NWRI CONTRIBUTION NO. 86-214

GAS CHROMATOGRAPHIC DETERMINATION OF BIS (2-ETHYLEHEXYL) PATHALATE AND DI-N-OCYTL PHTHALATE IN WATER SAMPLES (IN CONJUNCTION WITH THE ANALYSIS OF ORGANOCHLORINES)

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MANAGEMENT PERSPECTIVE

The National Water Quality Laboratory (NWQL) has expressed a need to expand their organic residue analysis, to include the quantitative analysis of bis(2-ethylhexyl) phthalate (BEHP) and di-n-octyl phthalate (DOP) in aqueous environmental samples. This method was developed in response to that need. The method described is compatible with the NWQL organochlorine residue analysis currently in use (Document 0112i).

The method incorporates the quantitative analysis of BEHP and DOP into the NWQL analytical scheme for the analysis of organochlorines by a modification of the dichloromethane extraction and silica gel clean up steps. This permits the simultaneous extraction of these phthalates and the organochlorines from aqueous samples and their contiguous elution from silica gel clean up columns for analysis by gas chromatography using electron capture detection.

PERSPECTIVE-GESTION

Le Laboratoire national de la qualité des eaux (LNQE) veut incorporer dans les analyses de résidus organiques la mesure quantitative du bis (éthylhexyl-2) phtalate et du di n-octyl phtalate dans des échantillons d'eau prélevés dans l'environnement. Pour répondre à cette demande, une méthode d'analyse a été élaborée. La technique décrite ici est compatible avec la méthode d'analyse des résidus organochlorés utilisée à l'heure actuelle au LNQE (document 0112i).

Pour mettre au point cette méthode d'analyse quantitative du bis (éthylhexyl-2) phtalate et du di n-octyl phtalate, on a modifié les procédés d'extraction au dichlorométhane et de nettoyage à la silice colloïdale du programme d'analyse des substances organochlorées du LNQE. De cette façon, on réussit à extraire simultanément des échantillons d'eau les phtalates et les substances organoclorées, en même temps qu'on procède à l'élution de la silice colloïdale dans les colonnes de nettoyage; on peut ensuite procéder à la chromatographie en phase gazeuse jumelée à l'analyse par capture d'électrons.

ABSTRACT

A method for the quantitative analysis of bis(2-ethylhexyl) phthalate and di-n-octyl phthalate in aqueous environmental samples is reported. The method enables the simultaneous extraction and clean up of organochlorines and phthalates for quantitative analysis. Natural water samples are solvent extracted with dichloromethane. The extract is concentrated and subjected to column chromatography on silica gel for clean up and for the separation of the organochlorines and phthalates into separate fractions. The column fractions collected are concentrated and quantitatively analyzed using capillary column gas chromatography with electron capture detection, which is sensitive to low picogram levels of phthalates. The method permits the quantitative determination of the two phthalates of interest in water samples at parts per trillion levels.

SOMMAIRE

Une méthode d'analyse quantitative du bis (éthylhexyl-2) phtalate et du di n-octyl phtalate dans des échantillons d'eau prélevés dans l'environnement est décrite ici. Cette méthode permet de procéder en même temps à l'extraction et au nettoyage de substances organochlorées et de phtalates afin de réaliser des analyses quantitatives. On soumet les échantillons d'eau naturelle à un procédé d'extraction au solvant dichlorométhane. L'extrait est concentré puis soumis à la silice colloïdale dans une colonne de chromatographie afin de nettoyer et de séparer en fractions les substances organochlorées et les phtalates. Par la suite, les fractions recueillies sont concentrées puis quantitativement analysées par la technique de chromatographie en phase gazeuse dans une colonne capillaire, en même temps qu'on procède à l'analyse par capture d'électrons; cette méthode permet de déceler les phtalates à l'échelle infiniment petite des picogrammes. Grâce à cette méthode, on peut mesurer en parties par billion les deux phtalates qui nous intéressent dans les échantillons d'eau.

1. INTRODUCTION

1.1 Properties and Occurrence

Phthalic acid esters (PAE's) are the condensation product of ortho-phthalic acid (1,2-benzenedicarboxylic acid) and aliphatic or aromatic alcohols. As a group these esters exhibit a wide range of polarities and vapor pressures. Generally, the solubility of these lipophylic compounds in water and their volatility is inversely related to the length of the ester side-chain. Among PAE's having isomeric side-chains, volatility increases and aqueous solubility decreases with the degree of branching of the alcohol moiety (Pierce et al., 1980 and Wolfe, Steen and Burns, 1980).

Phthalate esters are among the most ubiquitous manmade contaminants in the biosphere (Wolfe, Steen and Burns, 1980). They are used chiefly as platicizers and as such can constitute up to 40% by weight of finished plastic products (Autian, 1973). They migrate into the environment mainly through volatilization and leaching of the PAE's from plastics (Peakall, 1975).

BEHP is reportedly the most common and widely used PAE (Peakall, 1975) and it has been reported to be difficult to remove from industrial waste effluents (Rawlings, 1980) and from municipal wastewaters under advanced wastewater treatment (Reinhard et al., 1979).

1.2 Toxicity

Although the acute toxicity of PAE's to mammals is low, there is evidence that aquatic organisms concentrate PAE's and are more vulnerable to PAE's than warm blooded animals (Tepper, 1973). Disturbances of the reproduction and growth of aquatic species have been demonstrated in water containing low chronic PPB levels of PAE's (Mayer et al., 1973). The higher molecular weight PAE's, reportedly appear less toxic than the lower molecular weight esters (Pierce et al., 1980), however, this may be as a result of their lower solubility in water under the laboratory conditions that the toxicity tests were conducted. Fulvic acid, a natural component of soil and sediment has been found to complex PAE's, particularly higher molecular weight esters, to form water soluble complexes. In the natural environment, this complexation could increase the availability of the higher molecular weight PAE's (Mayer et al., 1973) and subsequently increase their detrimental affect on aquatic organisms.

The toxicity of PAE's to fish is much less than that of organochlorine pesticides (Peakall, 1975) and biomagnification of PAE's through the food chain is not expected to be as great as for organochlorines (OC's), since fish appear to be more capable of metabolizing and excreting PAE's (Pierce et al., 1980).

1.3 Environmental Significance

Six PAE's have been identified as priority pollutants by the American and Canadian governments; di-methyl phthalate (DMP), di-ethyl phthalate (DEP), di-n-butyl phthalate (DBP) butylbenzyl phthalate (BBP), bis(2-ethylhexyl) phthalate (BEHP), and di-n-octyl phthalate (DOP). Both governments, as part of the Great Lakes Water Quality Agreement of 1978, have set objectives limiting the concentration of these PAE's in the Great Lakes System.

The phthalate esters, BEHP and DOP have been proven the most persistent of the six PAE's identified as priority pollutants. They have been found less susceptible to alkaline hydrolysis at pH's and concentrations characteristic of aquatic ecosystems (Wolfe, Steen and Burns, 1980) and they have been found more resistant to biodegradation and metabolism than the lower molecular weight esters (Tabak et al., 1980 and Stalling et al., 1973 and Lokke et al., 1981).

From an evaluative model designed to assess the fate and transport of five of the priority pollutant PAE's in the aquatic environment, the accumulation of BEHP and DOP has been predicted to be the greatest and their distribution in the bottom sediment the highest because of their lower water solubility. The loss of BEHP through chemical or enzymatic degradation or volatilization was predicted to be insignificant and the greatest loss of DOP was predicted to be through volatilization (Wolfe, Burns and Steen, 1980).

1.4 Methdology

PAE's are volatile and readily separated and analyzed by gas chromatography using temperature programming. Gas chromatography with electron capture detection (GC/ECD) is desirable for the analysis of PAE's as well as OC's (Giam, 1976). Electron capture detection is reportedly more sensitive and specific than flame ionization detection (FID) for the chromatographic analysis of PAE's (Giam et al., 1975 and Weisenberg et al., 1975). However, PAE's have a lower electron capture response than organochlorine pesticides and PCB's. Therefore an efficient separation of PAE's from OC's is necessary prior to GC/ECD analysis in order to prevent interference from the co-extracted OC's (Webster et al., 1976). Adsorption (column) chromatography on florisil (Stalling et al., 1973 and Giam et al., 1975) and alumina (Burns et al., 1981 and Webster et al., 1976) has been employed for the separation of OC's and PAE's in environmental sample extracts.

This report describes a method developed for the silica gel fractionation and collection of PAE's and OC's in extracts of aqueous environmental samples. Silica gel is the adsorbent used by the NWQL for the clean up and fractionation of OC's and the method developed permits the simple extension of the current organochlorine methodology of the NWQL to include the GC/ECD analysis of BEHP and DOP in the analytical scheme.

Phthalate contamination of laboratory reagents and materials is well recognized as a major problem in the analysis of PAE's (Giam et al., 1975 and Ishida et al., 1980 and Singmaster et al., 1976 and Webster et al., 1976). Minimizing this background contamination is essential for the trace analysis of PAE's. Distilled-in-glass organic solvents that contain only parts per trillion levels of PAE's are commercially available (Bowers et al., 1981 and authors experience). These solvents are generally suitable for the trace analysis of PAE's, provided the degree of concentration of the solvents is not excessive. Numerous procedures, including washing with solvents and heating in a muffle furnace, have been reported for the decontamination of glassware and chromatographic reagents. It was found that muffling overnight at 600°C is not totally effective for the removal of PAE's, especially BEHP, from anhydrous sodium sulfate and washing with distilled-in-glass acetone prior to muffling is necessary.

Airborne PAE contaminants could significantly contaminate clean glassware in a relatively short period of time (Giam et al., 1975 and Singmaster et al., 1976). Therefore rinsing with distilled-in-glass acetone, just prior to use, is the most practical method of decontaminating glassware. The temperature of muffling is very important with chromatographic adsorbents because it affects their surface properties (Webster et al., 1976) and it has been suggested that silica gel, in particular, should not be heated above 300°C (Cassidy, 1951). Heating silica gel at 300°C for 12 hours is reported to result in the PAE contaminants being only "almost removed" (Ishida et al., 1980).

Washing silica gel with chloroform-methanol (1:1) is reportedly as effective as muffling at 300°C (Ishida et al., 1980). However, it was found that washing silica gel with organic solvents more polar than dichloromethane changed the chromatographic behaviour of the adsorbent. Prewashing the silica gel column with dichloromethane was found to be an effective means of removing BEHP and DOP contaminants from the silica gel without affecting the elution pattern of the OC's and PAE's during clean up.

2. SCOPE AND APPLICATION

2.1 The method covers the determination of bis(2-ethylhexyl) phthalate and di-n-octyl phthalate in natural aqueous samples, in conjunction with the analysis of polychlorinated biphenyls, organochlorine pesticides and chlorobenzenes.

2.2 The method may also be extended to include the determination of butylbenzyl phthalate (BBP) and di-n-butyl phthlate (DBP) under specific conditions (see 10.1 Clean Up and Fractionation).

2.3 Electron capture detection is very sensitive to PAE's which can be detected at levels of less than 10 pg with a linear response to at least 2000 pg (or 2 ng) of BEHP and DOP and 1000 pg (or 1 ng) of BBP and DBP.

2.4 The detection limit of the method is dependent upon the background level of the PAE's which has been found to be about 8±3 pg. Establishing the detection limit at two or three times the background level would set the detection limit at about 16 pg and 24 pg, respectively, using 1 µL injection volumes.

2.5 The practical detection limit of the method is dependent upon the concentration factor employed. Assuming a continuous large volume extractor is employed for the extraction of 50 litres of sample and the extract concentrated to 1 mL for quantitative analysis, then the practical detection limit would be 0.32 or 0.48 ng/L in the sample. Assuming only 1 L of sample is manually extracted and the extract concentrated to 1 mL, then the practical detection limit would be 16 or 23 ng/L (or 0.016 or 0.024 PPB) in the sample.

3. PRINCIPLE AND THEORY

3.1 Natural water samples are extracted with dichlormethane, either on site or in the laboratory using a large-sample extractor or conventional solvent extraction in separatory funnels.

3.2 The dichloromethane is evaporated under reduced pressure and the extract taken up in a small volume of iso-octane.

3.3 The iso-octane solution is subjected to adsorption chromatography on a silica gel column for the clean up and separation of the organochlorines and the phthalates.

3.4 The silica gel column is sequentially eluted with hexane and benzene for the collection of the organochlorines, followed by ethyl acetate for the collection of the phthalates.

3.5 The organic solvent is removed from the column eluates by vacuum distillation and replaced by a small volume of iso-octane.

3.6 The final iso-octane solutions are quantitatively analyzed by gas chromatography using a non-polar WCOT capillary column with splitless injection, temperature programming and electron capture detection.

4. INTERFERENCES AND STABILITY

4.1 Volatile electrophilic compounds can interfere with the GC/ECD quantitation of the PAE's. The electron capture detector is particularly sensitive to halogenated compounds and the relative response for organochlorine pesticides is 2 to 7 times greater than that for the PAE's.

4.2 An evaluation of the potential interference of organochlorine pesticides, polychlorinated biphenyls, technical chlordane,

toxaphene, polychlorinated diphenyl ethers, carbamate pesticides and phenols reveals that di-n-butyl phthalate, benzylbutyl phthalate and bis(2-ethylhexyl) phthalate are susceptible to interference and that this interference, which originates from components of technical chlordane, toxaphene and polychlorinated biphenyls, is removed by silica gel clean up.

4.3 PAE's are ubiquitous and found in all materials used in the analysis. All clean glassware should be rinsed with acetone (or dichloromethane) prior to use, especially disposable glassware such as Pasteur pipets and gas chromatograph vials. Only high purity glass distilled solvents are recommended and each bottle of solvent regardless of lot number should be analyzed for PAE's due to the possible contamination of the container itself. Care must be taken to follow the special procedures outlined in sections 8.4 and 10.1 for the removal of PAE's from the chromatographic materials used in the silica gel clean up. A blank should be run with each series of analysis, using the same lot of materials employed in the analysis of the samples.

4.4 Matrix interference may cause difficulties depending upon the source and nature of the sample. Highly coloured dichloromethane extracts may contain iso-octane insoluble co-extractants which could result in the loss of PAE's through occlusion, sorption or chemical interaction with the insoluble

material formed when the dichloromethane is replaced by iso-octane. This problem may be overcome by subjecting the concentrated dichloromethane extract to size exclusion chromatography, using an analytical size Water's μ-styragel column with a mobile phase of 1 mL/min dichlormethane and spectrophotometric detection of PAE's at 235 nm.

4.5 Iso-octane solutions of extracts are stable (prior to clean up) for at least three months stored on the bench (at room temperature and exposed to fluorescent room lighting) and for at least six months stored in the freezer at -20°C in the dark.

5. SAFETY PRECAUTIONS

5.1 All organic solvents should be used with care, avoiding inhalation of vapours and contact with skin.

5.2 Benzene is toxic, a known carcinogen and extremely flammable.

5.3 Dichloromethane is a suspected carcinogen and extremely volatile.

5.4 Acetone and hexane are very volatile and extremely flammable and inhalation may cause narcosis.

5.5 Ethylacetate and iso-octane are flammable.

5.6 All operations with these solvents should be performed in a suitable fumehood or canopy and the operator should wear suitable eye protection and follow established safety regulations.

6. SAMPLING PROCEDURE AND STORAGE

6.1 Ideally, water samples should be extracted on site, as soon as possible after collection. Otherwise, they should be stored refrigerated (4°C) in the dark using air tight containers with a minimum of head space in order to minimize losses through biodegradation, photolysis and volatilization which are reportedly the most significant processes accounting for the loss of PAE's from aquatic ecosystems (Wolfe, Steen and Burns, 1980).

6.2 Glass and teflon materials are recommended for the collection, storage and processing of water samples. Care should be taken to prevent the samples and extracts from coming into contact with plastics in order to avoid serious PAE contamination.

6.3 Care must be taken to ensure turbid water samples containing particulates are adequately mixed prior to subsampling because PAE's are reportedly adsorbed onto sediment particles through

van der Waals forces and hydrophobic interactions (Sullivan et al., 1982).

7. APPARATUS

7.1 All reusable glassware should be cleaned with chromic acid or a suitable detergent followed by adequate rinsing with reagent-grade water. Just before use, all glassware, including disposable glassware, should be rinsed with acetone.

7.2 A gas chromatograph equipped with a microprocessor/integrator, a Ni-63 electron capture detector, a split/splitless capillary injection system and automatic sampler/injector; Hewelett-Packard 5880A gas chromatograph complete with a 7671A automatic sampler, or equivalent.

7.3 Muffle furnace capable of maintaining 600°C; thermolyne type 1700 with 9.5"x8.5"x13.5" chamber or equivalent.

7.4 Laboratory oven capable of maintaining 130°C; Fisher Isotemp Gravity Convection Oven or equivalent.

7.5 Rotary Evaporator: Büchi Rotovapor R110 or equivalent.

7.6 Vortex-Evaporator; Buchler Vortex-Evaporator or equivalent.

7.7 Solvent-dispensing bottles of glass and teflon construction with variable 1 to 10 mL repetitive delivery volumes and 1 litre capacity; Oxford Automatic Pipetter model SA or equivalent.

7.8 Transfer micropipetter with variable 100 µL to 1000 µL dispensing volume; Finnpipette or equivalent and fitted with a short length of teflon tubing in order to accommodate disposable Pasteur pipets as tips.

7.9 Glassware:

- a large-sample extractor as per Neilson et al. (in press) or 2 L separatory funnels
- graduated cylinders, 2 L and 50 mL capacity
- Allihn filters with 10 cm x 4 cm ID reservoir and porosity B scintered glass frit (70-100 µ)
- round bottom flasks with 24/40 tapered stopper, 300, 100 and 50 mL capacity (cork rings to support flasks)
- graduated centrifuge tubes with teflon lined screw caps, 15 mL capacity
- glass chromatography columns with teflon stopcock, 35 cm x 1 cm ID.

8. REAGENTS AND MATERIALS

8.1 All solvents should be high quality, distilled in glass, pesticide grade; Burdick and Jackson or Caledon or equivalent. Each bottle should be checked for PAE contaminants before use.

8.1.1 Iso-octane

8.1.2 Hexane

8.1.3 Benzene

8.1.4 Dichloromethane

8.1.5 Ethyl acetate

8.1.6 Acetone

8.2 Reagent grade water; milli-Q quality or equivalent.

8.3 Anhydrous sodium sulfate; BDH AnalaR reagent or equivalent, heated to 600°C overnight and stored in a dessicator.

8.4 Adsorption column chromatography materials must be of highest purity and where indicated solvent washed before use to remove PAE contaminants.

8.4.1 Silanized glass wool; Chemical Research Services Inc. or equivalent, rinsed with acetone prior to use.

8.4.2 Anhydrous sodium sulfate; BDH AnalaR reagent or equivalent, washed with acetone in an Allihn filter, using 100 ml per 100 gm (6 cm plug) and dried at 130°C and then sintered overnight at 600°C and stored in a desiccator.

8.4.3 Silica gel; Woelm active silica gel 100-200 µm particle size (70-150 mesh), heated overnight at 130°C and deactivated with 3%

(w/w) reagent grade water and tumbled 3 hours (see 14.1 under Remarks).

8.5 Phthalate ester standards; analytical reference standards or highest purity possible. Available from the Quality Assurance Branch of the U.S. Environmental Protection Agency at concentrations of 5000 µg/mL in methanol.

8.6 Phthalate ester standard solution of 200 ng/mL in iso-octane.

- prepare an intermediate solution of 50 µg PAE's/mL of iso-octane by diluting 100 µL of the 5000 µg/mL stock solution to 10.0 mL with iso-octane.
- prepare a standard solution of 200 ng of PAE's/mL of iso-octane by diluting 200 µL of the 50 µg/mL intermediate standard solution to 50.0 mL with iso-octane.

8.7 A non-polar WCOT capillary column for gas chromatography; J&W Scientific 30 meter x 0.25 mm ID, DB-5 capillary column with 0.25 micron film thickness or equivalent.

9. EXTRACTION AND CONCENTRATION

9.1 Dichloromethane is superior to non-polar solvents for the extraction of PAE's (Arbin et al., 1980 and Weschler, 1981) and dichloromethane is the solvent employed by the WQNL for the extraction of organochlorines. The extraction technique

employed will depend upon the nature of the sample. For the extraction of ultra-trace levels, a large-sample extractor can be employed for the extraction of large volumes (50 L) of aqueous sample (Nielson et al., in press). Otherwise conventional solvent extraction of 1 to 2 litre volumes of aqeueous sample can be employed as per IWD Document 0112i.

9.2 Water samples, ideally, should be extracted at the sampling site without delay in order to prevent any loss of PAE's as a result of biodegradation. The biodegradation of PAE's, including BEHP, by mixed populations of aerobic micro-organisms in natural water samples has been reported (Saeger et al., 1976 and Schouten et al., 1979). Alternatively, dichloromethane can be added to the sample container, either in the laboratory or at the sampling site, and the sample thoroughly mixed with the dichloromethane at the time of collection, before transporting the sample back to the laboratory.

9.3 If water samples are not extracted at the time of collection, then the inside of glass storage vessels should be extracted with 50 mL of dichloromethane. This is necessary because PAE's in aqueous solutions are rapidly adsorbed onto glass surfaces (Sullivan et al., 1981). The extent of adsorption is inversely related to the aqueous solubility of the PAE (Sullivan et al., 1982) and therefore significant losses of BEHP and DOP may be expected upon storage of water samples in glass bottles.

9.4 If the dichlormethane extracts are dried by drawing them through an Allihn filter containing a 5 cm plug of anhydrous sodium sulfate (previously sintered overnight at 600°C), then 100 ml of acetone (or dichloromethane) solvent should be passed through first in order to remove phthalate contaminants. Extracts should not be concentrated by subjecting them to evaporation under a stream of nitrogen because of the possible introduction of PAE contaminants as a result of the outgassing of PAE's from plastic tubing, filters or molecular sieves.

10. CLEAN UP AND FRACTIONATION

10.1 The clean up of water sample extracts, together with the separation and collection of the organochlorine and phthalate fractions for GC/ECD quantitation is accomplished through adsorption chromatography on silica gel.

The most critical aspect of this part of the methodology is the removal of PAE contaminants from the chromatographic column and materials.

Phthalate ester contaminants cannot universally be removed from silica gel by solvent washing, without affecting the chromatographic behaviour of the silica gel. Dichloromethane is the strongest solvent that can be employed for prewashing silica gel columns without affecting the elution characteristics of the column. However, dichloromethane is only capable of stripping BEHP, DOP and the more non-polar PAE's from silica gel.

Therefore, in order to extend this methodology for the analsysis of the more polar PAE's, BBP and DBP, a source of silica gel, free of BBP and DBP contamination, must be used. Woelm, active silica (100-200 µm), packaged in 500 gm quantities in aluminum cans, has been found suitable for this purpose.

10.1.1 Prepare a 35 cm x 1 cm ID glass column using a plug of acetone rinsed, silanized glass wool, followed by 2.5 cm of anhydrous sodium sulfate (prewashed with acetone and sintered), 8 cm of 3% deactivated silica gel and a second 2.5 cm of anhydrous sodium sulfate (prewashed with acetone and sintered).

10.1.2 Prewash the column with 20 ml of dichloromethane followed by 20 ml of hexane. Ensure that once the column is wetted with solvents it is not allowed to dry past the top of the packing material. Discard the wash eluates and place a clean 100 mL round bottom flask under the column to collect the first fraction.

10.1.3 Using an acetone rinsed, disposable 9 inch Pasteur pipet, transfer the iso-octane concentrate of the extract to the head of the column.

10.1.4 Allow the meniscus of the extract concentrate to just disappear below the top of the upper layer of anhydrous sodium sulfate.

10.1.5 Rinse the flask that contained the extract concentrate with 5 mL of hexane and transfer the rinsings to the column.

10.1.6 Allow the hexane rinsings to drain down to the top layer of anhydrous sodium sulfate in the column.

10.1.7 Repeat steps 10.1.5 and 10.1.6.

10.1.8 Add 15 mL hexane eluant to the column and allow the hexane to drain down to the top layer of anhydrous sodium sulfate. Collect and label the hexane eluate along with the flask rinsings and the extract's iso-octane - FRACTION A.

10.1.9 Place a clean 50 mL round bottom flask under the column and elute the column with 20 mL of benzene. Allow the benzene to drain down to the top layer of anhydrous sodium sulfate and collect and label the eluate - FRACTION B.

10.1.10 Place a clean 15 mL screw cap centrifuge tube under the column and elute the column with 10 mL of ethyl acetate (see 14.2 under Remarks). Allow the column to totally drain dry with this eluant and collect and label the eluate - FRACTION C.

10.1.11 Add 10 mL of iso-octane to FRACTION A and FRACTION B and concentrate by vacuum distillation on a rotary evaporator to 3 mL. Do not concentrate to less than 3 mL.

10.1.12 Quantitatively transfer the 3 mL concentrates of FRACTION A and FRACTION B to graduated centrifuge tubes using an additional three rinsings of 2-3 mL iso-octane.

10.1.13 Place both fractions under a stream of nitrogen and evaporate to 1.0 ml using a water bath temperature of 30-35°C.

10.1.14 Evaporate FRACTION C just to dryness under reduced pressure using a vortex evaporator or a rotory evaporator using a temperature of 40°C.

10.1.15 Dissolve the residue of FRACTION C in 1.0 ml of iso-octane.

10.1.16 FRACTION A contains chlorobenzenes, PCB's, Heptachlor, Aldrin, Mirex and DDT metabolites (p,p'-DDE, o,p'-DDT, p,p'-TDE and p,p'-DDT).

10.1.17 FRACTION B contains α-BHC, Lindane, Heptachlor Epoxide, α-Chlordane, γ-chlordane, α-Endosulfan, β-Endosulfan, Dieldrin, Endrin, Methoxychlor, Toxaphene, Polyaromatic hydrocarbons and DDT metabolites (o,p'-TDE, p,p'-DDT and possibly o,p'-DDT and p,p'-DDT).

10.1.18 FRACTION C contains the phthalate esters.

11. QUANTITATIVE ANALYSIS

11.1 FRACTION A and FRACTION B are quantitatively analyzed for the OC's described in 10.1.16 and 10.1.17, respectively, using the gas chromatographic conditions described in NWQL Document 0112i.

11.2 The PAE's in FRACTION C are quantitatively analyzed for the PAE's using the gas chromatographic conditions employed for the analysis of toxaphene (Ryan et al., 1986).

Injection Volume:	1 µL
Injection Port:	splitless mode, 60 sec. purge activation time
Injection Port Temperature:	200°C
Detector:	Nickel-63 electron capture
Detector Temperature:	300°C
Detector Make-Up Gas:	Argon/methane (95/5) at 40 mL/min
Carrier Gas:	Hydrogen @ 1.0 mL/min (260°C), linear velocity 41.5-33.5 cm/sec, (80°-260°C)
Column Head Pressure:	10 psig
Initial Column Temperature:	80°C, hold for 3 min
Temperature Program Rate 1:	20°/min to 150°C
Temperature Program Rate 2:	2°/min to 260°C
Final Column Temperature:	260°C, hold for 10 min
Column:	30 metre x 0.25 mm ID, 0.25 µ film DB-5 capillary column (J&W Scientific, Inc.)

11.3 Calibrate the GC/ECD using a standard solution containing 200 PPB (µg/L) of the PAE's of interest in iso-octane. Be sure to correct for the blank values obtained for the analysis of iso-octane solvent. (Using the chromatographic conditions described in 11.2, the retention times for the six priority pollutant PAE's, DMP, DEP, DBP, BBP, BEHP and DOP should be approximately 9, 11, 22, 37, 45 and 52 min respectively).

11.4 Establish the reagent blank values for the PAE's of interest by concentrating the same volume of dichloromethane solvent used for the extraction of the water samples and subjecting it to the same clean-up procedure.

11.5 Analyze the PAE fraction (FRACTION C) of the water sample extracts, analyzing a standard before and after every five water sample extracts and running an iso-octane blank after every standard and extract.

11.6 Measure the peak height and/or peak area of the sample extract peaks whose retention times correspond to those of the PAE standards within ±0.03 min.

11.7 Correct the sample extract peak values for the reagent blank values obtained in step 11.4.

12. CALCULATIONS

12.1 Determine the correction factor (C_f) for each of the PAE's of interest, for the extraction technique employed, i.e. solvent extraction in separatory funnels or in a large-volume extractor. The correction factor is obtained by spiking a known amount of standard PAE's into the same volume of samples used in the analysis and running the spiked sample through the extraction, concentration, clean up and GC/ECD analyses. The level of the PAE spike should be at least double the indigenous concentration of that PAE in the sample. The correction factor is calculated by dividing the GC/ECD value (area or height) obtained for the spiked PAE in the sample extract by the value obtained for the same amount of PAE standard injected directly into the GC/ECD.

12.2 Calculate the concentration of individual PAE's in the aqueous sample using the following formula:

$$\text{aqueous concentration ng/L} = \frac{A_{ext}}{A_{std}} \times \frac{C_{std}}{C_f} \times \frac{V_{ext}}{V_{aqu}} \times 1000$$

where A_{ext} = peak area of height of a response in the sample extract

A_{std} = peak area or height of the corresponding PAE standard

C_{std} = ng/ml concentration of the PAE standard injected to give response A_{std}

C_f = correction factor to compensate for the loss of PAE during the processing of the sample (concentration and clean up)

V_{ext} = final volume (mL) of the iso-octane solution of FRACTION C

V_{aqu} = total volume in (mL) of the aqueous sample extracted.

12.3 Identification of PAE's can be reinforced by the GC/ECD analysis of sample extracts on two dissimilar non-polar capillary columns.

12.4 Final confirmation of the PAE's presence in sample extracts is achieved using GC/MS in the electron impact mode and monitoring the common mass ion of phthalate esters - m/e 149. Alternatively, chemical-ionization mass spectrometry can be employed to confirm the phthalate esters identity (Addison, 1979).

13. PRECISION AND ACCURACY

13.1 The standard deviation for the retention time of BEHP and DOP, based on ten replicate injections of standard solutions was less than ±0.01 minutes.

13.2 The relative standard deviation (RSD) of the peak area of BEHP and DOP based on ten replicate injections of 200 pg was 2.8% and 3.4% respectively (2.2 and 3.7% for peak height).

13.3 Based on recoveries obtained for aqueous samples containing 0.2 ng/ml, the recovery of BEHP and DOP should be 90% or better using either the large-volume extractor or conventional solvent extraction in separatory funnels.

14. REMARKS

14.1 The elution pattern of the organochlorines and PAE's must be determined for each batch of silica gel prepared.

14.2 If only BEHP and DOP are to be analyzed, then the 10 ml of ethyl acetate eluant for FRACTION C may be replaced with 20 ml of dichlormethane and the eluate collected in a 50 ml round bottom flask instead of a 15 ml centrifuge tube.

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