Persistence of 3-(Trifluoromethyl)-4-nitrophenol in Aquatic Environments

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■ Since 1958 the chemical 3-(trifluoromethyl)-4-nitrophenol (TFM) has been used to control the sea lamprey (*Petromyzon marinus*) in the United States–Canadian Great Lakes: Superior, Michigan, Huron, and Ontario. A study has been conducted to determine the degradability of TFM under laboratory conditions in aqueous (sediment-free) systems. No evidence of microbial degradation of TFM was found over test periods of up to 80 days. It appears that TFM may persist for extended periods of time in the Great Lakes. On the basis of past and present levels of usage of TFM, it is estimated that the concentration of TFM in Lake Superior water could approach, if TFM were a completely conservative chemical, 0.015 $\mu g/L$. This concentration is considerably less than the concentrations which have been found to be acutely and chronically toxic to aquatic life. It is concluded that the use of TFM as a sea lamprey larvicide does not represent a hazard to fish and other nontarget aquatic life in the Great Lakes.

Introduction

In 1958 the chemical 3-(trifluoromethyl)-4-nitrophenol (TFM) (Figure 1) was first used to control the predaceous sea lamprey (*Petromyzon marinus*) in Lake Superior, and this chemical is still used as the predominant means of controlling the lamprey in four of the Great Lakes: Superior, Michigan, Huron and Ontario (1, 2). From 1958 through 1976, 682 400 kg (active ingredient) of TFM has been used by Canada and the United States in these four Great Lakes (3, 4). This history of the development and use of TFM is recounted in a detailed review by Schnick (5).

The introduction of this newly developed pesticide into the environment raised many concerns, and this prompted numerous studies to assess the potential harm of this chemical to aquatic organisms. The majority of the studies centered on TFM uptake by plants and animals and its toxicological implications. Before the work reported in this paper, few studies examined the potential degradation of TFM in the environment.

Bioassay experiments (6) have shown that TFM remains toxic for long periods in aqueous systems; however, toxicity decreases in sediment–water systems. In sediment–water systems, irreversible sorption of TFM to sediments was reported (6–8). Olson and Marking (9) have examined the toxicity of TFM to various life stages of rainbow trout. The LC_{50} ranges from 0.53 to 40 mg/L, depending on the life stage, water hardness, and duration of exposure. They conclude that all of the life stages of rainbow trout tested are safe in minimal lampricidal concentrations of TFM.

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TFM is added to tributaries of the Great Lakes for the purpose of killing the maturing sea lamprey larva, which reside in the bottom sediment of the streams. The amount removed by sorption by the stream sediments is unknown at this time but is considered minimal. The remainder would be carried by the streams to the lakes. Within the lake, it is speculated that only a small part of the TFM residue would be taken up directly by lake sediments. Another part would be expected to be carried to the sediments as a result of sorption on the particulate matter present in the water column. The relative significance of sorption and transport of TFM to the sediments, for example, in the Lake Superior water column is unknown at this time. However, it is more than likely that most of the TFM added to the streams would likely remain in solution in the lake for long periods of time. This would be especially important in the more oligotrophic lakes such as Superior because of the very low particulate-matter concentrations in this lake. Because of this situation, it was decided that a study should be undertaken to determine the rate of biodegradation of TFM under conditions which would stimulate the behavior of TFM in the water column. This paper presents the results of a study designed to evaluate the degradation of TFM where aquatic sediments are not an influential factor.

Experimental Section

The biodegradability of TFM was determined by inoculating TFM solutions with microorganisms and analyzing the amount of TFM and possible degradation products remaining after various time intervals. In experiments where the uptake of dissolved oxygen (DO) was followed, the biochemical oxygen demand (BOD) procedure was employed (10). TFM residues were recovered from water by using 1:1 (v:v) benzene/hexane (11). TFM residue concentrations were determined by absorbance measurements of the TFM anion at a wavelength of 395 nm (12) and confirmed by gas–liquid-chromatographic analysis (GLC) (13).

Before determination of the A_{395} values, the samples were either centrifuged or filtered, to remove cellular material, but this procedure had no effect on the optical-density measurement. A check was made of some of the solutions for TFM degradation products by using a thin-layer-chromatographic (TLC) procedure developed by Lech (14).

GLC analysis of TFM was performed on a Varian Aerograph Model 1500 B equipped with a tritium foil detector. The usual operating conditions were as follows: column-temperature, 150 °C; injection-block temperature, 180 °C; detector temperature, 190 °C; column, 4 ft × 0.125 in. i.d. glass column packed with OV-101 (2%) and QF 1 (3%) on 100–200-mesh gas chrom Q; carrier gas (N_2) flow rate, 30 mL/min. The TFM molecule was derivatized with *tert*-butyldimethyldichlo-

$O^- \quad Na^+, H^+$
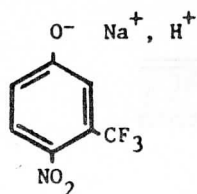
3-Trifluoromethyl-4-nitrophenol (TFM)

Appearance	rusty brown, crystalline powder
M.W.	207.13 (phenol form)
Melting Point	75–76°C
Solubility	5.0 g/l (24.5°C) as Na salt

Figure 1. Structure of TFM.

expt	Initial concn of TFM, mg/L	seed source [a]	variable of expt
1A	0.5	activated sludge	addition of 1.5 mg of glucose–glutamate
1B	5		
1C	10		
1D	50		
1E	0		
2	11	Lake Mendota water	2.5 g of glycerol added at day 21
3	11	raw sewage	2.5 g of glycerol added at day 17
4	11	activated sludge	2.5 g of glycerol added at day 17
5	5.2	raw sewage	
6	5.2	activated sludge	
7	5.2	Lake Mendota water	
8	5.2	TFM adapted raw sewage	
9	5.2	TFM adapted activated sludge	
10	5.2	TFM adapted Lake Mendota water	
11A	5.2	Lake Mendota water [b]	0.7 g of taconite tailings added per BOD bottle
11B	0.0		
11C	5.2		
11D	0.0		
12A	5.1	Lake Mendota [c] "deep hole" sediment and lake water	0.1 g of taconite tailings added per BOD bottle
12B	0.0		
12C	5.1		
12D	0.0		
13A	4.8	Ford River, Cedar River	
13B	0.9		

[a] 2 mL of seed source added per bottle. [b] Aerated with laboratory air 10 h before use. [c] Aerated with laboratory air 96 h before use.

rosilane/imidazole. R_T for the derivatized TFM was usually 2–3 min, and injection amounts ranged from 0.1 to 5 ng.

The possible formation of fluoride ion as a degradation product was followed by using a fluoride specific ion electrode (15). The possible degradation product, CO_2, was determined by using ring-labeled $[^{14}C]$TFM. The evolution of $[^{14}C]CO_2$ was determined by analyzing the radioactivity remaining in the reactor following N_2 gas stripping. Radioactivity was counted by using a Packard-Tricarb liquid scintillation counter.

Ring-labeled $[^{14}C]$TFM (activity, 3.66 mCi/mmol; 99% purity; Mallincrodt Chemical Works, St. Louis, MO) was obtained from the U.S. Fish and Wildlife Service through J. Lech, Marquette University, Milwaukee, WI. "Analytical-grade" TFM used in all experiments was in the acid form and was determined to be of 95% purity by using GLC techniques.

Nine of the experiments in this study were designed to test the availability of TFM as a sole C source for microorganisms in aqueous solutions containing dissolved O_2. Two experiments were designed to test the availability of TFM as a C source under anaerobic conditions. Also, three nutrient-enrichment-type studies were conducted. Table I describes the BOD-based experiments.

BOD Experiments. The biochemical oxygen demand (BOD) test was used for determining whether TFM was utilized by microorganisms as a sole C source. The microorganisms ("seed") were obtained from the Nine-Springs Domestic Wastewater Treatment Plant in Madison, WI, from Lake Mendota (Madison) water and sediment, and from water and sediment procured on sampling trips in waters previously treated with TFM (Ford and Cedar Rivers, Michigan), and they were a natural mixed flora. No effort was made to isolate and identify any microorganisms involved. Results of the experiments did indicate that microorganisms were present in the seed and that they were viable, and the assumption was that bacteria were primarily responsible for the observed activity. "Sediment" used for seed was the interstitial water of the sediment obtained by centrifuging the sediment. Additional information on procedures can be obtained from Thingvold (16).

To follow the uptake of DO, we used the biochemical oxygen demand procedure as described in APHA et al. (17) using seed water (source of microbe) and buffers as prescribed. The general procedure followed was to place a solution of TFM in a series of standard BOD incubation bottles along with sufficient non-C nutrients (seed water) and microorganisms (seed). Bottles were examined periodically for remaining O_2. Controls consisted of solutions with no TFM. At the end of the experiment, the following analyses were performed: F^-, pH, specific conductance, and A_{395}.

Three of the experiments test the degradability of TFM over time by using raw sewage, activated sludge, and Lake Mendota water as a source of seed. Three other experiments were conducted by using seed that had been exposed to relatively small amounts of TFM over a period of time in an effort to acclimate the microorganisms to TFM. The acclimation procedure used in this study is similar to that used by Pfeil and Lee (18), where the seed is exposed to the test (TFM) compound in a two-stage method. A 3-week period was used in an effort to adapt the microorganisms to TFM, and the amount of TFM exposed to the microorganisms was in the μg/L range.

Two of the experiments were designed to test whether additional surface area in the system would enhance the rate of degradation. The material used for this purpose was taconite tailings (processed iron ore obtained from Reserve Mining Company, Silver Bay, MN) with a size of ~2 μm. These tailings consist predominantly of iron, magnesium, silicates, and quartz. One set contained 0.7 g of taconite tailings per BOD bottle and Lake Mendota water for seed. The second set contained 0.14 g of taconite tailings per bottle and interstitial water from Lake Mendota sediment ("deep hole") used as seed. This latter experiment also served to assess the effect of using seed derived from lake sediment interstitial water.

Samples of water and sediment were collected from the Ford and Cedar Rivers. These river waters had been treated

with TFM in May 1971; the Ford River was treated with TFM in August 1972. A composite sample from the above sources, collected in October 1972, was used for seed.

Since some nitrophenols are known to have an impact on the respiration of miroorganisms, a study was conducted to determine the concentration level of TFM that would be inhibitory in microorganism respiration. The standard glucose–glutamate solution used to check the viability of seed in the BOD test (17) was used for this purpose. Concentrations of TFM used in this experiment were 0, 0.5, 5, 10, and 50 mg/L. The theoretical BOD of glucose–glutamate solution was 221 ± 13 mg/L. Concentrations of TFM at 10 and 50 mg/L had an inhibitory effect on the BOD of the glucose–glutamate solution. Therefore, the concentrations of TFM, used in this study in all but two experiments, were ~5 mg/L.

In order to check for the possibility of a longer-term effect of TFM on the seed microorganisms, an experiment was conducted in which the seed from three different sources, i.e., raw sewage, activated sludge, and Lake Mendota water, were exposed to TFM at 11.5 mg/L for a period of 17 or 21 days. At those times, glycerol (2 mg, 99% strength) was added to the BOD flasks. The results of this experiment show that there was very limited utilization of oxygen prior to the addition of glycerol (Figure 2). Shortly after glycerol addition, the dissolved-oxygen concentration dropped to zero in the flasks, indicating that the microorganisms from each of the sources of seed in each of the BOD flasks were viable even after prolonged exposure to TFM.

Nutrient-Enrichment Studies. If a compound is not directly available for microbial degradation, biotransformation

of the compound may still occur because of cometabolism (19, 20). Microbial activity caused by an easily metabolizable substance may result in the production of extracellular enzymes capable of transforming a persistent pesticide. In the following series of experiments, various nutrients that are easily metabolized by bacteria were added to TFM solutions. All solutions contained appropriate non-C nutrients and buffers identical with those of the BOD experiments.

The auxiliary C sources added in the three experiments were sucrose, glucose–glutamate, and sodium benzoate. The first two experiments with the added carbohydrates are more properly described as nutrient-enrichment studies, where the function of the C compond is to increase the bacterial population in the system. Concomitant with the increase in bacterial numbers is the increase in filial generation of bacteria which increases the possibility of producing a substrain of bacteria capable of degrading TFM. In contrast, a third experiment contained a compound similar to TFM, the function of which was to stimulate enzymes that may incidentally degrade TFM. All three experiments were run in duplicate under both aerobic and anaerobic conditions by bubbling filtered laboratory air or O_2-free N_2 gas. A second O_2-free control was used which contained TFM with no added C nutrient and was placed in a series of BOD bottles.

The degradation of TFM in the presence of a 100 mg/L sucrose solution was conducted by placing 10 L of a ~5 mg/L TFM solution in 20-L carboys and seeding it with 30 mL of Ford River water and sediment extract. The solution was kept in the dark and the experiments were run from 57 to 59 days.

In a similar experiment a glucose–glutamate mixture of 150 mg/L each was added to a 10 mg/L TFM solution prepared in Lake Mendota water. This water served as the source of seed for this study. [14C]TFM was added to these solutions to determine the efficiency of the liquid–liquid extraction procedure used to extract TFM for GLC analysis and of use in the analysis for TFM degradation products by TLC. All solutions were kept in the dark. The experiment was run for 38 days. GLC, TLC, and UV and visible spectrometry were used to determine any modification of the TFM molecule in both experiments.

The biodegradation (cometabolism) experiment, in which sodium benzoate was added, was conducted in 1000-mL Erlenmeyer flasks. Each flask was spiked with [14C]TFM for evaluating the extraction efficiency of TFM into an organic solvent, and also for the purpose of analyzing for TFM degradation products on TLC. All flasks were excluded from light and were maintained aerobic by aerating with laboratory air.

Three of the flasks contained a 10 mg/L solution of TFM using Lake Mendota water as the aqueous medium and as a source of seed. Before being used, the lake water was filtered through Whatman No. 2 filter paper to remove phyto- and zooplankton. Two flasks contained 10 mg/L TFM plus 25 mg/L sodium benzoate, two flasks contained 25 mg/L sodium benzoate, and two flasks contained 10 mg/L TFM in distilled water as a control for use as a standard for GLC analysis. The solutions were analyzed on day 0, 6, 13, 16, 26, and 29 of the experiment by GLC, TLC, and UV and visible spectroscopy to determine any modification of the TFM molecule.

Results

Figure 3 presents the results of the biodegradation studies presented in Table I. On the basis of DO utilization, F− release, visible and UV spectroscopy, and GLC analysis, no evidence was found to indicate that the potentially acclimated seed was capable of biodegrading TFM. A comparison of these results was made with those obtained by using nonacclimated seed organism in an analogous test. Overall, more DO was
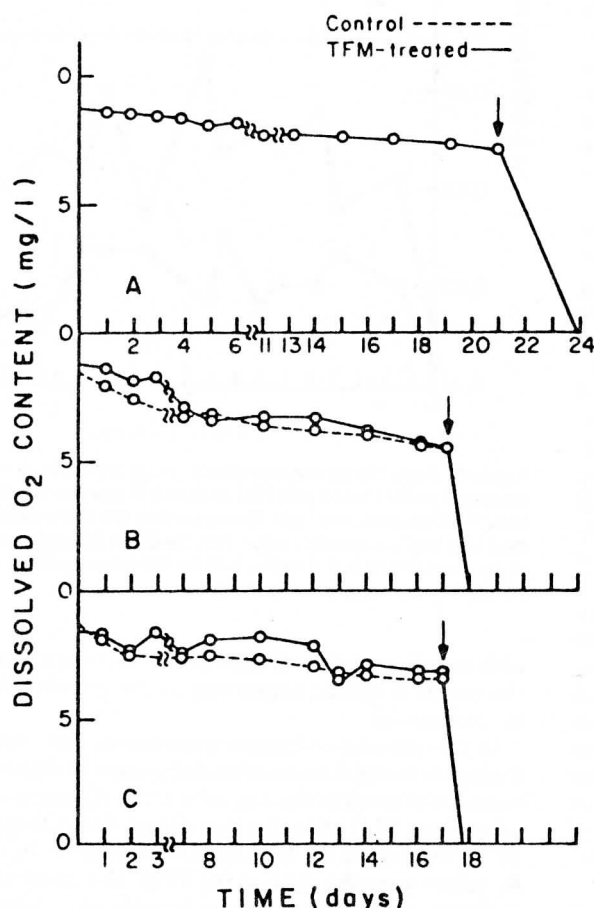
Figure 2. Effect of TFM on viability of microorganisms. Seed sources are (A) Lake Mendota water, (B) raw sewage, and (C) activated sludge. Glycerol (8280 mg/L) was added at the times indicated by the arrows.
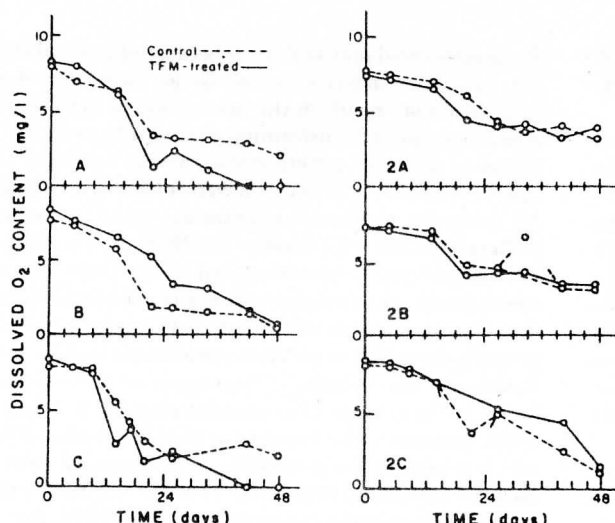
Figure 3. Effect of different types of seed sucrose on the dissolved-oxygen content of water treated with TFM (5.2 mg/L). Seed sources A–C were not previously exposed to TFM; sources 2A–2C were. Seed source for A and 2A was raw sewage, for B and 2B was activated sludge, and for C and 2C was Lake Mendota water.
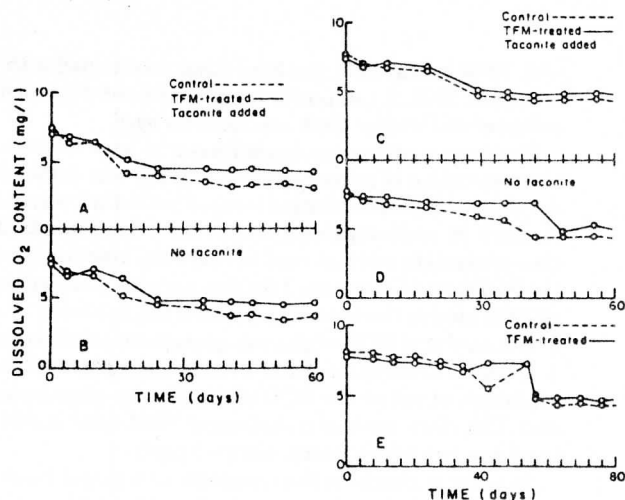

Figure 4. Effect of mixed-seed sources on the oxygen content of water treated with TFM (5.2 mg/L): (A and B) Lake Mendota water and taconite tailings; (C and D) Lake Mendota "deep-hole" sediment extract and taconite tailings; (E) Ford River, Cedar River, and Lake Michigan water.

depleted in the systems where nonacclimated seed was used, presumably because of the oxidation of organic material associated with the inoculum. Since the acclimated seed inoculum had been subject to aeration for 26 days before use, it was assumed that much of the organic material associated with the inoculum was oxidized before use in the tests.

The importance of measuring DO depletion in these systems is based on the premise that the hydroxylation of the aromatic ring is a necessary precursor step to the biodegradation of TFM. If TFM was degraded, based on F^- release and visible and UV analysis, and if this degradation was preceded by obvious O_2 consumption in the system, then it could be reasonable to conclude that TFM followed a degradation pathway similar to that of other aromatic compounds (21–24).

In the related series of experiments, microorganisms obtained from lake Mendota sediment extract and a mixed-seed source using water obtained from tributary streams previously treated with TFM also were unable to use TFM as a C source (Figure 4). Taconite tailings added to increase the total surface area within the bottle did not affect the results of the tests.

Bothwell et al. (8) reported that TFM was reduced to form the amino compound (R–TFM) in an anoxic, biologically active system. Neither the C-nutrient-enriched, anoxic systems nor systems using only TFM as a sole C source in this study showed this phenomenon. Figure 5, which shows the change in A_{395} values with time for a non-C-amended system, indicates that no loss of TFM occurred from the system, nor was TFM reduced by R–TFM. The system initially contained a small quantity of DO (1.2 mg/L) which was depleted within 7 days. Concomitant with the loss of DO in the system was the appearance of cloudiness (turbidity) in the TFM-containing flasks and the control, indicating the presence of increased numbers of microorganisms. Concomitant with the depletion of DO, the A_{395} value increased dramatically. This increase in absorbance subsided within 2 days to levels similar to those measured at the beginnning of the experiment. Visible and UV spectrometry and GLC analysis failed to reveal any alteration of the TFM molecule including the formation of R–TFM.

The addition of sucrose to seeded TFM solutions stimulated intensive microbial activity. Over the approximate 60-day period of the test, the average A_{395} values increased slightly,
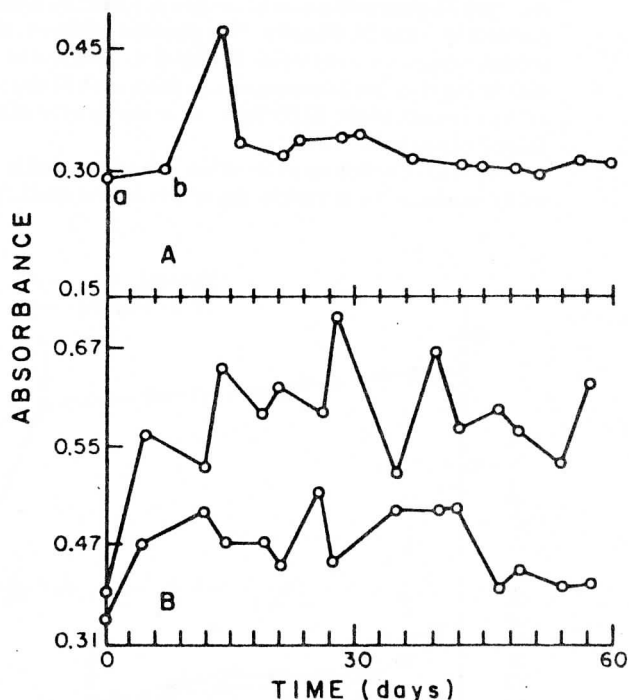
Figure 5. Absorbance measurements (A_{395}) with time. A represents anaerobic water treated with TFM and seed. B represents water treated with TFM, sucrose, and seed. The upper line in B is aerated water and the lower line is anaerobic water. In A the DO in the system at the start of the experiment is 1.2 mg/L; in B the DO in the system 7 days after the start of the experiment is 0 mg/L.

with wide fluctuations (Figure 5B). The cyclic appearance of the curves is related apparently to the growth cycle of the microorganism.

In the glucose–glutamate experiment, the results were analogous to the sucrose-amended system in that some fluctuation was noted in the A_{395} values. The R_f values of extracts taken from TLC plates for this system were the same as for TFM. Extracts of the TLC spots were analyzed by GLC, and R_t values were the same as for TFM. In this particular experiment, each extraction phase (organic and aqueous) of the several washings was counted for ^{14}C activity. No ^{14}C activity in excess of that found normally in the discarded phases was

measured. This supported the contention that the TFM molecule remained intact in these systems throughout the test period.

Cometabolism

Within 6 days, in the cometabolism test, the sodium benzoate was completely depleted from the system, as evidenced by the loss of the sorption band at 233 nm in the UV spectrum (Figure 6). Coincident with the depletion of the sodium benzoate, the sorption band for TFM in the UV shifted to a longer wavelength (as measured at pH 8.5) or disappeared (as measured at pH 3) (Figure 6). Also, a slight decrease in the molar absorptivity (A_{395}) occurred at this time. Within 24 h the UV and visible spectra for TFM returned to normal. GLC and TLC analysis at the time of the UV shift in the TFM spectra offered no clues to explain this phenomenon.

Discussion

TFM possesses all of the chemial features shown to impart persistence to organic compounds. Aromatic compounds are, in general, more resistant to biodegradation than nonaromatic compounds (25); metasubstituted phenols are more resistant than parasubstituted phenols (20, 26–28); and in particular, fluoro compounds are more resistant to degradation than chloro- or bromo-substituted compounds (19, 24). Also, it has been determined that oxygen-deficient aromatic ring structures must be hydroxylated (at least two OH groups per ring) to become potentially biodegradable (21, 22, 24).

Biodegradation of TFM, either directly or indirectly (cometabolism), in water systems may depend on the capability of microorgansms to oxygenate (hydroxylate) the TFM molecule. If can be speculated that oxygenation of the molecule may be limited because TFM is a highly polar compound possessing a high degree of resonant, electron localization which prevents the chemical from penetrating the bacterial cell wall. In the experiment where a sucrose amendment was used to stimulate microbial activity, no evidence was found that TFM was incorporated into the cell structure.

The sodium benzoate experiment was a system designed to degrade TFM through a cometabolic process. Enzymes involved in the degradation of a compound structurally similar to TFM might possibly degrade TFM as an incidental process. In no instance was evidence found that indicated TFM degrades either in the presence or absence of auxiliary C compounds or under aerobic or anaerobic conditions in sediment-free aqueous systems.

Extracellular oxygenation of TFM also may be inhibited by the high degree of electron localization. If metabolic processes are, in part, electrophilic (29), where resonant electrons of the benzene ring must be available for electrophilic substitution, the high electron-attracting character of the $-CF_3$ group may make the ring electrons in the vicinity of the $-CF_3$ group unavailable for reaction.

No F^- release was found in the TFM-treated systems, indicating that the C–F bonds were not broken. The existence of naturally occurring C–F bonds, along with consequent C–F lysing enzymes, in the environment are rare. Also, substitutes are generally left intact on the ring if hydroxylation takes place unimpeded, and they are displaced only after ring breakage has occurred (20, 29). It is more likely that C–C bonds of the ring would be preferentially cleaved to C–F bonds.

Subsequent to completion of this study, Bothwell et al. (8) reported on some studies which they had conducted on the degradation of TFM in natural water sediments. They found no degradation of TFM during a period of 2.5 months under aerobic conditions. They found some sorption of TFM to sediment material which was dependent on the sediment source.
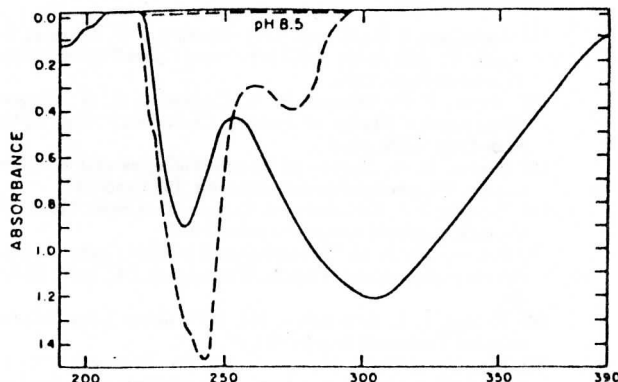
Figure 6. UV spectra showing the temporary disappearance of the sorption band of TFM at 233 nm, coincident with the degradation of sodium benzoate in the system containing both compounds, at pH 8.5 and 3.0.

All of the TFM used to date has been applied to streams tributary to Lakes Huron, Michigan, Superior, and Ontario. Within a short time of its application as a lampricide, some of the chemical is flushed into the lakes. It is in this environment that part of the TFM added to the tributaries must degrade. In general, it would be suspected that sediments in the Great Lakes, especially Lakes Superior, Michigan, and Ontario because of their great depth and low sediment content in the open waters, play a lesser role in the environmental chemistry of contaminants than most other water bodies. The experimental results presented herein show that in in vitro aqueous, sediment-free environments, TFM is persistent and resists biodegradation.

Thingvold (16) has computed the rate of buildup of TFM in Lake Superior water assuming a uniform rate of TFM input, uniform mixing, and that TFM is completely conservative within the lake with the only loss being through the outlet of the lake. He reported an expected concentration of TFM in Lake Superior water of 0.015 μg/L. This concentration is below the concentration which is believed to be acutely or even chronically toxic to aquatic life.

As discussed by Lee and Jones (30) biotransformation in the aqueous phase is just one reaction that must be considered in the hazard assessment of a chemical in the environment. While this study has shown that TFM does not degrade in a sediment-free water solution, one should not conclude that TFM would be conservative, i.e., unreactive, in the Great Lakes. The Great Lakes, especially the upper lakes, are in general relatively free of suspended inorganic sediment. It would be expected that some of the TFM added to tributaries would be sorbed by algae and their detrital remains and thereby be transported to the sediments. It appears that TFM may be degraded in sediments.

Another mechanism by which TFM would be lost from the upper waters of the Great Lakes is through photolysis. The work done thus far on the photolysis of TFM does not provide information that can be used to estimate the significance of this reaction mechanism in influencing the aqueous environmental chemistry of TFM in Great Lakes waters. While there are still a number of questions about the factors governing the persistence of TFM in the Great Lakes water column, it can be concluded that the past and near-future anticipated use of TFM does not represent a significant hazard to fish and other nontarget aquatic organisms in the Great Lakes, since the concentrations to be expected in the water column are well below those that have been shown or would be expected to be adverse to aquatic life other than the target organism.

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