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Trifluoroacetate, an Atmospheric Breakdown Product of Hydrofluorocarbon Refrigerants: Biomolecular Fate in Aquatic Organisms

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Aquatic organisms were monitored for their ability to incorporate trifluoroacetate (TFA), an atmospheric breakdown product of HFC and HCFC refrigerants containing a trifluoromethyl moiety. Because of the structural similarity of TFA to acetate, a biochemical intermediate and microbial nutrient, we suspected that organisms might use the fluorinated compound to synthesize biomolecules such as lipids and acetylated proteins. We exposed aquatic organisms (microbial communities, oligochaetes, macroinvertebrates, *Callitriche* sp., *Lemna* sp., and *Impatiens capensis*) to radioactive TFA ([^{14}C]F_3COOH) and examined them for distribution of radiolabel in different classes of biomolecules. The most label was found in oligochaetes and *I. capensis* leaves at 3 and 6 μg/g (as TFA), respectively, with the greatest proportion found in the protein fraction for each sample type. Aerobic microorganisms incorporated only a small fraction of the label (a few nanograms per gram as TFA), and the greatest proportion of label occurred in cell wall material. We have demonstrated that selected aquatic organisms spanning a range of trophic levels incorporated the xenobiotic TFA into their biomolecule fractions so that it was no longer extracted as TFA and thus was metabolically transformed.

Introduction

Hydrofluorocarbons (HFC) and hydrochlorofluorocarbons (HCFC) are currently in production and use as replacement products for CFC refrigerants, which are being phased out due to destruction of stratospheric ozone by their reactive chlorine byproducts (*1*−*4*). Trifluoroacetate (TFA) is formed by hydrolysis of atmospheric decomposition products of HFCs and HCFCs containing a trifluoromethyl group. Due to its high water solubility, refractory nature, and the large-scale, worldwide use of its parent compounds as refrigerants, computer models have estimated that concentrations of refrigerant-derived TFA in rainwater will reach levels ranging from 0.14 to 0.20 μg/L by 2010 (*5*). In poorly drained and evaporative water pools, such as seasonal wetlands, TFA concentrations could possibly exceed 100 μg/L (*6*). TFA originating from other sources has actually been measured in surface waters at concentrations ranging from 0.06 to 6.4 μg/L, the latter occurring in the Dead Sea (*7*). Because we measured active incorporation of TFA by aquatic microorganisms (Bott and Standley, unpublished), we explored its biological fate in aquatic organisms ranging from aerobic microbial communities to oligochaetes and streamside vegetation such as jewelweed (*Impatiens capensis*).

Trifluoroacetate is structurally similar to acetate, which is a biochemical intermediate and microbial nutrient; thus, we suspected that incorporated TFA might be used in synthesis of biomolecules such as lipids and acetylated proteins. Synthesis of fluorinated lipids such as ω-fluorooleic acid has been documented for the plant *Dichapetalum toxicarium* (ratsbane), and other species within the genus are also capable of synthesizing monofluoroacetate (*8*).

Metabolites of compounds containing a trifluoroethyl group similar to that of TFA have been linked to trifluoroacetylation of proteins in rats and humans (*9*−*11*). Halothane ($CF_3CHClBr$) and HCFC-123 (CF_3CHCl_2) were capable of trifluoroacetylating proteins in rats after metabolism of the parent compounds to the reactive trifluoroacetyl chloride (*11*). Halothane has been linked to a rare hepatitis in humans, a condition in which antibodies react against a trifluoroacetylated carboxylesterase in the liver (*10*), and at very high exposures, HCFC-123 and HFC-134a (CF_3CH_2F) have been linked to benign tumors of the pancreas and testes of rats (*12, 13*). Also, trifluoroacetyl protein adducts were measured in the liver of a Belgian worker chronically exposed to HCFC-123 (*14*).

These three analogous compounds (halothane, HCFC-123, and HFC-134a) share the basic structural characteristics, such as an ethylene backbone and three fluorines on one carbon, required as metabolic precursors of the reactive trifluoroacetyl metabolite. Trifluoroacetate also shares these structural characteristics; however, it may not be as easily transformed or incorporated by higher organisms. Satoh and co-workers (*10*) demonstrated that rats were unable to utilize TFA to form trifluoroacetylated proteins. Furthermore, toxic effects of fluroxene (2,2,2-trifluoroethyl vinyl ether) and its metabolite trifluoroethanol in mammals appeared to be caused by a reactive intermediate, such as the $CF_3C(OH)_2^+$ carbonium ion, rather than by TFA (*15*). There is little evidence to date of significant toxicity of TFA to either mammals or plants such as duckweed and algae (*16*−*18*), except for *Selenastrum capricornutum*, which may be more sensitive (*19*).

From early experiments, we determined that microbial communities were capable of incorporating TFA (Bott and Standley, unpublished), and thus we hypothesized that they might be capable of its metabolic transformation. Although conversion of TFA to the reactive trifluoroacetyl functionality was apparently not possible for rats (*10*), some organisms capable of acetate utilization as a substrate, such as acetoclastic bacteria, might be capable of that conversion. Using kinetic evidence of binding, Emptage (*20*) demonstrated that TFA was a substrate for the enzyme acetyl−Coenzyme A synthetase, although binding was very slow and measurable only at very high concentrations of enzyme (μM) and TFA (>1 g/L). As will be discussed below, this process may be the fundamental basis for incorporation of the radiolabel in aquatic organisms exposed to [^{14}C]TFA in our study. Organisms capable of transforming TFA may also act as a conduit for exposure to higher organisms through trophic transfer. To address these questions, we exposed aquatic organisms representative of numerous trophic levels to radioactive TFA and then fractionated the biomass to examine the association of radioactivity with various biomolecular constituents.

Experimental Section

Incorporation of [^{14}C]TFA by Aerobic Microbial Communities. Biological communities were pre-equilibrated by

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exposing them continuously to 30 μg/L TFA (as sodium salt) in flow-through mesocosms containing sediments from White Clay Creek (WCC, Chester Co., PA) and receiving WCC stream water. Five experiments were carried out with the microorganisms cultured from mesocosm sediments beginning after approximately 10 months exposure and spanning several years: (1) July 1993, (2) September 1993, (3) February 1994, (4) June 1994, and (5) March 1995. A few grains of sand collected from a mesocosm stream were inoculated into 1.6 L of 0.1% tryptone-yeast extract (TYE) broth containing radioactive TFA ([^{14}C]F_3COOH, sp. act. 54 mCi/mmol, synthesized by Amersham Radiochemicals, Arlington Heights, IL, and provided for our study by The DuPont Company) as the sodium salt. After 48 h of incubation at room temperature in a water bath shaker, the cells were harvested by centrifugation. Cultures yielded ~1−2 g wet weight of microbial biomass, which was sufficient for extraction and analysis of biomolecule fractions. Controls included non-[^{14}C]TFA exposed live cells and [^{14}C]TFA exposed "killed-cell" controls. The latter cultures were grown in parallel with the experimental cultures in 0.1% TYE but without [^{14}C]TFA. They were then fixed with formalin, and these fixed cells were exposed to radiolabeled TFA for the same duration as the [^{14}C]TFA exposure of live cells.

Experiments 1−3 were conducted to determine the distribution of label in biomolecule fractions, with experiment 2 carried out at both 7.5 and 15 μCi/L exposure levels (yielding concentrations of 10 and 20 μg/L TFA, respectively). Experiment 4 was conducted with mesocosm organisms and a TFA-tolerant isolate (isolated on potato dextrose agar containing 180 mg of TFA/mL) and involved exposure to either radiolabeled TFA or acetate (15 μCi/L) to compare uptake patterns. Finally, organisms in experiment 5 were exposed to 7.0 μCi/L of TLC-cleaned TFA (further discussion below). Except for experiment 1, samples were split to provide two replicates for each exposure type.

Because some radiolabel was found in biomolecule fractions of killed-cell controls, we tested the effectiveness of formalin treatment (final concentration 2%, held for 10−15 min prior to addition of TFA) with formalin-killed cells exposed to radiolabeled acetate. Cells grown in 0.1% TYE broth for 2 days were either autoclaved, fixed with formalin (2% final concentration), or left untreated ("live"), and then subsamples were exposed to [^{14}C]acetate. The original flasks were held for an additional 2 days to mimic the protocol used in the [^{14}C]TFA exposure experiments, after which subsamples were exposed to [^{14}C]acetate. Eight samples from each flask were transferred to shell vials: three were formalin fixed as a check on the prior treatment, the remaining five were untreated, and all were inoculated with 0.1 μCi of 2-[^{14}C]-acetate (sp. act. 55 mCi/mmol, New England Nuclear, Boston MA). After incubation for 2 h, samples were killed with 2 N H_2SO_4 and shaken on ice for 2 h. Cells were recovered by filtration onto 0.2-μm pore size membrane filters. Radioactivity recovered in biomass was determined by liquid scintillation counting.

Incorporation of [^{14}C]TFA by Macroorganisms. Biological communities were exposed to [^{14}C]TFA in two 3-L microcosms (0.5 m long, 10 cm diameter, Plexiglas cylinders with a 0.15 m long section cut from the top) equipped with submersible pumps (Teel Model 597, Grainger, Chicago, IL). Sediments from a TFA-exposed flow-through mesocosm and elmid beetle larvae collected from WCC were added to the microcosms along with 2 L of WCC stream water. At least 100 elmid larvae were added to each microcosm. Existing oligochaete densities in the sediment were sufficient for sampling purposes. Growths of *Lemna* sp. and *Callitriche* sp. developed naturally, and when an *Impatiens capensis* (jewelweed) plant grew in one system, we added a plant to the second. [^{14}C]TFA was added to the water in each system to provide a concentration of 40 μg/L (microcosms 3 and 4; 19 μCi/L). Two other microcosms were maintained as unamended controls (microcosms 1 and 2). The approximate maximum depths of sediment and water were 3.0 and 0.5 cm, respectively. [^{14}C]TFA exposures and mesocosm water volume were checked three times weekly. Water volume was brought to 2 L when needed with WCC stream water. [^{14}C]TFA levels in the water dropped to 80−85% of the added value during the first few days of the experiment (presumably through incorporation into sediment matrixes) and stayed at that level throughout the remainder of the experiment. Organisms were sampled for radioactive content after 34 days of exposure.

Uptake and Depuration of [^{14}C]TFA by Oligochaetes. A followup experiment was performed with oligochaetes collected from the microcosms approximately 3 months later. Worms were collected, gently washed, and transferred to trays of nonradioactive sediment to measure depuration over the next 2 days. Worms (three per sampling) were removed, washed, dried, and analyzed for radioactive content. As a control, animals not previously exposed were placed in [^{14}C]-TFA-contaminated sediments for 24 h to allow for external contamination and ingestion of sediment but minimal biotransformation of label by the worms. After this exposure, they too were transferred to unlabeled sediments and sampled for 96 h.

Isolation of Biomolecule Fractions. Cultured microorganisms and plant and animal samples were frozen immediately upon sampling and stored in an ultrafreezer at −80 °C until extraction of samples for four biomolecule fractions: (a) low molecular weight intermediates; (b) lipids; (c) proteins; and (d) residual materials, which included cell wall constituents and other insoluble components (methods after refs 21−23). Samples were homogenized and extracted with cold 10% (w/w) trichloroacetic acid. The solution was centrifuged and the supernatant reserved for determination of radioactivity recovered as low molecular weight compounds, which may have included unaltered TFA. The pellet was then extracted with a 1:2 chloroform:methanol solution to extract lipids. After centrifugation and filtration (necessary due to the buoyancy of some materials), the supernatant was concentrated by evaporation of the solvent under a stream of dry nitrogen gas and stored for analysis of ^{14}C-label recovered in lipids. The residual material was then extracted with hot 0.3 N NaOH to solubilize proteins. After acidification, the solution was centrifuged, the supernatant was reserved for protein analysis, and the pellet was analyzed for radioactivity in cell walls and other structural materials.

The potential for incorporation of unaltered TFA into the various biomolecule fractions through simple sorption rather than transformation was tested by spiking control oligochaete and jewelweed tissues with [^{14}C]TFA just prior to homogenization and extraction.

Radioactivity associated with each fraction was determined using liquid scintillation counting. Aliquots of the extract fraction were transferred to a cocktail appropriate for the solvent used for extraction (CytoScint for organic solvents and Ecolite for aqueous solutions, ICN, Irvine, CA), and samples were counted using a Beckman Model 3800 counter. The counting efficiency varied with extraction fluid, and a quench curve or empirical checks were used to determine DPM in each sample. Solvent blanks were used for background determinations. Radioactivity in the residual pellet was determined following combustion of the material in a sample oxidizer (Model 307, Packard Instruments, Naperville, IL).

Purity of the [^{14}C]TFA. The radiolabeled TFA used for experiments was listed as 99.6% pure by the manufacturer (Amersham, Arlington Heights, IL), as tested by high-pressure liquid chromatography (HPLC) with detection by absorbance

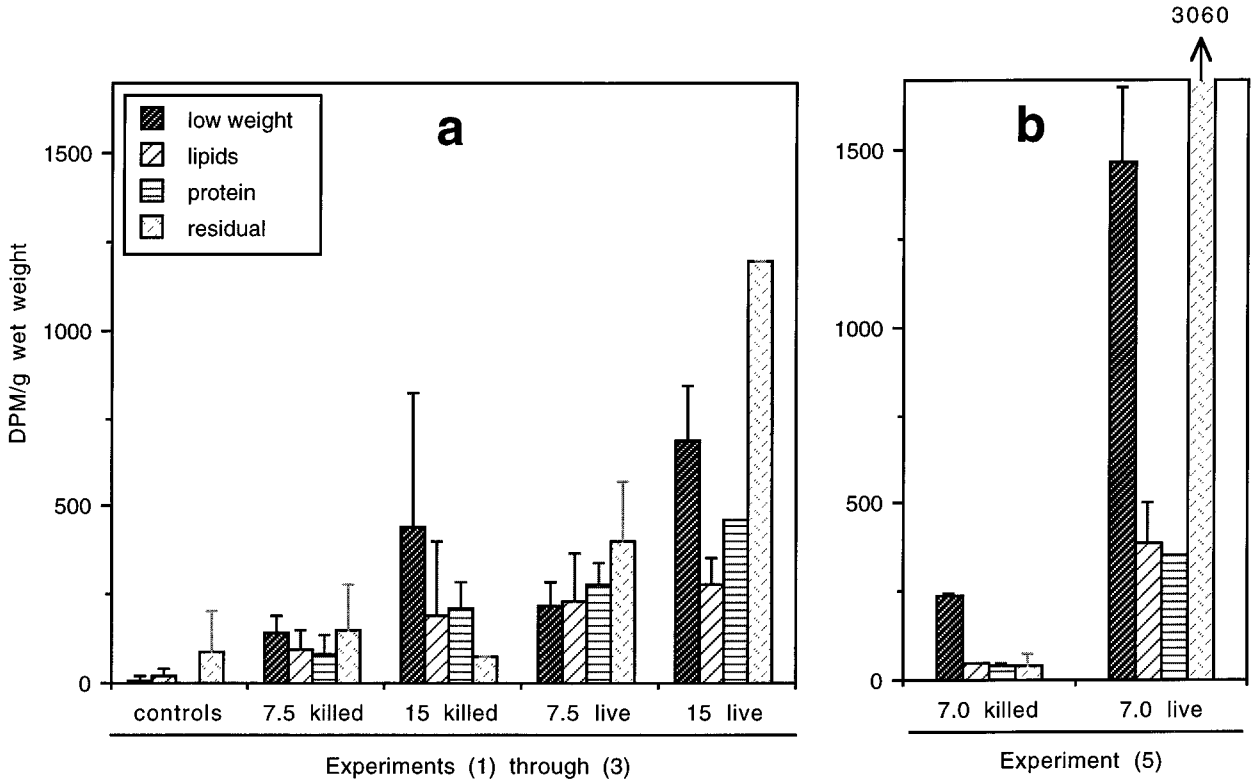

FIGURE 1. DPM/g tissue (wet weight) of radiolabel in biomolecule fractions of microbial communities grown in the presence of [^{14}C]TFA (live), communities exposed to [^{14}C]TFA after being fixed with formalin (killed), and control communities grown without [^{14}C]TFA: (a) communities sampled for experiments 1−3 and exposed to 7.5 and 15 μCi/L; (b) communities collected for experiment 5 and exposed to 7.0 μCi/L TLC-cleaned TFA. Error bars are standard deviations.

at 205 nm. We checked its purity by thin-layer chromatography (TLC) using silica gel (Baker) plates and the following mobile phases: (a) 10:1 hexane:acetone; (b) 1:1 hexane: acetone; and (c) 0.01 N HCl in methanol. Of 10 bands collected, most of the radioactivity was found in two bands: one at or near the origin for all three mobile phases and a second that moved just behind the solvent front when exposed to the first two mobile phases. A "TLC-clean" TFA standard was collected from the narrow band near the origin, and the region including and surrounding the "contaminant-(s)" band was collected to provide an extract of the isolated contaminant(s) (representing ~3% of the total radioactivity). We do not know the identity of the contaminant(s) at this time but suspect that byproduct(s) of TFA synthesis may be present and include a component such as a fluorinated ethanol due to its greater mobility than TFA in nonpolar mobile phases and the fact that it was not detected in the HPLC scan at 205 nm, a region where saturated species do not strongly absorb light. Unless otherwise noted, references to [^{14}C]TFA include this low level contaminant(s). Because uptake of the label was minimal, we considered it important to be certain that the contaminant was not the component being incorporated.

Sediments that were collected from the TFA mesocosm stream after exposure to nonradiolabeled TFA for ~900 d and frozen for 6 months were thawed and allowed to re-equilibrate with 40 μg of TFA/L for 1 week in a microcosm (experiment 5 described above). A few sand grains of this sediment were added to TYE broth in flasks containing either TLC-cleaned [^{14}C]TFA or contaminant(s) to determine whether the contaminant(s) contributed to results of incorporation into microbial biomolecule fractions. Microbial communities were exposed live or after being fixed with formalin. While uptake of contaminant(s) accounted for almost 40% of the total label incorporation by killed-cell controls, it accounted for less than 4% of total label incorporation by live cells. Thus, data presented in this paper were not corrected for the contaminant(s) incorporation. However, any correction would have accentuated the differences between live and killed samples and strengthened the conclusions regarding transformation of TFA.

Results and Discussion

Aquatic Bacterial Incorporation of [^{14}C]TFA. Microbial communities were collected periodically from mesocosms receiving long-term (2.5 yr) experimental exposure to 30 μg/L nonradiolabeled TFA for use in experiments. There was a trend toward increasing incorporation of label over the study period. Organisms in experiments 2 and 3 (averaged), 4, and 5 took up 1.7, 6.4, and 8.1 times the label as those in experiment 1, respectively. These results suggested either a slow increase in the ability of the cultured microbial community to metabolize TFA or a shift in population toward species with a greater ability to incorporate TFA.

Microbes collected for experiments 1−3 contained a total of 1100 DPM (5.0×10^{-4} μCi) or 2600 DPM (12×10^{-4} μCi) per gram of tissue after exposure to 7.5 or 15 μCi [^{14}C]TFA, respectively (sum of data for fractions of microbial biomass labeled 7.5 or 15 "live" in Figure 1a). This corresponded to an incorporation of label (as TFA) of 1.1 and 2.5 ng/g (wet weight) of microbial biomass from solutions containing 10 and 20 μg/L TFA, respectively. Microbes collected in 1995 (experiment 5, Figure 1b) incorporated approximately 4-fold more.

Control organisms grown in experiments 1−3 without radiolabeled TFA contained 120 DPM/g (after background correction). We are not certain of the source of this label, but it is low as compared to the radiolabel in exposed cells. Killed-cell controls (designed to measure abiotic binding of

TFA) for the same experiments contained 460 and 920 DPM/g for cells exposed to 7.5 and 15 μCi/L, respectively (labeled 7.5 and 15 "killed" in Figure 1a). Killed-cell controls from experiment 5 contained 360 DPM/g after exposure to 7.0 μCi/L of TLC-cleaned TFA (7.0 "killed" in Figure 1b). Total ^{14}C-label content was significantly elevated in cells exposed live to [^{14}C]TFA at each concentration over both the non-[^{14}C]TFA exposed controls and the respective killed-cell controls for all experiments (*t* test, $p < 0.05$). Furthermore, microbes grown in solutions containing 15 μCi contained approximately twice the label of those exposed to 7.5 μCi (experiment 2), demonstrating uptake of substrate in relation to exposure concentration. However, killed-cell controls contained significantly more total label than non-[^{14}C]TFA exposed controls (*t* test, $p < 0.05$).

Radiolabel content of experiments 1−3 live cells was significantly elevated over non-[^{14}C]TFA exposed controls in all four biomolecule fractions (Figure 1a, *t* tests, $p < 0.05$). Lipid, protein, and residual fractions of live cells contained elevated levels of radiolabel over killed-cell controls, but the levels were statistically significant only for lipid and protein fractions at the 7.5 μCi/L concentration and for the residual fraction at both concentrations (*t* tests, $p < 0.05$). Radiolabel (TLC-cleaned TFA) incorporation into biomolecule fractions of experiment 5 live cells was several times higher than incorporation of TFA by cells in earlier experiments (Figure 1b vs Figure 1a) and was significantly higher than the amount of label associated with killed-cell controls for all four biomolecule fractions. The greatest increases occurred in low weight and residual fractions (7- and 8-fold, respectively). As mentioned previously, this increase in label incorporation was probably due either to a shift in microbial community composition or their ability to incorporate TFA. Except for a slight increase in the low molecular weight fraction of experiment 5 killed cells (1.8-fold, *t* test, $p < 0.05$), association of label with biomolecule fractions of killed cells did not change significantly over the 2-year period (*t* test, $p > 0.05$). There was 14-fold greater incorporation of TLC-cleaned TFA in live cells than that associated with killed cells, and the patterns of incorporation were distinct (Figure 1). However, the distribution pattern of TFA-associated contaminant(s) in biomolecule fractions for live and killed cells was the same, suggesting that the contaminant(s) became cell-associated by abiotic processes (data not shown).

Live microbial cells in experiments 1−3 and 5 accumulated most of the radiolabel in the residual fraction, which included cell wall material. Conversely, most of the label in killed-cell controls was present in the low weight fraction, which may represent unaltered TFA accumulated by the microbes (Figure 1). Uptake into live cells was concentration dependent for all fractions except lipids (Figure 1a).

Association of radiolabeled TFA with formalin-fixed cells could be caused by several processes. The possibility that a portion of the bacteria survived treatment with formalin was ruled out by exposing formalin-killed and autoclaved cells to radiolabeled acetate, which is readily utilized. Radioactivity associated with biomass was only 1.8% and 2.0% for formalin and autoclaved cells, respectively. The level of [^{14}C]acetate associated with formalin fixed samples and those receiving additional formalin during the isotope exposure did not differ, confirming that the original treatment was sufficient to kill the cells. Thus, we concluded that the association of radiolabeled TFA with killed-cell controls also occurred through abiotic processes.

TFA from culture medium would not contribute appreciable radiolabel to that associated with killed cells. Because microbes were rinsed prior to extraction, they contained less than ~0.5 mL of the rinse solution during homogenization. Co-extraction of 0.5 mL of the original culture medium would have only contributed 2, 3, and 5 DPM/g to lipid, protein, and residual fractions, respectively, and any contribution from the rinse solution would be appreciably lower.

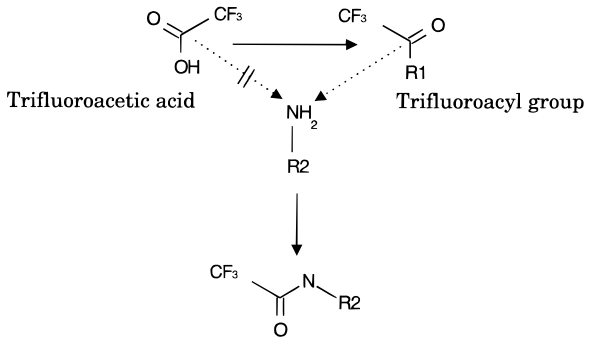

FIGURE 2. Hypothetical transformation pathways required for trifluoroacetylation of amines.

We also considered the possible abiotic binding of unaltered TFA onto proteins and cell wall (residual) materials of dead cells through condensation of the acid moiety to free amines of proteins, lipoproteins, and amino sugars; however, it is probable that the trifluoroacetic acid moiety must be converted to the more reactive trifluoroacetyl moiety prior to reaction due to the much lower reactivity of the carbonyl carbon in the acid, as shown in Figure 2. We attempted to acetylate the free amine on glucosamine (present in microbial cell walls) using trifluoroacetic acid and were unsuccessful (unpublished results). Furthermore, research on acetylation of the lysine component of proteins in rats also demonstrated no direct reaction with trifluoroacetic acid in rats (*10*). Acetylation of free amines in proteins only occurred in the rats after exposure to the more reactive trifluoroacetyl halide metabolites of halothane (*10*).

It is possible that TFA was binding to cellular and sedimentary material through electrostatic attraction processes or hydrogen bonding. In other experiments, acidification with H_2SO_4 slightly enhanced the association of radiolabel with cell material and sediment (data not shown). However, results from an experiment where samples were acidified with trichloroacetic acid to extract low weight compounds demonstrated that radiolabel was only extracted into the low weight fraction (see below). Thus, it is unlikely that weak associations such as hydrogen bonding could explain the presence of radiolabel in biomolecule fractions of killed cells. Association of radiolabel with biomolecule fractions of killed-cell controls requires further study, but binding was nearly always at a significantly lower level than incorporation of label by live cells.

The microbes incorporated a very low percentage of the radiolabel during the 48-h exposure period (~0.01−0.05% of the total label, excluding the low weight fraction due to the possible presence of unaltered TFA). In contrast, organisms exposed to the analogous acetate typically incorporate and respire as much as 75% of a similar concentration of [^{14}C]-acetate within a 2-h time period (Bott, unpublished). Microbes did not bioconcentrate TFA over exposure levels. If we assume that all label in the low weight fraction was attributable to unaltered TFA, a calculated bioconcentration factor (BCF = [μg of TFA/kg of organism] ÷ [μg of TFA/L of solution]) was approximately 0.02, demonstrating a strong impedance to uptake.

We can calculate an uptake rate of label of $2-8 \times 10^{-15}$ μg cell^{-1} d^{-1} (as TFA), which is 50−300-fold slower than microbial uptake rates in other experiments with attached benthic microbial communities (Bott and Standley, unpublished). The difference in uptake rates may be related to factors affecting growth rates and/or the selection of species.

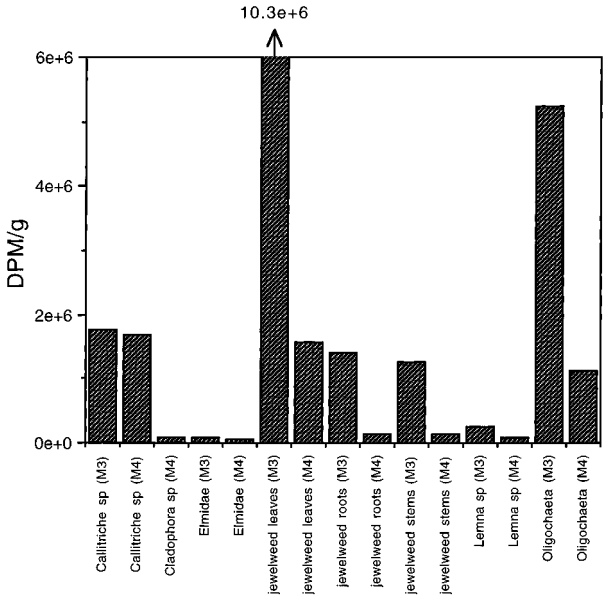

FIGURE 3. Total DPM/g tissue (wet weight) of radiolabel in individual samples of aquatic organisms reared in microcosms containing [^{14}C]TFA (log_{10}). (M3 = microcosm 3, M4 = microcosm 4.)

In this study, microbes were cultured under high nutrient conditions in batch cultures to produce cells for extraction.

Incorporation of [^{14}C]TFA by Macroorganisms. Incorporation of [^{14}C]TFA by aquatic organisms exposed in microcosms was taxon dependent, with oligochaetes and jewelweed containing the most radiolabel per gram (Figure 3). Thus, uptake appeared to be dependent on specific metabolic or trophic transfer pathways. As was done for microorganisms, we measured the distribution of radiolabel in the biomolecule fractions for organisms that accumulated the most label.

There was greater intermicrocosm variability of total label incorporation for sediment-exposed organisms, such as oligochaetes and jewelweed, than for organisms primarily associated with the water phase, such as *Callitriche* sp., *Lemna* sp., and elmid beetles (Figure 3). The jewelweed and oligochaetes reared in TFA-amended microcosm 3 (M3) had more label associated with them than those reared in TFA-amended microcosm 4 (M4), but the other species contained roughly the same amount of label in samples collected from both microcosms. These relationships paralleled the difference in TFA concentration in fine sediments collected from the two microcosms, with sediments in microcosm 3 containing twice the radiolabel per gram as those in microcosm 4 (7980 vs 3870 DPM/g dry weight, respectively). In a separate experiment (unpublished data), jewelweed plants reared in soils amended with water containing the same concentration of [^{14}C]TFA (20 μCi/L) incorporated comparable levels of label as those reared in the microcosms ($\sim 9 \times 10^6$ DPM/g), and thus uptake appeared to be related to pore water concentration and was independent of soil or sediment composition.

Incorporation of [^{14}C]TFA by Jewelweed. A greater amount of label became associated with jewelweed leaves than roots or stems (Figure 3). Because roots were the only part of the plant in contact with [^{14}C]TFA, label was apparently translocated to other parts of the plant. Thompson (*19*) measured incorporation of radiolabeled TFA by sunflowers, with the greatest accumulation occurring in the leaves. Thompson measured most of the radiolabel in a water extract that cochromatographed with TFA, although he considered his results "not conclusive" (*19*). In our study, most of the radiolabel in jewelweed leaves and roots was present in the protein fraction, with appreciable quantities of label also present in lipids of leaves and the residual fraction of roots (Figure 4). Incorporation of label into lipid fractions of leaves is understandable due to their higher wax content. Greater incorporation of radiolabeled TFA into the protein fraction than in lipids might be explained by the fewer molecular transformations needed to generate a molecular species capable of acetylating free amines in proteins than the number of transformations required to incorporate that

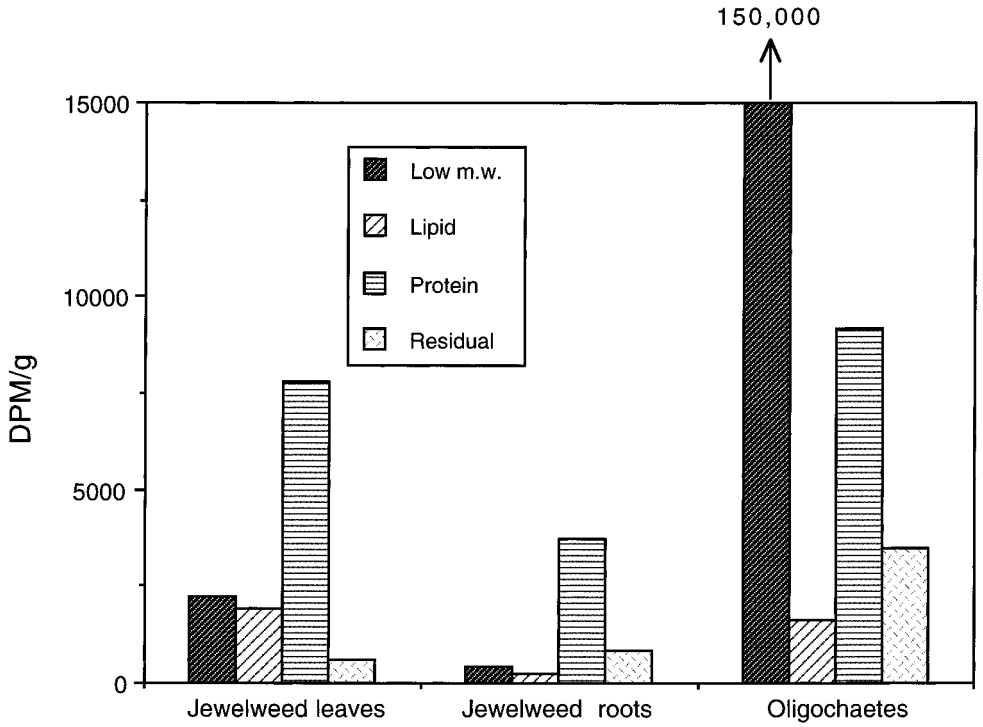

FIGURE 4. DPM/g measured in biomolecule fractions of jewelweed leaves and roots and oligochaetes reared in microcosms containing [^{14}C]TFA.

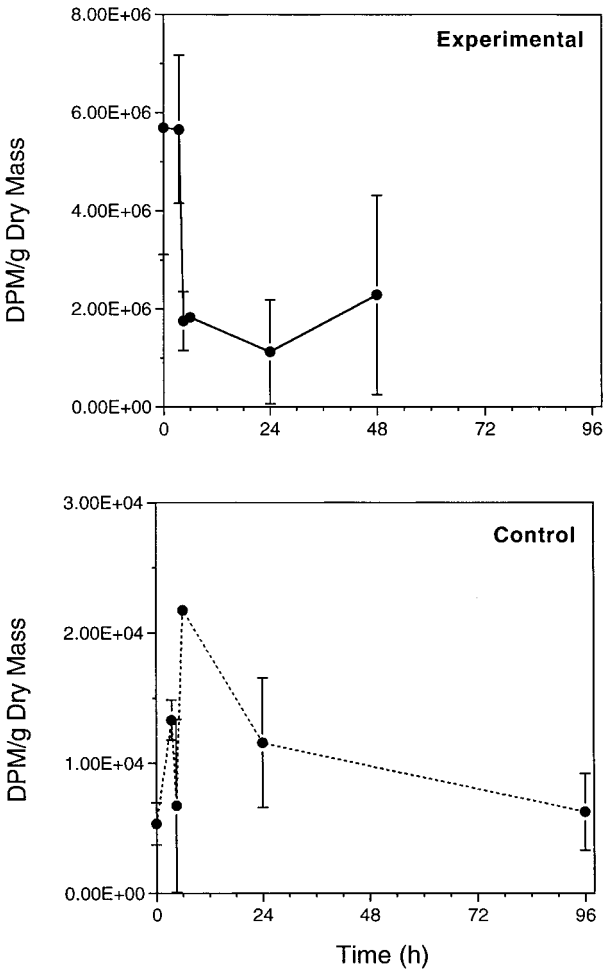

FIGURE 5. Loss of ^{14}C from oligochaetes transferred from [^{14}C]TFA-containing sediments to unamended sediments at t_0 (log_{10}). Error bars are standard deviations.

moiety in the synthesis of a fatty acid. An alternate explanation for the incorporation of label into the lipid fraction, besides actual synthesis of fatty acids incorporating the fluorinated moiety, may be acetylation of the protein component of lipoproteins. Molecular level investigations are required to document the mode of incorporation of TFA into biomolecule fractions.

Total radiolabel composition of subsamples of jewelweed tissues varied substantially (e.g., data in Figure 3 versus that in Figure 4); however, the variation occurred primarily within the low weight fraction where the radiolabel content ranged over several orders of magnitude and unaltered TFA was likely to be present. Radiolabel content in other biomolecule fractions of jewelweed were fairly constant.

Incorporation of [^{14}C]TFA by Oligochaetes. Oligochaetes extracted for determination of the distribution of radioactivity in biomolecules (Figure 4) contained substantially less label than those analyzed whole (Figure 3). However, over 90% of the label was present in the low molecular weight fraction, and as discussed above, radiolabel content of the low weight fraction may be more variable due to the presence of unaltered TFA. Given that oligochaetes burrow through sediments and would be exposed to high external concentrations of label, label associated with the animals could have been associated with sediment in the gut tract or sorbed to the animal integument.

The results of an experiment to differentiate between biomolecular incorporation versus abiotic sorption are shown in Figure 5. Three oligochaetes were sampled at each time (data from only two worms are reported for 48 h in the upper panel due to removal of an outlier). Those having long-term exposure to TFA lost radioactivity rapidly over the first 24 h after which the residual radioactivity was about 25% of the initial level. Control worms, exposed to TFA for only 24 h, contained radiolabel at 2 orders of magnitude lower concentration than oligochaetes exposed to [^{14}C]TFA for 3 months. We concluded from these experiments that oligochaetes can incorporate and transform substantial levels of TFA through their feeding activity and that approximately 25% of the total label associated with the worms (see Figures 3 and 4) is present as products of their metabolism rather than simple external sorption or radiolabel in gut contents.

Control Experiments Testing for the Potential of Experimental Artifacts. Because we do not yet have molecular proof of TFA transformation into biomolecules, additional experiments were carried out to confirm the nature of the processes of incorporation of TFA by microorganisms and higher trophic level aquatic biota.

Incorporation of Unbound [^{14}C]TFA into Biomolecule Fractions. Because label content of tissues was relatively low as compared to the amount of radioactivity present in the experimental solutions and uptake through processes such as partitioning rather than transformation might allow for incorporation of [^{14}C]TFA into certain biomolecule fractions, we carried out an experiment to determine whether appreciable quantities of unaltered [^{14}C]TFA could be co-extracted into the various biomolecule fractions. On average, 99.9% of the label added to oligochaete and jewelweed tissues just prior to extraction was measured in the low weight fraction as expected. Only 0.02, 0.03, and 0.05% of the remainder was extracted into the lipid, protein, and residual fractions, respectively. These proportions were insufficient to explain the extent of label incorporation as described above and thus exclude the possibility that simple bioaccumulation or concentration of unaltered TFA would have accounted for incorporation into biomolecule fractions.

The Potential for Uptake of Radiolabel after Defluorination of [^{14}C]TFA. Recent work by Visscher and co-workers (*24*) reported anaerobic bacterial defluorination of trifluoroacetate to form acetate. Although those results have not been confirmed (*25*) and because analytical detection limits were not low enough to rule out incorporation of acetate produced by defluorination of [^{14}C]TFA, we compared the distribution of label in biomolecule fractions of microbial cells exposed to [^{14}C]acetate to that of cells exposed to [^{14}C]TFA. Uptake of label by a mixed culture of microbes from a TFA mesocosm was compared to that of an isolate (isolate 1) that was also preacclimated to TFA and grew well on potato dextrose agar containing 180 mg of TFA/mL (although not using it as a sole carbon source). The mixed culture of mesocosm 3 organisms contained proportionately more label in protein fractions and less in lipids after exposure to [^{14}C]TFA than similar organisms reared in radiolabeled acetate (t test, $p < 0.05$, Figure 6). Isolate 1 organisms exposed to [^{14}C]TFA, on the other hand, contained significantly less label in proteins but more in the low weight fraction than those exposed to radiolabeled acetate (t test, $p < 0.05$, Figure 6). The significant differences between the distribution of label in tissues of cells utilizing acetate versus those utilizing TFA support the conclusion that the organisms were being exposed to TFA and not the defluorinated byproduct acetate. Utilization of acetate or TFA by isolate 1 was more easily differentiated than that by mesocosm 3 organisms and can be explained by averaging of distributions in the latter data caused by the variety of responses in a mixed population.

Significance and Implications

Selected aquatic organisms spanning several trophic levels incorporated radiolabel into biomolecule classes when

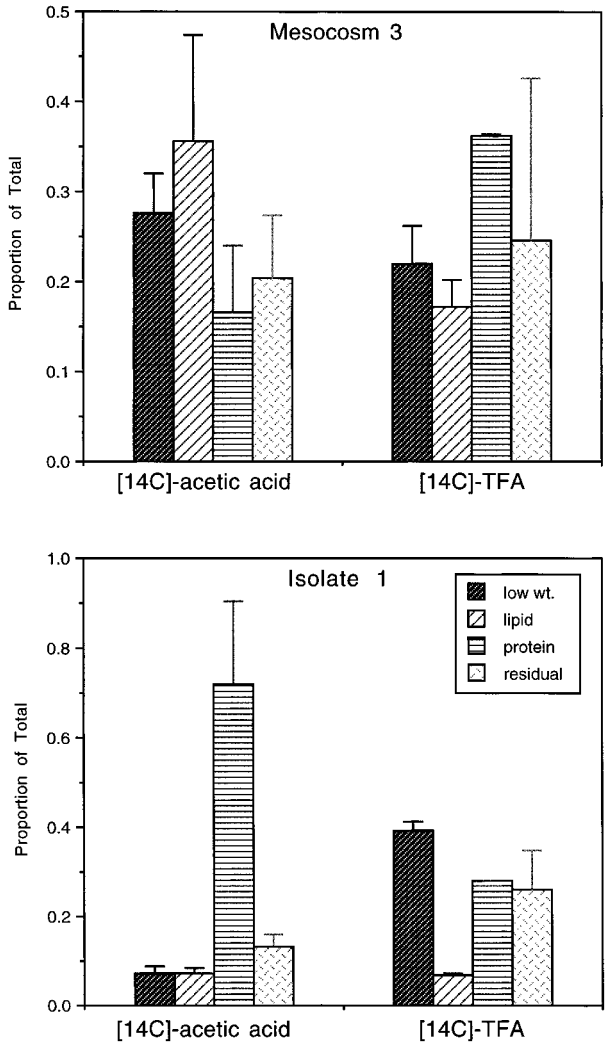

FIGURE 6. Experiment 4: DPM measured in biomolecule fractions of mixed populations of microbes sampled from mesocosm 3 (top) and an isolate (isolate 1, bottom), both of which were exposed to either radiolabeled TFA or acetate. Error bars are standard deviations.

exposed to [^{14}C]TFA. Whereas other manmade contaminants, such as polychlorinated biphenyls, are accumulated into lipid compartments of organisms, uptake of TFA does not appear to occur through simple dissolution into or bioaccumulation by tissues but rather through transformation of the molecule into biomolecules such as lipids, proteins, and cell wall materials. Because the analogous acetyl moiety is utilized to synthesize a variety of compounds ranging from fatty acids to acetylated proteins and the amino sugars present in microbial cell walls, incorporation of the structurally similar trifluoroacetyl moiety into all these classes is not inconceivable. The ability to incorporate the TFA molecule may be dependent on an organism's ability to convert the acid moiety to the more reactive acetyl moiety. An additional route of incorporation could be by uptake of pretransformed substances through trophic transfer.

Overall incorporation of the label was very low, occurring in the low ppb range for microbial communities and low ppm range for oligochaetes and jewelweed. Because our experiments were carried out at TFA concentrations 1−2 orders of magnitude higher than those expected in the aquatic environment, except perhaps for seasonal wetlands, the phenomenon we measured using a radiolabeled tracer would be difficult to monitor in environmental samples exposed to unlabeled TFA. Although we did not find substantial biological effects (Bott and Standley, unpublished), we did determine that the TFA molecule is not entirely inert but rather can be incorporated into the tissues of exposed organisms. Because trifluoroacetylation of proteins has been linked to health effects in humans and rats, incorporation of the TFA moiety into biomolecules must be researched further, with a priority being the molecular identification of fluorinated biomolecules.

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