A Rapid and Sensitive Method for Gas Chromatographic Analysis of the Selective Piscicide, "Squaxon"

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ABSTRACT

"Squaxon" (1,1'methylenedi-2 napthol; bis (2 hydroxy-1-napthyl) methane) was discovered by MacPhee and Ruelle (1968, 1969) to exhibit piscicidal properties that are highly species specific. The chemical is lethal to the Northern (Ptychocheilus oregonensis) and the Umpqua (P. umpquae) squawfish, freshwater cyprinids common to the Pacific Northwest, at water treatment concentrations of less than 100 parts per billion (ppb). Other species of fish, including the more desirable salmonids with which the squawfish competes ecologically, are unaffected until this dosage has been increased severalfold. Because squawfish are widely regarded as undesirable for food or sporting purposes, and because competition from squawfish has severly depleted salmonid populations in some areas (Thompson, 1959; Jeppson and Platts, 1959) the potential use of squaxon for fisheries improvement is quite obvious. Squaxon is currently being tested for this purpose on an experimental basis in selected areas in Idaho, Oregon and Washington, pending federal clearance for more widespread application.

Commercially formulated squaxon currently being field tested is dispersed as an ethanolic solution of the monosodium salt. The treatment solution consists of 39.5 per cent (by weight) 1,1' methylene-di-2 napthol dissolved in 60 per cent denatured ethyl alcohol. Traces of sodium hydroxide are also present in the commercial solution (Keating, 1972). We report here an analytical method by which petroleum ether-extracted, brominated squaxon can be quickly and easily detected in water and fish tissue by electron capture gas chromatography.

EXPERIMENTAL SECTION

Gas Chromatographic Conditions. A Micro-Tek 220 gas chromatograph (Micro-Tek Instruments Corp., Austin, Texas) equipped with a tritium foil parallel plate electron affinity detector was utilized for this study. A borosilicate glass, U shaped column, six feet in length with an outside diameter of 1/4 inch and an inside diameter of 5/32 inch was packed with 4 per cent SE-30, 6 per cent QF-1, on Chromosorb W.H.P., 80-100 mesh. The SE-30 column packing was prepared using a solvent/support ratio of 100/20 (milliliters per gram), and was placed in a column conditioner for 60 hours at 245° C. Liquid phases and solid support were obtained from Supelco, Bellefonte, Pennsylvania.

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Column temperature (isothermal) was 175° C, with the detector at 205° C and injection port at 220° C. Highly purified nitrogen was used as the carrier gas at a flow rate of 90 ml per minute. Off-column injection techniques were used, injecting the sample into a 4 inch, baffled glass insert placed directly above the columns.

Chemicals, Reagents and Glassware. 1,1'methylenidi-2-napthol (squaxon) monosodium salt analytical standard, 98.7 per cent, lot no. AD71-31, was supplied by the American Cyanamid Co., Princeton, New Jersey. Commercial squaxon monosodium salt ethanolic dispersion solution was obtained through the courtesy of American Cyanamid and the Idaho Department of Fish and Game. Petroleum ether, diethyl ether, hexane and methyl and ethyl alcohol of nanograde quality were obtained from Mallinckrodt Chemical Company, St. Louis, MO. Reagent grade potassium bromide and ferric ammonium sulfate were prepared by J. T. Baker Chemical Co., Phillipsburg, NJ. Reagent grade sulfuric acid was obtained from Dupont De Nemours and Co., Inc., Wilmington, Delaware. All distilled water used in the method was first extracted with nanograde hexane in order to eliminate possible interfering peaks during chromatographic analysis. To avoid contamination, all glassware was rinsed first with acetone and again with nanograde petroleum ether prior to use.

Brominating Solution. Stock brominating solution was prepared by dissolving 360 g of potassium bromide in 700 ml of preextracted, distilled water in a one liter volumetric flask. In a separate container, 110 ml of concentrated reagent sulfuric acid was carefully combined with 110 ml of distilled water. After allowing to cool, this mixture was then slowly added to the potassium bromide solution. Ten g of ferric ammonium sulfate were then added directly to the solution, the contents were mixed vigorously to insure maximum dissolution, and then allowed to cool and settle. As a final precaution against contamination, the final stock solution was again extracted with nanograde petroleum ether, using a ratio of 4 parts brominating solution to 1 part petroleum ether.

Squaxon Standard. Squaxon stock solution was prepared by dissolving 20 mg squaxon standard in 100 ml of nanograde methanol in a 100 ml volumetric flask. This solution is photosensitive and should be stored in a dark bottle. However, change in coloration does not appear to affect quantitative results. Squaxon working solution was prepared by diluting 1 ml of stock solution with nanograde methanol to a total volume of 50 ml. The final concentration of squaxon in the working solution was 4 nanograms per microliter.

Bromination of a standard was done as follows: One ml of working solution was taken to dryness in a 30 ml screw-cap glass tube. Twenty ml of the previously prepared brominating solution was then added, and the securely capped tube was mixed by gentle inversion and then placed on a rotary mixer at low speed (50-60 ppm) for ten minutes. The brominated squaxon was then extracted from this solution with nanograde petroleum ether. One ml of petroleum ether was added to the tube and the contents mixed on the rotary mixer for five minutes. The ether layer was then removed by disposable pipette and placed in a 13 ml centrifuge tube and the ether extraction was repeated once more. The two etheral fractions were combined and evaporated under nitrogen to a final volume of 1 ml. (Depending upon gas chromatographic capabilities and the particular analytical range desired, this 4 ng/ul solution may be diluted further with either petroleum ether or hexane, if so desired. However, routine utilization of the brominated standard in our laboratory has been at a final concentration of 100 picograms per microliter.) Six microliters of the final solution were then injected into the gas chromatograph.

Extraction from Water. Since squaxon has been shown to undergo some photodecomposition (Terriere, et al., 1970), all water samples were collected and stored by dark bottle techniques. Two hundred ml of water sample were placed in a 250 ml separatory funnel. One ml of 10% NaOH solution was then added, followed by 20 ml of diethyl ether containing 2 per cent ethanol. The contents were mixed vigorously for one minute and then allowed to separate. The aqueous layer was then drained into a clean container, and the ethereal portion placed in a 30 ml screw-cap glass tube. The aqueous phase was then transferred back into the original separatory funnel and extracted twice more in the same manner with ten ml aliquots of ethanolic ether. After partitioning and separation as before, the aqueous phase was then discarded and the three combined ether extracts were evaporated to dryness under a stream of dry nitrogen. Prior to evaporation, small quantities of water that occasionally formed at the bottom of the tube were removed by disposable pipette. As soon as the ethereal fractions had reached complete dryness, 20 ml of the previously prepared brominating solution were added and the tube was capped securely. The contents were slowly mixed by inversion, and then agitated gently at low speed (50-60 rpm) on a rotating mixer for ten minutes. The brominated squaxon was then recovered from this solution by extracting twice with 1 ml portions of petroleum ether, as described previously for the brominated standard, and a six microliter volume of the final ether concentrate was injected into the gas chromatograph.

Extraction from Fish Tissue. This report describes the analysis of squawfish muscle tissue as a representative sample. One gram of tissue was weighed out and placed in a small soxhlet thimble. This was inserted into a 125 ml soxhlet flask and extracted with 100 ml of petroleum ether for six complete cycles. The ether extract was then evaporated to dryness by placing the flask under a stream of dry air. Twenty ml of brominating solution were added to the flask and allowed to react for one hour, with intermittent mixing. The contents were then decanted into a 30 ml screw-cap glass tube, from which the brominated squaxon was then extracted with petroleum ether. One ml of petroleum ether was added to the tube, which was then capped securely and agitated on the rotary mixer at 50-60 rpm for 30 minutes. The contents were allowed to separate and as much as possible of the ether layer was removed by disposable pipette and placed in a 13 ml centrifuge tube. The extraction was then repeated once more with an additional 1 ml aliquot of petroleum ether. The combined ether extracts were concentrated to a 1 ml volume, and six microliters of this were injected into the gas chromatograph.

RESULTS AND DISCUSSION

Representative chromatographs illustrating the elution patterns of brominated squaxon standard in methyl alcohol are shown in Figure 1. The squaxon peak is clean and definitive in all cases, and the relationship between peak height and squaxon concentration is linear throughout all the various concentrations analyzed (50-300 nanograms).

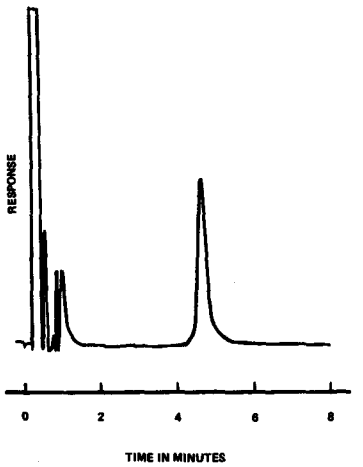

Figure 1(a) Brominated squaxon standard extracted from methyl alcohol. Peak value = 610 picograms.

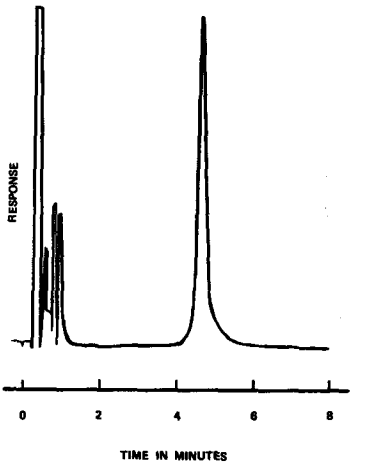

Figure 1(b) Brominated squaxon standard extracted from methyl alcohol. Peak value = 1220 picograms.

Figure 2 shows a chromatograph from an extracted water sample from a laboratory aquarium in which a Northern squawfish was experimentally poisoned with commercial squaxon ethanolic dispersion solution at a level of 10 ppm. Prior to analysis the water sample had been stored in a dark bottle at room temperature for two weeks. As with the standards seen in Figure 1, the extracted water sample results in a clear cut squaxon peak. Terriere, et al., (1970, 1973) have shown that squaxon decomposes rapidly in water in the presence of oxygen and light. Consequently, the recovery of only 20 parts per billion in the water sample is possibly due to this relative instability of the molecule. Minimum levels of detectability for squaxon in water using this procedure are currently at the level of 100 picograms (pg), or 85 parts per trillion for a 200 ml sample. Recovery has been 100 per cent, as based on a previously added 4 microgram quantity (20 parts per billion for a 200 ml water sample) of squaxon.

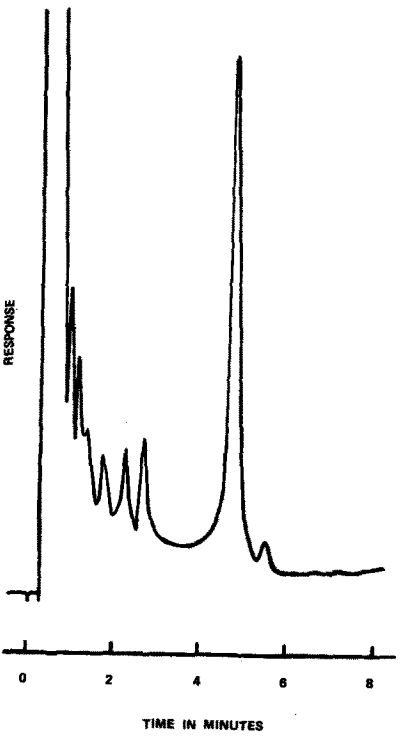

Figure 2. Brominated squaxon extracted from a 200 ml water sample. Peak value = 20 parts per billion.

Figure 3 shows a chromatograph obtained from the analysis of muscle tissue from a Northern squawfish (Ptychocheilus oregonensis) poisoned in the laboratory with the commercial ethanolic squaxon commercial dispersion solution. Although other small peaks are present near the injection point, the squaxon peak is clear and very definitive. However, because of the greater difficulties encountered thus far in cleanup, sensitivity of the method for squaxon in fish is currently somewhat more limited than in the case of water analysis. The minimum detectable limit for squaxon in fish tissue using this method is approximately 600 picograms, or 140 parts per billion (ppb) for a one gram tissue sample. Per cent recovery has averaged 85 per cent from a tissue spiked previously with a four microgram (or, 4 parts per million for a 1 g sample) quantity of squaxon. We are currently examining the suitability of the method for extracting squaxon from various other tissues from additional species of fish, as well as from other common species representative of aquatic ecosystems.

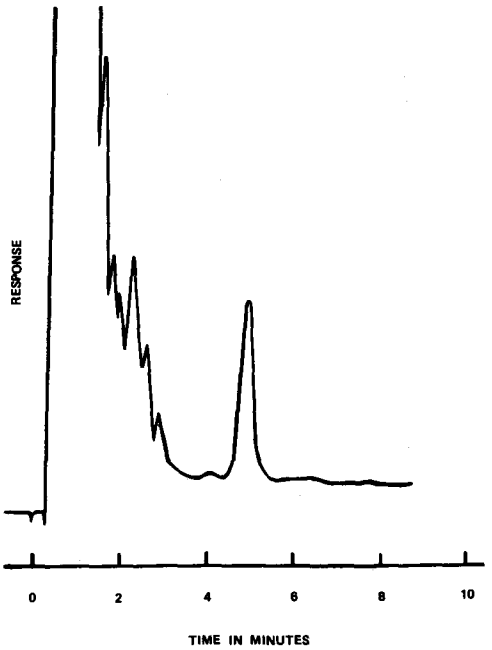

Figure 3. Brominated squaxon extracted from flesh of a Northern squawfish poisoned under laboratory conditions. Peak value = 147 parts per billion.

Terriere, et al., (1970) have successfully extracted squaxon from acidified water with both carbon tetrachloride and dichloromethane. The extracted residue is then reacted with diazo blue and the resulting color is read spectrophotometrically to determine the quantity of squaxon present. This colorimetric method permits analysis of water at the one microgram level of sensitivity. It is hoped that the gas chromatographic method reported here will provide a more sensitive means of complimenting their pioneer efforts in monitoring squaxon in the aquatic environment, should the chemical achieve more widespread use as a selective fish toxicant.

ACKNOWLEDGMENT

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