Detection and Measurement of Organic Lampricide Residues

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by

STACY L. DANIELS, LLOYD L. KEMPE, THOMAS J. BILLY,
and ALFRED M. BEETON

U.S. Bureau of Commercial Fisheries
Biological Laboratory
Ann Arbor, Michigan

and

Department of Chemical and Metallurgical Engineering
The University of Michigan
Ann Arbor, Michigan

Great Lakes Fishery Commission
Natural Resources Building
The University of Michigan
Ann Arbor, Michigan

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Stacy L. Daniels, Lloyd L. Kempe, Thomas J. Billy and Alfred M. Beeton

ABSTRACT

The selective lampricide, 3-trifluoromethyl-4-nitrophenol (TFM), and its synergist, 5,2'-dichloro-4'-nitrosalicylanilide (DCN), are separable from natural waters by anion exchange. The adsorbed compounds can then be recovered from the resin as concentrates by elution with selective; solvent mixtures. Measurements of the amounts of lampricides in the final concentrates can be made colorimetrically at 395 mμ for TFM and at 530 mμ for the safranin complex of DCN. TFM has also been separated for quantitative determination from homogenates of whole fish. The fish is first macerated in a blender and then hydrolyzed in hot, 3 N hydrochloric acid. The amount of background color, due to certain components of the fish in the hydrolysate, is reduced by one or a combination of three methods: (1) a series of three extractions with ether, methylene chloride, and benzene; (2) cation exchange followed by methylene chloride extraction; or (3) ether extraction followed by anion exchange and subsequent desorption with amyl acetate-acetic acid.

Introduction

Interest has been growing in the distribution and concentration of pesticides and organic nutrients that may be part of the microorganic content of natural waters, bottom sediments, and animal tissues. The Bureau of Commercial Fisheries Biological Laboratory in Ann Arbor, has undertaken a study of these organic compounds in waters of the Great Lakes region in connection with work on control of the sea lamprey for the Great Lakes Fishery Commission. The detection and measurement of the selective lampricide, 3-trifluoromethyl-4-nitrophenol (TFM), and a synergistic compound, 5,2'-dichloro-4'-nitrosalicylanilide (DCN-also termed Bayluscide by Strufe, 1962, and Howell, *et al.,* 1964), have been of particular concern. Ordinary methods of chemical analysis must be preceded by concentration because of the extremely

low levels of these lampricides to be expected as residues in the environment and/or fish.

The development of suitable analytical procedures for the separation, concentration, and detection of these lampricides has included a study of three techniques: adsorption onto activated carbon, adsorption onto ion-exchange resins, and solvent extraction. Activated-carbon adsorption was considered in an earlier paper (Daniels, et al., 1963); the procedure was a modification of a technique developed by the U. S. Public Health Service (Middleton, Rosen, and Burttschell, 1959). Ion-exchange and solvent extraction for the detection of lampricides in natural waters, bottom sediments, and fish tissues are described in the present paper.

The sodium salt of pure 3-trifluoromethyl-4-nitrophenol (TFM, mol. wt. 207.13) is a yellow-orange, crystalline solid that is soluble in water to the extent of approximately 5,000 ppm at 25° C. The free phenol has a melting point of 75° C. The 2-ethanolamine salt of 5,2'-dichloro-4'-nitrosalicylanilide (DCN, mol. wt. 370.2) is also yellow but is only sparingly soluble in water (230 ± 50 ppm at 25° C). The free compound melts at 216" C. Aqueous alkaline solutions of both compounds are intensely yellow; acidic solutions of TFM are colorless and those of DCN are faintly yellow.

Colorimetric methods have been developed for determining the quantity of TFM present in natural waters which possess conflicting background colors (Smith, Applegate, and Johnson, 1960; Ebel, 1962). DCN can be determined by direct colorimetric measurement of the potassium salt at 385 mμ or indirectly by complexing it with safranin dye, extracting into amyl acetate under basic conditions, and then measuring the color of the complex at 530 mμ (Strufe, 1962). The latter technique may be applicable for determining DCN in the presence of TFM since the latter compound has an absorption maximum at 395 mμ and does not form a complex with the safranin dye.

These colorimetric measurements, however, are limited to solutions containing about 0.1 ppm or greater. The lower limit for applicability of the test may be higher than 0.1 ppm if natural color and turbidity of water are significant. Measurements in samples containing lower levels of the lampricides are impossible without preliminary concentration of the dissolved components to detectable levels. Unfortunately, compounds other than the lampricides also may be concentrated to such a degree that the resulting background color obscures the determination (Shapiro, 1957, 1958). Some methods then must be employed to separate selectively and concentrate the lampricides.

Ion Exchange of TFM and DCN in Water

The low capacity and poor selectivity of activated carbon for the concentration of the lampricides prompted substitution of synthetic ion-exchange resins for this work. The equilibrium between aromatic acids and weak-base exchangers has been studied (Peterson and Gowen, 1953). Sorption of phenols by anion-exchange resins has been reported (Chasanov, Kunin, and McGarvey, 1956) and p-nitrophenol has been observed to exchange (Anderson and Hansen, 1955). The ethanolamine salt of DCN can be exchanged on a cationic resin (Strufe, 1961). Phenolic compounds form negatively charged phenolate ions in basic solution and therefore should exhibit anion exchange. The potassium salts of both TFM and DCN exhibit strong affinities for Dowex[1] 1, x 8, a quaternary ammonium anion-exchange resin in the hydroxide form. Mixtures of TFM and DCN have also been exchanged, and it appears that separation may be achieved by selective elution. The DCN-safranin complex also exchanges under basic conditions. The presence of buffer salts does not appear to reduce the effectiveness of any of the exchanges.

Recovery of TFM from concentrated solutions containing approximately 1,000 ppm of the technical grade TFM (approx. 70 percent 3-trifluoromethyl-4-nitrophenol on a dry basis) in distilled water by the use of anion-exchange resins, followed by elution with selective solvent mixtures, indicated the presence of a number of colored impurities. These other colored components, such as isomers of TFM, dinitro compounds, . . . , are probably structurally related to 3-trifluoromethyl-4-nitrophenol. The manufacturer has reported the composition of crystalline technical grade TFM to be approximately 70 percent 3-trifluoromethyl-4-nitrophenol and 20 percent other isomers, principally 3-trifluoromethyl-6-nitrophenol. This is not surprising since these two isomers are present as the primary nitration products of meta-phenol (Noller, 1958). The general applicability of anion exchange for concentrating TFM would therefore be improved by a better knowledge of the nature and quantities of these impurities.

All of the isomers of TFM have similar spectra in the visible region (Table 1). These spectra are comparable to those of the parent nitrophenols. The position of the nitro group appears to dictate the wavelength at which maximumabsorption occurs and the intensity of the color developed by a particular compound (Fig, 1). Other ring substitutions appear to have lesser influence on these characteristics. The type of solvent in which the spectra

[1]Trade names referred to in this publication do not imply endorsement of commercial products.

Table 1. - Absorption maxima (msx) and molar absorptivities (ϵ) for selected lampricides and related compounds

[All solutions are aqueous unless otherwise specified; values in parentheses are estimates]

Compound	Form	Absorption maxima (λmax)	Molar absorptivities (ϵ x 10^{-4}
2-nitrophenol[1]	Ortha isomer	415	0.528
3-nitrophenol[1]	Meta isomer	393	a.151
4-nitrophenol[1]	Para isomer	399	1.95
3-trifluoromethyl-2-nitrophenol	. . .	405	0.234
3-trifluoromethyl-4-nitrophenolZ	Recrystallized	395	1.313
3-trifluoromethyl-4-nitrophenols	70 percent	395	1.23
3-trifluoromethyl-4-nitrophenol[3]	98 percent	395	1.34
3-trifluoromethyl-5-nitrophenol	. . .	(393)	(0.151)
3-trifluoromethyl-6-nitrophenols	. . .	(408)	(0.522)
2-trifluoromethyl-4-nitrophenol[5]	. . .	(402)	(1.7)
5,2'-dichloro-4'-nitrosalicylanilide?	85 percent	335 375	1.0 1.16
5,2'-dichloro-4'-nitrosalicylanilide	Purified?	330 380	1.1 1.6
5,2'-dichloro-4'-nitrosalicylanilide		385	. . .
5,2'-dichloro-4'-nitrosalicylanilide	Safranin 0 complex	340 530	. . . . . .
5,2'-dichloro-4'-nitrosalicylanilide[9]	Safranin TH complex	530	. . .
2,7-diamino-3,6-dimethyl-10-pheny phenazine [10]	Safranin 0, 97 percent	520	3.26

[1] 0.01 N NaOH (Lang, 1962).
[2] 1 percent NaOH (Smith, Applegate and Johnson, 1961).
[3] 0.01 N KOH
[4] Estimated from data for m-nitrophenol (Lang, 1962).
[5] Estimated from data for the corresponding halo-nitrophenols (Smith, Applegate and Johnson, 1961).
[6] Methanol (Scbraufstatler, 1962).
[7] 0.01 N NaOH (Strufe, 1962).
[8] pH 9.0-9.5 KCl-KOH buffer extracted into amyl acetate.
[9] pH 9.0-9.5 KCl-KOH buffer extracted into amyl acetate (Strufe, 1962).
[10] pH 9.0-9.5 KCI-KOH buffer.

are determined is also important (Briegleb and Angerer, 1952; Lang, 1962).

The spectra of p-nitrophenol, 3-trifluoromethyl-4-nitrophenol (98 percent), and technical grade TFM (70 percent) can be compared (Fig. 2). The absorption maxima of all three are between 395 and 400 mμ. The magnitudes of the molar absorptivities vary somewhat; p-nitrophenol is the highest, followed by 98-percent and 70-percent 3-trifluoromethyl-4-nitrophenol, respectively. The low absorptivity of technical grade TFM (based on a

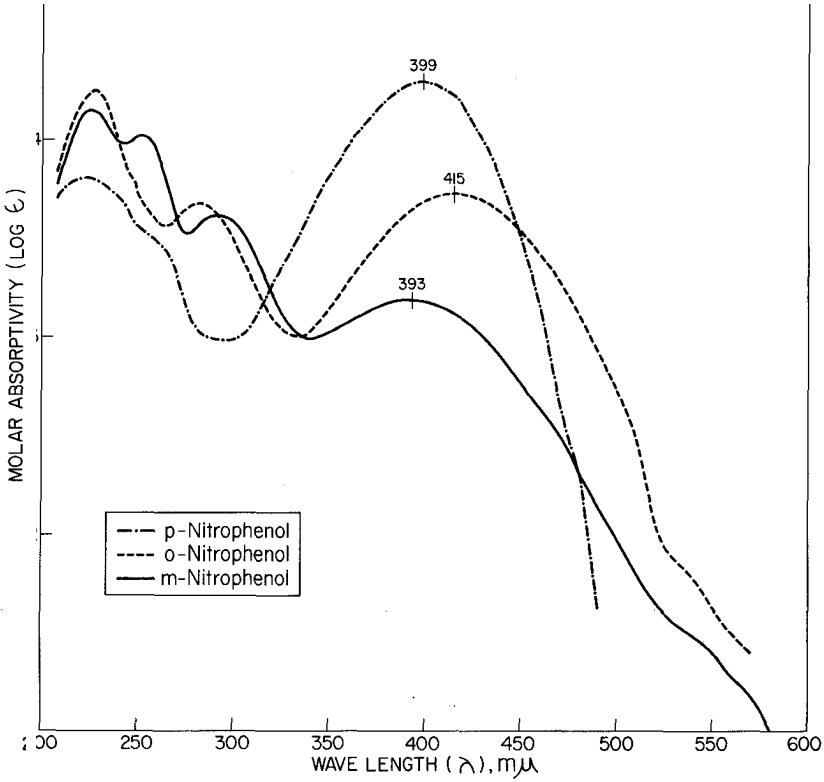


Figure I.-Visible absorption spectra for selected nitrophenols (data from Lang, 1962).

molecular weight of 207.13) probably is due to a small fraction of impurities, principally the ortho-nitro isomers of 3-trifluoromethyl phenol, i.e., 2-nitro- and 6-nitro-3-trifluoromethyl phenol, which have relatively low maxima compared to the para isomer. The maximum absorption of 3-trifluoromethyl-4-nitrophenol (98 percent) is lower than that of p-nitrophenol due to the additional substitution of the trifluoromethyl group on the aromatic ring.

The absorption spectrum of the synergist, DCN (based on a molecular weight of 382.2 for the potassium salt), is similar to TFM in the visible range, but has a much broader maximum near 375 mμ and a secondary peak at 335 mμ (Fig. 3). Distinction between TFM and DCN cannot be made on the basis of visible-absorption measurements in aqueous solutions containing both compounds because of the overlapping absorption peaks. The

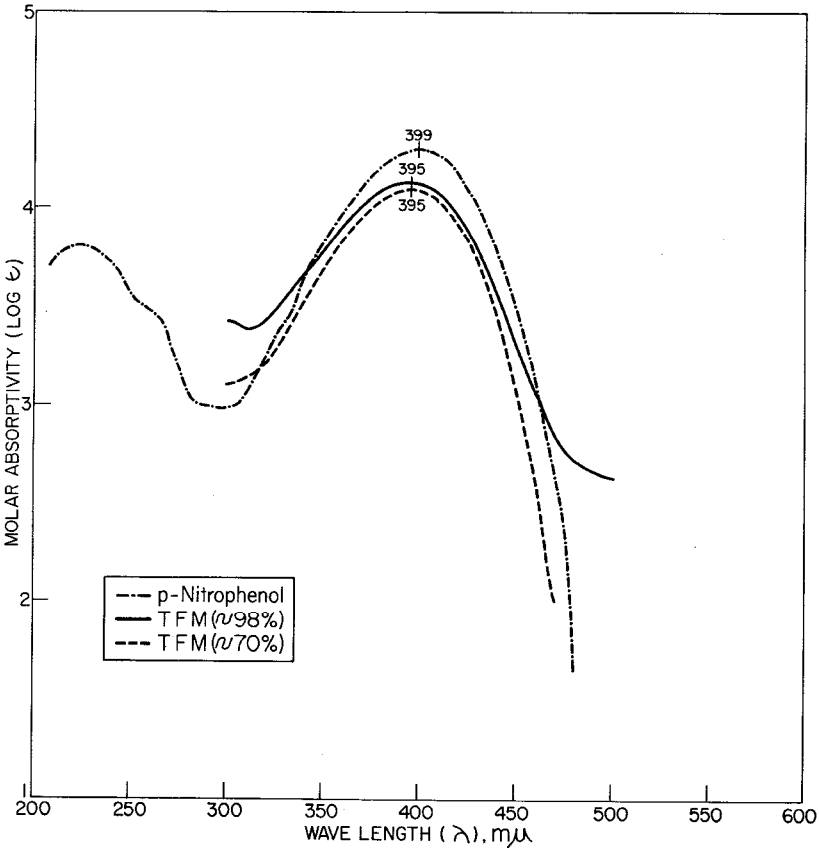


Figure 2. - Visible absorption spectrum of 3-trifluoromethyl-4-nitrophenol.

DCN-safranin dye complex exhibits a different spectrum possessing maxima at 335 and 530 mμ in amyl acetate solution. The complete visible spectrum of the DCN-safranin complex is not presented because of present uncertainty in the molar ratio. Because TFM does not complex with safranin it may be possible to determine DCN in the presence of TFM by using selective complexation and extraction into amyl acetate. Safranin and DCN separately are not appreciably soluble in amyl acetate.

A typical column, initially loaded with the technical TFM formulation, developed several distinctive bands of color upon elution with a solution of potassium hydroxide. The first two bands eluted from the column have been identified tentatively as the ortho-nitro isomers of 3-trifluoromethyl phenol, i.e., the

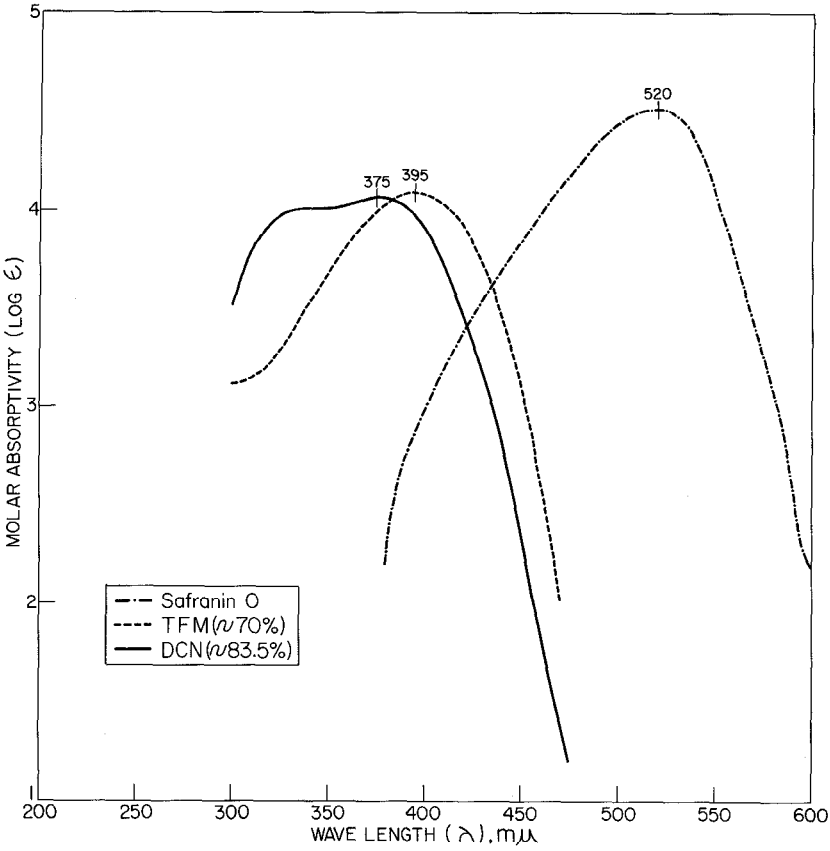
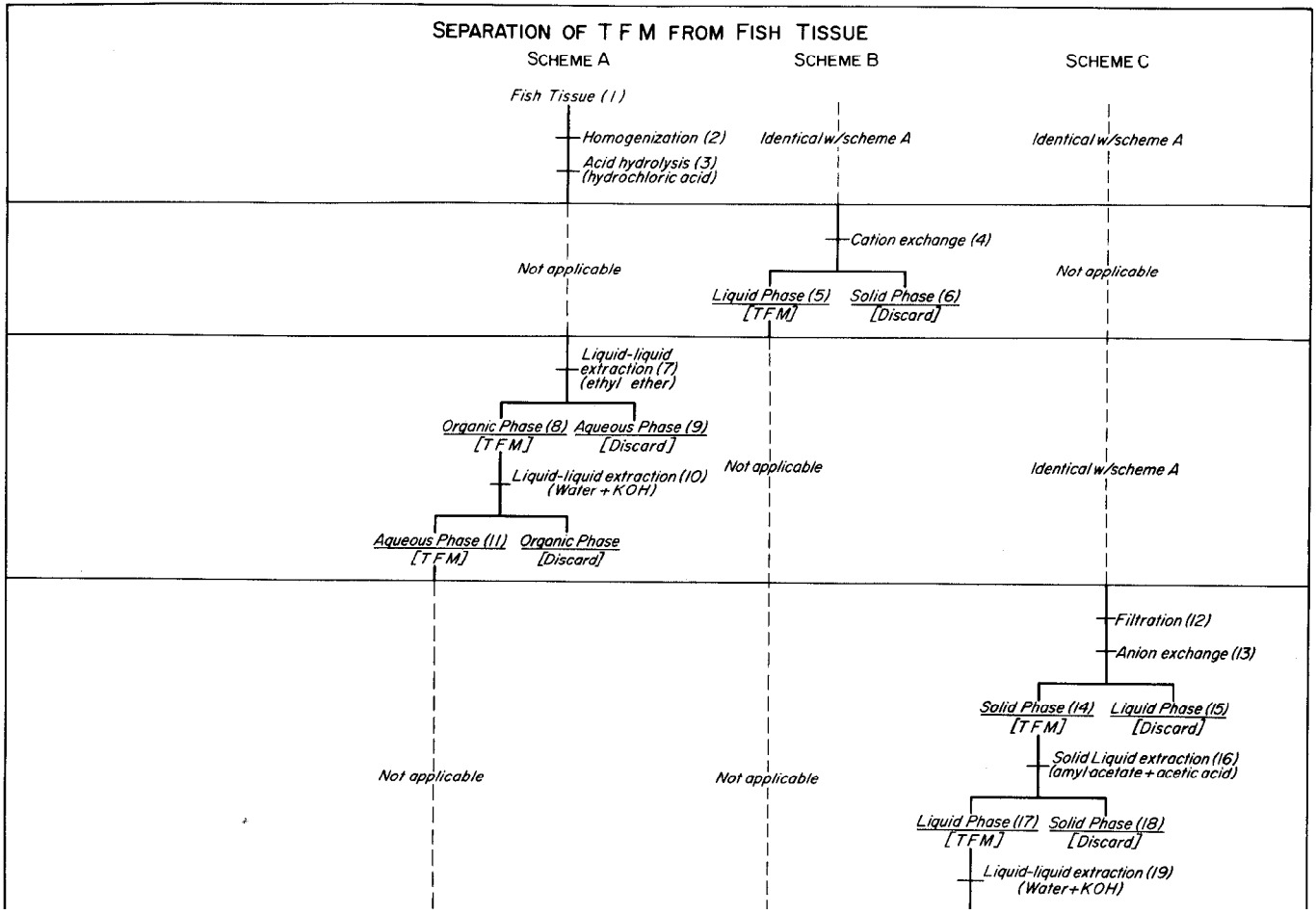


Figure 3. - Visible absorption spectrum of 5,2'-dichloro-4'-nitrosalicylanilide.

2-nitro and 6-nitro isomers. This conclusion was based on the order of elution and the absorption maxima. The spectrum of ortho-nitrophenol is distinct from the meta and para isomers as the maximum absorption is near 415 mμ (Table 1). The absorption maxima of the first two bands eluted from the column were both near 415 mμ. Ortho-nitrophenol migrates 6 to 10 times faster than either the meta or the para isomer when chromatogrammed on silica gel; benzene was the developing solvent and alkaline permanganate was introduced to the columns to detect the position of the phenols (Smith, 1963). A similar behavior of these isomers could be expected in ion-exchange chromatography.

Various components of the technical grade TFM can thus be separated by gradient-elution chromatography with anion-exchange


SEPARATION OF T F M FROM FISH TISSUE
SCHEME A
SCHEME B
SCHEME C
Fish Tissue (1)
Homogenization (2)
Acid hydrolysis (3)
(hydrochloric acid)
Identical w/scheme A
Identical w/scheme A
Cation exchange (4)
Not applicable
Liquid Phase (5) [TFM]
Solid Phase (6) [Discard]
Not applicable
Liquid-liquid extraction (7)
(ethyl ether)
Organic Phase (8) [TFM]
Aqueous Phase (9) [Discard]
Liquid-liquid extraction (10)
(Water + KOH)
Not applicable
Identical w/scheme A
Aqueous Phase (11) [TFM]
Organic Phase [Discard]
Filtration (12)
Anion exchange (13)
Solid Phase (14) [TFM]
Liquid Phase (15) [Discard]
Solid Liquid extraction (16)
(amyl acetate + acetic acid)
Not applicable
Not applicable
Liquid Phase (17) [TFM]
Solid Phase (18) [Discard]
Liquid-liquid extraction (19)
(Water + KOH)

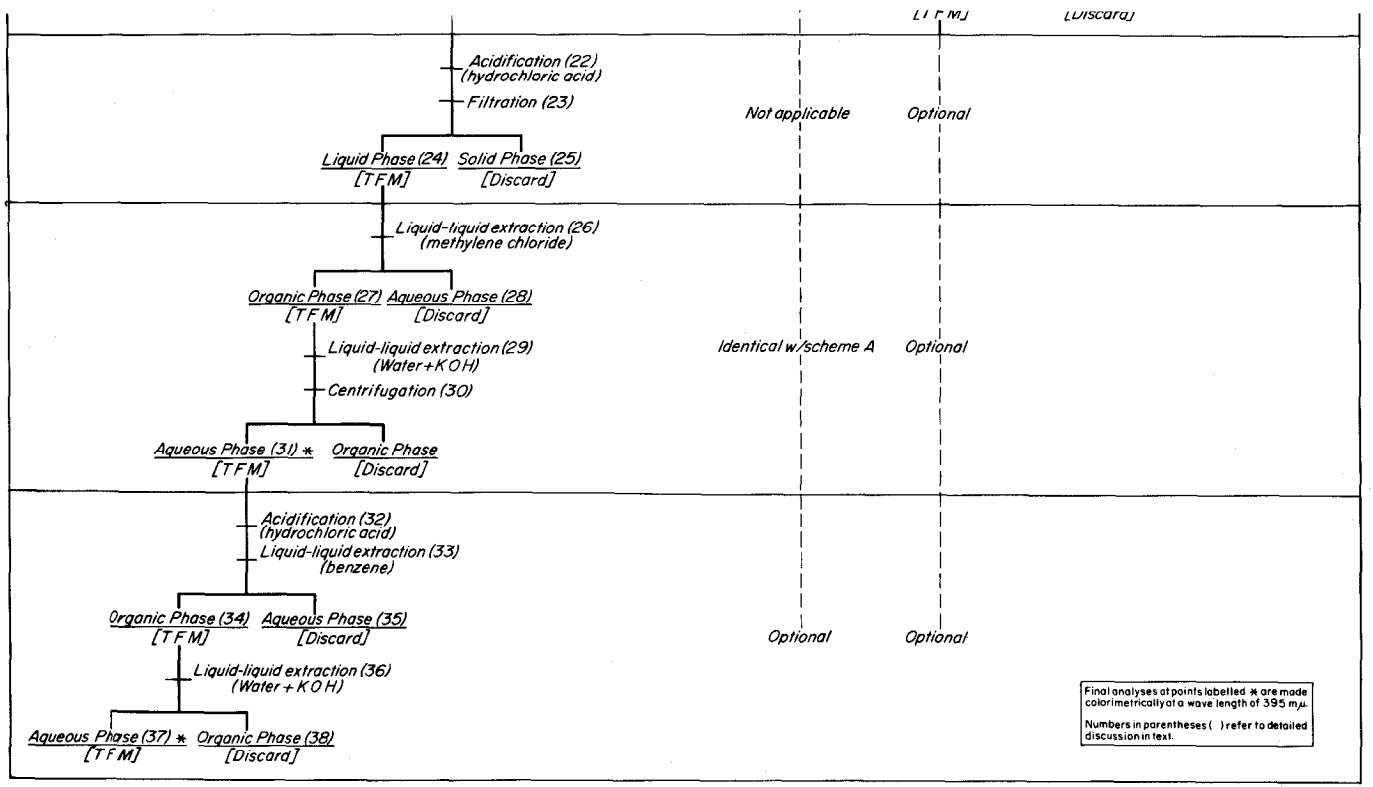


Figure 4. - Separation of TFM from fish tissue. Scheme A, solvent extraction; B, cation exchange; C, anion exchange.

resins. At present, only the absorption maxima of compounds producing color in alkaline solutions and their order of elution have been evaluated. Phenolic compounds can be differentiated by infrared analysis (Smith, Applegate and Johnson, 1961). Solutions of TFM (98 percent) in benzene have been measured to as low as 200 ppm concentration. A change of 0.1 in optical density (7.62 μ) corresponded to a change of 1,700 ppm in concentration.

Presence of other isomers, dinitro compounds, and similar reaction products has not been disproved. Until the question of relative composition is answered fully, analysis by visible absorption for 3-trifluoromethyl-4-nitrophenol is limited to its detection as a group of colored compounds whose absorption maxima in alkaline solution are approximately 395 mμ. The crude lampricide formulation will be termed TFM although the S-trifluoromethyl-4-nitro isomer is believed to be the most selective lampritidal component (Applegate, Howell, and Smith, 1958).

When dilute solutions containing 1 ppm or less of technical grade TFM were passed through a column of anion-exchange resin which was originally peach-colored, a thin yellow band formed at the top of the column. This band started to develop when as little as 50 micrograms of TFM had been adsorbed from solution. Desorption of TFM from the resin was accomplished by passing a small amount of amyl acetate, acidified with acetic acid (approximately 10 percent by volume) through the column. TFM solutions as low as 1 ppb in distilled water were concentrated sufficiently by this method to permit subsequent colorimetric determination of the TFM concentration. Anion exchange, therefore, appears to be applicable in concentrating dilute lampricides. It is expected that this technique will be useful for determining the concentrations of these compounds in natural waters.

Preliminary filtration of natural waters is often required to remove suspended solids that may clog anion-exchange columns. Adjustment of the pH of the samples to 8.5 with potassium hydroxide prior to anion exchange often produced precipitates, principally ferric hydroxide and calcium carbonate. Some naturally occurring organic material may also be coagulated. The precipitated material was removed by passing the collected water, after pH adjustment, through microporous filter disks (Gelman G-5 or Millipore HA, 0.45 μ pore size). The TFM lost by adsorption on the solids collected on the filter disks is assumed negligible because of the prevailing negative electrical charge.

All samples were adjusted to pH 8.5 with KOH, filtered, and then passed through l-gram beds of Dowex 1 x 8, 200/400 mesh, OH form, anion-exchange resin. Subsequent elution was made with amyl acetate (acetic acid 10 percent by volume). The column eluate was then extracted into basic water and TFM concentration

Table 2.-Colorimetric analysis of a natural water sampled before, during, and after treatment with TFM[1]

[Stream: Pentwater River, Oceana County, Michigan; basin: Lake Michigan; location: estuary above Pentwater Lake, T16N, R18W, S24]

Date	Days from treatment date	Before concentration by ion exchange		After concentration by ion exchange	
		Absorbance [2]	Concentration ppm[3]	Absorbance[2,4]	Concentration ppm[3]
April 16, 1964	-1	0.000	0.000	0.0000	0.0000
April 17, 1964	0	.078	1.31	.0629	1.06
April 20, 1964	+3	.003	.050	.0002	.0037
August 20, 1964	+122	.000	.000	.0000	.0000

[1] All absorbance values are averaged for two determinations except day +122.

2 Absorbance measured at 395 mμ blanked against sample taken before treatment commenced (day -1).

3 Equivalent concentration of technical grade TFM in ppm based on a molar absorptivity of ϵ = 12,300 cm^2/mol.

4 Absorbance corrected for tenfold concentration by anion exchange.

measured colorimetrically at 395 mμ. A layer of light-brown material remained adsorbed by the resin. A definite separation was thus effected. A typical analysis of stream samples includes colorimetric measurements before, during, and after treatment with TFM (Table 2). The final absorbance of the sample taken one day prior to treatment (day -1) was very low after pH adjustment and filtration. Measurements of the sample taken during treatment indicate a TFM recovery of approximately 80 percent after anion exchange, elution with amyl acetate-acetic acid, and back-extraction into basic water. A gradual reduction in absorbance has been observed for diluted solutions of TFM stored over a period of time and this factor may be important if such samples are not adequately preserved. The apparent, strong temperature dependency of the ionization constant for TFM also affects the magnitude of color development. The slight absorbance of the sample taken shortly after treatment was completed (day +3) may or may not be significant as a measure of residual TFM. Such low absorbance may only reflect variations in the background color or instrumental changes. A tenfold concentration was effected on the sample for which data are presented. A thousandfold concentration is practical, however, and often is desirable as the absorbance values may be in the extreme low range of the spectrophotometer.

A stream sample collected shortly before treatment is assumed to contain representative amounts of any naturally occurring colors that are present. A water sample taken at any time during treatment will be representative of the amount of chemical present in the water over this period if thorough mixing and uniform treatment concentrations of the lampricides can be assured, and if the uptake of lampricide by the bottom sediments is small compared to the total amount of chemical applied. The lampricide concentration tails off fairly rapidly after treatment is completed and samples taken 6 to 8 hours later must be concentrated prior to colorimetric analysis. A portion of this residium will also include any lampricide that has reversibly diffused into the sediment during treatment when a higher equilibrium concentration exists. The character of the residual concentration as influenced by this latter factor is not completely defined at the present time but its importance is recognized. Further work will be necessary to evaluate this process whereby residual lampricides are slowly released from bottom sediments into the water. Bottom sediments are also being examined for TFM by solvent extraction. Quantitation of the lampricides which have been initially adsorbed or otherwise removed from solution by fish, other aquatic organisms, or bottom sediments prior to analysis is augmented by a knowledge of the relative compositions of the technical grade chemicals introduced into the surrounding waters .

Solvent Extraction of TFM and DCN in Fish

A suitable technique for the release of the lampricides bound in fish tissues or bottom sediments requires that the TFM first be released into a separated soluble form for colorimetric determination of concentration. Three alternate schemes have been developed for the separation of TFM from fish tissue which are also broadly applicable to bottom sediments (Fig. 4, A,B,C). Numbers in parentheses in the figure refer to detailed discussion in the text.

The initial steps are identical in all three schemes. The fish tissue (1) to be analyzed is first homogenized (2) in water with a high-speed blender. The resulting slurry is hydrolyzed (3) by refluxing for 24 hours in 3 N hydrochloric acid. This hydrolyzed solution of soluble polypeptides, amino acids, insoluble oils, and acid-insoluble humin (Gortner, 1949) has a deep brown color. TFM in aqueous solution is not affected by this acid and heat treatment. Preliminary evidence indicates that the DCN molecule may be split by this severe treatment and the procedure at present is therefore limited to TFM. The hydrolyzed solution is allowed to cool, made up to volume, and aliquots taken according to the scheme employed.

The first scheme (Fig. 4A) is based on classical solvent-extraction methods. The distribution coefficient for TFM between polar and nonpolar solvents depends upon the pH of the solution. No detailed investigations have been conducted to determine the optimum pH range for extraction under these conditions; it appears, however, that an acid range of 2-4 and a basic range of 8-10 (below and above the pKa of 6.07) are suitable for TFM extraction from acid water to organic solvent and from organic solvent to basic water, respectively. In acidic solutions, TFM exists principally in its nonionized form and the distribution coefficient favors the organic solvent, ethyl ether (7). A water-solvent ratio of 10:1 is established and the aqueous acid solution (3) is extracted five times. The aqueous phase (9) is discarded. The combined ethyl ether extracts (8) are then extracted (10) with three portions of dilute KOH (volume ratio 3:1). The basic extracts (11) are combined and then warmed slightly to vaporize the ethyl ether from solution. Warming diminishes the amount of dissolved ether and reduces the possible effects of this solvent on the distribution coefficient of the next solvent-extraction pair.

At this point the basic sol&ion can either be brought up to volume and divided into aliquots, or carried through in its entirety to the next solvent-extraction step. Regardless of the procedure, the solution is again acidified (22) in the same manner as previously stated in the ether-extraction step. The acidified solution

is filtered (23) under vacuum with a Büchner funnel and Whatman No. 1 filter paper to remove any insoluble material. The filter paper and particulate matter (25) are then washed several times to insure complete removal of any retained TFM.

The filtered solution (24) is next extracted (26) five times with methylene chloride (volume ratio 10:0). The extracted aqueous phase (28) is discarded. The combined methylene chloride extracts (27) and washings are extracted (29) with dilute KOH in a manner similar to that in the earlier ether extraction. The basic aqueous phase (30) often takes on an opalescent color which prevents direct colorimetric analysis. Phase separation is also frequently hampered and appears to be a function of pH. It is therefore necessary to centrifuge this basic aqueous phase to achieve a good separation (31). Colorimetric analysis can be made at this point if the background color is sufficiently reduced in the blank determination containing fish but not TFM.

A third extraction accomplishes further separation of the lampricides from interfering background colors. The aqueous phase (31) from the previous extraction is first acidified (32) and can be used directly or made up to a measured volume and then divided into aliquots. The third extraction with benzene (33) is carried out in a manner similar to the earlier extractions with organic solvents (34-38), The basic aqueous extraction of the benzene phase is analyzed colorimetrically. In general, this method has achieved good results. The degree of concentration of minute residues is limited, however, by the various solvent proportions.

In the second procedure (Fig. 4B) an aliquot is passed through a prepared column of Dowex 50W x 8, 50/100 mesh, hydrogen form, cation-exchange resin (4). This step removes much of the residual color; material that is insoluble in acid solutions is also filtered out (6). The column is then washed with 3 N hydrochloric acid. Any TFM present passes through the column (5). The tacit assumption is made, however, that no TFM remains bound to the acid-insolubles, which usually constitute a small fraction of the total weight of hydrolyzed tissue, or to the ion-exchange resin. The pH of the solution is highly critical at this stage. If the pH is too low, nonionized TFM tends to "adsorb" onto the cation exchanger or the filtered solids and resists desorption during the subsequent washing with acidified water. This effect limits the general applicability of the test because recovery of the TFM has not always been quantitatively reproducible.

The combined effluents from the cation-exchange column are very faintly colored and still acidic. Under these conditions TFM exists as the nonionized phenol which ordinarily is not subject to cation exchange and is more soluble in nonpolar than in

polar solvents. The distribution coefficient between methylene chloride and acidic water greatly favors the organic phase. The aqueous solution which has previously been passed through a cation exchanger is next extracted with several portions of methylene chloride, which is immiscible with and denser than water. The combined methylene chloride extracts are then extracted with several portions of water adjusted to pH 10 with potassium hydroxide. TFM exists principally as the phenolate ion in solutions which are basic to its pKa of 6.07. In this condition TFM is more soluble in a polar than in a nonpolar solvent. If TFM is present in the organic phase, its presence will be dramatically demonstrated by its characteristic yellow color when it transfers to the alkaline water. The distribution between phases in this step so greatly favors the aqueous phase that three extractions may be sufficient for quantitation unless the final concentration is very high or unless the proportion of the phases is extremely adverse.

A third scheme (Fig. 4C), based upon anion-exchange as employed in the analysis of natural waters, combines the best features of the two previous ones. This scheme parallels the solvent-extraction procedure previously described (Fig. 4A) through the ether-extraction step. Suspended particles in the basic extract (11) from the ether extraction tend to produce partial clogging of the resin columns and are removed by passage through sintered glass filters (12) prior to exchange. The filtered solution is then brought up to volume and aliquots are taken to be passed through a column of an anion-exchange resin (13) prepared as follows:

The column containing Dowex 2 x 8, 200/400 mesh, anion-exchange resin in chloride form, is washed initially with distilled water and then with amyl acetate containing 10 percent acetic acid by volume. Conflicting colors that have been produced by the reaction of concentrated mineral acids with amyl acetate necessitate the substitution of acetic acid. Residual colors due to impurities that sometimes remain in the resin after manufacture and which may obscure the analysis are also removed by this washing. A 1 N solution of KOH is then passed through the column to insure conversion of the resin to the hydroxide form. This change is evidenced by a distinct color change from white (chloride form) to peach (hydroxide form). The resin column is now ready to receive the sample solution. The major portions of the colored components in the sample pass through and are not exchanged (15). A light brown color replaces the original peach color of the resin when fish hydrolysates are thus exchanged (14). Both TFM and DCN are exchanged rapidly and if present in sufficient quantity, form yellow bands at the very top of resin columns. These bands

are partially obscured, however, if the solutions to be exchanged contain a high proportion of exchangeable fish components.

After a solution containing fish hydrolysate and lampricide is passed through the anion-exchange column, the resin bed is washed with distilled water to remove any residual nonexchanged material. A solution (16) of amyl acetate, acidified with acetic acid (10 percent by volume) is then passed through the moist resin. A large distribution coefficient is evidenced for TFM between resin and amyl acetate under these conditions and the first 50 ml of eluent (17) contains essentially all of the TFM. Some difficulty may be experienced, however, if gas bubbles formed by the reaction between acid and exchanged carbonates disturb the resin column. The acidified amyl acetate selectively desorbs the lampricides and a brownish residue (18) of fish components is unaffected. Desorption of the lampricides from the anion-exchange resin is limited by the rate of diffusion from the interstices of the resin particles and the efficiency of solvent contact. A small-mesh resin and a low flow rate are important for best recovery. The amyl acetate phase is then extracted with basic water (19). The extracted organic phase (21) is discarded. The concentrations of the lampricides thus separated are measured colorimetrically (20) and compared to a fish blank, containing no lampricides, that is carried through the same treatment. Additional solvent extractions with methylene chloride and/or benzene are optional if further separation is considered necessary.

Analysis of bound lampricides in fish tissues by either solvent extraction or by combined cation exchange-solvent extraction requires further refinements to yield quantitative results. Resolution of the problems involved in reducing conflicting background colors in the final extracts and of recovering high percentages of the lampricides is necessary to insure highly sensitive methods. Background color, due to fish components, is reduced greatly in anion exchange as both TFM and DCN are adsorbed selectively by the anion-exchange resin and then desorbed by acidic amyl acetate. The anion-exchange procedure appears to possess the greatest potential for obtaining quantitative separation of lampricides from fish tissues. The procedure is fairly direct and the background color is the lowest of the three techniques followed. All of the procedures considered here are still under investigation, however, and may require modification for different species of fish, types of bottom sediment, or water samples.

Summary

1. Development of ion-exchange and solvent-extraction methods has been initiated for the analysis of natural waters,

bottom sediments, and fish tissues for the lampricide TFM and its synergistic compound DCN.

2. Natural waters were analyzed directly for their lampricide concentrations by anion exchange followed by elution with selective solvent mixtures.

3. Tissue homogenates of fish to which known quantities of TFM were added in the laboratory were analyzed for bound lampricides. The fish were first hydrolyzed in 3 N HCl; the background color was then reduced either by passing the hydrolysate through ion-exchange resins, by a series of solvent extractions, or by a combination of these techniques.

4. At present, detection and measurement are limited to the colored isomeric compounds present in technical grade TFM which absorb light at 395 mμ. DCN can be determined separately as the safranin dye complex at 530 mμ.

5. The methods employed for separating and selectively concentrating the lampricides to levels that can be determined colorimetrically are based on standard procedures applicable to other similar organic compounds.

Literature Cited

ANDERSON, R. E., and R. D. HANSEN

1955. Phenol sorption on ion exchange resins. Ind. Eng. Chem., 47: 71-75.

APPLEGATE, VERNON C., JOHN H. HOWELL, and MANNING A. SMITH

1958. Use of mononitrophenols containing halogens as selective sea lamprey larvicides. Science, 127: 336-338.

BRIEGLEB, G., and G. ANGERER

1952. Mesomerism of the nitrophenolate ions from the standpoint of a new effect in light absorption. Angew. Chemi., Bd. 64, S. 685-686.

CHASANOV, M. G., R. KUNIN, and F. McGARVEY

1956. Sorption of phenols by anion exchange. Ind. Eng. Chem., 48: 303-309.

DANIELS, S. L., L. L. KEMPE, E. S. GRAHAM, and A. M. BEETON

1963. Quantitation of microorganic compounds in waters of the Great Lakes by adsorption on activated carbon. Great Lakes Res. Div. Inst. Sci. Tech., Univ. of Mich., Pub. NO. 10, pp. 118-123.

EBEL, WESLEY J.

1962. A photoelectric amplifier as a dye detector. Great Lakes Fishery Commission, Tech. Rep. No. 4, pp. 19-26.

GORTNER, R. A.

1949. Outlines of Biochemistry. John Wiley & Sons, Inc., New York. 3rd. Ed., pp. 324-328.

HOWELL, JOHN H., EVERETT L. KING, Jr., ALLEN J. SMITH, and LEE H. HANSON

1964. Synergism of 5,2'-dichloro-4'-nitro-salicylanilide and 3-trifluormethyl-4-nitrophenol in a selective lamprey larvicide. Great Lakes Fishery Commission, Tech. Rep. No. 8, 21 pp.

LANG, L., Ed.

1962. Absorption spectra in the ultraviolet and visible region. Vol. III, Publishing House of the Hungarian Academy of Sciences. Budapest.

MIDDLETON, FRANCIS M., AARON A. ROSEN, and RICE H. BURTTSCHELL

1959. Manual for the recovery of organic chemicals in water. Robert A. Taft Sanitary Engineering Center, Cincinnati, Ohio, 52 pp.

NOLLER, CARL R.

1958. Chemistry of organic compounds. 2nd Ed., W. B. Saunders Co., Philadelphia, pp. 439-444, 507.

PETERSON, SIGFRED, and ELMER GOWEN

1953. Equilibria between aromatic acids and weak base anion exchangers. Ind. Eng. Chem., 45: 2574-2586.

SCHRAUFSTATLER, E.

1962. Chemical development of Bayluscide. Pflanzenschutz-nachrichten "Bayer," 15: 25-33.

SHAPIRO, JOSEPH

1957. Chemical and biological studies on the yellow organic acids of lake water. Limnol. & Oceanogr., 2: 161-179.

1958. Yellow acid-cation complexes in lake water. Science, 127: 702-704.

SMITH, B.

1963. Simple adsorption chromatographic method for the investigation of phenolic structures. Acta. Chem. Stand., 16: 843-848.

SMITH, MANNING A., VERNON C. APPLEGATE, and B.G.H. JOHNSON

1960. Colorimetric determination of halogenated nitrophenols added to streams as sea lamprey larvicides. Anal. Chem., 32: 1670-1676.

1961. Physical properties of some halo-nitrophenols. J. Chem. Eng. Data, 6: 607-608.

STRUFE, REIMER

1961. Field tests for thecolorimetric determinationof the molluscicide Bayer 73. Bull. World Health Org., Vol. 25, pp. 503-507.

1962. Determination of Bayluscide in field tests. Pflanzenschutznachrichten "Bayer," 15: 42-49.

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