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EVALUATION OF METHODS FOR ANALYSING TOXAPHENE IN WATER AND SEDIMENT SAMPLES

by

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MANAGEMENT PERSPECTIVE

At the request of the National Water Quality Laboratories, the quantitation of toxaphene from water and sediment was studied. Obtained recovery of toxaphene from water was quantitative but not from sediment. To achieve the latter goal, further development must be expended to make the method applicable to all types of sediment.

PERSPECTIVE GESTION

L'étude des méthodes de quantification du toxaphène en présence dans l'eau et les sédiments a été effectuée à la demande des Laboratoires nationaux de qualité de l'eau. Les taux de récupération obtenus se sont avéréssatisfaisants pour les échantillons d'eau mais non pour les sédiments. Pour ces derniers, il faudra développer de meilleures méthodes d'extraction qui pourront être appliquées à tous les types de sédiments.

ABSTRACT

This report describes a quantification method for toxaphene in water as well as an evaluation of various methods for the extraction of toxaphene spikes from a heavily contaminated sediment. The toxaphene quantitation from water gave acceptable recoveries (>80%). Using several extraction and cleanup procedures of toxaphene in sediment at the 10^{-7} g/g level (toxaphene to sediment), the recovery of toxaphene was less than 70%. Using solvents of varying polarity for extraction and column chromatography, different methods of extraction, and chromatography support materials with different activities did not enhance the recoveries.

SOMMAIRE

Dans cette étude nous décrivons une méthode servant à quantifier la présence de toxaphène dans l'eau et nous évaluons différentes méthodes d'extraction du toxaphène des sédiments fortement contaminés. Le taux de récupération du toxaphène dans l'eau s'est révélé acceptable, c'est-à-dire supérieur à 80 p. 100. Cependant, le taux de récupération à partir des sédiments (pour une concentration de 10^{-7}g de toxaphène par gramme de sédiments) est demeuré inférieur à 70 p. 100 malgré l'emploi de plusieurs méthodes d'extraction et de purification différentes. Même en utilisant des matériaux plus ou moins actifs et des solvants de polarités différentes pour l'extraction et la colonne chromatographique et en variant les méthodes d'extraction, nous n'avons pas pu obtenir un meilleur taux de récupération.

EVALUATION OF METHODS TO ANALYSE FOR TOXAPHENE IN WATER AND SEDIMENT SAMPLES

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INTRODUCTION

The methodology for analysis of toxaphene in fish tissue employing a modified technique commonly used for organochlorine determinations, has been developed (Ryan and Scott, 1985). The next step was to ascertain if the method was applicable to the two other major aquatic matrices, water and sediment. This was requested by the National Water Quality Laboratory. Previously (Ryan and Scott), we found that recoveries were low when analysing less than 200 ng/mL toxaphene. Using an electron capture detector and capillary column gas chromatography, less than 10^{-10} g of toxaphene can be detected. The 200 ng/mL is a practical lower limit, imposed by the cleanup procedures on the extracted samples. The other major complication in the analysis is interferences. However, some of these interferences can be measured during the toxaphene analysis. Gel permeation chromatography was used to eliminate lipids and other large molecules, while silica gel chromatography was used to separate classes of compounds. With the vast number of compounds sensitive to the electron capture detector, that exist in nature, the cleanup

procedures cannot eliminate all other unknown compounds from being collected in the toxaphene fraction of the cleanup. These other compounds can shift or alter peaks of interest during GC quantitation.

Essentially, variations of two documented methods for the extraction and cleanup of sediments were investigated (Analytical Methods Manual) (Wegen and Hofstee, 1982). During this evaluation, solvents of varying polarity and different solid absorbents were used for the extraction and cleanup procedures.

METHODS

Water samples of distilled water, Burlington municipal tap water and Hamilton Bay water were collected. As the Bay water is the receiving water for many industrial effluents and has indigenous biota, it was filtered through a 5 micron sieve before extraction. For all water types 1.0 L aliquots were placed in a 1-L separatory funnel with 200 g of reagent grade NaCl and shaken vigorously for 2 min with 25 mL of dichloromethane (Analar) then twice more with 15 mL of dichloromethane. The phases after each extraction were allowed to separate and the flask swirled if necessary to break up large bubbles at the liquid-liquid interface. The emulsion, when present, was taken into the organic phase. To the combined organic phase and emulsion, 15 mL of acetone was added to break up the emulsion. Any resulting

aqueous phase was drawn off and 2 g of anhydrous $MgSO_4$ was added to dry the solution. The dichloromethane was decanted into a 250 mL round bottom flask through a glass wool plug and the $MgSO_4$ was washed twices with 5 mL dichloromethane. Then 3 mL of isooctane was added and the solvent mixture taken down to dryness on a Rotovap. The residue was dissolved in 1 mL of isooctane.

Sediment was collected from Hamilton Harbour and allowed to air dry. This was then powdered in a large mortar with pestle then sieved through a No. 35 mesh screen. The sieved material was stored in clean, amber, small-mouthed bottles. To prepare samples, 5 or 10 g of the sediment material was weighed into a 250 mL wide-mouth bottle. Then 1 mL of a 1 µg/mL toxaphene in CH_2Cl_2 solution was added with an additional 15 mL of CH_2Cl_2 being added to provide a more homogeneous mixture. The sample bottles were placed in a vacuum dessicator and the solvent removed by application of vacuum. Blanks were prepared by either adding the toxaphene solution to a bottle or by just adding the sediment material. Any crust which formed in the bottle as a result of the evaporation of the solvent was broken up by a spatula.

Four methods of extraction were attempted. The first used a sonifier and is described in the Water Quality Branch Methods Manual. Initially, the extraction solvent was the recommended acetone-hexane mixture (1/1), but dichloromethane then hexane were used later to ascertain if either of these solvents would favour co-extractants.

Soxlet extraction was the second method used. In an ASTM 40-60 C type thimble, 5 g of anhydrous $MgSO_4$ was placed over the glass frit, then a 5 g sample of the sediment or spiked sediment was added. The sample bottle was washed three times with 25 mL of hexane. When toxaphene only was added, the toxaphene on the glass of the bottle was washed onto the $MgSO_4$ layer with hexane. Total volume of the hexane was 200 mL. The hexane was refluxed for 15 hrs, then the solvent was transferred to a 250 mL round bottom flask and 6 mL of isooctane added. The contents were taken to dryness on a Rotovap after which the residue was taken up in 1 mL of isooctane and transferred to a 15 mL centrifuge tube, the isooctane evaporated off and 10 mL cyclohexane: CH_2Cl_2 (1:1) was added prior to gel permeation and subsequent silica gel column chromatography (Ryan and Scott, 1985).

The third method was essentially that described by Wegen and Hoftsee (1981) with slight modifications in the sample size. A 5 g sample of the dried Hamilton Harbour sediment was wetted with 3 mL of water and shaken with 25 mL of acetone in a 250 mL round bottom flask for 35 min and allowed to settle overnight. The acetone phase was decanted through a glass wool plug into a 125 mL round bottom flask and the sediment shaken with 20 mL acetone for 30 min. After four hours the acetone was decanted off, the two acetone phases combined and then reduced to 20 mL volume on a Rotavap. This concentrate was placed into a 125 mL separatory funnel with 50 mL of water and shaken with 40, 20 and 20 mL of petroleum ether (30°-60°C). The emulsion at

the interface was taken into the ether phase. Dried Na_2SO_4 (5 gm) was added to dry the ether phase which was transferred to a 100 mL round bottom flask and the volume reduced to 0.5 mL.

For the cleanup, the concentrated ether phase was added to a chromatography column containing 10.0 g of alumina (Aluminum oxide, basic activity grade 1, Woelm, ICN Pharmaceuticals, GmbH & Co. which was dried at 150°C for 16 hours, then deactivated with 11% water). The column was rinsed with 15 mL of petroleum ether prior to adding the concentrate. Once the concentrate was added to the column, it was eluted with 20 mL of petroleum ether, then 20 mL 20:80 ethyl-ether: petroleum ether. Both fractions were collected separately and reduced to 1 mL by using a gentle stream of nitrogen.

A chromatography column was prepared by adding and tamping separately 0.5 g Na_2SO_4, 5 g silica gel (dried at 200°C for 16 hr) and an additional layer of 0.5 g of Na_2SO_4. This was washed with 15 mL of petroleum ether. Then the first fraction from the alumina column eluate was placed on the top of the column and eluted with 46 mL of petroleum ether. A second elution, using 40 mL of a 50:50 petroleum ether: CH_2Cl_2 was then performed. These two separate fractions were reduced to 1 mL under a gentle stream of nitrogen, then taken to dryness on a vortex evaporator. This cleanup procedure produced three final fractions. A (second fraction from the alumina cleanup), B (first fraction silica gel column) and C (second fraction from the silica gel column).

For the toxaphene blank, 10^{-6} g of toxaphene was dissolved in 20 mL of acetone, then 50 mL of water was added. This was extracted with the petroleum ether and separated into three fractions during the cleanup stage.

The fourth method of extraction utilized a large liquid water extractor (Goulden and Anthony, 1985) operated in an agitator mode. Five g of the spiked sediment was added to 1.5 L distilled water and 200 mL of CH_2Cl_2. The agitator was left on for 30 min, after which the phases were allowed to separate completely and the CH_2Cl_2 was drawn off. The CH_2Cl_2 was reduced to dryness on a vortex evaporator after concentrating to 10 mL. The extract was made up to 0.5 mL in petroleum ether (30°-60°C) and cleaned up by the procedure of Wegen and Hoftsee.

A Hewlett-Packard 5880 gas chromatograph was used for the quantification. An HP 7671 automatic sampler injected a 1 µL sample (in isooctane) into a split/splitless injector (200°C) which was in the splitless mode for 1 min. The hydrogen carrier gas (72 kPa), constant pressure) swept the sample onto a J&M DB-5 capillary column (30 m x 0.25 mm) which had a 0.25µ film thickness. The eluate of the column then passed into a $_{63}Ni$ electron capture detector maintained at 300°C and which was swept with Ar/Me (95%/5%) makeup gas at a constant pressure of 207 kPa. On the console, a threshold setting of 1 and peak width setting of 0.04 were used. The chromatograph was operated on a double ramp mode, with an initial temperature of 80°C which was

held for 3 min, then ramped to 150°C at a rate of 20°C/min. The rate was then decreased to 2°C/min to a maximum temperature of 260°C, and this temperature was held for 10 min. before cool down. The run time was 71.5 min. The computer integrator attached to the GC, produced results using two modes of operation. The first was with a constant baseline, as used in the fish analysis, which had set points outside the unresolved continuum of the toxaphene, and the other method was based on a constantly changing baseline defined by the minimum of the major peaks. The peaks of interest were those used in analysis of the fish tissue (Ryan and Scott, 1985). A typical trace of toxaphene is depicted in Fig. 1. The peak heights used for quantification are denoted by the small arrows.

RESULTS

i) Water

The CH_2Cl_2 extracts of the different water types when taken to dryness had a terpene-type aroma and the chromatographic traces contained many impurities as shown in Fig. 2 (a). Silica gel chromatography completely eliminated the aroma and the chromatographic background (Fig 2 (b)). The extraction results from the tap water and the lake water are listed in Table 1, with each result being an average of two.

Recoveries from lake water extractions are shown in Table 1. A recovery efficiency of greater than 90% was obtained for tap water as denoted by the results in column (a) of Table 1. In the same Table, columns (e) through (h) list the recoveries for individual lake water samples which are generally greater than 85%. Columns (i) and (j) list the averages for 4 and 5 sets of data. Initially, the emulsion resulting from the extraction of the lake water was treated separately from the organic phase. The toxaphene content of the organic phase and the emulsion are shown in columns (b) and (c). When these are combined (column d), the total is identical to the emulsion being included in the organic phase.

With recoveries greater than 85%, this method is suitable for analysing toxaphene in natural waters.

ii) Sediment

The method modified for toxaphene in fish and water was based on the method currently used for organochlorines (OCs) by the National Water Quality Laboratory. Accordingly, we endeavoured to use their extraction methodology for the sediment analysis of toxaphene (Analytical Methods Manual).

As the biobead column of the the gel permeation chromatography apparatus is irregularly cleaned and repacked, the effluent pattern of toxaphene had to be checked, with typical results being shown in Table 2. Once the eluent volume for toxaphene was known, the cleanup and subsequent analysis of the extracted sample were initiated.

(a) Sonification:

In accordance with the Analytical Methods Manual, 10 g of sediment was initially used. The first few samples taken through with this method yielded less than 60% recovery. As the recoveries were low, variations were made on the extraction method, namely by changing the solvent and the acidification of the sediment to pH 2. Both dichloromethane and hexane were used as extraction solvents. The CH_2Cl_2 extracted other material from the sediment which overwhelmed the toxaphene peaks in the resulting chromatograms. The hexane extracts gave results similar to the mixed solvent solution usually used (hexane: acetone). Increasing the acidity of the sediment to less than pH 2, resulted in the coextraction of other materials which obscured the toxaphene peaks. Another sample was spiked with WQB solution (Analytical Methods Manual) which contains several organochlorine pesticides. Coextracted material from the sediment obscured the anticipated peaks. As this solvent mixture of hexane and acetone extracted too much background material, the relatively nonpolar hexane was used as the solvent, and smaller sample size (5 g) was utilized. Employing these conditions, the individual peaks used for quantitation were not shifted or obscured, but the recoveries of compounds represented by these peaks within the sample varied considerably. In addition, as the sonification procedure extracted other compounds from the sediment, even using the nonpolar hexane, the elution of these compounds interferred with the integration, so the

baseline set points were extended by 1 min on either side of the original integration period, 19 to 51 min rather than 20 to 50 min. In addition, the changing baseline method was also used. The results from both these methods are given in Table 3. Nonquantitative amounts of the toxaphene were recovered (70%), as shown in Table 3. However, the straight baseline method resulted in lower recoveries than the changing baseline method.

(b) Soxlet Extraction

Two sizes of extractors were used, large (200 mL of solvent) and medium (150 mL of solvent). For all soxlet extractions, the solvent was hexane. The results are shown in Table 4, where about 65% of the toxaphene spike was recovered. These results were obtained using 5 gm of sediment material with the changing baseline method for integration and. Also, a blank containing toxaphene and no sediment was run with the results shown in Table 4. The recovery for the toxaphene blank was about 90%.

(c) Acetone Extraction

The results obtained using the Wegen and Hofstee method of extraction and cleanup are given in Table 5. Fraction A contains about 10% for several of the components while fraction B contained no toxaphene. Considerable amounts of toxaphene were found in

fraction C. However, by combining results from fractions A and C, only about 60% of the toxaphene was recovered by this method.

(d) Aqueous Extraction

Results from the continuous extraction of sediment with CH_2Cl_2 and water are given in Table 6. These values only represent the toxaphene peaks in fraction C. Fraction A in this method had to be diluted by a factor of 4 to give a reasonable chromatogram. The peaks of interest were obscured by other components extracted from the sediment.

DISCUSSION

The analysis of toxaphene in the water phase was a tractable problem. From previous work, we know the major difficulty is in the quantification (Scott and Ryan, 1985). The other possible area of concern is the effect of the emulsion during the extraction. As this emulsion can be taken with the organic phase, the analysis is not that difficult. Analysis of toxaphene from sediment is another matter. Chromatographic peaks used for quantifying toxaphene in fish tissue were distributed over the elution time of 30 to 45 minutes under the operating conditions of the gas chromatograph. Up to the elution time of 38 minutes for sediment samples, the recoveries of toxaphene are

because the peaks of interest are being interfered with by other compounds extracted from the sediment. When other common pesticides were added as spikes to the sediment samples, other co-extracted compounds in the sediment interferred with the quantitation of the chemicals from the spiking solutions.

For this study, solvents of varying polarity were employed, the most polar being acetone and the least being hexane. As expected, the more polar the solvent system, the more material extracted. Even with the least polar solvent too much interfering material was extracted from the sediment for a proper quantitation. During the clean up stage, silica gel of two activities were investigated (0% and 3% water) as was deactivated alumina (11% water). Although some separation was undoubtedly achieved, the column chromatography used here were not sufficient to provide adequate separation for the toxaphene quantitation at the 10^{-7} g/g (toxaphene to sediment) level. The extraction and cleanup methods used are shown in Appendix I.

Sediments can differ in a number of aspects including organic content. They can be composed of fine grained clays or thick black humic material. Treatment of sediment samples for analysis should be as uniform as possible, reducing any potential differences in techniques between laboratories using the same method. Ideally the method for a heavily polluted sediment should be the same as for a lightly polluted sample. The sediment that was used is considered to be an excellent example of a contaminated sediment. If a different, more pristine sediment were used to define the method, that method may

have produced erroneous results for a more heavily contaminated sediment. Until the interfering substances can be removed or accomodated prior to analysis, it is improbable that toxaphene can be analysed in sediment samples by a general method. Also the identification or removal of the interfering compounds was beyond the scope of the request which prompted this work.

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TABLE 1 Recoveries (in percent) of toxaphene from water

Retention Time (min)	Tap Water	Lake Water								
	(a)	Organic (Separate Emulsion) (b)	Emulsion (c)	Total (d)	(e)	(f)	(g)	(h)	ave4 (i)	ave5 (j)
32.26	95.7	78.7	10.7	89.4	86.0	82.8	89.2	90.8	87.2	87.6
34.65	92.0	74.9	11.3	86.2	83.4	80.8	88.4	88.4	85.3	85.4
35.85	95.0	75.9	10.3	86.2	84.9	81.6	88.2	90.0	86.2	86.2
36.54	97.0	81.4	18.6	100.0	108.5	104.6	102.0	101.0	104.0	103.2
37.11	92.4	77.0	10.5	87.5	84.3	81.4	89.6	89.6	86.2	86.2
38.79	91.1	7.0	9.5	86.5	85.3	80.7	88.7	89.7	85.8	85.9
41.42	90.0	77.6	10.9	88.5	85.3	82.8	85.3	89.7	85.8	86.3
42.57	90.1	77.0	10.6	87.5	85.7	82.3	88.2	92.2	86.4	86.6
43.09	90.8	75.7	10.2	85.9	85.8	84.5	87.3	90.3	87.0	86.8
43.54	91.1	76.8	10.7	86.6	87.4	85.2	85.8	91.0	87.4	87.3
43.83	88.8	76.8	10.1	86.9	85.1	83.0	88.2	88.7	86.3	86.4

ave4 is the average of columns (e), (f), (g) and (h).
ave5 is the average of columns (d), (e), (f), (g) and (h).

TABLE 2 Percentage of Toxaphene found in GPC eluate volumes

Retention Times (min)	GPC Eluate Volumes (mL)				
	140-160	160-180	180-200	200-220	Total
32.26	6.18	11.24	75.57	16.32	109.31
34.65	2.30	11.21	72.00	23.67	109.18
35.85	---	15.98	73.99	5.92	95.89
36.54	---	149.62	---	---	149.62
37.11	---	20.70	76.86	13.20	101.65
38.79	---	---	76.73	13.20	89.94
41.42	---	28.49	68.39	---	96.87
42.57	---	18.76	76.46	3.21	98.43
43.09	---	17.72	70.52	10.50	98.74
42.54	---	17.86	79.10	---	96.96
43.83	---	8.88	82.77	9.48	101.34

TABLE 3 **Sediment extraction by sonification using both baseline modes Peak Heights with standard deviation.**

Retention Time (min)	Changing Baseline Mode		% Recovery	Straight Line Mode		% Recovery
	Standard	Sediment		Standard	Sediment	
32.26	84.69±4.05	55.78±7.16	65.9	93.56±4.02	40.98±4.46	43.8
34.65	72.23±1.38	45.56±6.84	63.1	78.84±3.82	29.94±2.86	37.9
35.05	131.3±12.28	96.00±12.28	73.3	140.0±10.6	80.00±8.00	57.2
36.54	92.29±1.53	88.24±8.24	95.6	98.22±3.89	71.98±4.82	73.3
37.11	103.4±0.98	81.81±15.72	79.1	109.2±4.38	66.66±9.48	61.1
38.79	255.4±21.55	164.4±26.52	64.4	253.8±13.1	148.0±20.7	58.3
41.42	61.00±1.84	48.39±9.38	79.3	65.19±2.49	30.12±7.82	46.2
42.57	142.0±5.94	101.8±15.1	71.6	145.8±7.04	84.40±7.82	57.9
43.09	31.55±1.64	41.70±9.84	132.0	35.13±1.63	23.82±2.08	67.8
43.54	50.42±2.32	74.02±15.4	146.0	53.86±2.38	55.56±9.56	103.2
48.83	40.56±2.37			41.01±1.99		

TABLE 4 **Percent Recoveries of toxaphene from sediment using soxlet extractors** (%)

RT (min)	(a) Large Extractors		(b) Medium Extractor
	Sediment + Toxaphene	Toxaphene	Sediment + Toxaphene
32.26	60.4	90.9	69.9
34.65	73.2	94.3	67.1
35.85	80.4	81.6	75.9
36.54	96.6	93.0	91.5
37.11	98.2	82.1	77.8
38.79		89.4	100.7
41.42	(116.0)[a]	62.1	54.9
42.57	(273.0)[a]	71.1	61.2
43.09	(238.0)[a]	74.7	96.7
43.54		70.4	[a] (139.0)
43.85		83.1	

[a]Interference from co-extractants.

TABLE 5 Recovery of toxaphene using method of Wegen and Hoftsee (peak heights)

RT (min)	Standard Peak Height	Fraction A	Fraction C	Total	Percent
32.26	72.64	16.82	37.58	54.40	74.9
34.65	57.88	29.58	10.79	40.37	69.7
35.85	102.94	11.41	57.75	69.16	67.2
35.54	75.81		71.39	71.39	94.2
27.11	80.36		49.51	49.51	61.6
38.79	180.41	14.28	98.45	102.73	56.9
41.42	47.39		30.64	30.64	64.7
42.57	103.13	15.92	68.00	83.92	81.4
43.09	24.92		12.68	12.68	50.9
43.54	38.51	11.85	23.60	35.45	92.1
43.85	31.26	10.16	12.04	22.20	71.0

TABLE 6 Recoveries of toxaphene using extractor for sediment

RT (min)	Standard Peak Height	Fraction C from Extractor	% Recovery
32.26	72.64	27.93	38.5
34.65	57.88	5.41	9.4
35.85	102.94	41.83	40.6
36.54	75.81	54.45	71.8
37.11	80.36	32.46	40.4
38.79	180.41	107.44	59.6
41.42	47.39	18.29	38.5
42.57	103.13	45.04	43.7
43.09	24.92	13.05	52.4
43.54	38.51	12.29	31.9
43.85	31.26	9.42	30.1

CAPTIONS FOR FIGURES

Fig. 1 Typical chromatogram of toxaphene, where small arrows denote the peaks used for quantification.

Fig. 2 (a) Chromatogram of extracted tap water,

(b) Chromatogram of extracted tap water passed through silica gel cleanup column.

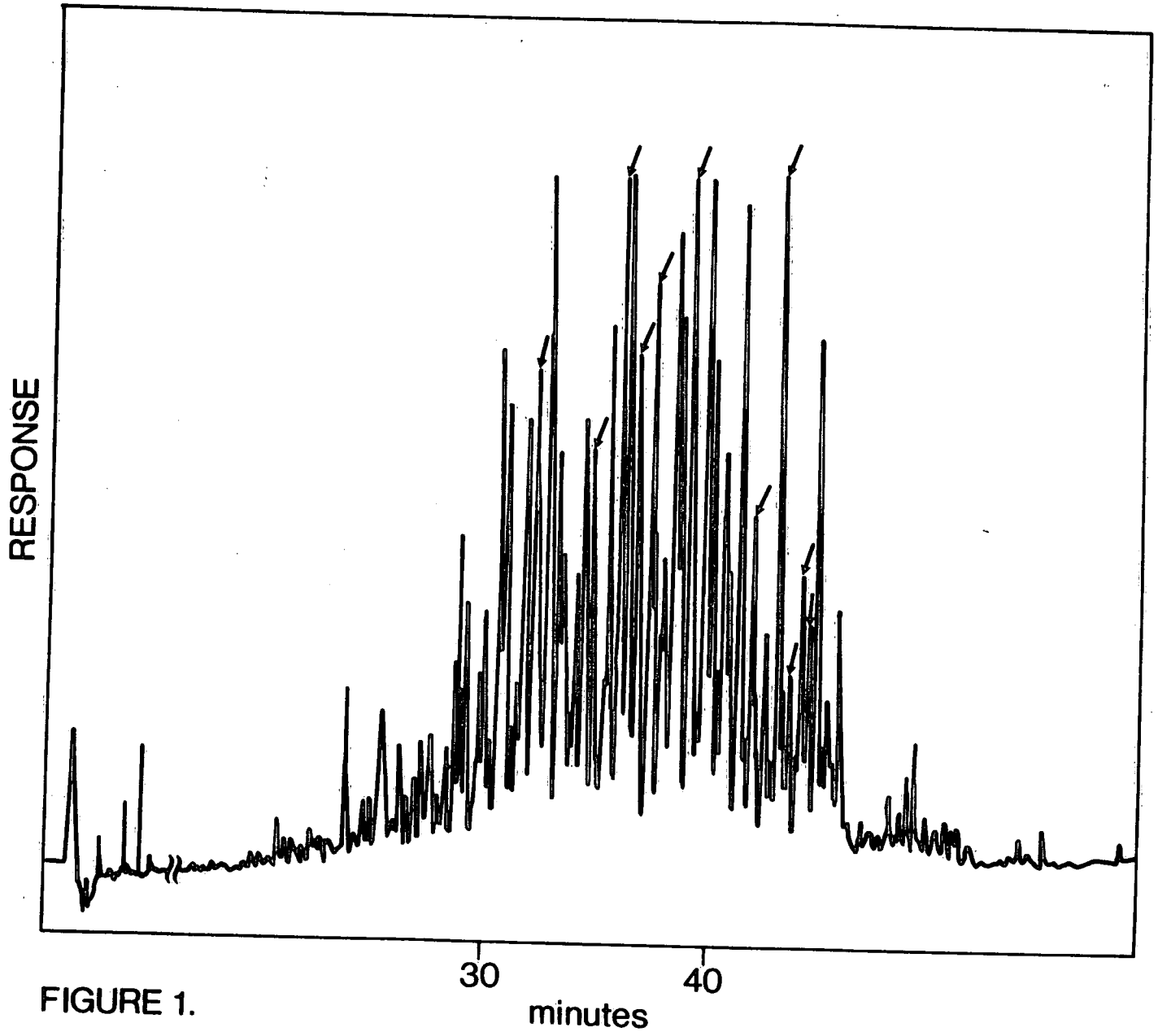

FIGURE 1.

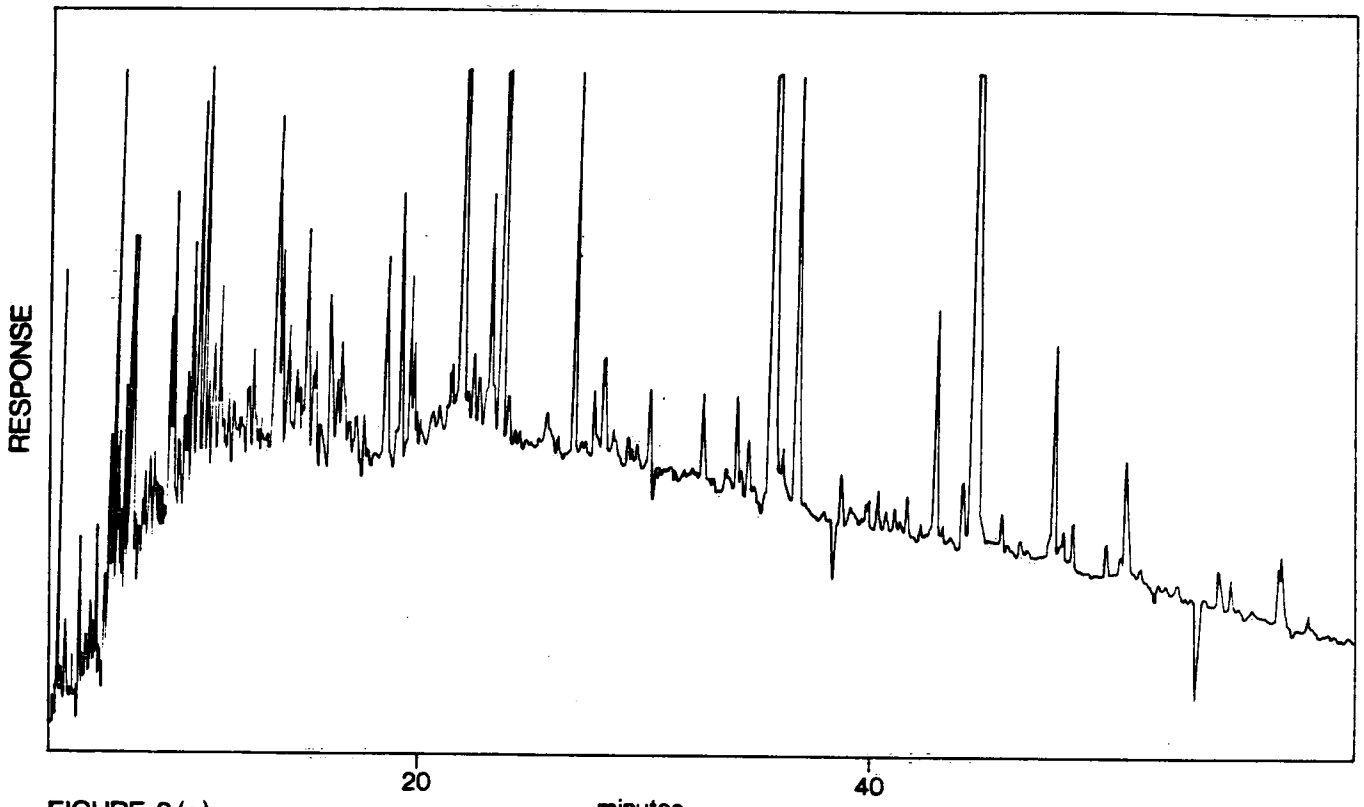

FIGURE 2 (a)

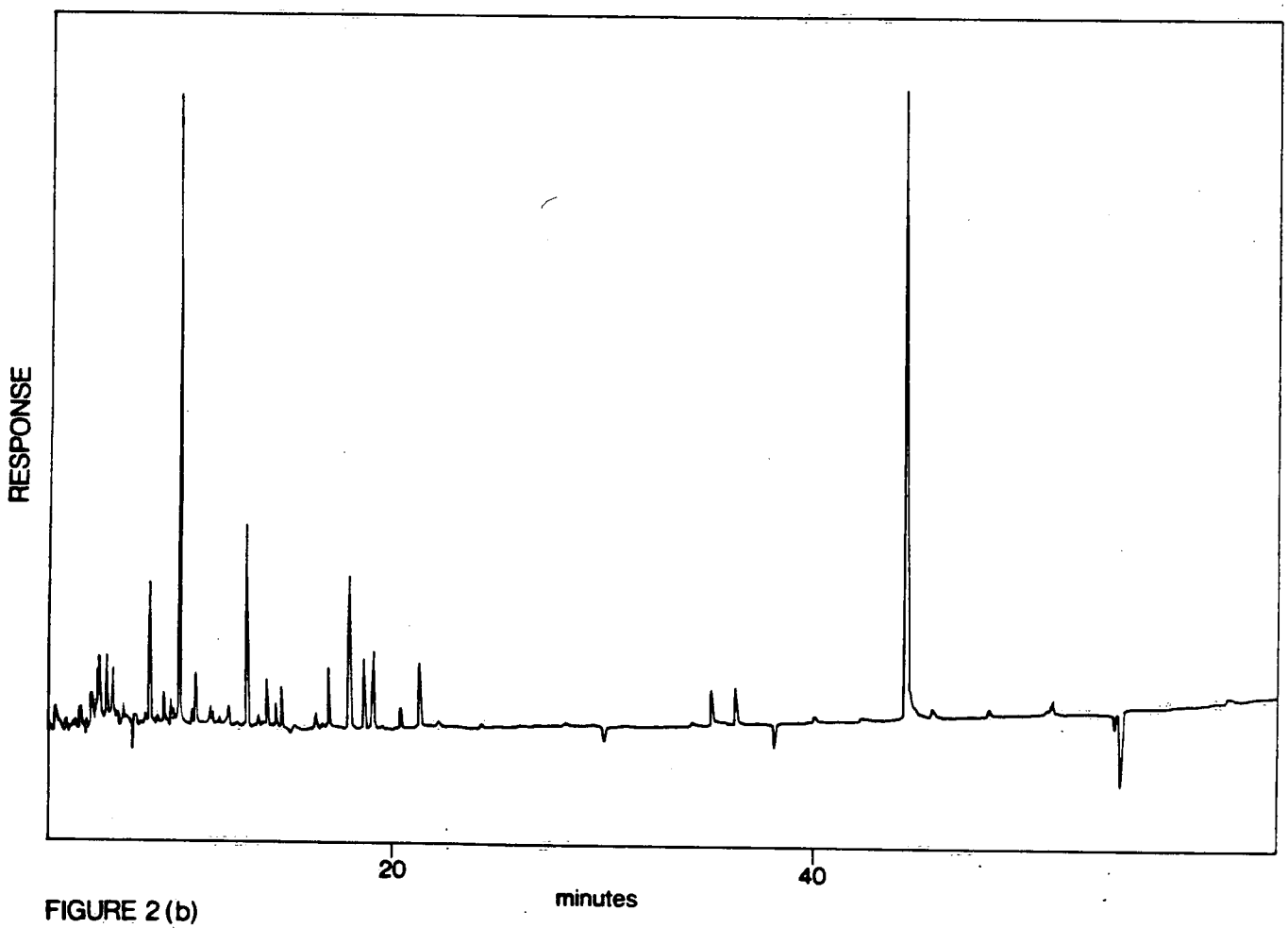

FIGURE 2 (b)

APPENDIX I
CLEAN UP AND EXTRACTION SCHEMES FOR TOXAPHENE IN SEDIMENT