

Toxicity Study of Perfluorohexanoic Acid (PFHxA) and Its Salts in Aquatic Organisms Zijin Guo, Hiroyuki Iwai

Abstract

Perfluorohexanoic acid (PFHxA) belongs to polyfluoroalkyl substances (PFAS). It is an environmental degradation product of C6-based fluorotelomer intermediates, used to produce various kinds of polymers. PFHxA is considered as a low toxic chemical compared to PFAS. Therefore, PFHxA is used as a replacement for long chain PFAS in industry site. However, the concerns of its ecotoxicity is still remained. Therefore, we performed exposure experiments by using two kinds of aquatic animals, medaka (Oryzias latipes) and rainbow trout (Oncorhynchus mykiss).

- 1. Fish Early-Life Stage Toxicity Test (ELS)(Oncorhynchus mykiss). <u>APFHx did not affect hatching and fish larval survival under exposure level of 10.1 mg/L.</u> The NOEC and LOEC of APFHx for post-hatch larval survival until Day 28 are both considered to be equal to or greater than 10.1 mg/L.
- 2. 96-hour acute toxicity study of APFHx and NaPFHx in Medaka LC50 of NaPFHx and APFHx was <u>3080mg/L</u> at and <u>1850mg/L</u>.
- 3. Fish Short Term Reproduction Assay of APFHx or NaPFHx in Medaka Both NaPFHx and APFHx did not show reproduction toxicity under tested concentration (10 mg/L and 100 mg/L)
- 4. Developmental toxicity test of APFHx with embryo of Zebrafish <u>APFHx induce morphological abnormalities at high concentrations (above 100 mg/L) and the effects was dose-dependent.</u>
- 5. Membrane transportation of APFHx in embryos of Zebrafish <u>APFHx was hardly found in Zebrafish embryos after exposure at low concentration.</u>

Methods

Chemical :

Perfluorohexaoic acid, ammonium salt (APFHx:CAS 21615-47-4) Perfluorohexaoic acid, sodium salt (NaPFHx:CAS 2923-26-4)

Experiment 1: Fish early –life stage toxicity test of APFHx

The rainbow trout, O. mykiss, early-life-stage (ELS) test was performed under flow-through conditions and in compliance with the OPPTS Biological Effect Test Guideline No. 850.1400. The test was conducted with a flow through test design.

Experiment 2: 96-hour acute toxicity study of APFHx and NaPFHx in Medaka

This test was carried out referring to the OECD TG 203, "Fish, acute toxicity test" . Test organism is Medaka (Oryzias latipes)

Experiment 3: Fish Short Term Reproduction Assay of APFHx or NaPFHx in Medaka

This test was carried out referring to the OECD TG 229, "Fish short term reproduction assay"

Experiment 4: Developmental toxicity test of APFHx with embryo of Zebrafish

This test was carried out referring to the OECD TG 236, "Fish embryo acute toxicity test" Positive control : sodium Valproate

Experiment 5: Measuring concentration of APFHx in zebrafish embryos/larvae

This study was conducted in reference to a literature below.

Nawaji, T., Mizoguchi, N., Ono, M., Matuura, Seki, M. and Teraoka, H. (2018): Comparing time-series of chemical concentrations in Zebrafish (Danio rerio) embryos/larvae exposed to teratogens with different hydrophoobicity; caffeine, sodium valproate, and diethylstilbestrol, J. Toxicol. Sci., 43, 267-273.

Results

1. Fish early-life stage toxicity test of APFHx

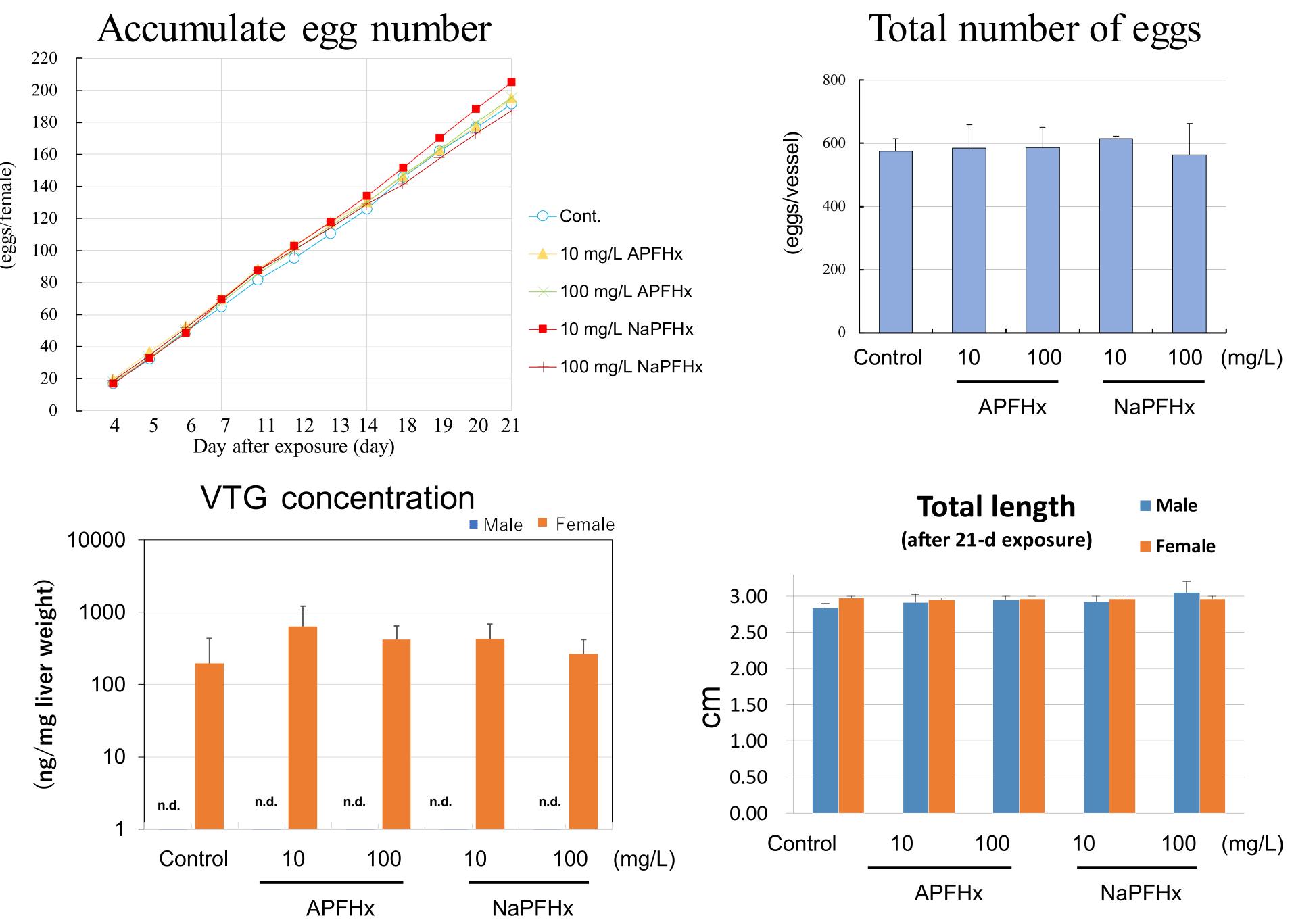
Table : Hatching success and fish larval survival Table : Total Length of and Dry Weight of Fish during the fish early-life stage test. 28 Days Post-Hatch.

Concentration (mg/L)	Hatched larvae (%)	Larvae surviving at 28days (%)	Concentration (mg/L)	Total Length of Fish (cm)	Dry Weight of Fish (g)
0	74	93	0	3.4 ± 0.23	0.0564 ± 0.0126
0.1	81	96	0.1	3.5 ± 0.17	0.0583 ± 0.0129
0.3	68	100	0.3	3.4 ± 0.20	0.0558 ± 0.0110
1	63	96	1	3.4 ± 0.19	0.0571 ± 0.0097
3	66	100	3	3.5 ± 0.15	0.0578 ± 0.0087
10	65	99	10	3.4 ± 0.19	0.0588 ± 0.0102

2. 96-hour acute toxicity study of APFHx and NaPFHx in Medaka

LC50 (96 hour exposure) 10000 100 _____ APFHx NaPFHx

3. Fish Short Term Reproduction Assay of APFHx or NaPFHx in Medaka



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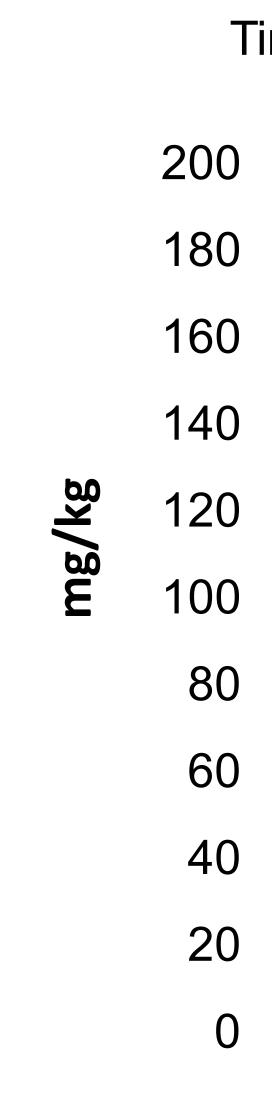
4. Developmental toxicity test of APFHx with embryo of Zebrafish

Mortality rate (%)					
Test group	Concentration	Cumulative number of dead (Mortality, %)			
iest group	(mg/L)	24hpf	48hpf	96hpf	120hpf
Control	0	0	0	0	0
Sodium Valproate	33.2	0	0	0	0
APFHx	100	0	0	0	0
APFHx	500	0	0	0	0
APFHx	1000	0	0	10	20
APFHx	1500	0	0	35	80
APFHx	2000	0	0	85	100

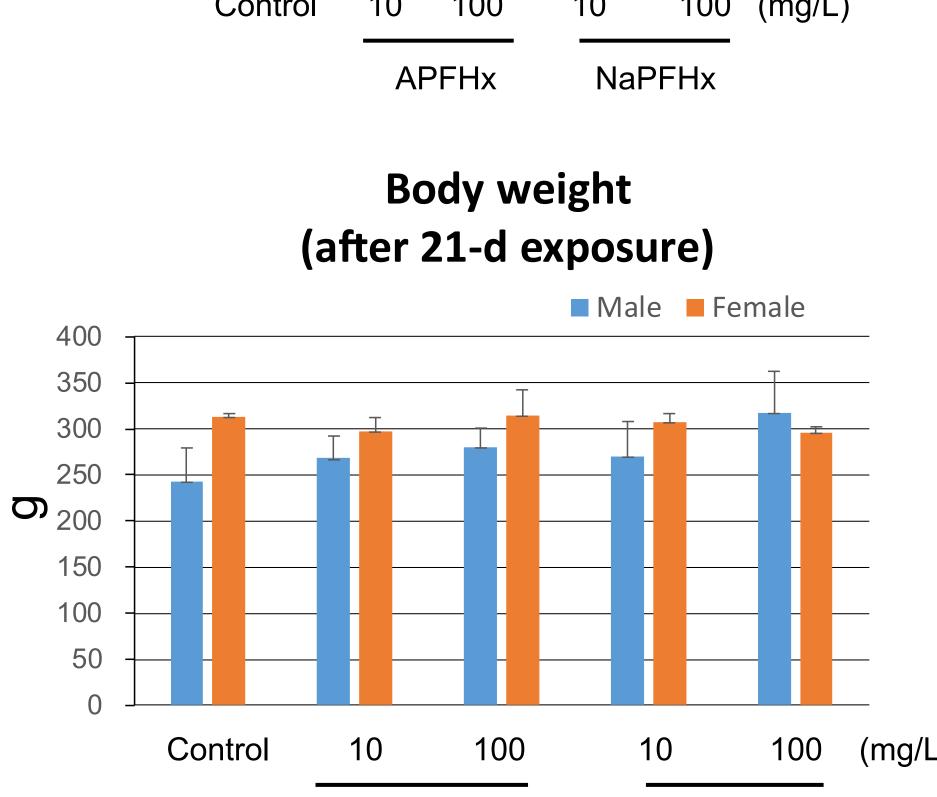
Frequency of morphological abnormalities

Test group	Concentration (mg/L)	Number of morphological assessment	Number of morphological abnormality	Frequency of morphological abnormality (%)
Control	0	20	2	10
Sodium Valproate	33.2	20	20	100
APFHx	100	20	1	5
APFHx	500	20	4	20
APFHx	1000	15	15	100
APFHx	1500	4	4	100
APFHx	2000	_	-	_

5. Membrane transportation of APFHx in embryos of Zebrafish



Fertility



Conclusions

Our data showed that PFHxA salts, APFHx and NaPFHx, do not induce adverse effects at the concentration under 100 mg/L in aquatic organisms. On the contrast, morphological abnormalities can be observed in zebrafish exposured with 100 mg/L APFHx, and frequency of morphological abnormality significantly increases while exposure level was above 500 mg/L. Membrane transportation analysis shows that traspotation efficiency is different between low dose exposure and high dose exposure. The peak amount in 50 mg/L APFHX exposed embryos is lower than that 50 mg/L APFHX exposed embryos. It migh cause the difference of morphological abnormalities between low and high dose exposed aquatic organisms.

In conclusion, PFHxA and its salt have no estrogen activity and no effect on reproduction to aquatic animals in our tested concentration lower than 100 mg/L.



Hatching rate

Test group	Concentration (mg/L)	Number of hatching	Hatchig rate (%)
Control	0	20	100
Sodium Valproate	33.2	20	100
APFHx	100	20	100
APFHx	500	20	100
APFHx	1000	18	90
APFHx	1500	18	90
	2000	10	00

Time-series of APFHx concentrations in embryos/larvae

