentrations of PFCs, mainly PFOS and PFOA in the environment, biota and humans as a result of their use in a number of commercial applications (Giesy and Kannan, 2002; Houde *et al.*, 2006; Kannan *et al.*, 2006). In May 2009, the 4th COP (Conference of the Parties) of Stockholm convention adopted amendments to Annex B of the Stockholm Convention on POPs which include PFOS, its salt and perfluorooctane sulfonyl

Received: 25 February 2010, Accepted: 1 September 2010
※To whom correspondence should be addressed.
Tel: +82-32-560-7181, Fax: +82-32-568-2037
E-mail: aubrey1@korea.kr



PFOS, perfluorooctane sulfonic acid, $C_8HF_{17}SO_3$ PFOA, perfluorooctanoic acid, $C_8HF_{15}O_2$

Fig. 1. Chemical structure of PFOS and PFOA.

fluoride (PFOSF).

Officially, Korea has no PFCs manufacture company. But based on the governmental survey in 2006, about 400 ton of PFCs related compounds were imported in Korea. The major usage of them was for ingredients of surfactant, catalyst, electronic materials, insulator and *etc* (NIER, 2006). In addition, the amounts of PFCs used as an ingredient of imported articles were not included in this survey. It means that Korea is not free from the environmental PFCs issues. The acute toxicity of PFCs to aquatic organisms has been known to be moderate to low (US EPA, 2000; Kennedy *et al.*, 2004). However, several studies recently reported that they are potential developmental toxicants (Thibodeaus *et al.*, 2003; Molina *et al.*, 2006; Yanai *et al.*, 2008) and shows chronic toxicity after long-term exposure (Ji *et al.*, 2008).

Earthworms are one of the most suitable animals in soil ecotoxicological tests as they are the most important biotic components in the soil. Among the earthworms, OECD and other authorities have recommended *Eisenia fetida* for the acute and sub-acute toxicity test of industrial chemicals (OECD, 1984; Peijnenbrg *et al.*, 1999; Jensen and Pedersen, 2006; Sanchez-Hernandes, 2006). To date, mortality, growth and reproduction have been the most common endpoints used in most of the studies on environmental toxicology. And avoidance test using earthworms has been widely used for the testing of organic chemicals, explosive materials, pesticides and heavy metals (Slimak, 1997; Langdon *et al.*, 2001; Reinecke *et al.*, 2002; Arnold *et al.*, 2003; Schaefer, 2003, 2004; Lukkari and Haimi, 2005; Garcia *et al.*, 2008). Recently, various kinds of biomarkers, such as lysosomal membrane stability measured by neutral red retention time and genetic effects assessed by comet assay are used as sub-lethal endpoints (Anna *et al.*, 2010).

Studies on the hazard of PFCs to the environment have been performed restrictively. Especially, very few studies are focused on the potential toxicity of PFCs on terrestrial organisms, such as soil dwelling invertebrates and terrestrial plants. Therefore, in the present study, we tried to evaluate the toxicity of PFOS and PFOA on the terrestrial environments using earthworms to fill the current gaps in knowledge of their environmental effects.

MATERIALS AND METHODS

1. Materials

PFOS and PFOA were obtained from Sigma Aldrich (St. Louis, MO, USA) and Wako (Japan), respectively (Fig. 1). PFOS was prepared by dissolving it in distilled water and PFOA was prepared by dissolving it in acetone.

2. Acute toxicity test using earthworm

We investigated the acute toxic effects of PFOS and PFOA using the terrestrial organism, *Eisenia fetida* according to the OECD test guideline 207 (Earthworm, Acute Toxicity Tests).

1) Test organisms

Earthworms, *Eisenia fetida* used in this study were raised at $20 \pm 2^{\circ}C$ in the continuous light condition. Earthworms were fed with the mixture of cow manure and cornflake once a week. Artificial soil for culture was prepared by mixing sphagnum peat : kaolin clay : air-dried quartz sand (1 : 2 : 7) according to the OECD

test guideline 207 and moisture content was adjusted as about 35%.

2) Test condition

Toxicity test was conducted at the same condition as breeding condition. Test animals with 300～600 mg of body weight (wet mass) were selected. Four replicates for each treatment group were carried out and each group was consisted of ten worms. For the exposure, solution of the test substance was mixed with artificial soil. Based on the results of the preliminary range-finding test, the concentrations of test chemicals for the definitive test were determined. In the definitive test, worms were exposed to the test substance for 14 days. During the test, there was no lethal organism in the control group so that this test could be regarded to meet the condition for the validity of the test.

3) Observation and treatment of results

The mortality of test organisms was determined after 7 and 14 day exposure. On the day of 7th and 14th, the living adult worms are observed and counted. Worms are classified as dead when they do not respond to a gentle mechanical stimulus to the front end. Symptoms of intoxication and distinct changes in behavior were also observed. At 0 day and 14 day, average live weight of worms, pH value and moisture content of artificial soil were determined. Worms were washed with de-ionized water prior to weighing and the excess water was removed by placing the worms briefly on filter paper. The wet mass of individual worms were weighed and expressed as average weight of worms. LC_{50} (50% lethal concentration) and its confidence limits were estimated using Probit analysis program (EPA600/4-85/013, 1985).

RESULTS AND DISCUSSION

Most PFCs are persistent in the environment and have been discovered as global pollutants in the air,

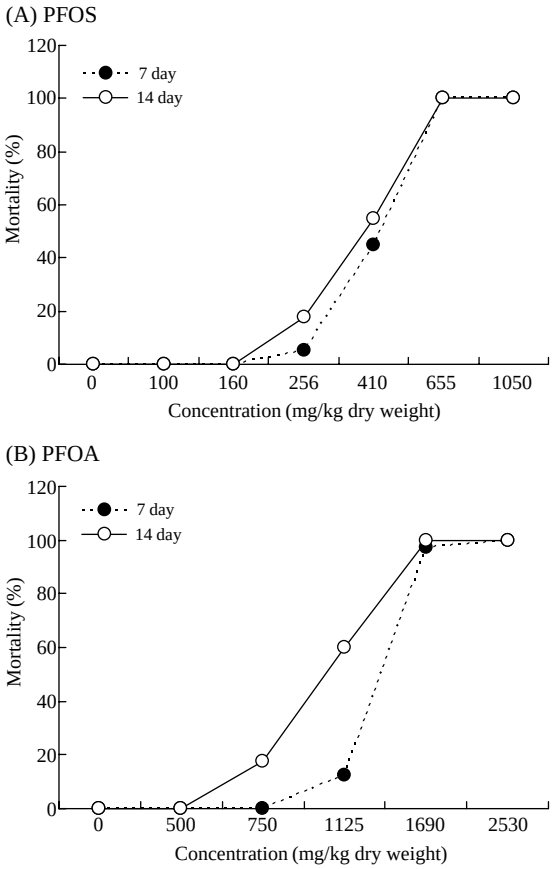


Fig. 2. Concentration-Effect Curve for PFOS and PFOA.

Table 1. Estimation of LC_{50} and NOEC values of PFCs in *Eisenia fetida* (Unit: mg/kg, dry weight)

Test substance	Exposure duration	LC_{50}	95% Confidence limits	NOEC*
PFOS	7 day	405.3	373.8～439.5	160.0
	14 day	365.4	333.6～400.2	160.0
PFOA	7 day	1,307.0	1,236.1～1,394.5	750.0
	14 day	1,000.8	926.2～1,081.5	500.0

*NOEC: The highest concentration causing no mortality

water and soil and even found in remote polar areas (Giesy and Kannan, 2002). PFOS have been determined in liver and blood samples from various mammals, birds, fish and humans (Bossi *et al.*, 2005; Letcher *et al.*, 2009).

In this study, PFOS and PFOA induced death of test organisms in a dose-dependent manner (Fig. 2). On the day of 14th the highest concentrations causing no mortality were 160 and 500 mg/kg (dry weight) in the PFOS and PFOA treated group. And the lowest concentrations of PFOS and PFOA causing 100% mortality were 655 and 1,690 mg/kg (dry weight), respectively. Based on these results, we calculated 14 day-LC_{50} values. LC_{50} values were estimated at the level of 365 and 1,000 mg/kg (dry weight) in the PFOS and PFOA-exposed group (Table 1). When we compared the toxicity of PFOS and PFOA, PFOS showed more potent effect in the view of lethal effect. PFOS was about 3 times more toxic to earthworms than PFOA.

The behavioral or pathological symptoms were also

Table 2. Changes of live weight in *Eisenia fetida* exposed to PFCs

Test substance	Nominal concentration (mg/kg, dry weight)	Live weight (mg)		Increasing rate (%)
		0 day	14 day	
PFOS	Control	0.482 ± 0.075	0.420 ± 0.066	−12.9
	100	0.430 ± 0.065	0.375 ± 0.065	−12.9
	160	0.431 ± 0.064	0.378 ± 0.060	−12.2
	256	0.426 ± 0.064	0.373 ± 0.060	−12.5
	410	0.430 ± 0.065	0.379 ± 0.058	−11.7
	655	0.436 ± 0.072	–	–
	1,050	0.406 ± 0.049	–	–
PFOA	Control	0.431 ± 0.069	0.374 ± 0.058	−13.2
	Solvent control	0.435 ± 0.066	0.379 ± 0.061	−12.9
	500	0.436 ± 0.065	0.383 ± 0.062	−12.2
	750	0.447 ± 0.058	0.389 ± 0.054	−13.0
	1,125	0.446 ± 0.061	0.367 ± 0.056	−17.7
	1,690	0.462 ± 0.058	–	–
	2,530	0.409 ± 0.068	–	–

Table 3. Changes of pH values and moisture content in artificial soil during the test

Test substance	Nominal concentration (mg/kg, dry weight)	pH value		Moisture content (%)	
		0 day	14 day	0 day	14 day
PFOS	Control	6.40	6.30	34.4	28.7
	100	6.43	6.34	34.1	31.9
	160	6.40	6.30	36.1	33.4
	256	6.43	6.26	35.6	32.3
	410	6.35	6.41	37.1	33.3
	655	6.33	–	36.5	–
	1,050	6.32	–	34.7	–
PFOA	Control	6.34	6.32	35.6	31.8
	Solvent control	6.39	6.31	39.2	33.6
	500	6.38	6.31	34.8	28.8
	750	6.40	6.37	37.4	31.5
	1,125	6.42	6.29	35.7	39.2
	1,690	6.31	–	34.3	–
	2,530	6.15	–	36.4	–

examined together with mortality. No behavioral changes or pathological symptoms were reported in the live worms (data not shown). In addition, we measured the live weight of worms at 0 day and 14 day. As shown in the Table 2, all of the test organisms showed slight weight loss (11.7～17.7%). However, the degree of weight loss in each exposed group has no significance to that of control group. These results suggested that PFOS and PFOA have no effects on the weight of earthworms.

Earthworm populations depend on both physiological and chemical properties of the soil, such as soil temperature, moisture, pH and salts. Especially, moisture content and acid level of soil are closely related with the inhabitation of earthworms, because they depend on dermal respiration and have no corneas (Grant, 1955). Therefore, moisture contents should be maintained to 35% during the test, and pH of artificial soil should be adjusted to 6.0±0.5. When we measured the pH value and moisture content at the start and end of the test, pH values were within pH 6.0±0.5 and moisture contents were maintained from 28.7% to 39.2% (Table 3). These results suggest that the present study is in accord with the validity criteria of the test, and that the toxicity of PFOS and PFOA is independent of the pH level.

In 2006 and 2007, National Institute of Environmental Research monitored the concentration level of PFOS and PFOA in the surface water, sediment and soil of industrial region, and 4 major water basins. The result showed that in all matrixes the concentrations of PFOS were higher than PFOA. In case of soil, the concentration of PFOS and PFOA was 0.42～0.73 and 0.13～0.25 (ng/g-dry weight), respectively (Cho *et al.*, 2009). Concentrations were comparable to those previously reported in other areas of Asia, in the level of 0.5 ng/g (Naile *et al.*, 2010). Based on the results of this study and other published literatures, it is suggested that the current PFOS and PFOA levels in soil may have no acute harmful ecological impact on the terrestrial environment. However, these results are based on the acute toxicity data. But the adverse effect occurred by sub-chronic or chronic exposure is an important factor to assess ecological risk assessment. Therefore, more studies on the long-term effects of PFOS and PFOA on the terrestrial fauna are needed for adequate ecological risk assessment of them.

REFERENCES

Arnold RE, Hodson ME, Black S and Davies NA. The influence of mineral solubility and soil solution concentration on the toxicity of copper to *Eisenia fetida* Savigny, Pedobiologia 2003; 47(5-6): 622-632.

Bossi R, Riget FF, Dietz R, Sonne C, Fauser P, Dam M and Vorkamp K. Preliminary screening of perfluorooctane sulfonate (PFOS) and other fluorochemicals in fish, birds and marine animals from Greenland and the Faroe Islands, Environ Pollut 2005; 136(2): 323-329.

Cho CR, Eom I, Kim EJ, Kim S, Choi K, Cho H and Yoon J. Evaluation of the level of PFOS and PFOA in environmental media from industrial area and major river basin, J Korean Soc Environ Anal 2009; 12(4): 296-306.

Garcia M, Römbke J, Torres de Brito M and Scheffczyk A. Effects of three pesticides n the avoidance behavior of earthworms in laboratory tests performed under temperature and tropical conditions, Environ Pollut 2008; 153(2): 450-456.

Giesy JP and Kannan K. Perfluorochemical surfactants in the environment, Environ Sci Technol 2002; 36(7): 146A-152A.

Giovanetti A, Fesenko S, Cozzella ML, Asencio LD and Sansone U. Bioaccumulation and biological effects in the earthworm *Eisenia fetida* exposed to natural and depleted uranium, J Environ Radioact 2010; 101(6): 509-516.

Grant WC Jr. Earthworm breeding farms, Science 1955; 121(3134): 107-108.

Houde M, Martin JW, Letcher RJ, Solomon KR and Muir DC. Biological monitoring of polyfluoroalkyl substances: a review, Environ Sci Technol 2006; 40(11): 3463-3473.

Jensen J and Pedersen MB. Ecological risk assessment of contaminated soil, Rev Environ Contam Toxicol 2006; 186: 73-105.

Ji K, Kim Y, Oh S, Ahn B, Jo H and Choi K. Toxicity of erfluorooctane sulfonic acid and perfluorooctanoic acid on freshwater macroinvertebrates (Daphia magna and Moina macrocopa) and fish (Oryaizs latipes), Environ Toxicol Chem 2008; 27(10): 2159-2168.

Kannan K, Perrotta E and Thomas NJ. Association between perfluorinated compounds and pathological conditions in southern sea otters, Environ Sci Technol 2006; 40(16): 4943-4948.

Kennedy GL, Butenhoff JL, Olsen GW, O'Connor JC, Seacat AM, Perkins RG, Biegel LB, Murphy SR and Farrar DG. The toxicology of perfluorooctanoate, Crit Rev Toxicol 2004; 34(4): 351-384.

Langdon CJ, Piearce TG, Meharg AA and Semple KT. Survival and behaviour of the earthworms Lumbricus rubellus and Dendrodrilus rubidus from arsenate contaminated and non-contaminated sites, Soil Biol Biochem 2001; 33(9): 1239-1244.

Letcher RJ, Bustnes JO, Diets R, Jenssen BM, Jørgensen EH, Sonne C, Verreault J, Vijayan MW and Gabrielsen GW. Exposure and effects assessment of persistent organohalogen contaminants in arctic wildlife and fish, Sci Total Environ 2010; 408(15): 2995-3043.

Lukkari T and Haimi J. Avoidance of Cu- and Zn- contaminated soil by three ecologically different earthworm species, Ecotoxicol Environ Saf 2005; 62(1): 35-41.

Molina ED, Balander R, Fitzgerald SD, Giesy JP, Kannan K, Mitchell R and Bursian SJ. Effects of air cell injection of perfluorooctane sulfonate before incubation on development of the white leghorn chicken (Gallus domesticus) embryo, Environ Toxicol Chem 2006; 25(1): 227-232.

Naile JE, Khim JS, Wang T, Chen C, Luo W, Kwon BO, Park J, Koh CH, Jones PD, Lu Y and Giesy JP. Perfluorinated compounds in water, sediment, soil and biota from estuarine and coastal areas of Korea, Environ Pollut 2010; 158(5): 1237-1244.

NIER (National Institute of Environmental Research), Korea. Ministry of Environment, National Inventory of Chemicals Statistics DB. Republic of Korea, 2006.

Organization for Economic Co-operation and Development. Guideline for testing of chemicals 207. Earthworm, Acute Toxicity Tests, 1984.

Peijnenburg WJ, Baerselman R, de Groot AC, Jager T, Posthuma L and Van Veen RP. Relating environmental availability to bioavailability: soiltype-dependent metal accumulation in the oligochaete Eisenia andrei, Ecotoxicol Environ Saf 1999; 44(3): 294-310.

Reinecke AJ, Maboeta MS, Vermeulen LA and Reinecke SA. Assessment of lead nitrate and Mancoseb toxicity in earthworms using the avoidance response, Bull Environ Contam Toxicol 2002; 68(6): 779-786.

Sanchez-Hernandez JC. Earthworm biomarkers in ecological risk assessment, Rev Environ Contam Toxicol 2006; 188: 85-126.

Schaefer M. Behaviour endpoints in earthworm ecotoxicology-evaluation of different test systems in soil toxicity assessment, J Soil Sediment 2003; 3(2): 79-84.

Schaefer M. Assessing 2,4,6-trinitrotoluene (TNT)-contaminated soil using three different earthworm test methods, Ecotoxicol Environ Saf 2004; 57(1): 74-80.

Slimak KM. Avoidance response as a sublethal effect of pesticides on Lumbricus terrestris (Oligochaeta), Soil Biol Biochem 1997; 29(3-4): 713-715.

Thibodeaux JR, Hanson RG, Rogers JM, Grey BE. Barbee BD, Ruchards JH, Butenhoff JL, Stevenson LA and Lau C. Exposure to perfluorooctane sulfonate during pregnancy in rat and mouse I: maternal and prenatal evaluations, Toxicol Sci 2003; 74(2): 369-381.

US EPA. Perfluorooctyl sulfonate; proposed significant new use rule, Federal Register 2000; 65(202): 62319-62333.

Yanai J, Dotan S, Goz R, Pinkas A, Seider FJ, Slotkin TA and Zimmerman F. Exposure of developing chicks to perfluorooctanoic acid induces defects in prehatch and early posthatch development, J Toxicol Environ Health A 2008; 71(2): 131-133.