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CHRONIC TOXICITY OF FLUOROTELOMER ACIDS TO *DAPHNIA MAGNA* AND *CHIRONOMUS DILUTUS*

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Abstract—Saturated and unsaturated fluorotelomer carboxylic acids (fluorotelomer acids: FTAs) represent important intermediates in the degradation of fluorotelomer alcohols to perfluorinated carboxylic acids (PFCAs). Recent studies have detected FTAs at low concentrations (ng/L) in precipitation and surface waters; however, information regarding chronic toxicity is lacking. The present study assessed the chronic toxicity of the 8:2 saturated fluorotelomer carboxylic acid (8:2 FTCA) to *Chironomus dilutus* and the 10:2 saturated and unsaturated fluorotelomer carboxylic acids (10:2 FTCA and 10:2 fluorotelomer unsaturated carboxylic acid [FTuCA]) to *Daphnia magna* in separate life-cycle tests. In *D. magna* tests the FTCA was consistently more toxic than the FTuCA. Lethal concentrations (LC50s) were 150 and >60 μg/L for FTuCA and FTCA, respectively. Reproduction was significantly reduced relative to the controls, with respective median effective concentrations (EC50s) for time to first brood and mean number of offspring/female of 287 and 214 μg/L for FTuCA and 50 and 48 μg/L for FTCA. In tests with *C. dilutus*, EC50s for survival and growth at 20 d were 2,610 and 1,250 μg/L. Total emergence and time to first emergence, the most sensitive endpoints, yielded EC50s of 440 and 890 μg/L. Few adults emerged and no reproduction occurred at the two highest concentrations (600 and 1540 μg/L). Mean number of eggs/female was not affected. These results represent the first chronic toxicity data for FTCAs and additional evidence that FTCAs are more toxic than some PFCAs. While the results indicate that current environmental concentrations of FTAs likely pose negligible risk to aquatic biota, additional quantification of FTAs in surface waters and assessment of their toxicity is needed before meaningful assessments of potential risks to aquatic biota are possible. Environ. Toxicol. Chem. 2010;29:1123–1131. © 2010 SETAC

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INTRODUCTION

Perfluorinated carboxylic acids (PFCAs) are recalcitrant global contaminants [1–5]. Perfluorinated carboxylic acids can be released into the environment directly via spills [2] or indirectly through chemical breakdown of consumer products or fugitive precursor compounds released during manufacturing processes. Fluorotelomer alcohols (FTOHs) have been identified as precursors that are potentially significant sources of global contamination of PFCAs [6–9]. Global annual production of FTOHs was approximately 5×10^6 kg for the years 2000 to 2002, approximately 40% of which occurred in North America [10,11]. The majority of FTOHs produced are incorporated into polymers for surface treatment applications on carpets, fabrics, leather, and paper products to repel soil, oil, and water, but they are also used in the manufacture of performance chemicals including fire-fighting foams, adhesives, cleaners, lubricants, and paints [12–14].

Fluorotelomer alcohols are linear perfluorinated chains of even numbers of carbon atoms attached to an ethanol group ($-CH_2CH_2OH$) and are distinguished by the ratio of fluorinated to hydrogenated carbon atoms (i.e., 8:2 FTOH). Fluorotelomer alcohols are likely released to the environment via degradation of the products into which they are incorporated and as fugitive emissions resulting from their manufacture and use in the synthesis of polymers [6,11,15]. Concentrations of FTOHs in the range of 11 to 171 pg/m^3 have been measured in the North American troposphere [8,16]. Fluorotelomer alcohols can be oxidized in the atmosphere to produce the corresponding saturated fluorotelomer carboxylic acids (FTCAs) [7,17]. The lower volatility and higher water solubility of the FTCAs compared to FTOHs suggest that atmospheric oxidation of the FTOHs will result in the deposition of FTCAs in surface waters. Indeed, the 8:2 FTCA, 10:2 FTCA, and 10:2 fluorotelomer unsaturated carboxylic acid (FTuCA) have been measured in precipitation in various urban sites in North America at concentrations up to 8.6 ng/L [18]. Fluorotelomer alcohols may also enter aquatic environments via wastewater treatment facilities as residuals or from the degradation of fluoropolymers used in surface treatment applications resulting from routine activities such as carpet and upholstery cleaning [6]. Recent studies show that FTOHs undergo indirect photolysis in aquatic environments, with FTCA as a major product and the corresponding FTuCA as a minor one [19]. Furthermore, microbial degradation of FTOHs in aqueous environments has been shown to result in significant production of corresponding FTCA and FTuCAs [6,9]. The worldwide occurrence of PFCAs in surface waters coupled with compelling evidence that FTOHs are a significant source of these compounds also strongly indicates the presence of FTCAs in this environmental matrix. Therefore, there is a need to quantify fluorotelomer acids (FTAs) in surface waters and to evaluate their potential effects in aquatic ecosystems. Although environmental exposure data are lacking, atmospheric concentrations [16,20] and those measured in precipitation [17] suggest that FTAs are likely present in surface waters at concentrations in the low ng/L range. These data suggest that

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toxicity tests should focus on long-term exposures to low concentrations to assess potential risks posed by FTAs to aquatic organisms.

The focus of the present study, therefore, was to assess the chronic toxicity of the 8:2 FTCA, and the 10:2 FTCA and FTuCA to two freshwater organisms, the pelagic microcrustacean *Daphnia magna* and the benthic macroinvertebrate *Chironomus dilutus*. Compounds and test organisms were chosen based on the results of previous acute studies with a suite of FTAs that found *D. magna* and *C. dilutus* to be most sensitive to these three FTAs [21].

MATERIALS AND METHODS

Test compounds

The three compounds assessed in the current study—8:2 FTCA, 10:2 FTCA, and 10:2 FTuCA (Table 1)—were synthesized at the University of Toronto using methods described by Achilefu et al. [22] and were >98% pure.

Test organisms

Original brood stocks of *D. magna* were obtained from Stantec Environmental Consulting with subsequent culturing in the laboratory following standard methods [23,24]. Colonies were reared in 250-ml glass beakers containing American Society for Testing and Materials (ASTM) reconstituted hard water [25] supplemented with sodium selenate and cyanacobalamin [23]. Cultures were maintained in a walk-in environmental chamber held at $20 \pm 2^{\circ}$C with a 16:8 h light:dark photoperiod. The colony was fed a diet consisting of a mixture of two species of green algae, *Chlorella vulgaris* and *Pseudokirchneriella subcapitata*, and a synthetic suspension of yeast, Cerophyll™, and trout chow (Yeast, Cereal Leaves, Flake-Fish Food, YCT). *Chironomus dilutus* egg cases were originally supplied by the U.S Environmental Protection Agency and subsequently cultured in the laboratory. Colonies were reared in 10-L glass aquaria containing washed, ashed (3 h at 550°C), and sieved medium-grain (250–499 μm) sand with overlying ASTM reconstituted hard water. Cultures were maintained in a walk-in environmental chamber at $23 \pm 2^{\circ}$C with a 16:8 h light:dark photoperiod and were fed 5 ml of ground Tetrafin™ (Tetra) fish flakes (56 g/L culture water) 5 d per week after partial water renewals.

Toxicity testing

Preparation of test solutions. Methanol was used as a solvent carrier to dissolve the FTAs in control reconstituted water. Just prior to starting each assay, concentrated methanol stocks of individual acids were prepared and then serially diluted with methanol to produce solvent stock solutions that could be added in equal volumes to reconstituted water to obtain final test solutions. Fresh test solutions were prepared on each assay renewal day using methanol stocks stored at 5°C. Methanol stocks were brought to room temperature and sonicated for approximately 15 min prior to each use to ensure that FTAs that may have crystallized during cold storage were redissolved before preparing the solutions. Polypropylene or polyethylene containers were used for testing, sampling, and preparing all solutions due to the propensity for perfluorinated compounds to adhere to glass [26].

Table 1. Nominal and mean (of four) measured actual concentrations ± standard error of the 8:2 saturated fluorotelomer carboxylic acid used in the *Chironomus dilutus* life-cycle assay

Nominal (mg/L)	Mean measured day 0[a] (mg/L)	Mean measured day 2[b] (mg/L)	TWA of measured concentrations[c] (mg/L)
0	0	0	0
0.3125	0.200 ± 0.030	0.043 ± 0.017	0.082
0.625	0.413 ± 0.029	0.273 ± 0.056	0.341
1.25	0.875 ± 0.092	0.435 ± 0.148	0.597
2.5	1.955 ± 0.090	1.175 ± 0.137	1.544
5	3.930 ± 0.498	2.220 ± 0.225	3.038
10	8.068 ± 1.233	3.645 ± 0.785	5.627

[a] Measured in freshly prepared test solutions prior to renewal, day 0.
[b] Measured in composite samples of 2-d-old solutions.
[c] Time-weighted average actual concentration.

Exposure system

Static-renewal exposures were employed in all life-cycle assays. Test solutions were partially renewed every 48 h in tests with *C. dilutus* and completely renewed three times weekly in *D. magna* tests (renewal procedures are described below). The experimental units in the *C. dilutus* assay were continuously aerated and mean concentrations of dissolved oxygen were consistently >7 mg/L for the duration the test, nearly three times the minimum requirement of 2.5 mg/L [27]. While replicates in the *D. magna* assays were not aerated, mean dissolved oxygen concentrations were approximately 6 mg/L throughout the test period, or double the minimum guideline requirements of 3 mg/L [25]. Regular residue sampling and analyses were conducted to account for potential variation in toxicant exposure concentrations throughout the study periods.

Life-cycle assay for D. magna

Daphnia life-cycle assays were conducted in accordance with standard methods [25]. Nominal concentrations used in the *D. magna* tests were 0, 1.9, 3.8, 7.5, 15, 30, and 60 μg/L for the 10:2 FTCA and 0, 9.4, 18.8, 37.6, 75, 150, and 300 μg/L for FTuCA, based on the results of previous acute assays [21]. Positive and negative (<300 μl/L methanol) controls were included. All assays were performed at an ambient air temperature of $20 \pm 2^{\circ}$C with a 16:8 h light:dark photoperiod. Five replicates were arranged in a randomized complete block design with eight treatments and five blocks. Tests were initiated with <24-h-old neonates that were randomly transferred to individual replicates containing 200 ml of test solution in 250 ml polypropylene beakers, from the lowest to the highest concentration in each block. Each replicate was administered a 156 μl aliquot of concentrated (4 times that fed to brood cultures) algal cell suspension (2 parts *P. subcapitata*: 1 part *C. vulgaris*) and 250 μl of YCT.

Maintenance of test and monitoring

Complete renewals of test solutions were performed three times weekly, beginning on the second day. On renewal days, fresh test solutions were prepared and added to a second set of replicates as described above. After feeding with algae and YCT, first-generation *D. magna* were carefully transferred via wide-bore pipette from old to new test solutions. The YCT was applied at a constant rate of 3.2 μl/ml/d, while daily algal rations varied with the age of the organisms: approximately 4.8×10^4 cells/ml on days 0 to 3, 5.1×10^4 cells/ml on days 4 to 5, 5.8×10^4 cells/ml on days 6 to 7, 7.7×10^4 cells/ml on days 8 to 9, 9.6×10^4 cells/ml on days 10 to 13, and 1.5×10^5 cells/ml on days 14 to 21. Mortality was recorded daily as were visual observations of behavior, level of activity, and general condition (color, size, and physical anomalies). Monitoring, removal, and enumeration of neonates were performed daily

until day 16 to ensure accurate determination of time to first brood. On renewal days, adults were transferred to new test solutions and the old test solutions were strained using nylon mesh to trap the neonates. On nonrenewal days, neonates were removed and counted using wide-bore pipettes. After day 16, neonates were removed and enumerated using the straining method on renewal days only. Water quality parameters were measured using a Sension model 156 Portable Multiparameter Meter (Hach) in new test solutions at the beginning of the 21-d assay and in old solutions at the middle and end of the test period. Water quality parameters remained relatively stable over the duration of both *D. magna* assays with mean values of 7.57 for pH, 116 $CaCO_3$/L for alkalinity, 188 mg $CaCO_3$/L for hardness, 545 μS/cm for conductivity, 19.7°C for temperature, and 6 mg/L for dissolved oxygen.

Evaluation of life-cycle endpoints

Endpoints measured in the *D. magna* life-cycle assay included survival of the first generation, time to first brood, number of adult reproduction days, total number of young, and number of young/adult reproduction day. The number of adult reproduction days was calculated by adding the number of surviving adult females in each replicate beginning on the first day of neonate production and continuing to the end of the assay. The total number of young was the sum of all live young produced in each replicate over the duration of the assay. The number of young/adult reproduction day was calculated as the total number of young divided by the number of adult reproduction days for each replicate.

Life-cycle assay for C. dilutus

The life-cycle assay was performed in a walk-in environmental chamber maintained at 23 ± 2°C with a photoperiod of 16:8 h light:dark and was conducted in general accordance with standard methods [27] for life-cycle toxicity testing using *C. dilutus*. Three days prior to test initiation, six large C-shaped egg cases were collected from adult mating chambers and transferred to Petri dishes containing control ASTM hard water. Hatching dishes were incubated in the environmental chamber until test initiation. Egg cases were examined under a dissecting microscope on days −2, −1, and 0 for viability, development, and hatch.

Nominal and actual concentrations for the *C. dilutus* life-cycle assay appear in Table 1. These concentrations were selected based on the results of previous acute assays with the 8:2 FTCA that generated approximate EC50 (growth) and LC50 values of 5.9 and 12.4 mg/L, respectively [21]. Positive and negative (<1.0 ml/L methanol) controls were included. Twelve beakers per treatment were set up the day before test initiation (day −1) by adding approximately 50 ml of clean culture sand to individual 250-ml polypropylene beakers. Overlying treatment solutions were then gently poured along the sides of each beaker to a volume of approximately 240 ml. Replicates were arranged in a randomized complete block design in the environmental chamber and allowed to settle for 3 h. Each replicate received 1.5 ml of Tetrafin fish flake slurry (4 g/L).

On the initial day of the assay (day 0), individual egg cases with hatched larvae were transferred to small (3-cm) glass Petri dishes containing fresh control water. Approximately 140 ml of overlying solution in each replicate was gently decanted and replaced with fresh test solution. Each replicate was then supplied with 1.5 ml of food and allowed to settle for approximately 3 h. Ten newly hatched larvae were collected and transferred using a dissecting microscope and pasture pipette into the water column of each replicate, beginning with the control and progressing to the highest concentration in each block.

Maintenance of test and monitoring

Partial renewals of test solutions were performed every 48 h, beginning on day 3. Approximately 140 ml of solution were slowly siphoned from each replicate using rubber tubing and then carefully replaced via gravity-fed flow through rubber tubing in order to minimize disturbance of the sediment and larvae. Each replicate received 1.5 ml of test food daily for the duration of the assay. Individual replicates were gently and continuously aerated commencing on day 3 until the end of the tests by bubbling air through rubber tubing to plastic pipette tips attached to the inside of each test vessel just below the solution surface.

Temperature and dissolved oxygen were measured in three randomly selected replicates per treatment every second day prior to each renewal. Other water quality parameters were measured in all new and 2-d-old (composites of five replicates per treatment) test solutions at the beginning, middle, and end of the assay. Water quality parameters remained stable throughout the assay, with mean values of 8.01 for pH, 125 mg $CaCO_3$/L for alkalinity, 186 mg $CaCO_3$/L for hardness, 544 μS/cm for conductivity, 21.8°C for temperature, and 7.4 mg/L for dissolved oxygen.

Peak female emergence in *C. dilutus* is delayed relative to that of males, necessitating a steady supply of viable males for mating purposes during later stages of the emergence period [28]. This was accomplished by adding four additional auxiliary replicates per treatment on day 10 of the test. Eggs for these replicates were collected on day 7 of the assay and set up and monitored as described previously.

Evaluation of life-cycle endpoints

Of the 12 initial replicates per treatment in the life-cycle test, four were randomly selected at 20 d to assess survival and growth. The remaining eight replicates were monitored for the duration of the assay for emergence and reproduction. On day 20, sediment and overlying solution from each growth/survival replicate was emptied into a glass sorting dish and all larvae removed using forceps. Live larvae from each replicate were placed in separate, ashed (2 h at 550°C) 42-ml aluminum weigh pans and dried at 60°C for approximately 24 h. Samples were then cooled to room temperature in a desiccator and weighed to estimate dry weight. Samples were then ashed at 550°C for 2 h and reweighed. Total ash-free dry weight was determined as the difference between the dry weight and ashed weight/replicate for each treatment.

Emergence traps were placed on the eight emergence/reproduction replicates on d 20. Detailed instructions for the construction and use of emergence traps, adult collecting dishes, reproduction-oviposition chambers (ROCs), and aspirators (for collecting adults) used in the current study are provided in Benoit et al. [28]. Emergence traps were placed on auxiliary replicates on day 30 of the assay.

Adults were collected daily from each emergence trap. Only adults that had broken completely free of the water surface were counted as having emerged [28]. One female from each treatment was transferred to an ROC containing control water and paired with one or more males from a replicate within the same treatment. In some cases individual males were used to mate more than one female from the same treatment. Dead adults and

pupae were removed daily from test vessels and ROCs. Mortality of adults and pupae was recorded daily.

Each ROC was monitored daily for egg production. Primary egg cases [28] were transferred to 3-cm diameter plastic Petri dishes containing control water and the number of eggs determined under a dissecting microscope, either by direct count or estimation using the ring count method [28]. Egg cases were incubated for 6 d in the environmental chamber, after which time the number of hatched eggs was determined by subtracting the number of unhatched eggs from the total number originally counted in each egg case.

Residue sampling and analyses

In the *D. magna* life-cycle test solutions were sampled once at the beginning, middle, and end of each 21-d period. In the *C. dilutus* life-cycle test solutions were sampled once at the beginning and end of the 60-d assay and on days 15 and 36. Prior to renewal, approximately 500 μl of freshly prepared solution was removed from each vessel in which new test solutions were mixed. This was followed 2 d later by the removal of 500 μl of the same (now 2 d old) solutions from composite samples comprised of aliquots from five randomly selected test vessels/treatment. Each 500-μl test solution sample was immediately placed in an equal volume of methanol in a 1.5 ml polypropylene flat top microcentrifuge tube (Fisher Scientific). Samples were stored at 5°C and shipped on ice to the University of Toronto for analysis.

Water samples were diluted 1:1 with methanol and stored at 4°C prior to analysis. All samples were analyzed using a Waters 616 pump with a 600 controller coupled to a Micromass Quattro Micro LC tandem mass spectrometer operating in negative electrospray ionization mode. Chromatographic separations were performed using an Ace® 3 C18 column (15 cm × 2.1 mm, 2.1 mm, 3 μm) (Life Sciences). Isocratic elution was used at a flow rate of 150 μl/min with mobile phase comprised of 75:25 10 mM ammonium acetate in methanol and 10 mM aqueous ammonium acetate. Samples were injected at a volume of 20 μl using a Waters 717 autosampler.

Mass spectral analysis was optimized by tuning the mass spectrometer using a 500 μg/L standard solution of each of the compounds of interest (Table 2). The capillary voltage was set to 3 kV while the cone voltage varied from 10 to 15 kV, depending on the compound. The dwell time was 0.2 s. The source block and desolvation temperatures were 110 and 250°C, respectively, while the desolvation gas flow rate was 250 L/h. Argon was used as the collision gas (3.0×10^{-3} mBar) and collision energies were varied for optimal detection of each compound analyzed.

External calibrations were used with standards ranging from 5 to 500 μg/L. Calibration curves were linear with r^2 values typically >0.99. Samples with concentrations <5 μg/L were concentrated using solid phase extraction (SPE) and eluted with 1 ml of methanol prior to analysis, while samples with concentrations >1,000 μg/L were further diluted before analysis.

Table 2. Optimized parameters for the measurement of saturated and unsaturated fluorotelomer carboxylic acids (FTCAs) in aqueous samples using liquid chromatography/double mass spectrometry

FTCA	Parent ion (m/z)	Daughter ion monitored (m/z)	Collision energy (eV)	Cone voltage (V)
8:2 FTCA	477	393	16	10
10:2 FTCA	577	493	15	14
10:2 FTuCA	557	493	15	15

Statistical analyses

Toxicity was evaluated using both linear and nonlinear regression techniques to generate lethal (LCX) and effect (ECX) concentrations resulting in 10, 25, and 50% (X = 10, 25, 50) differences in measured endpoints from control treatments. The LCX and ECX values for *C. dilutus* were calculated using time-weighted average (TWA) measured concentrations. Reliable residue data could not be obtained for the 10:2 FTCAs because of the very low concentrations used and detection limitations with analytical equipment at the time of the study [29]. Therefore, toxicity estimates for *D. magna* assays were calculated using nominal concentrations. Binomial (survival, emergence) data were evaluated using the Proc Probit procedure for probit analyses in the SAS System for Windows, Ver 8.2. Three models, normal, logistic, and Gompertz, were tested for best fit in the Proc Probit procedure. In cases where data either did not fit the probit model or where 95% confidence intervals could not be generated, data were subsequently analyzed using the nonparametric Trimmed Spearman-Karber Program, Ver 1.5 [30], to generate LC50 or EC50 values and corresponding confidence intervals. Growth and reproduction data were analyzed using nonlinear regression techniques described by Stephenson et al. [31]. Five models were tested for best fit using the Proc Nonlin procedure in SAS: linear, logistic, Gompertz, exponential, and hormetic. Scatter plots of raw data were first inspected to determine which of the models might be appropriate for describing concentration–response relationships. Final model determination was based on the value of the mean corrected coefficient of determination ($R_c^2 = 1$-[residual sum of squares/corrected sum of squares]) and on graphical interpretation of the fit of the model (residual vs predicted values). In some cases a lack of data points between the no-observed-effect concentration (NOEC) and the highest concentration tested precluded the use of nonlinear regression for generating point toxicity estimates; in these situations a linear interpolation method, the Inhibition Concentration (ICp) Program, Ver 2.0 [32], was used to generate point toxicity estimates. Statistically significant differences in treatment versus control responses were evaluated using a one-tailed, one-way analysis of variance at $p \leq 0.05$ with the Proc GLM procedure in SAS.

RESULTS

Daphnia magna

Responses were observed for all endpoints (survival, time to first brood, number of young/female reproduction day) for both the 10:2 FTCA and FTuCA life-cycle assays with *D. magna*, but only at the highest concentrations tested (Fig. 1; Table 3). Test acceptability criteria for control responses for survival (>80%) and reproduction (>60 neonates per female at the end of the test) were met. Based on the ICp linear interpolation method, the LC50 value for the 10:2 FTCA exceeded the highest concentration tested. For the 10:2 FTuCA, mortality data did not fit the probit model. Therefore, the Trimmed Spearman-Karber method was used to estimate an LC50 of 150 μg/L (Table 3), which was also the NOEC.

The time to first brood was approximately 8 d for the control and the first five concentrations for both 10:2 FTCA assays, but increased to 18 and >21 d at the highest concentrations for the 10:2 FTCA (60 μg/L) and 10:2 FTuCA (300 μg/L) (Fig. 1B). Adults exposed to 300 μg/L of the 10:2 FTuCA did not produce any young (Fig. 1B,C). The EC50 values for time to first brood

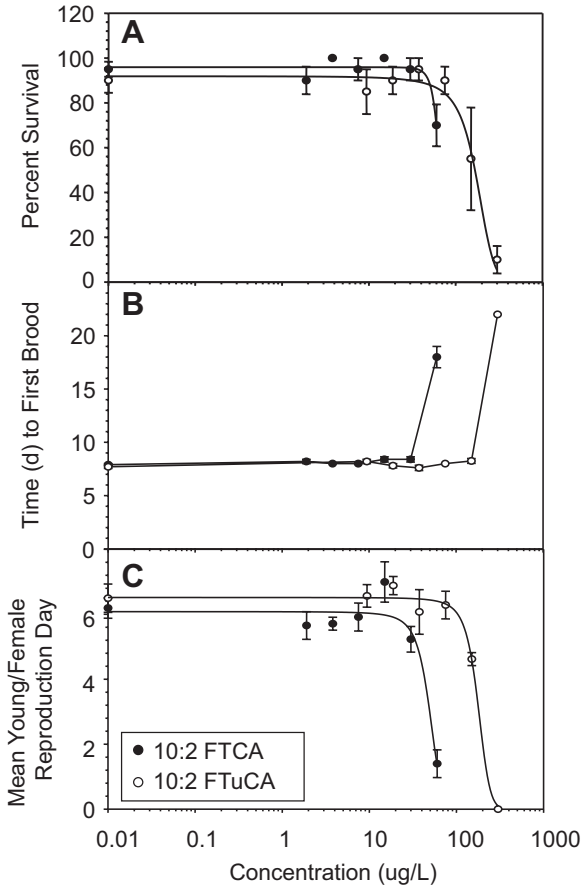

Fig. 1. Responses of *Daphnia magna* to 21-d exposures of the 10:2 saturated (10:2 fluorotelomer carboxylic acid) and unsaturated (10:2 FTuCA) fluorotelomer carboxylic acids. Error bars represent the standard error of the mean. Endpoints measured include (**A**) survival, (**B**) time to first brood, and (**C**) mean number of young/female reproductive day.

for the 10:2 FTCA and 10:2 FTuCA were 50 μg/L and 287 μg/L, respectively, with corresponding NOEC values of 30 μg/L and 150 μg/L. The mean number of young produced per female reproduction day was relatively consistent at six to seven for the control and the first four concentrations in both 10:2 FTCA assays. For the 10:2 FTCA, this decreased to approximately 5 and one young per female reproduction day at 30 μg/L and 60 μg/L, respectively (Fig. 1C). In 10:2 FTuCA exposures, young per female reproduction day decreased from five at 150 μg/L to zero at 300 μg/L (Fig. 1C). The EC50 values for mean number of young produced per female reproduction day for the 10:2 FTCA and 10:2 FTuCA were 48 μg/L and 214 μg/L, respectively, with corresponding NOEC values of 30 μg/L and 75 μg/L.

Chironomus dilutus

Responses to the 8:2 FTCA were observed at 20 d for both survival (Fig. 2A) and growth (Fig. 2B). Test acceptability criteria for control survival (>70%) and growth (>0.48 mg/individual) were met. Mean 20-d survival declined at concentrations >340 μg/L, from just over 76% in the controls to 0% at 5630 μg/L. Corresponding 95% confidence intervals (CIs) for LC values could not be determined using the Probit model. Therefore, the Trimmed Spearman-Karber method was used to estimate an LC50 value (with 95% CI) of 2,150 (1,780, 2,580) μg/L, which closely agreed with the point estimate generated by the probit model (2,160 μg/L; Table 4). The corresponding NOEC for survival was 1,540 μg/L. Mean total ash-free dry weight followed a similar trend, decreasing from 6.41 mg in the controls to 0.43 mg in the 3,030 μg/L treatment. The EC50 for growth was 1,250 μg/L, with a corresponding NOEC of 340 μg/L.

Emergence of *C. dilutus* was significantly reduced in all treatments ≥340 μg/L (Fig. 3). Emergence declined progressively relative to controls from approximately 59% of mean control emergence at 0.34 mg/L, to 34%, 16%, and 2% at 600, 1,540, and 3,040 μg/L, respectively (Fig. 3). A lone male emerged and two pupae were recovered in the 3,040 μg/L treatment; no adults emerged from the 5,630 μg/L treatment nor were any pupae observed. The EC values for total percent emergence and time to first emergence appear in Table 4. The data for total percent emergence did not fit the probit model. Therefore, the EC50 for this endpoint, 440 μg/L, was determined using the Trimmed Spearman-Karber method, which does not provide EC25 or EC10 estimates (Table 4). The NOEC for total emergence was 80 μg/L.

Low emergence in treatments ≥340 μg/L resulted in a limited number of adults available for mating, which, in turn, limited our capacity to assess reproduction (Table 5). The percentage of emerged females that oviposited ranged from approximately 42% in the controls to approximately 30% in the 340 μg/L treatment (Table 5). Of the females that did emerge in the 600 and 1,540 μg/L treatments, none produced egg masses. Reproduction was not observed in the 3,040 and 5,630 μg/L treatments due to lack of female and adult emergence, respectively. No significant difference existed in the mean number of eggs/egg mass between the control and lowest two treatments (80 and 340 μg/L; Table 5). Similarly, no significant difference in the mean number of eggs/female was observed between the

Table 3. Lethal and effect concentrations (LC and EC) of the 10:2 saturated (10:2 FTCA) and unsaturated (10:2 FTuCA) fluorotelomer carboxylic acids causing an *x*% (*x* = 10, 25, 50) change in the endpoint measured in *Daphnia magna* life-cycle assays

FTCA	Endpoint	EC/LC10 (μg/L)	EC/LC25 (μg/L)	EC/LC50 (μg/L)	Model[a]
10:2 FTuCA	Mortality	NC [b]	NC	150 (120, 200)	TSK
	Time to first brood	204 (197, 208)[c]	235 (230, 238)	287 (284, 288)	ICp
	Young/female reproductive day	117 (2, 164)	171 (150, 183)	214 (199, 221)	ICp
10:2 FTCA	Mortality	33 (12, 54)	60 (43, 111)	>60	Probit
	Time to first brood	33 (31, 34)	40 (37, 41)	50 (46, 54)	ICp
	Young/female reproductive day	33 (0, 34)	39 (34, 41)	48 (44, 52)	Icp

Values in parentheses represent 95% confidence intervals for each point estimate. Values preceded by > indicate estimate exceeds the highest concentration tested.

[a] TSK = Trimmed Spearman-Karber method. ICp = Inhibition concentration linear interpolation method.

[b] NC = not calculated.

[c] Values in parentheses represent 95% confidence intervals for each point estimate.

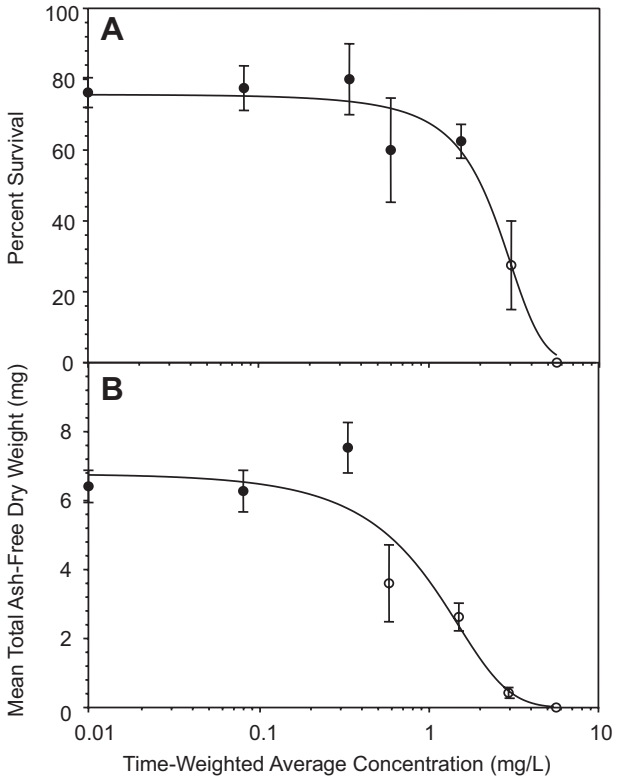

Fig. 2. Percent total survival (**A**) and growth (**B**) of *Chironomus dilutus* larvae after 20-d exposure to the 8:2 saturated fluorotelomer carboxylic acid. Error bars represent the standard error of the mean. Open circle indicates a response that is significantly lower ($p \leq 0.05$) than control. Control acceptability criterion is 70%.

control and two lowest concentrations, even though approximately 39% fewer eggs were produced in the two treatments (Table 5).

The number of viable eggs that could be evaluated for hatchability was reduced because of low and delayed emergence of males from auxiliary beakers (i.e., no mating) and, for irregular egg masses, because of the need to use acid to separate the eggs from the gelatinous egg (this process kills the eggs) to facilitate counting [28]. For example, of the 20 egg cases produced in the control replicates (Table 5), only 11 could be assessed for hatchability because two were unfertilized and seven were acid-counted [28]. Of the 11 viable control egg cases, percent hatch was approximately 95%. No hatch was observed in the single viable egg mass in the 80 μg/L treatment (out of seven) (Table 5). None of the six cases in the 340 μg/L treatment (Table 5) were monitored for hatch because four were unfertilized and two were acid-counted.

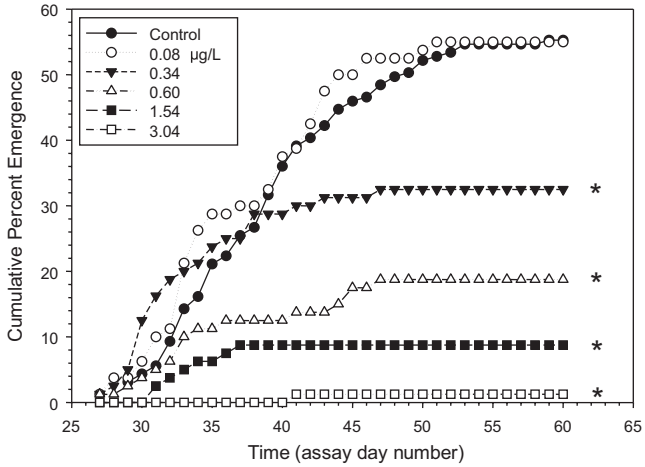

Fig. 3. Percent cumulative emergence of *Chironomus dilutus* exposed to the 8:2 saturated fluorotelomer carboxylic acid (8:2 fluorotelomer carboxylic acid). Asterisk indicates a response that is significantly lower ($p \leq 0.05$) than control. Control acceptability criterion is 50%.

Table 5. Reproductive endpoints (± standard error) of the mean for *Chironomus dilutus* exposed to the 8:2 saturated fluorotelomer carboxylic acid (8:2 FTCA) in the life-cycle assay

Endpoint	Treatment concentration (mg/L)				
	0[a]	0.08	0.34	0.60	1.54
Total females emerged	51	29	14	5	3
Total egg masses	20	7	6	0	0
Females ovipositing (%)	42 ± 8.8	31 ± 12.2	30 ± 19.1	0	0
Mean eggs/egg mass	1,222 ± 85	1,027 ± 142	1,035 ± 236	NA	NA
Mean eggs/female	527 ± 117	319 ± 134	318 ± 217	0	0
Mean hatch (%)	95 ± 1.7	0	NA	NA	NA

Only treatments from which at least one female emerged are included.
[a] Combined total for blank and solvent controls.
NA = not applicable.

DISCUSSION

Ongoing investigations of the sources of global contamination by PFCAs have generated a strong body of evidence for FTOHs as the precursors. Furthermore, environmental fate data and elucidation of chemical and biological degradation pathways of the FTOHs indicate potential continuous and cumulative releases of FTAs to aquatic environments. In conjunction with previous acute (10-d) studies [21], the results of the present chronic toxicity studies represent the first reports of the toxicity of FTA to aquatic organisms. Chronic toxicity thresholds were, with one exception, lower than those determined in acute exposures. For example, EC50 and LC50 values of 1,250 μg/

Table 4. Lethal and effect concentrations (LC and EC) of the 8:2 saturated fluorotelomer carboxylic acid (8:2 FTCA) causing an *x*% ($x = 10, 25, 50$) change in the endpoint measured in the life-cycle assay with *Chironomus dilutus*

Endpoint	EC/LC10 (mg/L)	EC/LC25 (mg/L)	EC/LC50 (mg/L)	Model[a] r^2
20-d survival	0.79 (NC[b])	1.76 (NC)	2.61 (NC)	Probit
20-d growth	0.47 (0.05, 0.90)[c]	0.78 (0.35, 1.20)	1.25 (0.84, 1.66)	Logistic 0.79
Total % emergence	NC	NC	0.44 (0.38, 0.52)	TSK[b]
Time to 1st emergence	0.19 (0, 0.46)	0.44 (0.11, 0.77)	0.89 (0.49, 1.30)	Logistic 0.64

[a] TSK = Trimmed Spearman-Karber method.
[b] NC = not calculated.
[c] Values in parentheses represent 95% confidence intervals for each point estimate.

L and 2,610 μg/L for *C. dilutus* growth and survival in the life-cycle assay were nearly 5 times lower than those generated in the acute exposures (EC50 = 5,860 μg/L, LC50 = 12,420 μg/L), indicating either a higher sensitivity of the younger life stage exposed in the chronic assay (<24-h-old larvae) versus acute exposures (10-d-old larvae) or the longer exposure period in the chronic test. Similarly, the chronic LC50 of 150 μg/L calculated for the 10:2 FTuCA in the *D. magna* life-cycle assay was approximately 5.5 times lower than the 96-h acute LC50 (833 μg/L [21]). Because initial exposures in both chronic and acute *Daphnia* assays involved neonates of the same age (<24 h), differential toxicity reflects differences in exposure duration. An acute-to-chronic ratio of 10 is commonly applied in toxicology to predict chronic toxicity thresholds from acute toxicity data and to generate water quality guidelines where chronic data are lacking [33]. This generalization overestimates chronic toxicity of the 10:2 FTAs to *D. magna*, but would provide an additional small margin of safety if used to generate water quality criteria for these compounds.

The chronic LC50 for the 10:2 FTCA exceeded the highest concentration tested in the *Daphnia* life-cycle assay. However, a comparison of LC25 values shows the chronic threshold of 60 μg/L to be nearly twice the acute LC25 of 35 μg/L [21], and represents the one exception where a chronic point estimate was greater than the corresponding acute value. The most likely explanation for this anomaly is the use of nominal rather than actual TWA concentrations in calculations of chronic toxicity thresholds for the *D. magna* assays. As shown in Table 3, actual TWA concentrations for the 8:2 FTCA were approximately half those of corresponding nominal concentrations. This was due in part to lower initial actual concentrations in freshly prepared test solutions but mainly reflected decreasing aqueous concentrations between renewal periods (Table 3). Assuming that actual TWA concentrations of the 10:2 FTCA are similarly reduced compared with nominal concentrations, actual concentrations would be expected to be approximately half of the corresponding nominal concentrations. The chronic LC25 value of 60 μg/L for the 10:2 s-FTCA, if reduced by half, is then slightly lower than the acute LC25 value. Thus, it seems reasonable to assume that the nominal chronic toxicity thresholds reported here for *D. magna* underestimate actual toxicity.

Results of the life-cycle assays of 10:2 FTA in *D. magna* agree with previous findings of higher acute toxicity with the saturated versus unsaturated forms of the FTAs [21]. Acute LC and EC50 values for survival and immobility were approximately one order of magnitude larger for the 10:2 FTuCA (279 and 833 μg/L) than the 10:2 FTCA (25 and 63 μg/L). Differences in chronic endpoints were less pronounced, with EC50 values for time to first brood and young/female reproduction day approximately six and 4.5 times higher, respectively, for the 10:2 FTuCA versus the 10:2 FTCA. The higher toxicity observed with FTCA versus FTuCAs could potentially be because the test organisms were exposed to both forms of the acid as well as their metabolites during laboratory assays initiated with only FTCA. Organisms exposed to FTUCA are subject to at least two fewer metabolites, FTCA and hydrogen fluoride (HF). In fact, organisms may be exposed to even fewer metabolites because FTuCA and HF are not necessarily the sole metabolites of FTCA degradation [6]. The fluoride ions generated when FTCA is degraded to its corresponding FTuCA, either in aqueous solution or in vivo, may result in differential toxicity of FTCA and FTuCA. The LC50 values from the literature for aquatic invertebrates exposed to fluoride range from 12 to 352 mg/L, depending on the species, exposure duration, water hardness, and temperature [34]. However, if the FTCAs are degraded in vivo to their FTuCA counterparts, fluoride ions generated within the organism might elicit greater toxicity than documented for aqueous exposures.

Point estimates of reproductive thresholds for the 10:2 FTCA in the *D. magna* life-cycle assay were similar (≈50 μg/L) and both time to first brood and mean young/female reproductive day were more sensitive endpoints than mortality (LC50 of >60 μg/L). In contrast, mortality was the most sensitive endpoint for the 10:2 FTuCA assay, with an LC50 of 150 μg/L, while EC50 values for mean young/female and time to first brood were approximately 214 and 287 μg/L, respectively.

The most sensitive endpoint in the *C. dilutus* life-cycle assay was total emergence (EC50 ≈40 μg/L). The apparent sensitivity of emergence in the present study contrasts with results from other chronic assays with *C. dilutus*. For example, in a life-cycle assay with the related 8-fluorocarbon (8-FC) perfluorinated acid (PFOS), emergence, survival, and growth were equally sensitive endpoints [35] and emergence was actually the least sensitive endpoint measured in a *C. dilutus* life-cycle exposure to zinc [36]. Midges utilize surface tension to facilitate successful transfer across the air–water interface during emergence; a reduction in surface tension due to the presence of surfactants could therefore decrease emergence success. Although the proportion of midges that attempted to emerge but failed (as indicated by partial emergences or drowned adults) was not assessed in the present study, Takamura [37] found significantly reduced emergence of several species of midges exposed to a hydrocarbon surfactant.

Low adult emergence in treatments ≥340 μg/L and limited availability of viable eggs for determination of hatch limited the evaluation of reproductive endpoints in the *C. dilutus* life-cycle assay. Other studies have shown mean number of eggs/female to be a sensitive endpoint for assessing toxicity, while embryo viability (% hatch) tends to be relatively insensitive [28,36]. In contrast, neither of these endpoints showed concentration-responses in a *C. dilutus* life-cycle assay with PFOS [35].

With the exception of PFOS toxicity to *C. dilutus* [35], the toxicity thresholds of the FTCAs are considerably lower than those reported for PFAs in other aquatic organisms [35,38–40]. However, the toxicity thresholds generated for the 8:2 FTCA in the current *C. dilutus* life-cycle assay are five to 28 times greater than those measured in a previous study with PFOS and *C. dilutus* [35]. In the present study, EC50 and LC50 values for emergence, growth, and survival were approximately 440, 1,250, and 2,610 μg/L, while those for the PFOS life-cycle assay were 94.5, 93.8, and 92.2 μg/L, respectively [35]. Because the length of the perfluorinated carbon chain of PFOS and the 8:2 FTCA molecules are equal, it can be concluded that the higher toxicity observed with PFOS is the result of differences in the structure of the attached hydrophile [35]. In the PFOS study, larvae exposed to higher concentrations tended to be pale, apparently losing the red pigmentation associated with hemoglobin. Additionally, larvae were often observed undulating on the sediment surface, a behavior that may indicate oxygen stress. These observations led to the hypothesis that PFOS may have been interfering, directly or indirectly, with the function of hemoglobin and a recent study in chickens has shown that two genes regulating hemoglobin activity experienced an approximately twofold decrease in activity following exposure to PFOS [41]. However, the above behavior was not observed in the current assay with the 8:2 FTCA. Although larvae were occasionally observed on the sediment surface, the

undulatory motion was not apparent and they generally did not remain on the surface of the sediment for extended periods of time. Hence, there is no evidence in this case to suggest that toxicity from the 8:2 FTCA was the result of interference with hemoglobin. Based on the current understanding of the mechanism of toxicity for PFOS, and the similarity of PFCA and FTA structures, plausible mechanisms of action include those reported for PFCAs, including interference with cellular processes such as gap junctional intercellular communication [42], energy metabolism [39], and mitochondrial respiration [43], among others.

The extent to which the results of these studies can be used to evaluate the risks of FTAs to aquatic ecosystems is constrained by the absence of reliable exposure data. However, recent studies of the environmental sources, fate, and degradation of fluorotelomer compounds [6,7,10,15] coupled with concentration data for FTAs in rain [17] and FTOHs in surface waters [41], indicate that FTAs are present in surface waters in the ng/L range. Furthermore, because FTCAs are intermediates in the pathway leading to the formation of PFCAs, it is unlikely that concentrations of individual FTAs will be greater than current measured concentrations of PFCAs, which are found in the ng/L to μg/L range in surface waters [44]. In general, toxicity thresholds generated in chronic exposures to FTAs for both *D. magna* and *C. dilutus* were in the μg/L to low mg/L range. Based on predicted exposure concentrations in aquatic systems, these data suggest that currently measured concentrations of FTAs are not likely to pose significant risks to either of these species. However, characterization of FTA exposures in aquatic ecosystems is required before more definitive conclusions regarding potential risks can be made.

CONCLUSION

The data presented in the present study represent the first chronic toxicity data for FTAs. The chronic toxicity threshold ranges for FTAs to *D. magna* (low-mid μg/L) and *C. dilutus* (mid μg/L-low mg/L) are considerably lower than thresholds reported for many of the PFCA compounds (i.e., PFOA) that have attracted scientific and regulatory attention. The findings, therefore, indicate the need to consider intermediates of the environmental degradation of PFCA precursors in future risk assessments. The results indicate that current environmental concentrations of individual FTAs likely pose negligible risk to aquatic biota. However, there remains a critical need for additional quantification of the FTAs in surface waters and assessment of their toxicity in order to undertake a meaningful assessment of potential risks to aquatic biota.

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