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Aquatic hazard, bioaccumulation and screening risk assessment for ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoate

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HIGHLIGHTS

- The fluoropolymer industry is moving to alternative polymerization processing aid with more favorable environmental profiles.
- One candidate replacement chemical is ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoate.
- Aquatic toxicity studies indicate ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoate is not classified for aquatic hazard.
- A bioconcentration study with common carp indicates the substance is of low concern for bioconcentration.
- PNEC comparisons with reported surface water concentrations suggest the risk to aquatic organisms is low.

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ABSTRACT

The fluoropolymer manufacturing industry is moving to alternative polymerization processing aid technologies with more favorable toxicological and environmental profiles as part of a commitment to curtail the use of long-chain perfluoroalkyl acids (PFAAs). To facilitate the environmental product stewardship assessment and premanufacture notification (PMN) process for a candidate replacement chemical, we conducted acute and chronic aquatic toxicity tests to evaluate the toxicity of ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoate ($C_6HF_{11}O_3.H_3N$) or the acid form of the substance to the cladoceran, *Daphnia magna*, the green alga, *Pseudokirchneriella subcapitata*, and a number of freshwater fish species including the rainbow trout, *Oncorhynchus mykiss*, In addition, testing with the common carp, *Cyprinus carpio,* was conducted to determine the bioconcentration potential of the acid form of the compound. Based on the relevant criteria in current regulatory frameworks, the results of the aquatic toxicity and bioconcentration studies indicate the substance is of low concern for aquatic hazard and bioconcentration in aquatic organisms. Evaluation of environmental monitoring data in conjunction with the predicted no effect concentration (PNEC) based on the available data suggest low risk to aquatic organisms.

1. Introduction

Fluorinated substances with more favorable properties, most notably rapid elimination from living systems, are being developed as replacements for long-chain perfluoroalkyl acids (PFAAs) such as perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) [Ritter 2010, Buck 2011, USEPA 2010/15]. Examples of such alternatives include per- and poly-fluoroalkyl ether carboxylates [Buck 2011, 2015, Gordon 2011] that have replaced ammonium perfluorooctanoate, APFO, in its primary historical use as a fluoropolymer polymerization processing aid [Feiring, 1994]. Wang et al. (2013) discussed a number of fluorinated alternatives and summarized the available knowledge on their environmental release, persistence and exposure of humans and biota. These same authors identified data gaps for these fluorinated alternatives including the lack of sufficient hazard data for environmental assessment.

Ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoate (henceforth referred to as the test substance) was developed by DuPont as a replacement polymerization processing aid for use

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in fluoropolymer manufacturing. At an ambient temperature of 20 °C, the ammonium salt of this substance is a solid while the acid form is a liquid. Fluoropolymer manufacturing utilizes an aqueous solution of the substance as a polymerization processing aid with either capture for re-use or thermal destruction of the substance during fluoropolymer processing [Brothers patent US20090281261]. Aqueous fluoropolymer dispersions that contain polymerization processing aid may be used to coat surfaces used to make non-stick cookware. In this process, the fluoropolymer is sintered onto the substrate surface at temperatures >265 °C which leads to destruction of the polymerization processing aid (i.e., the polymerization processing aid decomposes at 150–160 °C, ECHA, 2011).

The current study assessed the acute and chronic toxicity and bioconcentration of the test substance using common aquatic test species. The objective was to generate aquatic hazard data for algae, *Daphnia* and fish according to current regulatory guidelines and using Good Laboratory Practice (GLP). These data inform substance classification and labeling according to European Union Directive 67/548/EEC as well as the Globally Harmonized System on Classification [UNEP 2012] and were used for derivation of a Predicted No Effect Concentration (PNEC) to aid in the evaluation of potential risk to aquatic organisms [ECHA 2008].

2. Materials and methods

2.1. Test substance

The test substance, ammonium, 2, 3, 3, 3-tetrafluoro-2-(heptafluoropropoxy)-propanoate, [$CF_3CF_2CF_2OCF(CF_3)COOH\bullet NH_3$, CAS# 62037-80-3], and the corresponding acid 2, 3, 3, 3-tetrafluoro-2-(heptafluoropropoxy)-propanoic acid, [$CF_3CF_2CF_2OCF(CF_3)COOH$, CAS# 13252-13-6] were prepared, characterized and provided by DuPont Chemicals and Fluoroproducts, Wilmington, DE. The properties of the test substance, an aqueous solution of the test substance and the acid form of the test substance are shown in Table 1.

2.2. Acute aquatic toxicity

The acute aquatic toxicity of the test substance was evaluated in studies utilizing the rainbow trout (*Oncorhynchus mykiss*), Japanese medaka (*Oryzias latipes*), rare gudgeon (*Gobiocypris rarus*), the freshwater invertebrate, *Daphnia magna*, and the freshwater green alga, *Pseudokirchneriella subcapitata,* as the test species. Bioconcentration testing with the common carp and acute testing with medaka were conducted using the acid form of the test substance while acute testing with rare gudgeon was conducted using both the acid and ammonium salt forms of the test substance. All other tests were conducted using the ammonium salt form of the test substance. These studies were conducted using Good Laboratory Practices (GLP) and in conformance with OECD test guidelines 203 (fish), 202 (*Daphnia*), and 201 (algae) and USEPA OPPTS test guidelines 850.1010 (*Daphnia*), 850.1075 (fish) and 850.5400 (algae), respectively. All study endpoints are reported based on measured test substance concentrations. Based on the results of range-finding studies, acute toxicity tests were conducted as limit tests typically using a nominal test concentration of 120 or 150 mg/L.

2.3. D. magna 21-d reproduction

The chronic toxicity of the test substance to the cladoceran, *D. magna*, was assessed in a 21-d static renewal test (MWF renewals) according to OECD test guideline 211. A well water control and nominal concentrations of 2.5, 5, 10, 20, and 40 mg/L (mean, measured concentrations were 2.13, 4.17, 8.13, 16.2, and 33.0 mg/L) were tested in 250 mL beakers containing 200 mL of test solution. Each test concentration or dilution water control was replicated 10 times with one neonate per replicate. First instar daphnids, < 24 h old, were randomly assigned to the test chambers. Daphnids were fed *P. subcapitata* and YCT at final concentrations of approximately 62,500 cells/mL and 2.1 mL/L of test solution, respectively, on a daily basis.

2.4. Rainbow trout 90-d early life-stage

The study was conducted under GLP and in conformance with OECD 210 and USEPA OPPTS 850.1400 test guidelines. Nominal test substance concentrations tested ranged from 0.63 to 10 mg/L (mean measured concentrations of 0.65, 1.08, 2.16, 4.66 and 8.89 mg/L). A total of 80 embryos were exposed per concentration (20 embryos per embryo cup, 2 cups per replicate, two replicates per concentration at test start) using an intermittent-flow, proportional diluter system. On day 46, after swim-up had begun in

Table 1
Physical–chemical properties of the test substances used for aquatic toxicity and bioconcentration testing.

Property[a]	Test substance ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoate salt	Ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoate salt	Test substance[b] 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoic acid
Purity	Aqueous solution ~85wt% in water	Ammonium salt dried powder 99.4% pure	Protonated Acid 98% pure
Molecular Formula	$C_6F_{11}O_3^- \cdot H_4N^+$	$C_6HF_{11}O_3 \cdot H_3N$	$C_6HF_{11}O_3$
Molecular weight	347.08	347.08	330.05
CAS number	62037-80-3	62037-80-3	13252-13-6
Appearance	Clear colorless liquid	Solid powder	Clear, colorless liquid
Melting point °C			<−40
Sublimation point °C		~130–140	
Boiling point °C			129
Decomposition point °C		~150–160	
Dissociation constant (pKa)	3.8		2.8
Density (g/mL, 20 °C)	1.569	~1.7	1.690
Aqueous Surface tension mN/m (1 g/L)	66.3		59.4
Vapor pressure (Pa, 20 °C)		~0.01	100–300
Solubility (g/L)	>200		
K_{ow}			2.0 (ChemSilico) 1.34 (SPARC)
Hydrolysis	Stable (pH 4, 7, 9 at 50 °C)		

[a] ECHA (2011).
[b] Rare gudgeon and medaka acute tests and common carp bioconcentration (uptake only) test.

the control, the fingerlings were thinned to a total of 30 fish per concentration (15 fish per replicate, 2 replicates per concentration). Analytical verification of test substance concentrations was conducted on day -1, day 0, once weekly thereafter, and at test end. Test solutions were supplied to each replicate test chamber at a rate of approximately 1.25 L of test solution per test chamber each hour, resulting in approximately 5 test solution volume additions of 6 L (30 L total) every 24 h. Embryos and alevins were held in relative darkness until day 30 and then held under a 16L (237–371 lux):8D photoperiod that included 30 min of transitional light (4–159 lux) between light and dark intervals through test end. Test solutions were maintained between 11.8 and 12.9 °C (mean 12.5 °C). On day 46, surviving alevins and fingerlings were thinned to15 per replicate tank using random numbers and feeding initiated using newly-hatched brine shrimp (*Artemia* sp., *ad libitum,* San Francisco Bay Brand, Newark, California) 2–3 times daily. On days 53–89, one daily feeding was supplemented with trout chow (Aqua Max® Starter Fingerling 300 5D03, PMI® Nutrition International, LLC). Daily observations were made for assessment of number of dead eggs, first and last day of hatching, first day of swim-up, and survival and abnormalities post-hatching. Standard length and blotted wet weight of surviving fingerlings were determined at test end.

2.5. Common carp bioconcentration

Bioconcentration of the acid form of the test substance (purity 99.6%) was evaluated using common carp, *Cyprinus carpio*. The study design complied with Japanese new chemical substance testing guidelines which are similar to USEPA OPPTS, 850.1730 and OECD TG 305 test guidelines. Carp were exposed to the test substance under flow-through conditions for 28 days during the uptake (exposure) phase of the study. Two aqueous test concentrations (nominal 20 and 200 μg/L, mean measured 20 and 198 μg/L) and dechlorinated Yokohama municipal tap water (dilution water control) were used for testing with 28 fish per exposure treatment and 12 fish in the control. Dissolved oxygen was maintained between 7.2 and 8.4 mg/L, temperature was 24.0–24.6 °C and pH ranged from 7.4 to 8.0 during testing. Fish were fed at 2% fish wet weight per day. Two samples of two fish were sampled from each test substance treatment on days 4, 7, 15, 21 and 28 and processed for test substance analysis. Average lipid content of the fish was 3.3% at test initiation and 3.8% at test end.

2.5.1. Chemical analyses

The chemical analysis scheme for aqueous samples from the rainbow trout early-life stage study is provided here as an example of the typical analytical scheme used for all studies. Samples were analyzed by HPLC/MS/MS using an Agilent Model 1100 HPLC with a Micromass Quattro Micro MS instrument and MassLynx/QuanLynx v. 4.0 software. A Zorbax® SB-C8 LC column, 100 × 2.1 mm, 3.5 μm particle size, was used for analysis with a 0.400 mL/min flow rate at 35 °C and isocratic elution using a 50% Nanopure water, 20 mM ammonium acetate:50% methanol, 20 mM ammonium acetate mobile phase (test solution samples). Fish tissue samples were analyzed using gradient elution with 20 mM ammonium formate; formic acid (1000:1):methanol mobile phase. The injection volume was 5 μL for whole fish tissue samples and 10 μL for aqueous test solution samples. The typical LOD and LOQ for aqueous samples were determined to be 0.009 mg/L and 0.100 mg/L, respectively. The LOD for fish tissue analyses was 0.001 mg/L.

2.6. Data analyses

Acute study endpoints were calculated using the probit procedure and SAS® software (2000). For algal studies, healthy cell counts were used to calculate the E_bC_{50} (biomass), E_yC_{50} (yield), E_rC_{50} (growth rate) and associated NOEC values. The statistical methods used for data analysis followed the approaches outlined in OECD statistical guidance for aquatic toxicity tests (OECD 2006). All statistical tests were calculated at a significance level of $\alpha = 0.05$. Predicted No Effect Concentration (PNEC) calculation for evaluating aquatic risk followed REACH Chapter 10 (ECHA, 2008) guidance. For the bioconcentration study, steady state was defined as three consecutive measurement days over which there was no statistically significant increase in mean tissue residues in whole fish as recommended in the test guidelines.

3. Results

3.1. Acute aquatic toxicity

During algae, daphnid and fish testing with the test substance, the dilution water quality was acceptable, all chemical and physical parameters were within the expected ranges, and all relevant OECD test acceptance criteria were fulfilled.

3.1.1. Fish (e.g., rainbow trout)

The study was conducted with a 120 mg/L nominal (mean measured 96.9 mg/L) limit test substance concentration and a dilution water control at a mean temperature of 11.8 °C (range of 11.7–12.4 °C). Fish in the dilution water control ranged from 4.3 to 4.9 cm in standard length (mean 4.47 cm), and 0.941–1.493 g in wet weight, blotted dry (mean 1.068 g) at test end. Control loading at test end was 0.534 g/L. No mortality was seen at the mean measured 96.9 mg/L limit test concentration or in the control at the end of the 96-h limit test. The 96-h LC_{50}, based on the mean measured limit test concentration was >96.9 mg/L. The rainbow trout results are discussed here since they are representative of the acute toxicity tests with medaka (96-hr LC50 > 100 mg/L, nominal) and rare gudgeon (96-hr LC50 value > 150 mg/L nominal for acid and ammonium salt).

3.1.2. Daphnia magna

The study was conducted with a nominal 120 mg/L (mean measured 102 mg/L) test substance concentration and a dilution water control at a mean temperature of 20.4 °C (range of 20.1–20.7 °C). No immobility or sublethal effects were observed in the control or the mean measured 102 mg/L test substance limit concentration at the end of the 48-h limit test. The 48-h EC_{50}, based on the mean measured limit test concentration and immobility, was >102 mg/L.

3.1.3. Pseudokirchneriella subcapitata

A single abiotic stability control replicate, six blank (synthetic algal assay procedure medium, AAP) control replicates and six nominal limit test concentration replicates of 120 mg/L (mean measured 106 mg/L) were used for testing with a mean lighting intensity of 5890 lux (range of 5650–6080 lux), a mean temperature of 23.9 °C (range of 23.8–24.0 °C), and a shaking speed of 95 rpm. Test solutions were not renewed. Inhibition of cell growth expressed as biomass (cell number), area under the growth curve, and average specific growth rate of *P. subcapitata* exposed to a mean, measured limit test concentration of 107 mg/L for 72 h was -2, -4, and 0%, respectively. Healthy cell counts increased in the blank control by at least a factor of 16 in 72 h, the coefficient of variation of average specific growth rates during the whole test period (0–72 h) in blank control replicates did not exceed 7%, and the mean coefficient of variation for section-by-section specific growth rates (days 0–1, 1–2, and 2–3) in the blank control replicates did not exceed 35%, thereby satisfying the appropriate test

acceptance criteria. No statistically significant growth inhibition was observed at the mean measured limit test concentration and the 72 h EC_{50} and NOEC values for all endpoints were >107 mg/L.

3.2. D. magna 21-d reproduction

Nominal test substance concentrations used for the study were 2.5, 5, 10, 20, and 40 mg/L with corresponding mean, measured concentrations of 2.13, 4.17, 8.13, 16.2, and 33.0 mg/L. Test substance was not detected in the dilution water (well water) control during the study. Total alkalinity, EDTA hardness, and conductivity values for the dilution water control and high test substance solution during the study ranged from 86 to 90 mg/L as $CaCO_3$, 125–176 mg/L as $CaCO_3$, and 290–340 μmhos/cm, respectively. The overall temperature ranged from 19.3 to 21.0 °C and pH ranged from 7.5 to 8.2. The minimum dissolved oxygen concentration was 7.3 mg/L or 80.2% of saturation at 20 °C throughout the study.

Summary data for the study are presented in Table 2. Percent survival of adult daphnids in the dilution water control was 100% at the end of the study. Surviving adult daphnids in the dilution water control produced an average of 143.7 total live young at the end of 21 days. No ephippia were seen at any test substance concentration or in the dilution water control. The NOEC for adult *D. magna* survival, length of surviving adults, first day of reproduction, number of immobile neonates, and number of live young on day 21 were >33 mg/L, > 33 mg/L, > 33 mg/L, 8.13 mg/L and 4.17 mg/L, respectively.

3.3. Rainbow trout 90-d early life-stage

Nominal test substance concentrations selected for the 90-day trout early life-stage study were 0.63, 1.25, 2.50, 5.00, and 10.0 mg/L with corresponding mean, measured concentrations of 0.651, 1.08, 2.16, 4.66, and 8.89 mg/L. Temperature and pH of the test substance and dilution water control solutions ranged from 11.8 to 12.9 °C (mean 12.5 °C) and 7.4 to 8.3, respectively. Dissolved oxygen concentrations ranged between 5.8 and 10.3 mg/L over the duration of the study. Based on mean measured test substance concentrations, the 90-day EC_{50} values and NOEC values (except last day of hatching) for endpoints evaluated were greater than 8.89 mg/L. The statistically calculated NOEC value for last day of hatching was 1.08 mg/L. However, evaluation of the data indicated that mean last day of hatching ranged from 24 days in the control to 23 days in the highest three test concentrations (Table 3). Based on the lack of any other significant effects at test concentrations less than or equal to 8.89 mg/L, the slight decrease in last day of hatching is not believed to be a significant biological effect. Therefore, the overall study NOEC is 8.89 mg/L.

3.4. Common carp bioconcentration

Common carp were exposed to the acid form of the test substance under flow-through conditions for 28 days with no depuration phase. Mean measured aqueous exposure concentrations for the test substance at the low and high test concentrations were 0.020 mg/L and 0.198 mg/L. Uptake phase concentrations of the test substance in whole fish were considered to have reached steady-state based on the lack of increase in tissue concentrations with time during the 28-day exposure phase. All bioconcentration factors (BCF) were <30 for both test concentrations evaluated during the study (Table 4).

4. Discussion

The test substance was developed by DuPont as an alternative to long-chain perfluoroalkyl substances for use as a polymerization processing aid in fluoropolymer manufacturing. Wang et al. (2013) suggested that the lack of sufficient hazard data for environmental assessment was a data gap for a number of alternative fluorinated substances including the substance discussed in the current study. The results of this effort help to fill a number of the identified data gaps for the test substance and provide the data necessary to help develop a screening aquatic risk assessment for the test substance.

The results of acute and chronic aquatic testing conducted during this investigation demonstrate that the ammonium salt form of the test substance, as well as the acid form of the substance, exhibit low acute and chronic aquatic toxicity. The reported acute test endpoints are above the European Union and Global Harmonized System (GHS) classification limit of an LC/EC50 of 100 mg/l. In addition, based on the acute endpoints, global GHS and U.S. EPA would classify the test substance as being of low concern for acute hazard in aquatic ecosystems (UNEP 2008, Smerchek et al., 1995). Similarly, the NOEC values from the green algae study with *P. subcapitata*, the chronic life-cycle reproduction study with the cladoceran, *D. magna*, and the chronic early-life-stage study with the rainbow trout, *O. mykiss*, demonstrate that the test substance exhibits low chronic toxicity hazard for aquatic organisms (UNEP, 2012).

The ammonium salt of the test substance is expected to be completely dissociated in water resulting in the production of ammonium ion (NH_4^+). The equilibrium in water between the two forms of ammonia, un-ionized ammonia (NH_3) and ammonium ion (NH_4^+), is controlled primarily by pH and temperature. Therefore, ammonia speciation determines ammonia toxicity since un-ionized ammonia is typically more toxic to aquatic organisms than ammonium ion (USEPA, 1999). The estimated concentrations of un-ionized ammonia at the EC50/LC50/NOEC values established for the test substance in the *D. magna* and rainbow trout tests are presented in Table 5 along with the appropriate acute and chronic endpoints for un-ionized ammonia. Analysis of the potential contribution of the ammonia to the toxicity of the test substance suggests that it could play a limited role in the acute toxicity of the test substance (Table 5). A more substantive role in observed toxicity in the rainbow trout ELS test may be indicated based on the

Table 2
Summary results from a 21-day reproduction test with *Daphnia magna*.

Mean measured test substance concentration (mg/L)	Adult survival, %	Mean first day of reproduction	Mean live Young[a]	Total immobile Young[a]	Mean adult length (mm)
Control	100	9.1	143	0	4.3
2.13	100	9.5	132	2	4.5
4.17	100	9.2	137	9	4.4
8.13	90	9.0	126[b]	3	4.3
16.2	100	9.2	124[b]	12	4.4
33.0	100	9.6	122[b]	8	4.4

[a] – per surviving parental organism.
[b] – statistically significant from control.

Table 3
Hatching, survival and growth data from a 90-day rainbow trout early life-stage test.

Mean measured test substance concentration (mg/L)	Replicate	First day of hatching	Last day of hatching	Percent hatching	Percent survival at Swimup	Percent survival at test end	Mean length (cm) at test end	Mean wet Wt (g) at test end
Control	1	21	24	75	67	93	4.9	1.68
	2	20	24	95	74	93	4.8	1.67
	3	21	24	85	77	–	–	–
	4	21	24	70	93	–	–	–
	Mean[a]	21	24	81	77	93	4.9	1.68
0.65	1	20	24	70	64	93	4.9	1.78
	2	21	25	95	68	100	4.9	1.70
	3	21	24	75	93	–	–	–
	4	21	24	100	50	–	–	–
	Mean[a]	21	24	85	69	97	4.9	1.74
1.08	1	21	24	80	75	100	4.9	1.73
	2	21	24	90	67	93	5.1	1.86
	3	20	23	80	88	–	–	–
	4	20	23	70	64	–	–	–
	Mean[a]	21	24	80	73	97	5.0	1.80
2.16	1	21	23	95	84	100	4.9	1.70
	2	21	23	80	69	100	4.9	1.70
	3	20	23	85	71	–	–	–
	4	21	23	85	65	–	–	–
	Mean[a]	21	23	86	72	100	4.9	1.70
4.66	1	21	23	90	67	100	4.8	1.70
	2	21	23	80	69	100	4.7	1.62
	3	20	23	85	59	–	–	–
	4	21	23	75	60	–	–	–
	Mean[a]	21	23	83	64	100	4.8	1.68
8.89	1	21	23	80	81	100	4.9	1.76
	2	21	23	80	81	100	4.9	1.73
	3	20	23	80	75	–	–	–
	4	21	23	90	72	–	–	–
	Mean[a]	21	23	83	77	100	4.9	1.75

[a] – calculated as mean of replicate values.

Table 4
Concentrations of the test substance in water and fish and calculated bioconcentration factors from a 28-day bioconcentration test.

Day	Replicate	Fish no. 1 Wt[a], g	Fish no. 2 Wt[a], g	Mean water Conc., mg/L	Fish tissue Conc., mg/Kg	BCF
Low concentration						
4	1	5.02	5.51	0.0188	<0.55	<30
4	2	4.16	6.54	0.0188	<0.55	<30
7	1	4.03	5.22	0.0195	<0.55	<29
7	2	4.20	4.16	0.0195	<0.55	<29
15	1	6.81	6.84	0.0193	<0.55	<29
15	2	4.79	8.10	0.0193	<0.55	<29
21	1	5.11	7.35	0.0198	<0.55	<28
21	2	6.70	9.22	0.0198	<0.55	<28
28	1	9.77	6.45	0.0198	<0.55	<28
28	2	8.07	7.53	0.0198	<0.55	<28
High concentration						
4	1	3.96	6.10	0.194	<0.55	<3
4	2	3.95	5.79	0.194	<0.55	<3
7	1	4.02	6.63	0.199	<0.55	<3
7	2	6.34	4.70	0.199	<0.55	<3
15	1	6.31	4.50	0.202	<0.55	<3
15	2	5.38	6.60	0.202	<0.55	<3
21	1	7.93	7.44	0.199	<0.55	<3
21	2	7.56	8.92	0.199	<0.55	<3
28	1	8.17	5.44	0.198	<0.55	<3
28	2	7.46	6.81	0.198	<0.55	<3

[a] Mean fish weight at test start was ~5 g.

calculated concentration of un-ionized ammonia in that study.

In order to extrapolate a PNEC (Predicted No Effect Concentration), three trophic levels were taken into consideration; the chronic NOEC values measured for rainbow trout (8.89 mg/l), *Daphnia* (4.17 mg/l) and algae (107 mg/l). Applying a safety factor of 10X to the lowest reported chronic endpoint, as suggested in the ECHA guidance (2008), the resulting PNEC value for the test substance is 0.417 mg/l. Recently, Heydebreck et al. (2015) reported the results of environmental monitoring studies that included determination of 2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)propanoic acid in water samples from coastal and riverine areas of Germany and China. Concentrations in water at the European monitoring sites ranged from not detected to a high value of 86.1 ng/L with typical values < 3 ng/L while concentrations in water at the monitoring sites in China ranged from not detected to a high value of 3.1 ug/L with typical values generally <100 ng/L.

Table 5
Calculated concentrations of unionized ammonia (NH_3) in acute and chronic toxicity test with *Daphnia magna* and rainbow trout.

Daphnia acute test			Trout acute test		
	Range	Calculation		Range	Calculation
Temp.°C	20.1–20.7	20.7	Temp.°C	11.7–12.4	12.4
pH	7.6–8.0	8.0	pH	7.2–7.7	7.7
% Free NH_3	4%		% Free NH_3	1.1%	
48-h EC50 (mg/l)		>102 (M)	96-h LC50 96 h (mg/l)		>96.9 (M)
[NH_3–N] (mg/l)		0.21	[NH_3–N] (mg/l)		0.06
96-h NH_3–N 48-h EC50 (mg/l), (USEPA, 1999)		0.53–4.9	96-h NH_3–N LC50 (mg/l), (USEPA, 1999)		0.16–1.1
Daphnia chronic test			**Trout ELS Test**		
Temp.°C	20–21	21	Temp.°C	11.1–14.4	13
pH	7.5–8.2	8.2	pH	7.37–8.04	7.8
% Free NH_3		6.3%	% Free NH_3	4.5%	
21-d NOEC (repro) (mg/l)		4.17 (M)	NOEC (mg/l)		1.08 (M)
[NH_3–N] (mg/l)		0.01	[NH_3–N] (mg/l)		0.003
NH_3–N NOEC (reprod., mg/l), (Gersich and Hopkins, 1986)		0.42	NH_3–N NOEC (mg/l), (Calamari et al., 1981)		0.025

Calculations were based on the following.
% test substance ammonium = 0.052%.
% free ammonia (NH_3) = $100/(1 + 10^{\circ}(pKa\text{-}pH))$.
where pKa = 9.245 + 0.0324 (25-T).
where T = °C.
(M) – endpoint based on mean measured test concentrations.

Comparison of the calculated PNEC with the limited number of surface water concentrations currently available from Heydebreck et al. (2015) suggest the risk to aquatic organisms from the test substance is low. The risk quotient (i.e., PEC/PNEC ratio) for the highest reported environmental concentration from China is 0.007 which is multiple orders of magnitude below a level that would suggest potential risk to aquatic organisms. In addition, testing with the common carp indicates that the substance is unlikely to bioconcentrate in aquatic organisms. However, it is also important to note that the results of the carp bioconcentration study do not inform the questions of potential dietary bioaccumulation and trophic biomagnification. The present evaluation also did not consider a variety of other potential effects, e.g., endocrine toxicity, neurotoxicity, immunotoxicity, behavior toxicity, etc. or effects on reproduction in aquatic organisms other than *D. magna* although an assessment factor (safety factor) of 10 was applied to the lowest available chronic reproduction endpoint to develop the PNEC used for the screening risk assessment.

While aquatic hazard and risk are important aspects of the environmental assessment of chemicals, additional data are needed to assess potential effects in terrestrial species, particularly in avian species, as well as the potential for human health effects as evaluated in mammalian toxicology studies. To address these areas, extensive additional investigations on the test substance have been conducted (Buck, 2015). These studies evaluated the absorption, distribution, metabolism and excretion of ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoate in rats, mice and the cynomolgus monkey (Gannon et al., 2016), two-year chronic toxicity and carcinogenicity in rats (Caverly Rae et al., 2015), and reproductive toxicity and bioaccumulation in bobwhite quail (Hoke et al., in preparation) to provide the data for environmental and human health assessments.

5.0. Conclusion

The acute and chronic aquatic toxicity and bioconcentration tests reported here were conducted in conformance with OECD, EU, and U.S. EPA test guidelines under GLP. These tests utilized the ammonium salt to develop acute and chronic aquatic hazard data with freshwater test species while the acid form was used in acute tests with two additional fish species as well as a fish bioconcentration/uptake study. Based on current regulatory frameworks, the available aquatic toxicity and bioconcentration data indicate that ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)
-propanoate is not classified for aquatic hazard under either global GHS or European CLP legislation, that it is unlikely to bioconcentrate in aquatic organisms, and that it poses low risk to aquatic organisms.

Disclaimers: Animal welfare act compliance

This study complied with all applicable sections of the Final Rules of the Animal Welfare Act regulations (9 CFR) and the Guidelines from the Guide for the Care and Use of Laboratory Animals (NRC 1996) and the American Veterinary Medical Association (AVMA), 2007 Guidelines on Euthanasia. DuPont Haskell Global Centers is accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC) International.

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