# Use of 3-Trifluormethyl-4-nitropheno as a Selective Sea Lamprey Larvicide

Great Lakes Fishery Commission

TECHNICAL REPORT No. 1
May 1961

The Great Lakes Fishery Commission was established by the Convention on Great Lakes Fisheries, between Canada and the United States, ratified on October 11, 1955. It was organized in April, 1956 and assumed its duties as set forth in the Convention on July 1, 1956. The Commission has two major responsibilities: the first, to develop co-ordinated programs of research in the Great Lakes and, on the basis of the findings, recommend measures which will permit the maximum sustained productivity of stocks of fish of common concern; the second, to formulate and implement a program to eradicate or minimize sea lamprey populations in the Great Lakes. The Commission is also required to publish or authorize the publication of scientific or other information obtained in the performance of its duties.

A report describing the Commission actions and the progress of the sea lamprey program, carried out by contracts with the United States Bureau of Commercial Fisheries and the Fisheries Research Board of Canada, is published annually. Included in this report is a summary of the work of federal, state and provincial agencies co-operating in a program to restore lake trout. There is also an outline of the fishery investigations under way in the Great Lakes. However, the need for a more detailed presentation of certain information has led to the establishment of a Technical Report Series, of which the following report on the use of chemicals to control sea lamprey is the first.




1319 N. University Ave.
Ann Arbor, Michigan

# USE OF 3-TRIFLUORMETHYL-4-NITROPHENOL AS A SELECTIVE SEA LAMPREY LARVICIDE

by

VERNON C. APPLEGATE, JOHN H. HOWELL, JAMES W. MOFFETT

United States Bureau of Commercial Fisheries

B. G. H. Johnson

Fisheries Research Board of Canada

and

MANNING A. SMITH

Dept. of Chemistry, Bucknell University

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GREAT LAKES FISHERY COMMISSION
1451 Green Road
P.O. Box 640
Ann Arbor, Michigan

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# USE OF 3-TRIFLUORMETHYL-4-NITROPHENOL AS A SELECTIVE SEA LAMPREY LARVICIDE

The recent discovery of a group of chemical compounds that are significantly more toxic to sea lampreys than to other aquatic organisms offers promise of an early and effective control of this pest. The sea lamprey has all but destroyed the lake trout populations of Lakes Huron and Michigan. In Lake Superior, production of the lake trout fishery has declined to record low levels. Only a rapid and drastic reduction in sea lamprey predation can save the lake trout population there. Other species of food and game fishes have suffered severe decreases from persistent attack by the lamprey.

The sea lamprey spends only a small portion of its life as a parasite in the Great Lakes. The fully grown and sexually mature adults migrate into streams to spawn and thereafter die. The eggs hatch in a week to 10 days and the larvae remain in the stream bottom for 5 years or longer before metamorphosis into the adult form. Following this transformation the young lampreys migrate downstream to the lakes to begin their parasitic existence. The life cycle of the sea lamprey has been described in detail elsewhere (Applegate 1950; Applegate and Moffett 1955).

Control of the adult lampreys distributed throughout a body of open water as large as one of the Great Lakes, by known and available techniques, is not feasible. Fortunately, this pest can be attacked effectively at those stages in its life cycle when it is concentrated in restricted areas. Various devices have been developed which prevent spawning by blocking the streams below the spawning grounds. Electrical weirs, that repel or destroy the lampreys, have been used (Applegate, Smith, and Nielsen 1952; Erkkila, Smith, and McLain 1956). A serious shortcoming of this control method is the time required to achieve the desired effect. Even though the adults have been destroyed before spawning, 5 or more generations of larval lampreys are already in the stream-enough to provide an annual supply of parasitic adults for an equal period of time.

Almost all the larvae of the sea lamprey live in the spawning streams. Treatment of these streams with selectively toxic chemicals that kill the larvae provides immediate reduction of all generations in the population before they become parasites. Control of the species can thus be achieved without a delay of several years.

Applegate, Howell, and Smith (1958) reported the differential toxic effects among larval lampreys and fishes of 6 mononitrophenols containing halogens. Subsequently, 4 additional compounds in this chemical group were discovered that display similar selectively toxic properties. All of these halogenated mononitrophenols have certain characteristics in common. They have from 1 to 3 halogens substituted in the ring, or in an aliphatic radical attached to the ring. Only one nitro group is present. They differ in the number and type of halogen atoms (F, Cl, or Br) attached to the phenol molecule and in the positions of the nitro and halo groups relative to the hydroxyl group. The 10 selectively toxic nitrophenols are:

2-Bromo-4-nitrophenol
3-Bromo-4-nitrophenol
2-Chloro-4-nitrophenol
5-Chloro-2-nitrophenol
3-Fluoro-4-nitrophenol
2,5-Dichloro-4-nitrophenol
3,4,6-Trichloro-2-nitrophenol
3-Trifluormethyl-2-nitrophenol
2-Trifluormethyl-4-nitrophenol
3-Trilluormethyl-4-nitrophenol

Although all of these compounds are more toxic to lampreys than to most other aquatic organisms, certain of them are more desirable for practical applications because of their physical and chemical properties, ease of handling in the field, effectiveness at low concentrations, and cost. Of all the compounds, 3-trifluormethyl-4-nitrophenol met these requirements most closely and hence was selected at an early date for development for field use. This report is concerned with the laboratory tests, field trials, and early operational treatments that were undertaken with this compound.

## 3-TRIFLUORMETHYL-4-NITROPHENOL (TFM)

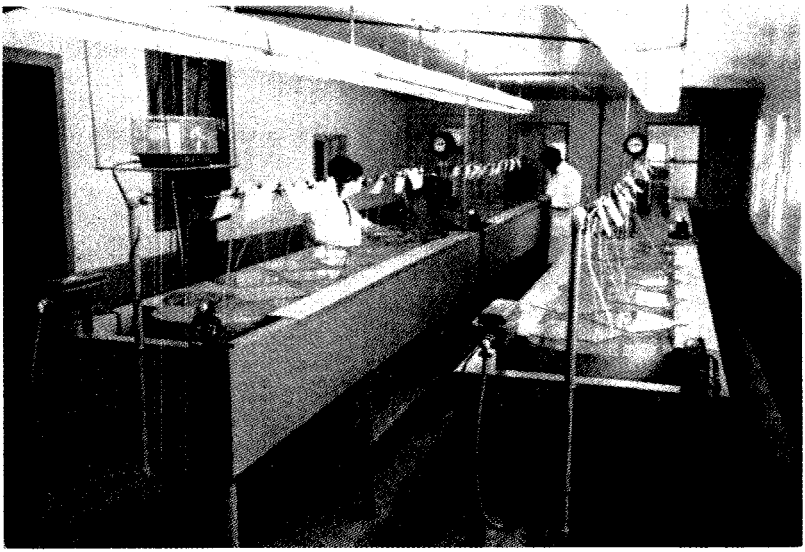
TFM is a crystalline solid at room temperature, yellow to orange in color in its pure form, and light brown in technical-grade preparations. It is sparingly soluble in water (0.498 g. TFM/100 g. $H_2O$ at 24.50 C.) but highly soluble in most organic solvents. Aqueous solutions of TFM are acidic (pK - 6.07 $\pm$ 0.03) and form salts, called phenolates, in the presence of alkalis. Phenolates of the alkali metals are weak bases. The free phenol is colorless in acid solution but deep yellow in base solution. The compound is highly stable and resistant to biochemical attack. It is not detoxified by any known natural process.

TFM is selectively toxic among larval lampreys and fishes not only in the form of its free phenol but also as its sodium salt. Indeed, the two forms of the phenol appear to have nearly identical toxic properties (Applegate, Howell, and Smith 1958) . The solubility of the sodium phenolate in water is much greater than that of the free phenol. Since a larvicide is best applied in the field as a water-miscible liquid formulation, only the phenolates are practical because their greater solubilities permit the preparation of more highly concentrated stock solutions. All laboratory and field experiments reported here were conducted with various formulations of the sodium salt of TFM.

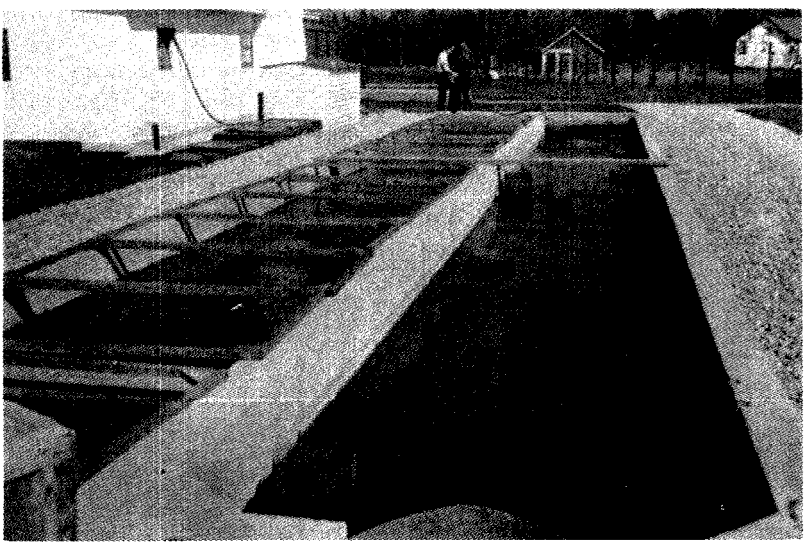
Subsequent to these experiments we found that certain mixed amine salts of TFM are as effective in their biological properties as the sodium salts. The amine salts have more desirable physical properties than do the sodium salts, permitting commercial preparations of greater stock strength with better stability of the solutions when stored at low temperatures. Commercial formulations of the amine salts will be used extensively in stream treatments in the future.

All laboratory tests were conducted with a formulation of the sodium salt of TFM prepared from a purified sample of the compound (a commercial, technical-grade sample recrystallized from benzene-petroleum ether, m.p. 75°–76° C.). Dimethyl formamide and water were used as solvents. A commercial, technical-grade preparation (91 percent pure) was used in raceway (simulated stream) tests of the compound. This material was converted to its sodium salt and formulated as an aqueous solution containing 30 percent by weight of the active ingredient. A commercial formulation of the sodium salt of TFM called Lamprecid 2770[1] containing 45 percent by weight of the active ingredient was utilized in all experimental stream treatments reported here.

[1] Registered Trademark of Hostachem Corp., a division of Farbewerke Hoechst AG.



Constant temperature baths utilized in conducting bioassay tests. The four independantly controlled baths shown can hold 68 ten-liter test jars.



Simulated stream test of TFM in a running-water raceway at the Hammond Bay Laboratory. Cylindrical screen cages contain either larval lampreys or small test fishes.

## BIOASSAY METHODS

Laboratory facilities and equipment were similar to those described by Applegate, Howell, Hall, and Smith (1957). Animals were placed in lo-liter glass battery jars (IO-inch diameter), each containing 6 liters of test solution. The suitability of this test volume was determined experimentally. The test jars were aerated by means of standard stone air-breakers. Oxygen levels in the jars during tests were maintained at near-saturation. Temperatures were held constant by immersion of the test jars in a specially constructed water bath.

Test animals were larvae of the sea lamprey *(Petromyzon marinus)* and fingerling rainbow trout *(Salmo gairdneri)*. Lamprey larvae ranged from 3.5 to 5.0 inches and rainbow trout from 4.5 to 5.5 inches in total length. All larvae were collected from a restricted area of the Ocqueoc River, Presque Isle County, Michigan. The rainbow trout were obtained as a single lot of fish from a hatchery of the Michigan Department of Conservation.

In the typical test, 3 specimens of either larval lampreys or rainbow trout were placed in each test container. After the animals were tempered and acclimated to the test temperature, appropriate amounts of TFM in aqueous solutions were added to produce the desired concentrations. Sixteen simultaneous replications were run with a total of 48 individuals of each species at each concentration. In certain assays conducted with test waters of varying characteristics from a group of tributaries of the upper Great Lakes, 2 specimens of each test species were placed in each jar and only 8 replications were made at each concentration. This reduction in test animals and replications was dictated by the limited volume of test water that could be transported over long distances.

Dilution waters were obtained from several sources depending on the purpose of the experiments. Tests to determine the biological activity of TFM at 3 temperature levels were made with a single lot of water taken from Hammond Bay of Lake Huron. This water was stored under semi-refrigerated conditions in a concrete tank which had an inert coating on its interior walls. The physical and chemical characteristics of the water did not change significantly during storage and use. Average values during the period were: oxygen, 13.0 ppm; $CO_2$, 2.2 ppm; methyl-orange alkalinity, 109 ppm; phenolphthalein alkalinity, 0.0 ppm; pH, 7.7; conductivity, 182.3 mMhos/18° C. The biological activity of TFM in waters of differing physical and chemical properties was tested in dilution waters collected from a series of widely separated tributaries of Lakes Huron, Michigan, and Superior, that were known from preliminary experiments to differ broadly in water quality. The properties of these waters are indicated elsewhere in this report.

Physical and chemical analyses of dilution waters were made immediately before their use in all tests. Dissolved oxygen was checked frequently during the tests to insure an adequate concentration. All physical and chemical determinations were made according to the procedures set forth in the ninth edition of "Standard Methods for the Examination of Water and Sewage." TFM concentrations in all test containers were checked colorimetrically by the method described in Smith, Applegate, and Johnson (1960).

The laboratory tests were conducted for a 24-hour period. The responses of the test animals were observed frequently, usually at 30-minute intervals. In experiments where the test animals were slow to die, observations were made at l-hour intervals. Arithmetic dosage intervals were employed in all laboratory work. These data were sufficiently descriptive to permit rapid development of techniques for the application of TFM in streams.

## BIOASSAY RESULTS

Earlier investigations established that TFM is acutely toxic to larval lampreys at low concentrations and that at these concentrations it is non-toxic to other fishes inhabiting the same waters. The chemical also destroyed the larvae in a relatively short time. It exhibited the most definitive action on larvae and functioned at the lowest concentration of any of the nitrophenols tested. It was further established that the differential toxicity displayed by TFM covered a sufficiently broad range of concentrations to permit its regulated application in streams to destroy lamprey larvae without concurrent damage to other fish (Applegate, Howell, and Smith 1958). To extend these preliminary findings, experiments were conducted in the laboratory to determine the effect of certain physical and chemical properties of natural waters upon the biological activity of TFM.

The effect of water temperature upon the toxicity of the chemical was explored by performing tests at 35°, 45°, and 55" F. The results indicated very little difference in the activity of TFM at these 3 temperatures. The minimum lethal dose for larval lampreys (concentration killing 100 percent of the test larvae within 24 hours) was 2.0 ppm at all three temperatures (Table 1, Figure 1). 'The velocity of death of the larvae slowed, however, as the temperature was lowered (Table 2). A small reduction was detected in the toxicity of TFM to rainbow trout as water temperature declined (Table 1, Figure 1). Maximum allowable dosages (concentration killing approximately 25 percent of the rainbow trout within 24 hours) were 8.9 ppm at 55° F., 9.3 ppm at 45° F., and 9.7 ppm at 35° F. These values were derived from the curves in Figure 1. The velocity of death of the rainbow trout was also slowed by lowered temperatures (Table 3).

TABLE 1. - Mortalities of larval sea lampreys and rainbow trout exposed to various concentrations of the sodium salt of 3-trifluormethyl-4-nitrophenol at water temperatures of 35°, 45°, and 55°F.
[Test period-24 hours]

| Concentration of TFM (ppm) | Percentage mortality | | | | | |
|---|---|---|---|---|---|---|
| | at 35°F. | | at 45°F. | | at 55°F. | |
| | Larval sea lampreys | Rainbow trout | Larval sea lampreys | Rainbow trout | Larval sea lampreys | Rainbow trout |
| 0.75 | 0.0 | | 2.1 | | 0.0 | . |
| 1.0 | 4.2 | | 8.3 | | 4.2 | |
| 1.25 | 25.0 | | 29.2 | | 27.1 | |
| 1.5 | 45.8 | . | 47.9 | | 47.9 | |
| 1.75 | 89.6 | | 83.3 | | 83.3 | . |
| 2.0 | 100.0 | | 100.0 | | 100.0 | |
| 3.0 | 100.0 | | 100.0 | | 100.0 | |
| 4.0 | | | | | | |
| 5.0 | | . | | | | |
| 6.0 | | | | | | |
| 7.0 | | | | | . | 0.0 |
| 8.0 | | 0.0 | . | 0.0 | | 12.5 |
| 9.0 | | 6.3 | | 18.8 | | 26.7 |
| 10.0 | | 31.2 | | 37.5 | | 43.8 |
| 11.0 | | 56.3 | | 62.5 | | 63.8 |
| 12.0 | | 75.0 | | 82.3 | | 87.5 |
| 13.0 | | 86.3 | | 100.0 | | 100.0 |
| 14.0 | | 100.0 | | | | |

TABLE Z.-Velocity of death of larval sea lampreys exposed to various concentrations of the sodium salt of 3-trifluormethyl-4-nitrophenol at water temperatures of 35°, 45°, and 55°F.

[Test period-24 hours]

| Concentration of TFM (ppm) | 35°F. | | | | 45°F. | | | | 55°F. | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Percentage mortality | Time to death (hours) | | | Percentage mortality | Time to death (hours) | | | Percentage mortality | Time to death (hours) | | |
| | | Min. | Aver. | Max. | | Min. | Aver. | Max. | | Min. | Aver. | Max. |
| 0.75 | 0.0 | | | | 2 . 1 | | (21.0) | | 0.0 | | . . | . . |
| 1.0 | 4.2 | 17.0 | 19.0 | 22.0 | 8.3 | 17.0 | 18.0 | 19.0 | 4.2 | 17.0 | 19.0 | 21.0 |
| 1.25 | 25.0 | 13.0 | 17.8 | 22.0 | 29.2 | 13.0 | 16.9 | 20.0 | 27.1 | 6.0 | 15.2 | 21.0 |
| 1.50 | 45.8 | 11.0 | 16.4 | 22.0 | 47.9 | 10.0 | 15.3 | 22.0 | 47.9 | 8.0 | 14.4 | 22.0 |
| 1.75 | 89.6 | 10.0 | 15.3 | 21.0 | 83.3 | 10.0 | 14.7 | 22.0 | 83.3 | 6.0 | 13.2 | 21.0 |
| 2.0 | 100.0 | 9.0 | 14.0 | 20.0 | 100.0 | 6.0 | 11.8 | 20.0 | 100.0 | 5.0 | 10.4 | 16.0 |
| 3.0 | 100.0 | 7.0 | 10.3 | 12.0 | 100.0 | 5.0 | 7.3 | 12.0 | 100.0 | 5.0 | 6.9 | 10.0 |
| 5.0 | 100.0 | 5.0 | 6.3 | 7.5 | 100.0 | 3.0 | 4.5 | 5.5 | 100.0 | 2.5 | 3.8 | 7.0 |
| 7.0 | 100.0 | 4.5 | 5.6 | 7.5 | 100.0 | 3.0 | 4.0 | 5.5 | 100.0 | 2.0 | 3.1 | 4.5 |
| 9.0 | 100.0 | 3.0 | 4.5 | 5.5 | 100.0 | 2.0 | 3.3 | 4.0 | 100.0 | 1.5 | 2.7 | 3.5 |
| 11.0 | 100.0 | 3.0 | 4.2 | 5.0 | 100.0 | 2.0 | 2.6 | 3.5 | 100.0 | 1.5 | 2.2 | 3.0 |

TABLE S.-Velocity of death of rainbow trout fingerlings exposed to various concentrations of the sodium salt of 3-trifluormethyl-4-nitrophenol at water temperatures of 35°, 45°, and 55°F.
[Test period-24 hours]

| Concentration of TFM (ppm) | 35°F. | | | | 45°F. | | | | 55°F. | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Percentage mortality | Time to death (hours) | | | Percentage mortality | Time to death (hours) | | | Percentage mortality | Time to death (hours) | | |
| | | Min. | Aver. | Max. | | Min. | Aver. | Max. | | Min. | Aver. | Max. |
| 8 | 0.0 | .. | .. | .. | 0.0 | .. | .. | .. | 12.5 | 13.5 | 16.8 | 20.0 |
| 9 | 6.3 | .. | (9.5) | .. | 18.8 | 14.0 | 17.6 | 19.0 | 26.7 | 1.0 | 11.1 | 21.5 |
| 10 | 31.2 | 10.0 | 15.4 | 20.0 | 37.5 | 4.0 | 13.8 | 20.0 | 43.8 | 1.5 | 9.5 | 22.0 |
| 11 | 56.3 | 6.5 | 14.6 | 20.5 | 62.5 | 4.5 | 12.4 | 20.0 | 68.8 | 1.0 | 5.0 | 11.5 |
| 12 | 75.0 | 6.0 | 11.5 | 19.5 | 82.3 | 2.5 | 8.0 | 19.0 | 87.5 | 1.5 | 4.6 | 7.0 |
| 13 | 86.3 | 6.5 | 10.2 | 17.5 | 100.0 | 2.3 | 5.4 | 13.3 | .. | .. | .. | .. |
| 14 | 100.0 | 6.5 | 10.0 | 18.0 | 100.0 | 2.3 | 4.4 | 11.3 | .. | .. | .. | .. |

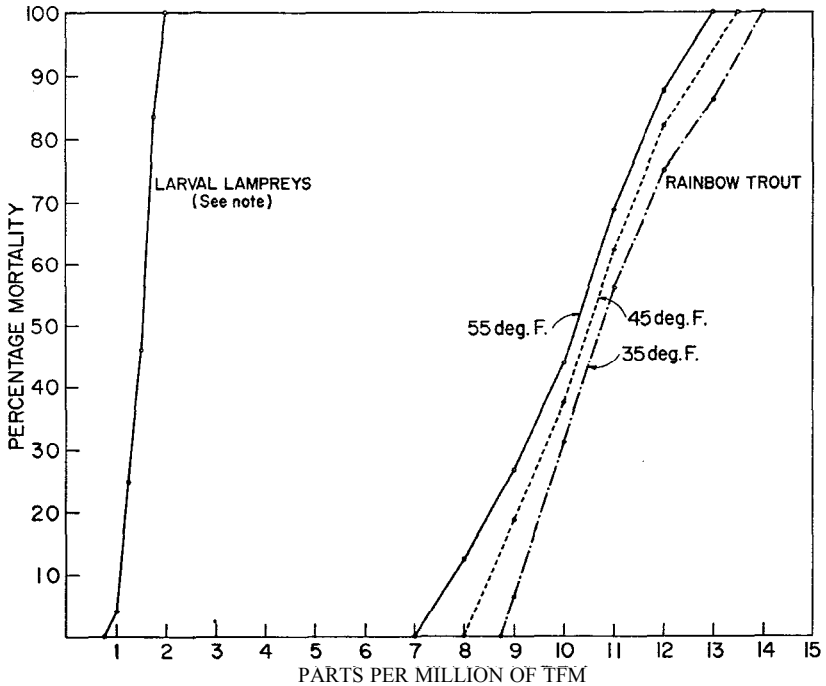



FIGURE 1. - Mortalities of sea lamprey larvae and rainbow trout fingerlings exposed to various concentrations of TFM at water temperatures of 35°, 45°, and 55°F.

NOTE: Responses of the larval lampreys are plotted only for a water temperature of 55°F. Mortalities of larvae at lower temperatures were so similar to those at 53°F. that separate curves could not be drawn (see Table 1).

The utility of the chemical as a selective larvicide is not impaired by low water temperatures. The differential toxic effects are, in fact, somewhat improved as water temperature drops to near freezing. As temperature is lowered from 55° to 35° F., mortalities caused by the compound among rainbow trout are slightly lessened whereas those among the larval lampreys remain essentially the same.

Certain characteristics of natural waters, other than temperature, influence the toxic effects of many chemicals upon aquatic organisms. Among these factors are pH, alkalinity, hardness, dissolved oxygen, and turbidity. Bioassays were conducted with dilution waters obtained from 16 tributaries of Lakes Huron, Michigan and Superior that varied widely in pH, conductivity, and alkalinity. Among them, they represented nearly the entire range of these characteristics found in streams infested with sea lamprey larvae. In these tests, temperature was held constant at 55° F.

The results of these bioassays are summarized in Table 4 where pertinent physical and chemical properties of the waters are also given. The responses of the test animals are reported in terms of minimum lethal dosages for lampreys and maximum allowable dosages for rainbow trout.

The toxicity of TFM is strongly influenced by water hardness and pH. The chemical is most effective in soft, acid waters where the minimum lethal concentrations were as low as 0.5 ppm. As pH, conductivity, and alkalinity of the waters increased, the dosage requirements of TFM to effect a loo-percent kill of larval lampreys increased. In the hardest and most alkaline waters tested, the minimum lethal concentration for the larvae was 8.0 ppm. The changes in the toxicity of TMF to rainbow trout were comparable. The differential toxic action of the compound was retained regardless of its level of activity in any given water, i.e., the increases in the minimum lethal concentration for larval lampreys and in the maximum allowable dose for trout were roughly proportional. Treatment of any stream with TFM is not precluded because of variations in hardness and pH, but, quantities of TFM required for effective treatment may be considerably greater in hard, alkaline waters thin in soft and acid waters.

No clear-cut relation could be demonstrated between the biological activity of TFM and these properties of the water. Measurements of pH, alkalinity, and conductivity, which reflect in some measure the ionic composition of the natural waters, are only broadly correlated with the biological activity of TFM. It is presumed that the toxicity of TFM is affected by a combination of ions present in the water; the most important probably are those of calcium and hydrogen. Prediction of the precise toxicity of TFM from analyses of stream water is not possible, however, at present.

The effects of dissolved oxygen on the toxicity of TFM to larval lampreys and fishes were not evaluated. Such information is not critical since concentrations of dissolved oxygen are relatively high in the great majority of streams inhabited by sea lamprey larvae. Neither has the effect of turbidity upon the toxicity of the compound been explored but results of several simulated stream trials and actual stream treatments in turbid water gave no indication at all that turbidity affected the toxicity of TFM.

The biological activity of the halo-mononitrophenols in waters of the upper Great Lakes varies seasonally. TFM is most effective during late fall, winter, and early spring. Its toxicity diminishes during late spring and is at a minimum in July and August. The compound then becomes more toxic again during the early fall. The degree of seasonal change in toxicity varies according to location around the three upper lakes; in some areas it is negligible but in others the change is pronounced. The decline in biological activity of TFM in summer in

TABLE 4. - Variation in the biological activity of the sodium salt of 3-trifluormethyl-4-nitrophenol in waters of differing physical and chemical characteristics.

[Tests conducted for a 24-hour period at a water temperature of 55°F.]

| Source of test water | Minimum lethal dose of TFM[1] (ppm) | Maximum allowable dose of TFM2 (ppm) | Properties of test water | | | | |
|---|---|---|---|---|---|---|---|
| | | | Conductivity (*m*Mhos/18° C.) | $CO_2$ (ppm) | phth alk. (ppm) | M. 0. alk. (ppm) | PH |
| Boyne River, Ontario | 0.5 | 2.25 | 36.6 | 2.7 | 0.0 | 17.0 | 6.8 |
| Goulais River, Ontario | 0.5 | 2.5 | 52.6 | 3.5 | 0.0 | 28.0 | 6.9 |
| Big Carp River, Ontario | 0.5 | 2.75 | 64.2 | 4.5 | 0.0 | 28.0 | 6.7 |
| Stokely River, Ontario | 0.75 | 2.75 | 62.4 | 3.6 | 0.0 | 37.0 | 7.0 |
| Bad River, Wisconsin | 1.0 | 4.5 | 79.7 | 2.5 | 0.0 | 47.0 | 7.3 |
| Brule River, Wisconsin | 1.0 | 4.5 | 96.9 | 2.3 | 0.0 | 57.0 | 7.4 |
| Two Hearted River, Michigan | 1.5 | 6.0 | 83.7 | 3.6 | 0.0 | 70.0 | 7.3 |
| Gravel River, Ontario | 1.5 | 4.75 | 114.2 | 4.2 | 0.0 | 83.0 | 7.3 |
| White River, Wisconsin | 1.5 | 5.75 | 139.0 | 1.8 | 0.0 | 90.0 | 7.7 |
| Sucker River, Michigan | 2.0 | 8.5 | 109.5 | 1.6 | 0.0 | 68.0 | 7.6 |
| No. Br., Fish Creek, Wisconsin. | 2.0 | 8.0 | 132.1 | 2.1 | 0.0 | 83.0 | 7.6 |
| Pere Marquette River, Michigan | 3.0 | 9.0 | 297.4 | 3.8 | tr. | 156.0 | 7.6 |
| Jordan River, Michigan | 5.0 | 17.0 | 307.1 | 1.4 | 0.0 | 179.0 | 8.1 |
| Nottawasaga River, Ontario | 5.0 | >15.0 | 381.4 | 1.8 | 8.0 | 255.0 | 8.1 |
| Pipestone Creek, Michigan | 6.0 | >17.0 | 408.5 | 2.1 | 0.0 | 205.0 | 8.0 |
| Kewaunee River, Wisconsin | 8.0 | >29.0 | 380.8 | 1.4 | 5.0 | 217.0 | 8.1 |

1 Minimum dose required to kill 100 percent of the larval lampreys.

2 Dose required to kill 25 ± percent of the rainbow trout.

streams of certain areas is sufficient to render impractical the treatment of these watersheds in this season because of the high cost of the chemicals required to give the dosage levels needed to kill the larvae. For economic reasons, therefore, nearly all stream treatments are undertaken during the fall, winter, and spring. A thorough study of this phenomenon and its possible causes is presently underway.

The physiological effects of TFM upon test lampreys have not been completely explored. This chemical causes an excoriation of the respiratory epithelium accompanied by rupture of this tissue and enlargement of the blood vessels supplying it. Severe hemorrhaging from the respiratory capillaries often fills the gill pouches with blood. The compound is also hypotensive in its effect upon the larvae. Blood vessels become dilated, particularly the large posterior cardinal veins which lie on the dorsal wall of the coelom. These vessels, as well as other elements of the venous system, become greatly engorged with blood. These observations suggest that the lampreys suffer a general circulatory collapse aggravated by suffocation due to the presence of excessive mucus and blood in the gill pouches. Lamprey larvae appear first to be irritated, then to be narcotized, by the toxicant. Respiration becomes irregular and responses to stimuli weaken.

Rainbow trout exposed to high concentrations of TFM exhibit different symptoms. Irritability increases to the time of death. The fish exhibit symptoms of anoxia such as surfacing movements and distended gill covers. The gills give no evidences of hemorrhaging but instead become coated with coagulated mucus. This coating doubtless contributes to the suffocation of the fish.

## RACEWAY TEST METHODS

Experiments were made with TFM in a running-water raceway to provide an intermediate stage of testing of the chemical which would approach natural stream conditions more closely than the conventional bioassay tests. These trials also yielded information on the action of TFM under continuous water flow which required the regulated metering of the chemical, as an aqueous solution of its sodium salt, into the "stream."

The raceways are concrete troughs, 65 feet long, 6 feet wide and 30 inches deep, Water from Lake Huron is delivered to the head of the race through a surge tank which stabilizes the flow. The volume of water discharged into the experimental "stream" is measured through a "V" weir.

An artificial stream bed was constructed on the floor of the raceway for each test with materials taken from the beds of local rivers. The topography of this artificial stream provided a small mixing pool at the head of the race, followed by a shallow gravel riffle, and then a

pool about 18 inches deep. A second shallow-water area, usually of sandy materials, was located about midway along the race. Below this point was another silt-bottomed pool. At the foot of the latter pool, a final, shallow riffle was created of sandy or silt materials. Water dropped into a waste flume from the foot of this last riffle.

Test specimens were placed in this artificial stream sufficiently in advance of a treatment to allow them to become adjusted to it. Larger fishes such as the trouts, bullheads, adult suckers, large rock bass, and mature sea lampreys moved unrestricted within the 65-foot-long test area. Smaller fishes were placed in cylindrical screen cages at various points throughout the "stream." Larval lampreys were kept in similar screen cages set deeply enough into the bottom to permit the larvae to establish themselves in burrows. Restriction of the smaller fishes and the larval lampreys to these cages permitted more rapid and accurate determination of mortalities. About 15 species were employed in each test. These usually included sea lampreys (2 or more life-history stages), 3 trout species, miscellaneous "panfish," "rough fish," forage minnows, turtles, crawfish, and aquatic insects.

Each experimental treatment was conducted for 24 hours. The TFM was metered into the water by an electric-motor-driven, dual-piston fluid-proportioning device. This machine pumped from a concentrated stock solution and delivered the concentrate, diluted with flush water, to a perforated pipe located in the mixing pool at the head of the raceway. The concentration of the larvicide in the raceway was controlled by regulating the concentration of the stock solution and the feed rate of the proportioning equipment. The technique was essentially that planned for treatment of natural streams.

During a test, observations were made of all animals at intervals of 2 hours or less. Water temperatures were recorded at the head and foot of the raceway at 2-hour intervals. The fluid-proportioning device was checked at least hourly to assure that it was feeding at an accurate rate.

## RACEWAY TEST RESULTS

Eight raceway or simulated stream tests were conducted, each at a different concentration of TFM. Test concentrations ranged from 1.5 to 13.0 ppm. Results obtained are summarized in Table 5. The common and scientific names of test animals used are listed in Table 6.

Under conditions that simulated treatment of a stream, the differential toxic effects of TFM among lampreys and rainbow trout were essentially the same as those displayed in bioassay tests. The mortality of larval lampreys was 100 percent at concentrations as low as 3.0 ppm. At 1.5 ppm, the next lower concentration tested, 91.5 percent of the larvae were killed. Rainbow trout were not affected by a 24-hour exposure to concentrations as high as 11.0 ppm.

TABLE 5. - Toxicity of an aqueous solution of the sodium salt of 3-Trifiuornrethyl-4-nitrophenol to sea lampreys (*Petromyzon marinus*), fishes, aquatic insects, crayfish, and turtles as determined by simulated stream tests in a running-water raceway.

[Tests at indicated concentrations were conducted for a 24-hour period at various water temperatures ranging from 45" to 55°F. Mean water temperatures extant during testing at each concentration are listed below.]

| Concentration of TFM (ppm) | Mean water temperature (F.°) | Percentage mortality of test species (Number of specimens of each species utilized indicated parenthetically beneath each mortality datum; total number of test animals utilized—1,749.) | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Larval sea lampreys | Adult sea lampreys (mature upstream migrants) | Adult sea lampreys (recently transformed) | Rainbow trout | Brook trout | Brown trout | Rock bass | Sunfishes | Yellow perch | White suckers (adults) | White suckers (immature) | Bullheads | Common shiners | Lake chubs | Creek chubs | Longnose dace | Central mudminnows | Logperch | Mottled sculpins | Turtles | Crayfish | Caddisfly larvae |
| 1.5 | 47.4 | 91.5 (153) | 0.0 (5) | 0.0 (9) | 0.0 (10) | 0.0 (10) | 0.0 (10) | 0.0 (1) | 0.0 (5) | 0.0 (6) | 0.0 (5) | 0.0 (6) | 0.0 (8) | 40.0 (5) | ... | 0.0 (1) | 0.0 (7) | ..... | 0.0 (2) | ..... | ... | ... | ... |
| 3.0 | 45.9 | 100.0 (150) | 100.0 (5) | 100.0 (5) | 0.0 (10) | 0.0 (10) | 0.0 (10) | ... | 0.0 (11) | 0.0 (6) | 0.0 (5) | 0.0 (5) | 0.0 (15) | 0.0 (20) | 0.0 (9) | 0.0 (1) | 0.0 (20) | ..... | ..... | 0.0 (5) | 0.0 (1) | 0.0 (10) | ... |
| 5.0 | 45.2 | 100.0 (149) | 100.0 (5) | 100.0 (10) | 0.0 (10) | 0.0 (10) | 0.0 (10) | ... | 0.0 (12) | 0.0 (8) | 0.0 (5) | 0.0 (2) | 66.6 (12) | 0.0 (10) | 0.0 (5) | ... | 0.0 (5) | 0.0 (5) | 100.0 (1) | 0.0 (10) | 0.0 (2) | 0.0 (15) | 0.0 (3) |
| 7.0 | 49.3 | 100.0 (150) | 100.0 (5) | 100.0 (10) | 0.0 (10) | 0.0 (10) | 0.0 (10) | 0.0 (3) | 0.0 (8) | 12.5 (8) | 0 0 (5) | 28.5 (7) | 100.0 (11) | 0.0 (16) | 0.0 (3) | 0.0 (1) | 16.6 (6) | 0.0 (5) | ..... | 0.0 (7) | ... | 0.0 (8) | 0.0 (4) |
| 9.0 | 54.4 | 100.0 (150) | 100.0 (5) | ..... | 0.0 (10) | 0.0 (10) | 10.0 (10) | 0.0 (1) | 10.0 (10) | 50.8 (12) | 80.0 (5) | 50.0 (10) | 100.0 (11) | 10.0 (10) | ... | 0.0 (1) | 100.0 (10) | ..... | 100.0 (1) | ..... | ... | 0.0 (3) | 0.0 (4) |
| 10.0 | 43.6 | 100.0 (149) | 100.0 (5) | ..... | 0.0 (10) | 0.0 (10) | 10.0 (10) | 0.0 (8) | 100.0 (5) | 100.0 (10) | 80.0 (5) | 100.0 (8) | 93.3 (15) | 100.0 (10) | ... | ... | 100.0 (13) | 100.0 (5) | 100.0 (1) | 100.0 (4) | 0.0 (1) | 0.0 (5) | 0.0 (15) |
| 11.0 | 45.9 | ..... | ..... | ..... | 0.0 (10) | 0.0 (10) | 0.0 (10) | 0.0 (5) | ..... | ..... | .... | ..... | ..... | ..... | ... | ... | ..... | ..... | ..... | ..... | ... | 0.0 (5) | 0.0 (10) |
| 13.0 | 54.2 | ..... | ..... | ..... | 80.0 (10) | 0.0 (10) | 50.0 (10) | ... | ..... | ..... | .... | ..... | ..... | ..... | ... | ... | ..... | ..... | ..... | ..... | ... | ... | ... |

TABLE 6. - Common and scientific names of test animals used in toxicity tests of the sodium salt of 3-trifluormethyl-4-nitrophenol under simulated stream conditions.

| Common name | Scientific name |
|---|---|
| Brook trout | *Salvelinus fontinalis* |
| Brown trout | Salmo *trutta* |
| Bullheads | *Ictalurus melas, I. nebulosus* |
| Caddisfly larvae | Limnophilidae (primarily *Astenophylax* ssp.) |
| Central mudminnow | *Umbra limi* |
| Common shiner | *Notropis cornutus* |
| Crayfish | *Cambarus* ssp. |
| Creek chub | *Semotilus atromaculatus* |
| Lake chub | *Hybopsis plumbea* |
| Logperch | *Percina caprodes* |
| Longnose dace | *Rhinichthys cataractae* |
| Mottled sculpin | *Cottus bairdi* |
| Rainbow trout | Salmo *gairdneri* |
| Rock bass | *Ambloplites rupestris* |
| Sea lamprey | *Petromyzon marinus* |
| Sunfish | *Lepomis macrochirus, L. gibbosus* |
| Turtles | *Chrysemys picta, Chelydra serpentina* |
| White suckers | *Catostomus commersoni* |
| Yellow perch | *Perca flavescens* |

Particularly valuable was the finding that mature, adult sea lampreys (upstream migrants of the spawning run) and recently-transformed lampreys (downstream migrants) were also killed by TFM at concentrations as low as 3.0 ppm. Treatment of streams with TFM during the spring spawning run of mature adults or during the fall and winter downstream movement of recently-transformed individuals will destroy at least part of these migrant populations along with the populations of larvae.

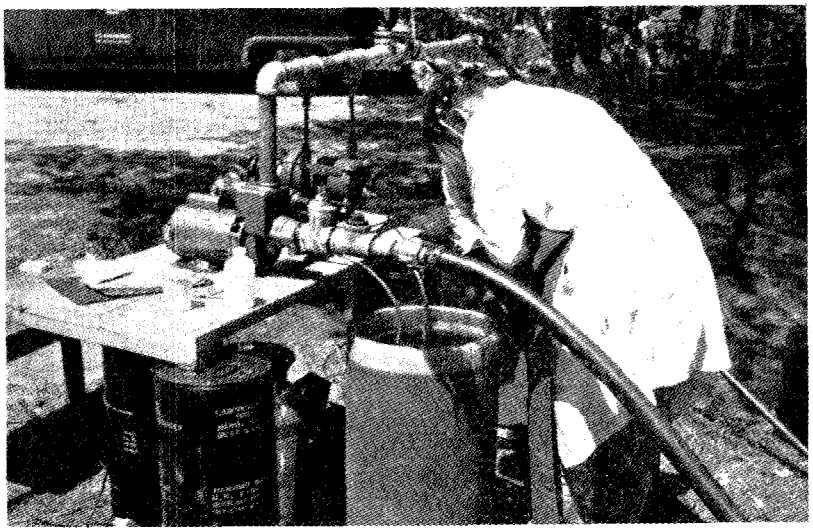
Brook and brown trout, and certain panfish tolerated TFM as well as the rainbow trout. Brook trout were not harmed by exposure to 13.0 ppm, the highest concentration tested. Significant mortalities did not occur among brown trout and rock bass until the concentration exceeded 9.0 ppm. Yellow perch were affected at concentrations

above 7.0 ppm. Where survival of these several species is desired, maximum concentrations of the chemical in streams can be reduced to protect them without altering the effectiveness of the larvicide treatment.

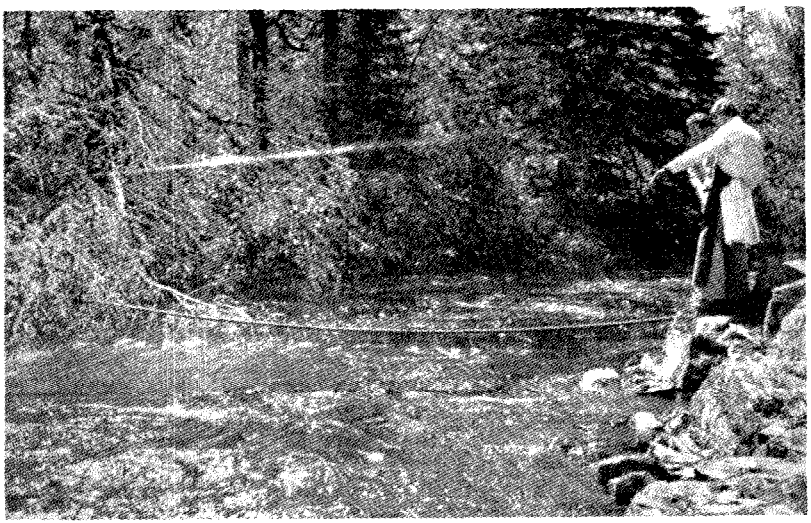
Among the so-called rough fishes, significant mortalities of adult suckers first occurred at concentrations of 7.0 ppm. Immature suckers were slightly less tolerant of the chemical than were the adults. Bullheads died in significant numbers when the concentration exceeded 3.0 ppm. They appear to be as susceptible to TFM as do the lampreys.

Forage fishes, such as shiners, chubs, dace, and sculpins were generally unaffected except at the highest concentrations of TFM used. Logperch, however, were killed by concentrations as low as 5.0 ppm.

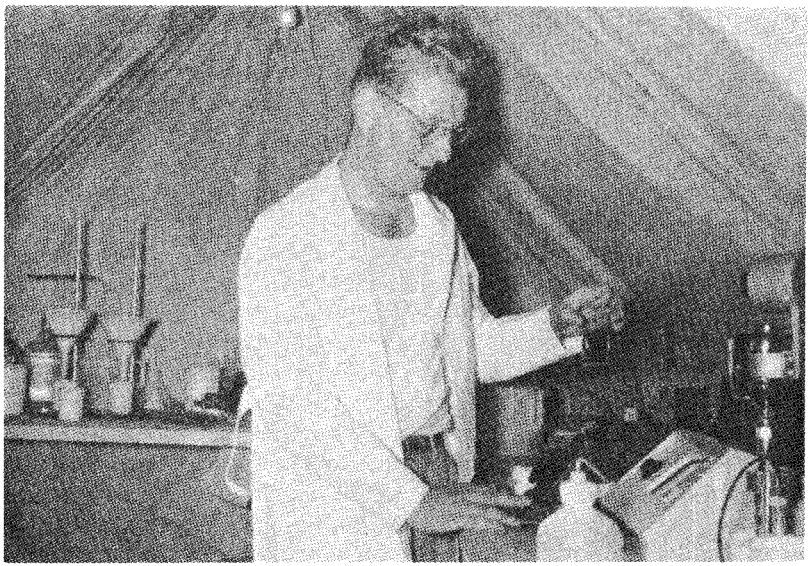
Crayfish and caddisfly larvae were not affected by any concentration of the chemical used. Turtles likewise were unaffected.



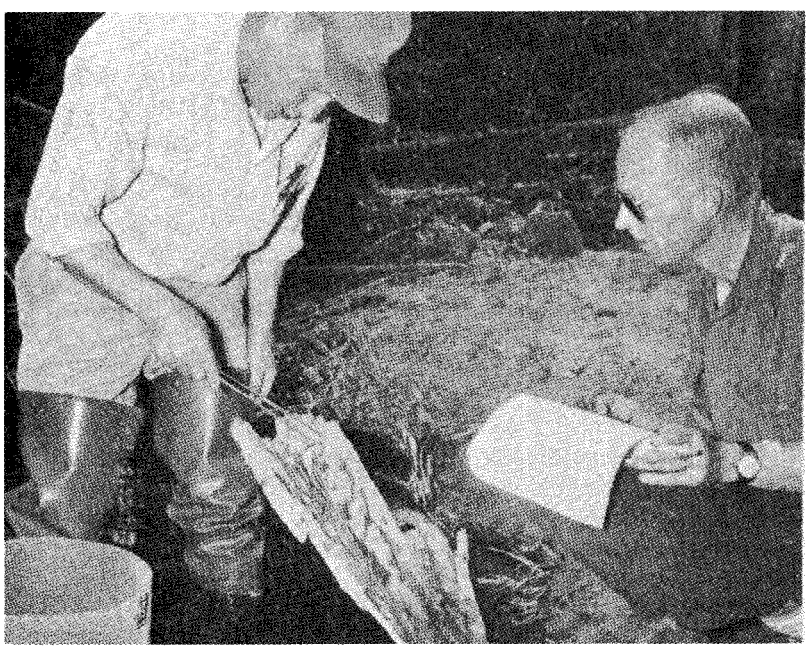
Chemical feeding device in operation at the Silver River. Stock jar being filled with formulated TFM. Smaller hose carries mixture of water and chemical to perforated discharge pipe on bed of stream.



Perforated pipe discharging mixture of formulated TFM and water into the Silver River. Pipe has been raised above water level only for demonstration purposes.



Determining concentration of TFM in a stream water sample by colorimetric analysis.



Observers checking mortalities of caged larval lampreys during treatment of Mosquito River.

## EXPERIMENTAL STREAM TREATMENTS

The raceway tests demonstrated that TFM could be metered successfully into running water to effect a complete kill of larval lampreys without damage to most other aquatic animals. Three tributaries of Lake Superior, infested with sea lamprey larvae, were selected for experimental applications of TFM to test the laboratory findings. These watersheds varied greatly in physical characteristics, ease of access, and length of stream requiring treatment. They were chosen for these reasons and because they offered unique possibilities for well-controlled experimentation. The three streams were: Mosquito River, Alger County, Michigan; Silver River, Baraga County, Michigan; and Pancake River watershed, District of Algoma, Ontario. Each experimental treatment is discussed separately in subsequent sections.

Foremost among the requirements for the successful application of TFM in streams are: (1) the precise determination of the minimum lethal dosage for larval lampreys and the maximum allowable dosage for a selected fish species in a particular water; (2) an accurate, controllable device ‘for the metering of small amounts of a liquid formulation into the stream; and, (3) a precise method of analysing the treated water mass for TFM so that control of the concentration may be achieved.

Since the amount of TFM and the time required to treat a given stream cannot yet be determined from chemical analyses of the water, each application is based on pre-treatment bioassays conducted with water from the stream. All applications of TFM were immediately preceded by one or more such bioassays performed under conditions that were anticipated would exist in the stream at the time of treatment. Bioassay methods were essentially those described earlier in this report. Larval lampreys and rainbow trout were utilized as test animals. Only 4 replications were run at each concentration to restrict to a reasonable quantity the amount of test water needed at the laboratory. These bioassays were adequate for the accurate determinations of minimum lethal and maximum allowable dosages. Subsequent to the experiments reported here, a mobile laboratory has been constructed for pre-treatment bioassays. Placed at the site of each application, this facility greatly simplifies the performance of these necessary tests.

The efficient application of TFM requires a highly accurate and controllable pumping system that can be transported easily to remote locations. Two types of fluid-proportioning pumps have been used with varying degrees of success. In general, these pumps are arranged to feed a concentrated stock solution of TFM into a pipe containing a stream of water drawn from the river by a centrifugal pump. The initial dilution of TFM in this circulating system results in a more efficient dilution of the chemical in the river than does the direct intro-

duction of undiluted formulation. The introduction of the diluted TFM into the river through a perforated pipe under considerable pressure aids in mixing the chemical with the water. Experiences with these fluid-proportioning systems are described in the reports of each stream treatment.

The pre-treatment bioassays show the concentration of TFM required to treat effectively a given stream. Stream flow is then determined at the point of introduction of the chemical and at the stream mouth to give some measure of the dilution of the toxicant which will occur over this reach of the watershed. From these data, the rate at which TFM must be fed into the stream is calculated, as follows:

$$F' = \frac{C \times F}{0.03713 \times C'},$$

where F' = rate of feed in U.S. gallons per hour
$F$ = volume of flow of the stream in cfs at the point of introduction of the chemical
C' = concentration of stock solution in grams per liter
C = concentration of TFM in ppm desired in the stream at point of introduction

Because of inacuracies inherent in both the measurement of volume of flow and in the feed-rate adjustment of the pump, the initial concentration of the toxicant in the water is only an approximation of that desired. Furthermore, any change in the volume during treatment will alter the concentration of TFM. It is frequently necessary not only to adjust the initial feed rate but also to make subsequent changes to compensate for variations in stream flow. Obviously, a precise means of determining the concentration of TFM at any point in the stream is required. Accurate measurement of the amount of TFM was made by a method of colorimetric analysis based on the natural yellow color of the nitrophenols. Simply stated, the procedure involves the comparison of samples of treated stream water with standard solutions of TFM in a Klett-Summerson photoelectric colorimeter. This technique is rapid and accurate under field conditions; it has been described by Smith, Applegate, and Johnson (1960).

## TREATMENT OF THE MOSQUITO RIVER

The Mosquito River in Alger County, Michigan, was treated on May 14, 1958, with the formulation of TFM known as Lamprecid 2770. The larvicide introduced into the river at a natural falls

about 1¾ miles above the mouth. Sea lamprey larvae were found only below this barrier. Just prior to the treatment, the river had a discharge at the mouth of 39.8 cfs.

Pre-treatment studies of the stream included: a determination of the distribution of larval lampreys with an estimate of their numbers, and the determination of the kinds and relative abundance of fishes, and other vertebrate and invertebrate aquatic organisms; a check of rate of water movement and other hydraulic features of the stream, including the increase of flow through the reach to be treated; determination of chemical and other physical properties of the creek water at various locations in the watershed; the recording of water-temperature fluctuations for a 'i-day period prior to the experiment; and, bioassay tests at the Hammond Bay Laboratory with water and larvae from the stream for the purpose of determining the minimum lethal and maximum allowable concentrations of the chemical (Table 7).

Prior to the treatment, larval lampreys were placed in 40 cages which were distributed in the river among 3 stations. These cages contained a total of 1,000 larvae. The condition of these larvae and the numbers dead at hourly intervals during the treatment were recorded.

A quantitative estimate of the effect of the treatment upon resident sea lamprey larvae was sought by intensive sampling of 4 marked areas of larval habitat. Two of these areas were fished with an electrical shocker and larvae removed before treatment; the remaining 2 areas were 'examined by the same method after treatment.

Lamprecid 2770 was applied to the river for 9 hours. A proportioning device called a Proportioneers Triplex Chem-O-Feeder was used. This feeder, which was equipped with Lucite heads and fittings, polyethylene tubing and neoprene diaphragm, check valves, and washers, was manifolded into the discharge side of a circulating pump. The circulating pump picked up untreated water from the stream and returned it (approximately 11 gpm) plus the chemical through a perforated pipe laid on the stream bottom. Pressure in the delivery pipe was maintained at approximately 20 psi to insure good mixing at the point of introduction.

It was intended to produce a concentration of 6 ppm of Lamprecid 2770 in the river. A mistake in the adjustment of the length of piston stroke at the beginning of the test resulted in a near doubling of the feed rate. This mistake was detected by analyses of treated water samples and was corrected in less than ½ hour. During the remainder of the test considerable difficulty was experienced in maintaining a consistent feed rate. Examination of the "feeder heads" after the trial indicated considerable crystallization and fragmentation of the lucite material. Fragments of lucite had almost completely clogged

TABLE 7.—Some physical and chemical properties of the waters of the streams treated experimentally with TFM and the biological activity of the larvicide in these waters as determined from pre-treatment bioassays.

[Tests conducted for a 24-hour period at water temperatures as indicated below.]

| Source of test water | Test temperature (F.°) | Minimum lethal dose of TFM [1] (ppm) | Maximum allowable dose of TFM [2] (ppm) | Properties of test water | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Conductivity (*m*Mhos/18° C.) | $CO_2$ (ppm) | phth alk. (ppm) | M. O. alk. (ppm) | pH |
| Mosquito River, Alger County, Michigan | 55 | 2.0 | 9.0 | 161.3 | <1.0 | 0.0 | 163.3 | 8.0 |
| Silver River, Baraga County, Michigan | 55 | 1.0 | 4.5 | 60.3 | <1.0 | 0.0 | 34.0 | 7.3 |
| Pancake River, District of Algoma, Ontario | 60 | 1.0 | 4.0 | 48.3 | 3.7 | 0.0 | 29.0 | 6.9 |
| Gimlet Creek, [3] District of Algoma, Ontario | 60 | 1.0 | 3.0 | 35.9 | 1.6 | 0.0 | 25.0 | 7.1 |

[1] Minimum dose required to kill 100 percent of the larval lampreys.

[2] Dose required to kill 25 ± percent of the rainbow trout.

[3] Tributary to the Pancake River.

the discharge check valves of all 3 heads. Other pump-component materials (Polyethylene and Neoprene) were not affected adversely.

Analyses of the treated stream water indicated that the larvicide was applied initially at a concentration of about 12 parts per million for a period not exceeding 30 minutes. Thereafter, the average concentration was in the order of 5.5 parts per million. The total amount of the formulation used was 45½ gallons. The total weight of the formulation applied was 385 pounds; the weight of active ingredient contained in this amount of formulation was approximately 163 pounds.

All larval lampreys confined to cages were dead after 7¾ hours of exposure to the chemical. Nearly complete kills of these control specimens were observed in a much shorter period; 91 percent were dead in 3½ hours, 97 percent in 4½ hours, and 99 percent had died within 5½ hours.

Dead and dying larvae were seen in the treated area of the stream after about 2 hours of exposure to the larvicide. Post-treatment examination of the marked sample areas revealed no live larvae. In addition to the latter areas, the larval habitat in some 1,400 linear feet of stream was examined intensively with electric shockers. This distance represented more than 15 percent of the entire area of stream treated and included 4,569 square feet of larval habitat. Within this area no sea lamprey larvae and only 4 live American brook lampreys (*Lampetra lamottei) were found.*

Only 7 species of fishes were found in the Mosquito River (Table 8). Brook trout and resident rainbow trout were common and a sub-

TABLE 8. - Common and scientific names of lampreys and fishes found in the Mosquito River, Alger County, Michigan.

| Common name | Scientific name |
|---|---|
| American brook lamprey | *Lampetra lamottei* |
| Blacknose dace | *Rhynichthys atratulus* |
| Brook stickleback | *Eucalia inconstans* |
| Brook trout | *Salvelinus fontinalis* |
| Central mudminnow | *Umbra limi* |
| Mottled sculpin | *Cottus bairdi* |
| Rainbow trout | *Salmo gairdneri* |
| Sea lamprey | *Petromyzon marinus* |
| Spottail shiner | *Notropis hudsonius* |

stantial number of lake-run rainbow trout were present, or migrating into the stream, during the time of the test. Other resident fishes consisted of shiners, dace, mudminnows, sculpins, and sticklebacks. Dragonflies, mayflies, caddisflies, stoneflies, dipterans, and aquatic earthworms were also present.

During the trial and in post-treatment examinations of the stream only one fish was found which could conceivably have been harmed by the larvicide. This fish was a large, lake-run rainbow trout which bore 2 fresh lamprey scars and was spent. Other lake-run rainbow trout which had been building nests and spawning prior to the application remained more or less quietly on or near their redds while the chemical was in the river. Authentic records were obtained of the capture of 4 lake-run fish on lures during the treatment. No effect of the larvicide upon the aquatic invertebrates was observed.

## TREATMENT OF THE SILVER RIVER

The Silver River, Baraga County, Michigan, was treated with Lamprecid 2770 on June 11, 1958. The larvicide was introduced into the river just above a natural falls some 4½ miles upstream from the mouth. Spawning-run sea lampreys reportedly cannot pass over these falls and larval sea lampreys were found only below this barrier. Just prior to the treatment, the river had a discharge at the mouth of 80.0 cfs.

Pre-treatment examination of the stream was similar to that of the Mosquito River. The relative numbers and distribution of larval lampreys were determined, as were the kinds and relative abundance of fishes and of other vertebrates and invertebrates. Water movement and flow patterns were checked with fluorescein dye. Volume of flow was determined and water analyses were made at various points in the area to be treated. Water temperatures were recorded for 2 weeks prior to the test. At the laboratory, bioassay tests were conducted in water and with larvae from the river (Table 7).

Prior to the treatment, a total of 1,205 larval lampreys were placed in the river in 50 cages distributed among 5 stations. The condition of these larvae and the numbers dead were recorded at hourly intervals during the treatment.

The effect of the application upon resident sea lamprey larvae was evaluated by intensive pre- and post-treatment sampling of over 100,000 square feet of larval habitat. Approximately one-half of this area was examined with electric shockers before treatment and all larvae captured were removed from the stream; the remaining area was examined by the same method after treatment.

Lamprecid 2770 was applied to the river for 13½ hours with a Proportioneers Model 1106D direct-displacement, dual-piston chemical

feeder. Both discharges of this pump were manifolded into the discharge side of a circulating pump. The latter picked up untreated water above the point of introduction and delivered this water plus the chemical into the stream through a perforated pipe laid across the stream bed. Pressure in the delivery pipe was maintained at 20 psi.

Application of the larvicide started at a feed rate intended to produce a concentration of 3.0 ppm of the active ingredient in the stream immediately below the point of introduction. Analyses of treated stream water indicated a concentration of 2.8 ppm in this locale. This deficiency was so small that it did not warrant adjustment of the feeder. The initial rate was therefore maintained without interruption throughout the entire period of application. A total of 128 gallons of formulation was used. The total weight of the formulation applied was 1,280 pounds; the weight of active ingredient contained in this amount of formulation was 583.5 pounds.

All larval lampreys confined to cages were dead after 8½ hours of exposure to the chemical. Nearly complete kills of these control specimens were observed in a much shorter period; 85.3 percent were dead in 4 hours, 97.7 percent in 5 hours, and 98.6 percent had died within 6 hours.

Some dead and dying larvae were seen in the treated areas of the stream after about 1½ hours of exposure to the larvicide. Subsequently, many thousands were seen dead in the river.

Estuarine conditions in this river had not been encountered before. A long, sluggish estuary is present that merges gradually into the narrow head of a finger-like bay. During the spring and summer seiches, which occur at less than hourly intervals, alter the water level of bay and estuary to a considerable degree. It was considered possible that the cyclic, tidal-like in-flows of water would dilute the treated discharge from the river to a concentration below the lethal level. This possibility was important since the estuarine waters were inhabited by a great number of larvae; they were also known to be present in the shallower waters at the head of the bay.

Analyses of water samples from the estuary and bay indicated that concentrations of the larvicide were initially erratic in this area. Subsequently, a uniform concentration of 2.2 ppm became established in the entire zone and to a point approximately ½ mile out in the bay. This condition persisted for many hours. Inspections on foot and by boat revealed many dead larvae lying on the bed of the bay out to the limit of visibility (depths of about 6 feet).

The post-treatment examinations with electrical shockers covered approximately 50,000 square feet of larval habitat in the river proper and in the estuary. In the latter area the examination extended to depths of 4 feet but also included other parts of the channel that were periodically above water level after the seiches ebbed. Only 1

live larval lamprey was found. This individual was situated within the entrance of a small tributary of the river proper.

The Silver River is known to contain 25 species of fishes, in addition to 4 species of lampreys (Table 9). At the time of treatment, brook trout and rainbow trout (both resident and migrant) were present. White suckers and bullheads were migrating into the stream. The most abundant forage species (resident and migrant), were spottail shiners, blacknose dace, longnose dace, logperch, Johnny darters, and sculpins.

Among all fishes present, only the logperch was affected in any significant degree by the larvicide. Occasional individuals of most species present succumbed to the toxin. Post-treatment examination of the stream indicated that even the logperch, seemingly most susceptible to the chemical, was still present in substantial numbers. Other species seemed to be as abundant as they were prior to the application.

No significant effect of the chemical was observed upon the aquatic invertebrates in the stream except for one species of mayfly, which appeared to be stimulated to a premature "hatching."

## TREATMENT OF THE PANCAKE RIVER AND ITS TRIBUTARY, GIMLET CREEK

The Pancake River and Gimlet Creek were treated concurrently on August 26 and 27, 1958, with Lamprecid 2770. The larvicide was introduced into the Pancake River about 12 miles above its mouth. The river has 3 natural falls, only one of which was considered an effective barrier to spawning-run sea lampreys, between the point of introduction of the larvicide and the stream mouth. TFM was introduced into Gimlet Creek about 3 miles above its confluence with the Pancake River just above a series of 6 beaver dams. Immediately prior to the treatments, the total discharge of the Pancake River was 37.0 cfs and that of Gimlet Creek was 5.7 cfs. Pretreatment examinations of the streams included all of the procedures outlined for the previous stream trials of TFM (Table 7).

Before the experiment was started, 1,200 larval lampreys were placed in the two rivers in 50 cages; 30 cages were divided among 3 stations in the Pancake River and 20 among 4 stations in Gimlet Creek. The condition of the larvae and the numbers dead were recorded at hourly intervals during the treatment.

The application of Lamprecid 2770 was begun in Gimlet Creek on August 26 and in the Pancake River on August 27, about 36 hours later. This interval was established, on the basis of the timing of downstream movements of dye, so that the two treated water masses would reach the confluence of the streams simultaneously. The feeders at

TABLE 9. - Common and scientific names of lampreys and fishes found in the Silver River, Baraga County, Michigan.

| Common name | Scientific name |
|---|---|
| American brook lamprey | *Lampetra lamottei* |
| American smelt | *Osmerus mordax* |
| Blacknose dace | *Rhynichthys atratulus* |
| Brook Stickleback | *Eucalia inconstans* |
| Brook trout | *Salvelinus fontinalis* |
| Brown bullhead | *Ictalurus nebulosus* |
| Burbot | *Lota lota* |
| Common shiner | *Notropis cornutus* |
| Creek chub | *Semotilus atromaculatus* |
| Golden shiner | *Notemigonus crysoleucas* |
| Hornyhead chub | *Hybopsis biguttata* |
| Johnny darter | *Etheostoma nigrum* |
| Logperch | *Percina caprodes* |
| Longnose dace | *Rhinichthys cataractae* |
| Longnose sucker | *Catostomus Catostomus* |
| Mottled sculpin | *Cottus bairdi* |
| Northern brook lamprey | *Ichthyomyzon* fossor |
| Northern pike | *Esox lucius* |
| Rainbow trout | *Salmo gairdneri* |
| Redhorse sucker | *Moxostoma* sp. |
| Rock bass | *Ambloplites rupestris* |
| Sea lamprey | *Petromyzon marinus* |
| Silver lamprey | *Ichthyomyzon unicuspis* |
| Smallmouth bass | *Micropterus dolomieui* |
| Spottail shiner | *Notropis hudsonius* |
| Trout-perch | *Percopis omiscomaycus* |
| Walleye | *Stizostedion v. vitreum* |
| White sucker | *Catostomus commersoni* |
| Yellow perch | *Perca flavescens* |

both points of introduction were the direct-displacement, dual-piston, type previously tested at the Silver River. Application techniques with this equipment were identical to those of the earlier test.

The introductions of larvicide, both in Gimlet Creek and in Pancake River, were begun at rates intended to produce concentrations of 3.0 ppm of the active ingredient in the streams immediately below the points of application. Analysis of the treated water in Gimlet Creek showed an initial concentration of 4.8 ppm; the feed rate was reduced to give a concentration of 2.5 to 3.3 ppm for the duration of the treatment. A total of 6 gallons of formulation, containing 28 pounds of active ingredient, was applied over a period of 12 hours in Gimlet Creek. Analysis of the treated water in the Pancake River showed an initial concentration of 3.7 ppm at the point of introduction; the feed rate was reduced to give a concentration of 2.4 to 2.6 ppm for the duration of the treatment. A total of 33 gallons of formulation, containing 155 pounds of the active ingredient, was applied in Pancake River over a period of 12 hours.

The captive larval lampreys nearest the feeder site in Gimlet Creek were dead by the end of 5 hours of exposure to the toxicant; those located nearest the mouth were dead after 8½ hours. The caged larvae nearest the feeder site in the Pancake River were dead after 5 hours of exposure to the chemical and those near the mouth were dead after about 7 hours, with the exception of larvae in two cages located in a semi-isolated part of the estuary. The incomplete effectiveness of the treatment in the latter area (approximately 1,000 square yards) is attributed to the restricted exchange of water between it and the estuary proper. There was a complete kill among caged larval lampreys at all other stations.

Dead and dying lamprey larvae were observed in Gimlet Creek and Pancake River within 2 to 3 hours of the beginning of the respective treatments. Estimates of the total number of dead lamprey larvae ranged between ½ and ¾ million. Immediately after the treatments, several thousand dead larval lampreys were collected, and identified. Roughly one-third were sea lampreys. In the Pancake River, this species occurred only below the lowermost falls (about 3 miles from the mouth), but in Gimlet Creek dead sea lamprey larvae were found between the uppermost beaver dam and the feeder site.

Post-treatment examinations of at least 50 percent of the larval habitat of both the Pancake River and Gimlet Creek were made with electric shockers. The survey of the Pancake River was confined to the area in which dead sea lamprey larvae had been found. In Gimlet Creek, 38 live larval lampreys were taken during the post-treatment shocker survey-all of them were American brook lamprey and all were found in isolated oxbows, or in the mouth of one small tributary. In the Pancake River, 3 live American brook lampreys were recovered

near the mouth of Gimlet Creek. The semi-isolated bay in the estuary of the Pancake River yielded 10 live larvae of *Ichthyomyzon* sp. and 7 of the American brook lamprey. No live sea lamprey larvae were recovered after the treatments of Gimlet Creek and Pancake River.

At least 18 species of bony fishes were known to occur within the Pancake River system (Table 10). At the time of treatment, rainbow trout, brook trout, white suckers, sculpins, burbot, and several species of minnows and of darters were present. The sculpins appeared to suffer fairly heavy mortalities from the toxicant. A few longnose dace and burbot were killed as were a very small number of rainbow trout.

TABLE 10. - Common and scientific names of lampreys and fishes found in the pancake River watershed, District of Algoma, Ontario.

| Common name | Scientific name |
|---|---|
| American brook lamprey | *Lampetra lamottei* |
| American smelt | *Osmerus mordax* |
| Brook stickleback | *Eucalia inconstans* |
| Brook trout | *Salvelinus fontinalis* |
| Burbot | *Lota lota* |
| Common shiner | *Notropis cornutus* |
| Johnny darter | *Etheostoma nigrum* |
| Lake chub | *Hybopsis plumbea* |
| Logperch | *Percina caprodes* |
| Longnose dace | *Rhynichthys cataractae* |
| Mottled sculpin | *Cottus bairdi* |
| Ninespine stickleback | *Pungitius pungitius* |
| Northern pike | *Esox lucius* |
| Rainbow trout | *Salmo gairdneri* |
| Rock bass | *Ambloplites rupestris* |
| Sea lamprey | *Petromyzon marinus* |
| Silver lamprey | *Ichthyomyzon unicuspis* |
| Spottail shiner | *Notropis hudsonius* |
| Trout-perch | *Percopsis omiscomaycus* |
| White sucker | *Catostomus commersoni* |
| Yellow perch | *Perca flavescens* |

The applications of larvicide caused a premature emergence of mayfly nymphs, but did not appear to affect other groups of invertebrates seriously. For no species, other than the lampreys, did the mortality appear to be so severe that the animal could not restore its numbers rapidly by natural means.

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## LITERATURE CITED

APPLEGATE, VERNON C.
1950. Natural history of the sea lamprey *(Petromyzon* marinus) in Michigan. U. S. Fish and Wildlife Serv., Spec. Sci. Rept.: Fish. No. 55, 237 pp.

APPLEGATE, VERNON C., BERNARD R. SMITH, and WILLIS L. NIELSEN
1952. Use of electricity in the control of sea lampreys: Electra-mechanical weirs and traps and electrical barriers. U. S. Fish and Wildlife Serv., Spec. Sci. Rept.: Fish. No. 92, 52 pp.

APPLEGATE, VERNON C. and JAMES W. MOFFETT
1955. The sea lamprey. Scientific American, Vol. 192, No. 4, pp. 36-41.

APPLEGATE, VERNON C., JOHN H. HOWELL, A. E. HALL, and MANNING A. SMITH
1957. Toxicity of 4,346 chemicals to larval lampreys and fishes. U. S. Fish and Wildlife Serv., Spec. Sci. Rept.: Fish. No. 207, 157 pp.
1958. Use of mononitrophenols containing halogens as selective sea lamprey larvicides. Science, Vol. 127, No. 3294, pp. 336338.

ERKKILA, LEO F., BERNARD R. SMITH, and ALBERTON L. MCLAIN
1956. Sea lamprey control on the Great Lakes-1953 and 1954. U. S. Fish and Wildlife Serv., Spec. Sci. Rept.: Fish. No. 175, 27 pp.

SMITH, MANNING A., VERNON C. APPLEGATE, and B. G. H. JOHNSON
1960. Colorimetric determination of halogenated nitrophenols added to streams as sea lamprey larvicides.
Analytical Chemistry, Vol. 32, No. 12, pp. 1,670-1,675.

# APPENDIX I

## TOXICITY OF TFM TO MAMMALS

The effect of TFM on mammals had to be learned before large quantities of concentrated formulations could be handled or dilutions of the chemical introduced safely into public waters. TFM was found to be highly toxic, both internally and externally, to mammals in its crystalline form and as a concentrated stock solution. Tests performed by the Wisconsin Alumni Research Foundation with a 20-percent (by weight) formulation of TFM showed an acute oral LD 50 for the rabbit of approximately 0.16 g./kg. The acute dermal LD 50 was approximately 1.6 g./kg. The primary skin irritation index was approximately 2.6, i.e., that of a moderate skin irritant. TFM is essentially innocuous as an eye irritant at least at stream concentrations. Extreme care must be exercised in handling concentrated forms of TFM. Protective clothing, rubber gloves, and face masks or goggles are necessary for the minimum protection of handlers.

The very low concentrations of TFM required to kill larval lampreys in streams are harmless to wildlife and livestock. Game biologists of the Michigan Department of Conservation tested the effects of a 13 ppm solution of the sodium salt of TFM on deer. Penned animals exposed to treated drinking water for 3 days showed no ill effects, either short- or long-term. Other test deer, exposed to a choice of both treated and untreated drinking water, actually showed a preference for water containing the chemical.

The effects of the consumption of TFM-treated drinking water upon the milk, milk products, and general health of dairy cows were determined by a veterinary, Dr. Albert Dobias, of Cheboygan, Michigan, who conducted experiments with 6 1,000-lb. cows (5 Holstein and 1 Durham) and 4 Holstein calves approximately 10 months old. Two Holsteins and the Durham cow were used as test animals and 3 Holsteins served as controls. In addition, 2 Holstein calves were used as test animals and 2 as controls. Test cows and calves were exposed for 24 hours to water containing 13 ppm of the sodium salt of TFM. It was prepared from the commercial formulation known as Lamprecid 2770 and represented the maximum concentration of TFM likely to be used in streams.

Urine samples were taken from all animals and milk samples from the cows prior to exposure of some to the TFM-treated water and at intervals of 12, 24, 48, 120, and 240 hours during and after exposure; these samples were refrigerated and subsequently analyzed for TFM. Urinalysis for sugar, albumen, acetone, and blood was conducted by use of an Ames diagnostic kit for both test and control cows.

Other data recorded were: milk production, temperature, pulse, and respiration. The water consumption by both test and control animals was measured during the 24-hour exposure.

No harmful effects upon the animals were observed. The test animals drank less of the chemically treated water than the control animals did of the untreated water. This difference may have been due to the objectionable taste or smell of the-chemical. Milk production did not drop nor did the milk have any detectable off-odor. All animals appeared to be normal during and after the study. Analyses of the milk and urine samples, conducted by the Dow Chemical Company, revealed that small quantities of TFM were excreted in the urine. The phenol could not be detected in the milk even with ultrasensitive analytical procedures.