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ANALYTICAL METHOD FOR TOXAPHENE IN FISH TISSUE

by

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MANAGEMENT PERSPECTIVE

The methodology for toxaphene analysis in fish tissue, described in this report, was initiated at the request of the National Water Quality Laboratories. Toxaphene is a high priority chemical found in environmental samples from the Great Lakes area. The procedure used by the NWQL for the analysis of other organochlorines was modified with the emphasis being placed on the quantitation of toxaphene, a multicomponent mixture. Using gas chromatography, 10^{-11} g of toxaphene can be detected, but only amounts in excess of 2×10^{-10} g/g of sample can be quantified.

PERSPECTIVE GESTION

La méthode de dosage du toxaphène dans les tissus des poissons, décrite dans ce rapport, a été instituée à la demande du Laboratoire national de la qualité des eaux. Le toxaphène est une substance chimique d'intérêt très prioritaire présente dans des échantillons environnementaux prélevés dans la région des Grands Lacs. La méthode suivie par le Laboratoire national de la qualité des eaux pour l'analyse d'autres composés organochlorés a été modifiée principalement en vue de doser le toxaphène, un mélange à plusieurs composants. A l'aide de la chromatographie en phase gazeuse, on peut déceler 10^{-11} g de toxaphène, mais on peut seulement déterminer des quantités de plus de 2×10^{-10} g/g d'échantillon.

MÉTHODE DE DOSAGE DU TOXAPHÈNE DANS LE POISSON

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RÉSUMÉ

Une méthode par chromatographie en phase gazeuse à capture d'électrons a été mise au point pour doser le toxaphène dans les tissus des poissons. Les étapes de nettoyage étaient des modifications d'une méthode existante utilisée pour doser les composés organochlorés dans des échantillons environnementaux. On a surtout cherché à isoler, dans le chromatogramme complexe du toxaphène, des pics qui n'étaient pas perturbés par d'autres composés organochlorés, qui permettaient d'obtenir un taux de récupération quantitatif à une concentration de 0,2 ppm et qui donnaient des résultats reproductibles. Pour le dosage, on a choisi onze pics qui respectaient ces exigences dans la plage de concentrations de 0,2 - 5 µg/mL avec un taux de récupération quantitatif ($>$ 85 %). La justesse était généralement supérieure à 6 %.

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ABSTRACT

An electron-capture-gas chromatographic method has been developed for the analysis of toxaphene in fish tissue. The cleanup steps were modifications of an existing method used to determine organochlorine concentrations in environmental samples. Emphasis was placed in selecting peaks from the complex chromatogram of toxaphene which were not interferred with by other OC's, which gave quantitative recoveries at 0.2 ppm and yielded reproducible results. Eleven peaks were selected for the quantitation which followed these requirements in the concentration range of 0.2 µg/mL to 5 µg/mL with quantitative recoveries (>85%). Precision was generally better than 6%.

INTRODUCTION

Toxaphene, produced from the chlorination of camphene, has been found in significant levels in the fish from the Great Lakes area (Onuska and Terry, 1985). The finding of toxaphene in these fish is surprising as the pesticide is not extensively used in the Great Lakes

basin. To assess the extent of the possible problem arising from the presence of toxaphene in the food web, an accurate analytical method must be used. There are several methods that are in use in analytical laboratories and undoubtedly each has its own advantage. However, the thrust of this work is to use a slightly modified method that is currently being utilized for analyzing chlorinated pesticides in a routine analytical laboratory (Analytical Methods Manual).

The effort was divided into two sections. First was to ascertain the range, precision and reproducibility of the analysis, taking the compound through the cleanup procedures, and determining the percentage loss of the constituent peaks. The second section involved the extraction and quantification of toxaphene from fish tissue.

Toxaphene, the formulated pesticide, is a complex mixture of norbornyl-type halogenated compounds. The complexity of the mixture is reflected in the chromatogram of toxaphene shown in Fig. 1. The shape of the chromatogram suggests many of the peaks are sitting on a continuum of unresolved components. This chromatogram was obtained by using a modern (1985) gas chromatographic system including fused silica capillary column and electron capture detector. With less sensitive instrumentation (Hughes et al., 1970), the number of resolved peaks is considerably lower.

Rubick et al, 1982 reported on a method that utilized a number of mixed solvent column chromatographic techniques. Quantitation was performed by summing selected glc peaks. As their report is the most recent state-of-the-art document, we were interested in checking on several aspects, not to criticize the work, but to enhance our understanding of the behavior of toxaphene. We wanted to determine the behavior of the individual contributing peaks over a range of concentrations and examine the recovery of these peaks compared to standards over the same concentration range. Once this was known, the results could be applied to the fish tissue. In addition, a selection of other common organochlorine compounds were added. This permitted selection of toxaphene peaks that were not interferred with so that the toxaphene and the OC's may be determined at the same time. The resulting method was then applied to the results from the fish tissue. This method is given in the following.

METHODS

(a) Materials :

The solvents acetone, cyclohexane and methylene chloride were distilled in glass quality supplied by Caledon Chemicals. Hexane,

benzene and iso-octane were high purity solvents supplied by Burdeck and Jackson Co. Ltd., Michigan. The toxaphene standard was supplied by the National Bureau of Standards, Washington, D.C. Interferences were contained in four solutions. The first designated as WQB, contained the organochlorines listed in Table 1. The table also shows the concentration of the OC's. The second solution was a mixture of 1242, 1254 and 1260 PCB's at a total concentration of 200 pg/L. The third solution contained technical grade chlordane, made up from standards purchased from Polyscience Corp., Niles, Ill. The fourth solution, denoted as WQA, contained aldrin, DDE, hexachlorobenzene, DDO, DDP, mirex and heptachlor epoxide at concentrations of 5 to 40 pg/L. The compounds in WQA did not provide any additional interferences to the analysis. All solutions were made up in iso-octane.

Anhydrous Na_2SO_4 (Analar from BDH Chemicals, Canada) was dried overnight at 600°C and allowed to cool under dry conditions. Silica gel (Woelm Phama, GmbH & Co., Eschwege) 70 - 150 mesh, was dried overnight at 120°C, 3% water added, tumbled, then stored under anhydrous conditions. Homogenized fish tissue from various lakes in Ontario as well as homogenized yearling fish tissue from a hatchery near Maple, Ontario were obtained from M. Whittle of G.L.B.L., Fisheries and Oceans, Canada.

(a) **Equipment** :

For large amounts of solvent, concentration of the samples was accomplished using a Bucchi Rotavap R110, and for 10 mL or less, a Buchler Vortex Evaporator was used. The heater on both instruments were set at 40°C to facilitate the volatilization of solvents and solvent mixtures.

Gel permeation chromatography was performed on an automated GPC Autoprep 1001 chromatograph, with the column packed with SX-3 Bio Beads gel resin. In the initial set of runs, the 2.5 cm i.d. column was 48 cm long, but after repacking and adding more resin, the column was 60 cm in length. A 1:1 mixture of cyclohexane and dichloromethane was the mobile phase, eluting at a rate of 5 mL/min. The first 150 mL (for the 48 cm column) or 180 mL (60 cm column) were discarded and the next 60 mL were collected for further processing.

A Hewlett Packard 5880 gas chromatograph equipped with an H.P. 7671 automatic sampler, a split/splitless injector, capillary column and an electron-capture detector was used exclusively for all the analyses. For these analyses, a 30 m x 0.25 mm J&W Scientific, non-polar, DB-5 capillary column with a 0.25 μ film thickness was utilized. The chromatograph was operated on a double ramp mode, with an initial temperature of 80°C which was maintained for 3 min after

injection, then ramped to 150°C at a rate of 20°C/min. Then the rate was decreased to 2°C/min until the maximum temperature of 260° was reached and this temperature was held for 10 min before cool-down was initiated. The run time was 71.5 min. A 1 uL sample was introduced in the splitless mode into an injector set at 200°C, and after 1 min the valve automatically changed to split mode. The $_{63}$Ni E.C. detector was maintained at 300°C. The hydrogen carrier gas was maintained at a constant pressure of 72 kPa and the argon/methane make-up gas (95/5) at a constant pressure of 207 kPa. On the instrument console, a threshold setting of 1 and a peak width greater than 0.04 were deemed to be the optimal settings. Between samples containing toxaphene or interferences, iso-octane was injected.

Preparation of Samples

(a) **Toxaphene Solutions** :

A concentrated stock solution was prepared from which aliquots were taken to make up solutions of 10.0, 1.0, 0.2 and 0.1 mg/mL toxaphene in iso-octane. A minimum of 10 chromatograms at each concentration were run. Also, solutions at each concentration of toxaphene were prepared and to these were added the solutions containing the interferences. This was done by adding 2 mL aliquots of the WQA, WQB, technical grade chlordane and PCB's solutions and 2 mL of the appropriate toxaphene solution. These were made up in 15

mL centrifuge tubes, taken to dryness, and made up to 10 mL volume with 1:1 cyclohexane, dichloromethane mixture. Then 5 mL of each solution was injected into the GPC.

(b) **Fish Samples** :

Fish tissue (5 g) was weighed into a 100 mL glass beaker then 3 grams of anhydrous Na_2SO_4, for a fish to salt ratio of 5:3. After 40 mL of dichloromethane was added to this mixture, the probe of the Polytron homogenizer was inserted into the beaker for extraction. With the speed setting of 5, the solution was stirred for 1 min. After the suspension settled, it was decanted through a 5 cm plug of anhydrous Na_2SO_4 contained in an Allihn filter fitted into a 250 mL round bottom flask using suction. This step was repeated twice using fresh dichloromethane. Then the filter was washed with 2 x 10 mL fresh dichloromethane and vacuum was continued until the cake dried. The solvent in the filtrate was removed using a rotovap and the contents transferred to a 15 mL centrifuge tube with the 1:1 cyclohexane: dichloromethane solution, with the final volume being 10 mL. The tubes had to be centrifuged before placing on the GCP if there was any particulate material in the tubes. As it is recommended that no more than 1 g of lipid be passed through the GPC at a time, the round bottom flask was weighed before and after transferring the fish lipid phase to the centrifuge tubes. If necessary, the lipid phase was diluted.

In addition to the above, each fish tissue type was spiked with 1.0 ng/mg of toxaphene.

Treatment of Samples

At this point, all samples were treated the same. The first step was to use GPC which was operated according to the manufacturer's instructions. The samples were collected in 100 mL round bottom flasks, the delivery tubes shaken into the flasks and the tips washed with dichloromethane. Iso-octane (3 mL) was added as keeper, and the flasks taken to dryness on the rotovap. After laying the flasks on their sides for 5 min, 1 mL of iso-octane was added to dissolve the residue in the flask, in readiness for the next step.

A 1.25 cm i.d. chromatographic column was prepared by adding a 2.5 cm portion of Na_2SO_4 over a glass wool plug. Then an 8 cm layer of silica gel was added and this was gently tapped to eliminate air spaces. Finally an additional 2.5 cm layer of Na_2SO_4 was added. Prior to adding the sample, 20 mL of hexane was passed down the column, then with the meniscus of the hexane just below the air-Na_2SO_4 interface, the sample was quantitatively transferred to the chromatographic column using a 22.5 cm glass disposable Pasteur pipette. This was placed on the top of the column material by passing the sample down the column until the top of the solvent was just below the top of the solid packing in the column. Then 5 mL of hexane was used to

rinse the 100 mL flask and this was transferred to the column using the Pasteur pipette. Once the top of the hexane phase reached the top of the solid phase, an additional 20 mL of hexane was added. When the hexane phase disappeared below the top of the solid phase, 10 mL of benzene was added. All the benzene was collected in a 15 mL centrifuge tube, to which 1 mL of iso-octane was added. The contents of the tube were then taken down to dryness on the vortex evaporator. Then the residue was taken up to a known volume of isooctane, usually 1 or 10 mL, ready for gas chromatographic analysis, and the tube securely capped to prevent evaporation if analysis was to be delayed.

Chromatographic Analysis

The equipment utilized was capable of several baseline settings for integrating chromatograms. Only two were selected for further investigation. The first was a flat baseline with set points at 19 and 50 min. The second, denoted as the default mode, constructs the baseline from the minima between peaks in the chromatogram. Between the times specified, there were about 120 individual peaks when an initial concentration of 1 µg/mL was used. To reduce the chromatographic output to a manageable level, only those peaks which contributed greater than 0.4% to the total area were retained for further consideration. Thirty-three peaks were initially selected for inspection with their behavior during cleanup procedures being noted. Those peaks which were quantitatively recovered and not interferred

with by the proximity of other added contaminants were retained. This resulted in 11 peaks being used. To ensure that these 11 peaks reflected the pattern of toxaphene, the results of these peaks were compared, when possible, to the original 33 peaks with very good correlation between the results. If the retention time of a particular peak was shifted by 0.02 min. relative to the retention times of the other selected peaks, the peak was not used. These results will be presented later.

Retention times given in the text are based on either the retention times of methoxychlor or alpha-chlordane.

RESULTS

(a) **Gel Permeation Chromatography** :

Utilizing the fractionation mode of the GPC, fractions of a toxaphene standard were collected every 10 mL. The first 13 fractions were combined and analyzed with the other fractions being analyzed individually. The toxaphene elutes in the 150 to 210 mL volume of solvent. When the column was repacked and made longer, another calibration was necessary, and the "dump" cycle had to be extended to 180 mL with a collection time of 12 min or 60 mL.

(b) **Silica Gel Chromatography**

A total of 40 mL of solvent was used to elute the toxaphene from the silica gel column, the first 25 mL being hexane and the remainder benzene. Samples were collected every 5 mL and analyzed. Chromatograms showed that 97% of the toxaphene was eluted in the first 10 mL of the benzene.

(c) **Standard Solutions** :

The retention times relative to chlordane and methoxychlor, used in the following tables are those which were adjudged to provide the best results from consideration of the data from the fish samples. Listed in Table 2 are the peak area and heights as well as the 95% confidence limits for the concentrations of 10.0, 1.0, 0.2 and 0.1 µg/mL for both baseline settings examined. Inspection of this data can reveal several important details. The values indicate a linear trend over the concentration range used. If the value of the 95% confidence limits are divided by the mean of the height or the area, a better fit results for the analysis using the constant baseline. The greatest scatter occurs for values derived from the changing baseline. The best fit was found for the values for peak height when the baseline was constant.

Table 3 lists the peak areas and heights for the toxaphene samples containing the interferences. These samples had been passed through the GPC and silica gel columns. There is a linear relationship between the concentrations of 10.0, 1.0, 0.5 and 0.2 µg/mL. The values for 0.1 µg/mL are low compared to the others. If the recovered values are compared to the standards as a percentage, the recoveries are good for 10.0, 1.0 and 0.2 µg/mL, but range between 97 and 37% for the 0.1 µg/mL runs, as shown in Table 3. This indicates that at low concentrations, peak height or peak area cannot be linearly extrapolated. Also, this variability in the recoveries reflects the fact that there are differences between the compounds that comprise the toxaphene mixture, and that not all of these compounds behave the same under the cleanup conditions.

The results arising out of the changing baseline method were known to produce less precise data, but the results from this method were still collected. This was done so as to provide an adequate alternative in case other future samples were not amenable to quantitation by the flatbaseline method. By having an appreciation of the precision of the other method, a measure of the toxaphene concentration can still be calculated.

(d) **Selection of Reference Retention Peaks** :

In a contaminated environment, a fish will take up many chemicals, including those of direct interest. When such fish tissue is analysed, those other compounds, chlordane, dieldrin, DDT, etc., are often present in chromatograms and their positions on the chromatograms are well known by the laboratory staff where such analysis is being conducted. The cleanup procedures used for this study were not intended to remove them, and their presence can be used to denote the retention times of peaks of interest. Two compounds, methoxychlor and α-chlordane were selected and the retention times of toxaphene peaks relative to these two compounds were determined. Originally the peak for p,p'-DDE was used as a reference peak, but it was overwhelmed by a relatively minor peak in toxaphene at the 10 ppm level causing a shift in the retention times relative to this peak.

(e) **Effect of Reducing the Number of Peaks in Quantification**

Table 5 lists the peaks initially considered for quantification and which of them were eliminated. There were initially 45 peaks that contributed greater than 0.4% to the area under the chromatographic trace. Recoveries of less than 80% during the cleanup steps reduced the number of peaks to 33. An additional six peaks were rejected from consideration because of the proximity of interference peaks. A

further eight peaks were eliminated from further consideration because of evidence that each was part of a composite peak. This left 19 peaks that could be used in the quantitation. Eight other peaks were rejected for a variety of reasons.

In Table 6, for each row, the values for the concentration are relatively constant, producing the same value for 11 peaks as for 33 peaks. There is also little difference between the results for the standard and the cleaned up standard. Addition of the maximum concentration of interferences found in fish from the Great Lakes increased the concentration values by about 13% at the 0.2 µg/mL level and a maximum of 1.2% at the 1 µg/mL level when only 11 peaks are considered. For the samples used in this part of the study, maximum concentrations of interferences were added and a straight baseline was used in the quantification. These interferences would tend to increase the area under the chromatogram, producing enhanced readings. When normal concentrations of interferences were added in the other part of this study, there was no noticeable increase in the peak heights. The values in this table show that the quantification using the selected 11 of the many peaks in toxaphene, produce reliable results.

(f) **Fish Analysis and Rational**

In standard solutions, the chromatographic peaks arising from toxaphene are quite easy to work with. In environmental samples, this is not necessarily the case. Not all of the components of toxaphene denoted by the peaks will behave similarly in the environment and not all will be absorbed and retained by the organism in similar amounts. Accordingly, a single peak should not be used to quantify toxaphene, particularly when not all peaks are found in an environmental sample. The peaks that we have extensively studied are only a small percentage of a more complex mixture, and the absolute percentages will change between formulations. When using selected peaks to analyse the mixture, the result will be an indirect measure of the total toxaphene concentration.

In Table 7 are listed the results from the Lake Opeongo fish and fortified Lake Opeongo fish tissue. Comparison of the results from the fish tissue, fortified fish tissue and standard solutions passed through the cleanup procedure which were analysed on the same day, shows that the recovery of toxaphene is quantitative (>85%). The concentration of toxaphene in the fish tissue may be calculated in two ways. In both instances, the height of each comparable peak in the standard solution must be known. In the first method, the height of the peak in the test fish is divided by the height of the standard

peak (often with a concentration factor). These values are summed and divided by the number of peaks considered to yield a concentration. Alternatively, the peak heights for the fish tissue are summed as are the heights of the similar peaks in the standard. The total heights from the fish tissue are then divided by the total heights from the standards. This result is then multiplied by the concentration of the standard to produce the concentration of toxaphene in the fish. Using the first method, a concentration of 248 ng/n is calculated and the second method produces a concentration of 253 ng/g. This is a difference of 5 ng/g or within 2% of the mean. In Table 8 are the results from the fish tissue from Lake Ontario and Lake Superior. Comparison of the concentration of toxaphene for each fish from the two methods of calculation shows that the values agree within 2% for each fish. The other entry in Table 8 is from a yearling trout raised in a hatchery near Maple Ontario. All fish tissue samples were processed with blanks to ensure that there was no contamination from the equipment and in the cleanup steps. No trace of toxaphene or interferences were found in the chromatograms of these samples. Also blank solvent samples were interspersed with the other samples and no residual toxaphene was carried over by the gas chromatograph.

These results are interesting. The hatchery fish shows least contamination by toxaphene, although there is a recognizeable pattern of this material in the chromatogram. The source of the toxaphene

could possibly be from the food the fish was fed. At 100 ng/mL or below, the toxaphene is difficult to quantify. Tissue from the Lake Opeongo fish contained a measureable amount of toxaphene. This fish resided in what is generally considered a pristine environment, unaffected by industrial or agricultural effluents. Fish tissue from a Lake Ontario trout exhibited another aspect of the difficulty of determining the concentration of toxaphene. No peak was observed at -0.17 min. relative to methoxychlor. By having several peaks to quantify the substance, the loss of one peak is not catastrophic. The concentration of toxaphene is greater than that in the Lake Opeongo fish but less than the Lake Superior fish. Lake Superior is the most oligotrophic of the Great Lakes and should be the least contaminated. However, toxaphene had been used as a lampricide in Lake Superior and this relatively persistent chemical may have accumulated in the fish tissue.

From the fish extracts, measures of other organochlorine pesticides present in the fish tissue were also calculated. These results are shown in Table 9. As reported in this table, there was little contamination of the hatchery fish by chlordane, DDT, dieldrin or endrin. Results for the fish from Lake Opeongo are a factor of 10 greater than the hatchery fish although this fish was considered to be existing in a pristine environment. Values measured from extracts derived from Lake Ontario fish are higher than those from Lake Opeongo but are less than found in the extracts from the Lake Superior fish.

The minimal detectable amounts of toxaphene were 10^{-11} g for standards but the minimal reproduceable amount of toxaphene after the cleanup stage measured at the detector was 2×10^{-10} g.

ACKNOWLEDGEMENTS

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Fig. 1 Chromatogram of toxaphene

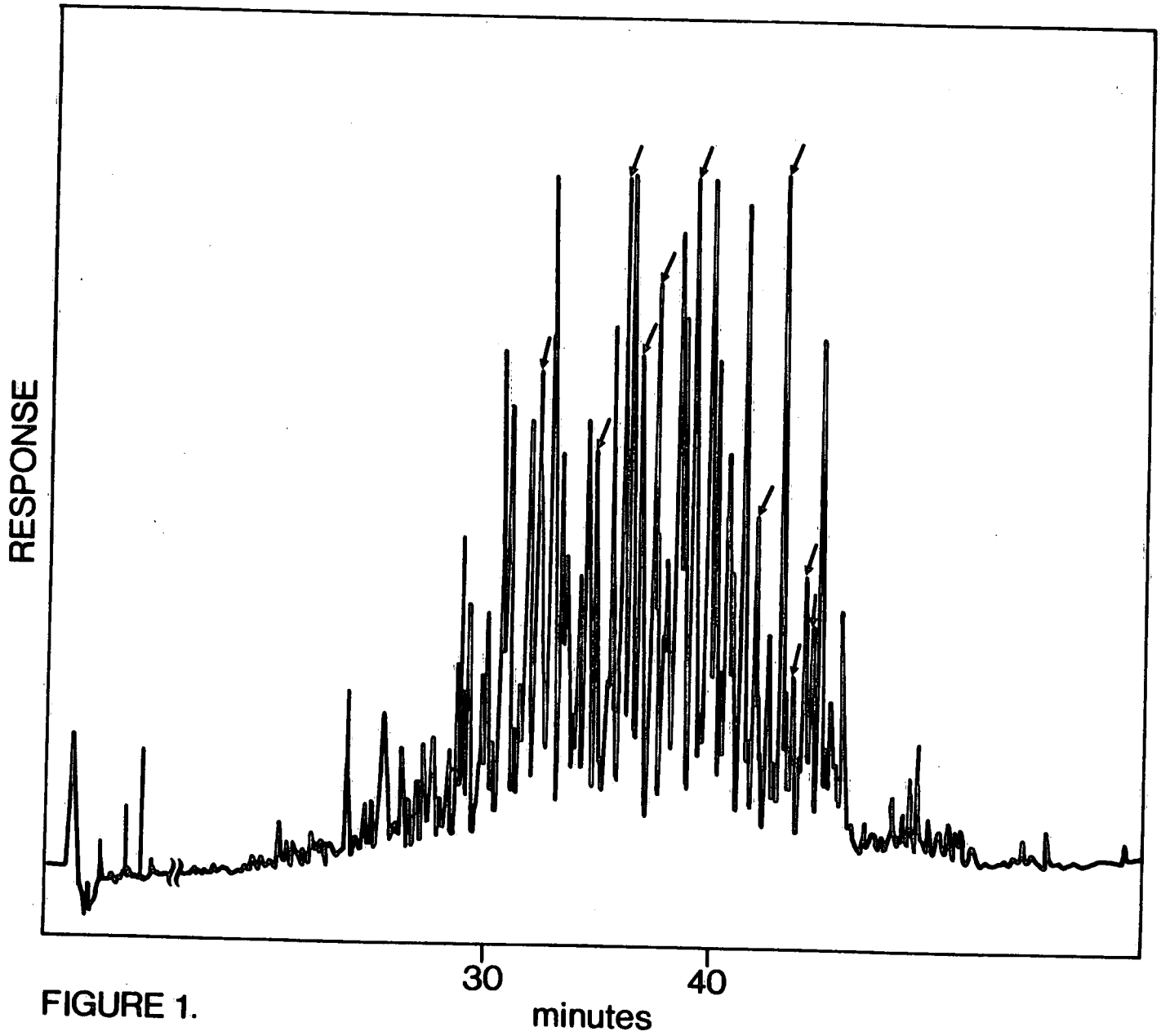

FIGURE 1.

Table 1. Interferences and Their Concentrations in WQB Stock Solution

Interference	Concentration (pg/L)
Lindane	5
Heptachlor epoxide	5
α-chlordane	10
β-chlordane	10
p,p-DDE	20
o,p-DDT	30
Methoxychlor	100
Endosulphan	10
Dieldrin	20
β-endosulphan	30
Endrin	20
p,p-DDT	20
di, tri, tetra and penta substituted chlorinated benzenes	40 Immaterial as they do not interfere with analysis

Table 2. Chromatographic Data of Selected Toxaphene Peaks of Standard Solutions with 95% Confidence limits. Retention Times Relative to (i) methoxychlor or (ii) α-Chlordane.

RT (min) (i)	RT (min) (ii)	10 µg/mL	1 µg/mL	0.2 µg/mL	0.1 µg/mL
(a) Peak Heights with Constant Baseline					
-9.32	4.72	618.7± 23.1	75.61± 4.95	18.42±0.43	7.30±0.23
-6.94	7.10	510.0± 17.9	64.69± 3.89	14.99±0.32	5.71±0.19
-5.72	8.32	905.2± 31.7	116.13± 7.18	25.91±0.60	10.31±0.23
-5.03	9.01	639.0± 27.8	79.11± 4.93	19.63±0.40	8.02±0.28
-4.46	9.57	700.6± 30.5	88.78± 5.84	20.49±0.42	8.00±0.26
-2.78	11.25	1873.0± 80.9	188.79±16.62	44.10±0.84	17.59±0.50
0.04	14.04	427.0± 17.0	54.85± 3.02	12.74±0.22	4.81±0.13
1.00	15.04	913.6± 45.3	118.28± 8.64	26.82±0.69	10.53±0.33
1.54	15.55	238.2± 10.3	35.19± 0.98	6.87±0.50	2.34±0.08
1.97	16.01	353.5± 16.0	46.13± 2.70	10.52±0.22	3.79±0.11
2.28	16.29	291.7± 13.6	38.30± 2.15	8.67±0.17	3.02±0.23
(b) Peak Areas using Constant Baseline					
-9.32	4.72	7453.1±237.9	544.37±49.62	221.66±5.45	80.77±4.62
-6.94	7.10	5066.6±165.9	636.99±35.30	201.13±4.47	51.97±1.91
-5.72	8.32	8060.1±281.1	1019.32±57.93	234.09±5.21	87.27±2.93
-5.03	9.01	6866.3±282.1	900.18±50.20	114.13±2.43	36.65±1.71
-4.46	9.57	6785.2±246.9	859.89±49.87	198.21±4.52	69.34±2.93
-2.78	11.25	15102.9±704.6	1858.13±138.4	447.73±9.24	176.49±4.72
0.04	14.04	3958.1±197.6	505.16±27.24	116.85±2.22	42.80±1.96
1.00	15.04	6866.3±282.1	788.50±22.35	201.13±4.47	74.05±2.51
1.54	15.55	2019.1± 85.2	297.64± 8.44	54.76±1.30	15.87±0.81
1.97	16.01	3925.8±163.9	513.77±28.43	114.13±2.43	36.65±1.71
2.28	16.29	254.93± 10.4	338.65±19.97	73.21±1.72	21.92±1.23
(c) Peak Heights using Variable Baseline					
-9.32	4.72	603.4±19.7	72.57± 3.34	16.89±0.67	6.68±0.48
-6.94	7.10	496.0±15.7	61.15± 3.21	13.95±0.50	5.19±0.17
-5.72	8.32	891.4±29.1	113.03± 5.41	24.44±0.69	9.96±0.28
-5.03	9.01	625.3±25.2	76.24± 3.67	18.58±0.46	8.31±0.28
-4.46	9.57	687.0±28.3	86.12± 4.43	19.77±0.44	8.86±0.36
-2.78	11.25	1559.7±78.5	187.81±14.60	43.08±0.73	19.35±0.65
0.04	14.04	412.1±15.3	52.80± 1.87	12.06±0.63	3.96±0.38
1.00	15.04	900.8±43.8	116.62± 7.65	25.82±0.39	10.25±0.31
1.54	15.55	225.5±10.1	32.24± 1.22	6.14±0.24	2.19±0.55
1.97	16.01	340.8±14.9	42.96± 3.19	9.88±0.39	3.85±0.12
2.28	16.29	27.92±1.23	36.63± 1.34	8.15±0.33	2.88±0.10
(d) Peak Areas using Variable Baseline					
-9.32	4.72	7127.8±193.2	840.15±43.34	183.92±13.20	68.05±1.87
-6.94	7.10	4814.0±233.9	573.99±52.68	118.82± 9.03	42.67±1.61
-5.72	8.32	7783.4±233.9	956.87±47.01	197.76±12.59	78.40±2.03
-5.03	9.01	6852.9±210.4	826.96±48.12	183.51± 8.42	88.69±5.87
-4.46	9.57	6483.9±200.8	799.35±38.82	173.51± 5.18	86.64±7.06
-2.78	11.25	14719.5±640.9	1790.89±98.93	417.83± 6.79	227.96±19.7
0.04	14.04	3755.3±171.5	473.24±16.63	105.06± 3.15	27.67±6.19
1.00	15.04	6627.8±248.8	858.04±46.41	182.11± 7.96	69.59±1.99
1.54	15.55	2019.8± 85.2	252.49±18.89	43.98± 2.82	13.40±0.35
1.97	16.01	4909.0±153.2	481.59±15.20	103.13± 4.71	37.17±1.24
2.28	16.29	2639.5±554.1	313.13±10.83	63.04± 5.30	19.89 0.89

Table 3. Chromatographic Data of Toxaphene Peaks from Solutions that had Added Inferences and had been Processed through the Cleanup Steps. Retention Times Relative to Methoxychlor or α-Chlordane.

RT (min) (i)	RT (min) (ii)	10 µg/mL	1 µg/mL	0.5 µg/mL	0.2 µg/mL	0.1 µg/mL
(a) Peak Heights with Constant Baseline						
-9.32	4.72	694.9± 22.3	83.85± 2.55	47.46±0.74	16.64±0.74	5.04±0.29
-6.94	7.10	543.5± 17.2	62.82± 2.23	36.43±0.80	14.30±0.80	4.12±0.80
-5.72	8.32	1047.5± 41.3	121.40± 4.55	69.79±0.75	23.78±0.75	8.31±0.29
-5.03	9.01	725.2± 27.5	92.64± 2.39	54.95±30.33	45.74±30.33	12.20±1.56
-4.46	9.57	803.1± 27.8	94.84± 3.25	54.95±0.79	19.29±0.79	6.72±0.31
-2.78	11.25	1731.2± 75.5	213.28± 8.14	117.87±1.55	38.70±1.55	14.11±0.50
0.04	14.04	483.1± 15.6	58.24± 1.95	35.09±0.63	10.63±0.63	-
1.00	15.04	1056.2± 37.9	127.24± 4.43	73.66±0.63	25.46±0.63	10.09±0.54
1.54	15.55	265.4± 91.5	30.68± 0.98	18.54±0.50	6.12±0.50	1.72±0.38
1.97	16.01	396.9± 13.1	46.46± 1.51	28.35±0.54	9.64+0.54	2.37±0.36
2.28	16.29	329.1± 11.0	73.11±68.52	23.26±0.49	7.66+0.49	1.75±0.23
(b) Peak Areas using Constant Baseline						
-9.32	4.72	5443.7±496.2	586.04±18.07	333.41±20.72	112.11±5.76	32.21±1.48
-6.94	7.10	5444.7±173.9	614.58±19.99	416.34±27.12	164.43+11.53	32.46±3.70
-5.72	8.32	9037.7±296.6	1049.37±33.13	629.28±44.97	222.65+10.06	69.59±4.09
-5.03	9.01	7976.7±263.9	1099.74±45.74	610.98±55.03	428.55±22.85	109.3 ±15.42
-4.46	9.57	7594.9±266.9	886.88±27.72	537.05±37.32	187.62±16.64	53.48±4.49
-2.78	11.25	13442.5±461.2	1639.06±60.97	937.98±55.10	324.49±15.09	102.58±5.27
0.04	14.04	4089.1±381.1	417.22±19.83	289.53±21.27	89.95± 3.48	-
1.00	15.04	7894.8±252.1	892.81±30.02	536.31±35.18	187.89±6.84	69.01±5.29
1.54	15.55	2266.1± 85.2	249.44± 8.59	156.08± 9.06	46.86±7.14	8.39±5.35
1.97	16.01	4385.5±151.1	488.83±26.43	316.28±12.33	103.89±8.24	18.78±3.80
2.28	16.29	270.91± 9.69	288.61±11.08	186.40±14.37	95.27±6.20	8.49±2.35
(c) Peak Heights using Variable Baseline						
-9.32	4.72	631.9±35.6	81.29± 2.74	44.94±4.06	14.18±0.47	5.70±0.25
-6.94	7.10	486.9±27.8	60.34± 2.60	36.45±3.52	11.73±0.62	4.65±0.25
-5.72	8.32	986.1±40.1	188.96± 4.12	53.76±7.30	21.72+0.76	9.65±0.39
-5.03	9.01	667.7±31.7	90.22± 2.34	51.19±5.47	48.55±37.20	14.09±1.86
-4.46	9.57	760.0±30.9	92.42± 2.94	52.69±4.38	17.46±0.68	8.82±0.76
-2.78	11.25	1696.7±66.7	210.82± 7.59	115.60±7.09	37.29±1.12	15.23±0.83
0.04	14.04	446.1±23.0	55.74± 2.04	33.05±2.56	9.73±3.12	-
1.00	15.04	1034.0±41.0	124.70± 4.25	71.67±5.39	24.35±0.80	9.99±0.49
1.54	15.55	244.9±10.1	28.24± 1.22	16.37±1.30	5.53±0.17	2.01±0.23
1.97	16.01	374.4±13.3	43.29± 2.21	26.38±2.28	9.22±0.35	2.81±0.22
2.28	16.29	32.7±32.9	34.74± 2.53	21.28±1.91	7.24±0.28	1.84±0.04
(d) Peak Areas using Variable Baseline						
-9.32	4.72	4755.4±583.1	561.23±24.15	309.72±29.93	98.97±13.73	37.25±1.98
-6.94	7.10	4423.1±348.2	570.42±41.56	366.25±48.64	111.82±10.59	43.98±5.14
-5.72	8.32	7943.4±511.6	999.72±43.41	580.57±61.07	178.84± 9.64	97.07±6.08
-5.03	9.01	6685.8±566.9	1032.30±48.07	550.66±72.40	410.19±27.38	60.25±23.50
-4.46	9.57	6657.6±430.1	832.51±40.86	487.07±51.73	154.98±10.71	98.97±15.23
-2.78	11.25	2597.6±442.5	1588.18±54.45	891.37±63.23	290.02±13.51	26.84±14.50
0.04	14.04	3596.2±427.5	387.11±19.94	261.66±26.06	106.32± 3.25	18.37±2.27
1.00	15.04	7393.7±277.1	853.51±30.16	490.79±54.53	172.36±10.00	67.66±3.90
1.54	15.55	1908.6±113.5	210.75±14.55	120.82±13.69	38.43± 1.59	11.91±1.70
1.97	16.01	3965.1±166.7	435.37±43.25	279.04±27.56	95.82± 3.73	23.19±3.08
2.28	16.29	2414.2±105.1	251.00±27.33	161.27±17.31	52.09± 3.24	23.19±0.36

Table 4. Percent Recoveries of Toxaphene.

RT	10 µg/mL	1.0 µg/mL	0.2 µg/mL	0.1 µg/mL
(a) Peak Heights with Constant Baseline				
-9.32	112%	111%	90.3%	69.0%
-6.94	107%	96.8%	95.4%	72.1%
-5.72	116%	105%	91.8%	80.6%
-5.03	113%	117%	233%	152%
-4.46	115%	107%	94.1%	84.0%
-2.78	92.4%	113%	87.8%	80.2%
0.04	113%	106%	83.4%	-
1.00	114%	108%	94.9%	95.8%
1.97	113%	101%	91.6%	62.5%
1.54	111%	87.2%	89.1%	73.5%
2.28	113%	190%	88.4%	57.9%
(b) Peak Areas with Constant Baseline				
-9.32	73.0%	108%	50.6%	39.9%
-6.94	107.5%	96.5%	81.8%	62.5%
-5.72	112%	103%	95.1%	79.7%
-5.03	116%	122%	375%	29.8%
-4.46	112%	103%	94.7%	77.1%
-2.78	89.0%	88.2%	72.5%	57.2%
-0.04	103%	113%	77.0%	93.2%
1.00	115%	82.6%	93.4%	-
1.54	112%	83.8%	85.6%	52.9%
1.97	112%	95.1%	91.0%	51.2%
2.28	106%	85.2%	130%	38.7%
(c) Peak Heights with Variable Baseline				
-9.32	105%	112%	84.0%	85.3%
-6.94	98.2%	98.7%	84.1%	89.6%
-5.72	111%	167%	88.9%	96.9%
-5.03	107%	118%	241%	170%%
-4.46	111%	107%	88.3%	99.5%
-2.78	109%	112%	86.6%	78.7%
0.04	109%	106%	76.4%	-
1.00	115%	107%	94.3%	97.4%
1.54	109%	87.6%	90.0%	91.8%
1.97	110%	101%	93.3%	73.0%
2.28	117%	94.8%	88.8%	63.9%
(d) Peak Areas with Variable Baseline				
-9.32	66.7%	66.7%	53.8%	54.7%
-6.94	91.9%	99.4%	94.1%	103%
-5.72	102%	104%	90.4%	124%
-5.03	97.6%	125%	224%	67.9%
-4.46	103%	103%	82.6%	114%
-2.78	85.6%	85.6%	69.4%	55.6%
0.04	95.8%	95.8%	101%	-
1.00	112%	99.5%	94.6%	97.3%
1.54	94.5%	94.5%	87.4%	88.9%
1.97	80.8%	90.4%	92.9%	62.4%
2.28	91.5%	91.5%	82.6%	62.4%

Table 5. List of Peaks Considered for Quantitation and Reasons for Their Elimination.

Retention Time (min)	
32.26	
34.65	
35.85	
36.54	Selected peaks
37.11	
38.79	
41.42	
42.57	
43.09	
43.54	
43.83	
30.71	Technical chlordane interference
32.67	Too close to another toxaphene peak
38.36	Composite peak in fish tissue
40.47	Composite peak in toxaphene
42.01	Composite peak in fish tissue
42.18	Too close to another toxaphene peak
42.77	Interference from technical chlordane
44.23	Composite peak in fish tissue
29.99	
32.81	
33.97	Suspected composite peaks
37.27	
39.48	
39.97	
42.27	
29.03	Technical chlordane interference
29.23	Dieldrin interference
30.04	Dieldrin interference
32.14	Technical chlordane interference
35.33	Technical chlordane interference
38.17	Technical chlordane interference
30.96	
31.22	
31.75	
32.97	Poor Recoveries
33.20	
33.40	
34.33	
36.13	
37.59	
39.70	
40.29	
45.15	

Table 6. Effect of using Decreasing Number of Peaks to Quantify Toxaphene (with standard deviation).

Concentration and Treatment	Calculated Concentration (ng/g)			
	33 Peaks	27 Peaks	19 Peaks	11 Peaks
0.2 ppm	188± 10	186± 10	187±10	185±11
0.2 ppm+cleanup	186± 11	187± 12	190±11	192±11
0.2 ppm+cleanup + interferences	307±204	256±126	252±63	254±69
1.0 ppm	978± 6	977± 6	977±6	975± 5
1.0 ppm+cleanup	988± 32	990±33	992±35	1006±19
1.0 ppm+cleanup + interferences	1076±161	1033±87	1013±49	1031±32
10.0 ppm	16069±1634	16003±1709	16119±1491	16146±1076
10 ppm+cleanup	16516±1859	16444±1948	16618±1554	16980±1167

Table 7. Recoveries and Concentration of Toxaphene from Fish Tissue, Lake Opeongo Trout.

RRT (min)	Peak Height of Sample	Peak Height of Fortified Fish Sample	Difference in Heights	Standard Peak Height	% Recovery	Concentration (ng/g)
-9.32	18.65	106.05	87.40	91.42	95.6	204
-6.94	8.57	75.61	67.04	73.37	91.4	117
-5.72	52.98	192.47	139.49	134.25	103.9	395
-5.03	50.39	145.11	94.72	91.94	103.0	548
-4.46	21.51	124.82	103.31	104.78	98.6	205
-2.78	81.06	330.21	249.15	237.74	104.8	341
0.00	11.58	71.80	60.22	62.28	97.7	186
1.00	72.44	211.17	138.73	137.07	101.2	528
1.54	18.45	49.78	31.33	34.81	90.0	530
1.97	8.41	59.06	50.65	51.39	98.6	164
2.28	16.92	57.52	40.60	42.42	95.7	399
Totals						
	342.31			1061.47	98.2	
Concentration (ng/g)						
	323					329

Table 8. Concentration of Toxaphene from Fish Tissue.

	Hatchery Yearling		Lake Ontario		Lake Superior	
RRT (min)	Peak Height	Concentration (ng/g)	Peak Height	Concentration (ng/g)	Height	Concentration (ng/g)
-9.32			28.96	353	27.29	333
-6.94			16.37	223	19.72	269
-5.72	1.44	11	88.16	684	116.70	905
-5.03	4.32	47	111.28	1221	130.05	1415
-4.46			25.29	251	47.24	468
-2.78	3.97	17	81.82	384	188.22	884
0.00			19.02	307	43.04	694
1.00	8.41	61	111.66	815	265.09	1927
1.54	1.02	29	17.32	520	30.83	926
1.97			5.04	98	9.58	186
2.28			14.25	348	33.33	815
Total						
	19.16		519.17		840.09	
Concentration	(ng/g)					
	31	33	489	473	791	802

Table 9. Summary of Organo Chlorines in Fish (ug/g).

	Lake Opeongo	Lake Superior	Lake Ontario
α -Chlordane	0.01	0.01	0.04
γ -Chlordane	0.02	0.03	0.10
Dieldrin	0.03	0.04	0.16
p,p^0-DDE	0.29	1.50	3.42
o,p^0-TDE		0.12	
o,p^0-DDT	0.06	0.99	0.29
Total DDT	0.41	1.72	4.04
% Lipid	12	13	21