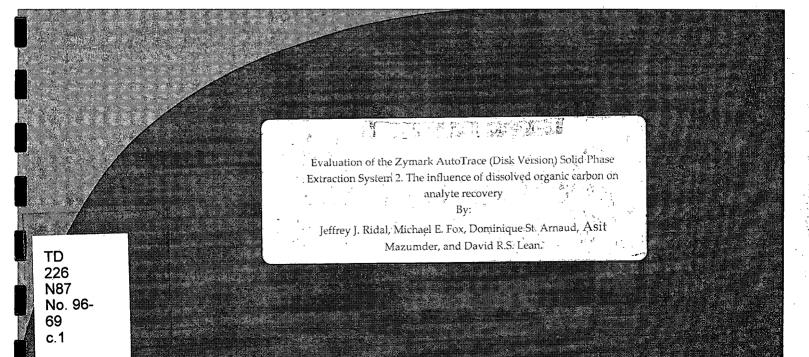
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Environment Canada Water Science and Technology Directorate

Direction générale des sciences et de la technologie, eau Environnement Canada



Evaluation of the Zymark AutoTrace™ (Disk Version)

Solid Phase Extraction System

2. The influence of dissolved organic carbon on analyte recovery.

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Evaluation of the Zymark AutoTrace[™] (Disc Version) Solid Phase Extraction System 2. The Influence of Dissolved Organic Carbon on Analyte Recovery.

MANAGEMENT PERSPECTIVE

Issue

Current procedures for the extraction of contaminants from water are labour intensive, time consuming and produce large volumes of waste solvents. Restrictions on the use, transport and disposal of hazardous solvents are expected to become more stringent. New procedures are needed to address these issues.

Background

Recent advances in laboratory instrumentation have resulted in commercially available instruments utilising processes such as solid phase extraction (SPE). This technique has the potential to replace traditional liquid-liquid extraction of organic contaminants from water with substantial savings in time and solvents consumed. Dissolved organic carbon (DOC) in natural waters may cause low recoveries of analytes. This phenomenon has not been adequately investigated.

Status

In the first stage (NWRI Contribution 94-45) we evaluated the Zymark Autotrace SPE system under laboratory conditions with solutions of analytes dissolved in distilled water. In this study we moved to field conditions with natural waters containing moderate to high concentrations of dissolved organic carbon. At the highest DOC levels investigated, recoveries of target analytes were approximately 20% less than those achieved by neutral pH liquid-liquid extraction. Thus, further work is required for the successful extraction of a full range of natural waters.

Next Steps

The next stage of the study will utilise cartridge style adsorbents rather than the disk adsorbents used in this report. The increased capacity of these cartridges is expected to allow the successful extraction of larger volumes of high DOC natural waters.

ABSTRACT

The influence of dissolved organic carbon (DOC) from a Canadian Shield humic lake on the recovery of selected organochlorine pesticides by a solid phase disk extraction (SPE) method has been investigated. We tested a semi-automated Zymark AutoTrace™ instrument fitted with SPEC™ disks. In the first of two experiments, we examined recoveries of selected organochlorine pesticides from fortified distilled and high DOC lake water. Mean recoveries of analytes from distilled (62 \pm 5%) and lake water (55 \pm 7%) were lower by SPE than by neutral pH liquid-liquid extraction, which averaged 75 ± 11% and 76 \pm 12%. When volume of lake water samples processed was increased from 750 to 3000 mL, recoveries generally decreased. High DOC samples benefitted from additional dichloromethane extractions of disks, as up to 12% additional analytes were recovered. Breakthrough, assessed from liquid-liquid extraction of sample effluent, was low for distilled water samples (<1%) but increased for lake water samples (6-37.5%). An additional 2-11% of analytes were recovered by solvent rinses of the containers. In a second experiment, we tested the extraction efficiency of the SPE method to recover contaminants from a standard volume of fortified distilled water after treating extraction disks to various amounts of DOC from filtered, unfortified lake water. Mean recovery of the spiked solution was lower from DOC treated disks (48 \pm 8%) than from untreated disks (62

 \pm 5%). These results indicate that analysts need to assess DOC as a factor affecting measurements of organochlorine pesticides from aquatic systems with this SPE technique.

INTRODUCTION

Solid phase extraction methods are attractive alternatives to liquid-liquid extraction (LLE) for the determination of organic contaminants in natural waters. SPE methods use greatly reduced amounts of solvents, require less intermediate laboratory processing, are more adaptable to semi or full-automation, and offer the promise of greater reproducibility than traditional liquid-liquid extraction methods. A discussion of recent developments in LLE and SPE methods can be found in Part 1 of this contribution series (Sullivan et al., 1994).

A semi-automated SPE apparatus, the Zymark AutoTrace® fitted with SPEC® C_{18} -bonded glass fibre disks, has been previously tested for completeness of extraction of selected organochlorine contaminants from spiked Milli-Q water (Sullivan et al., 1994). The goal of that work was to establish optimum experimental settings for parameters such as loading rates, disk drying times, and choice of extraction solvents. However, we expected Milli-Q water not to accurately represent environmental samples, and in particular that the DOC content of the sample may affect the performance of the SPE technique investigated. Dissolved organic matter may lower extraction efficiencies of pesticides for SPE methods by competing for available sorption sites on the solid phase material. SPE may not extract pesticides associated with organic macromolecules (Eadie et al. 1990, Kulovaara 1993). For example, decreased recoveries of pesticides dissolved in a humic acid solution compared with HPLC-grade water have been observed for C₁₈ bonded-phase silica cartridges (Johnson et al. 1991). Here we present results from investigations of the recoveries of organochlorine contaminants spiked into lake water with varying levels of DOC.

MATERIALS AND METHODS

Solvents used in this study were pesticide grade (Burdick and Jackson). Glassware was cleaned by soaking in laboratory-grade detergent, rinsing with distilled water, and drying in an oven at 200°C overnight. Glassware was solvent rinsed immediately prior to use. Lake water from Lac Cromwell (a 10 ha humic lake located 100 km north of Montreal, see Table 1 for chemical characteristics) was sampled by submersible pump into solvent-rinsed 20 L stainless steel cans, and pressure-filtered using 1 µm pore-sized glass fibre filters (147 mm, combusted; Gelman). Subsamples of filtered lac Cromwell water and laboratory grade distilled water were taken from the cans into pre-combusted 100 mL glass vials, acidified to pH 3 with reagent grade phosphoric acid, and stored at 4°C prior to DOC analysis. Analyses were performed with a high temperature combustion instrument (Shimadzu) using standard protocols.

Table 2 gives a list of compounds studied and the levels of these contaminants in the spiked lake water. We supplemented the organochlorine test solution used in earlier laboratory studies (solution I, Sullivan et al., 1994, Macguire et al., 1995) with a spiking solution containing other organochlorine (OC) pesticides of interest (solution 2). All pesticides had stated minimum purities >95%. OC solutions were mixed into methanol, and equal amounts added to 18 L both of distilled and lake water to give a 0.5% methanol final concentration. An additional 18 L of lake water was spiked with 90 mL of methanol only to give a 0.5% solution. These solutions were stirred slowly with electric mixers for 1 hour prior to commencing the experiments.

After stirring, duplicate 500 mL subsamples of all solutions were liquid-liquid extracted by shaking with DCM (3 x 30 mL) in a 1 L separatory funnel. Upon completion of the experiments, containers holding solutions of OC spiked water were emptied and the containers were twice rinsed with 100 mL of DCM to assess potential loss to container walls.

Solvent rinses of SPEC disks, system settings and extraction procedures followed those recommended by Sullivan et al. 1994. Briefly, disks are rinsed with DCM and ethylether/pentane solvents, and wetted with methanol prior to sample processing. To remove residual water after sample processing, disks were dried in a desiccator for 2 hours prior to extraction. All disks were then extracted with 2 x 5 mL of 15% ethyl ether in pentane (EEP) solvent. In the present work, selected disks were also eluted with an additional 2 x 3 mL of DCM. In order to make sample processing more time efficient, we divided the AutoTrace program into three separate sections: (1) Load Samples; (2) Rinse Disks (to remove residual water after loading samples); (3) Elute Disks. Thus, during the time one set of 6 samples were drying in the desiccator, a second set of samples was loaded onto the disks.

Samples extracted by liquid-liquid technique were concentrated by rotary evaporation after addition of 1 mL iso-octane keeper. Care was taken not to exceed 30°C bath temperature in an attempt to maximize recovery of volatile components. Liquid-liquid extracted samples were cleaned-up on a mini-column of 2% deactivated Florisil. All SPE samples were analysed without further cleanup. Gas chromatographic analysis on an HP 5890 used splitless injections on DB-5 and HP50 30-m bonded phase columns with dual electron capture detectors and hydrogen carrier gas using conditions described in Sullivan et al. (1994). Some decomposition of DDT was noted in the chromatograms, likely owing to thermal decomposition in the inlet of the gas chromatograph. DDT values were calculated as the sum of p,p-DDT and p,p-DDE. Results for the di- and tri-chlorobenzenes were compromised by interference peaks on both GC columns and are not reported here.

Two experiments were used to test the effects of sample DOC on sample recovery. Experiment 1 compared analyte recoveries from distilled and lake water solutions. We also tested the effect of increasing the lake water sample volume on the recoveries of analytes. Experiment 2 tested recoveries for a 500 mL aliquot of OC-spiked distilled water after disks were treated with increasing amounts of lake water and DOC. This goal of this

experiment was to provide information on the degree to which DOC in lake water is responsible for saturating available sites on the C_{18} disk.

Quality Control: Analytical blanks were assessed by extracting SPEC disks after solvents rinses, and after treating the disks with 500 mL aliquots of unfortified distilled or lake water. In all cases, blanks values were <<1% of spiked water concentrations. Losses due to sample handling and clean-up procedures was investigated for the SPE method by adding the spike solution onto pre-conditioned SPEC disks in the Zymark disk holders. The LLE method recovery was checked by analysing for contaminants spiked directly into DCM.

RESULTS AND DISCUSSION

Spike Recoveries. Results for the recovery of sample spikes directly into DCM or onto pre-conditioned SPEC disks (while in disk holders) are shown in Table 3. Recoveries of the spikes from DCM were almost quantitative with mean value = $102 \pm 6\%$, and ranged from 88-109%. These results indicate that work up losses for LLE extraction samples should be small with the methods used. Lower recoveries were obtained for the spikes directly onto SPEC disks which averaged $90 \pm 11\%$ and ranged between 63-112%. The anomolous low value of 63% was obtained for α -HCH. The generally lower recoveries with the SPE method suggest that small but significant losses may occur during normal processing of samples with the techniques used here. Losses with the AutoTrace system may occur through incomplete flushing of extraction solvent from the lines or disk holders.

EXPERIMENT 1

In Figure 1 we compared SPE recoveries of analytes from the same volumes of OCfortified distilled and lake water. Results from liquid-liquid extraction of the distilled and lake water solutions were very similar (±4%) for all analytes and average values are

shown. LLE recoveries averaged $75 \pm 11\%$ and ranged from 58 - 98%. SPE recoveries of same volume (750 mL) distilled and lake water samples were $62 \pm 5\%$ (range 52-68%) and $55 \pm 7\%$ (range 46-67%), respectively. Standard deviations of replicate samples ranged from 1.4 - 9.8% for LLE results and from 1.6 - 14.7% for SPE samples. Earlier work using OCs spiked into high purity Milli-Q water found 64-91% extraction of analytes from the SPEC disks using 15% ethyl ether/pentane with standard deviations of analyses ranging from 1.4-12.5% (Sullivan et al., 1994). Lower recoveries for the laboratory-grade distilled water may be the result of higher levels of DOC in this water than the high purity Milli-Q water.

Figure 2 shows analyte recoveries when the volume of OC-fortified lake water was increased from 750 to 1500, 2000 and 3000 mL. Each sample sequence therefore increased the total DOC loaded onto the disks. It was generally found that SPE recoveries decreased with sample volumes greater than 750 mL or 9 mg DOC. Average recoveries and range of results from extractions for the 1500 mL (i.e. 18 mg DOC), 2000 mL (24 mg DOC), and 3000 mL (36 mg DOC) samples were $35 \pm 14\%$ (17-59%), 36 ± 10 (18-50%), and $32 \pm 9\%$ (19-44%), respectively. Recoveries decreased systematically with increasing sample volume in the cases of methoxychlor, DDT and mirex, however rather similar recoveries were found for sample volumes of 1500 mL (18 mg) or greater for the majority of analytes.

As shown in Table 4, additional extractions of disks with DCM recovered up to 12% more of OCs with highest recoveries found for samples with the highest DOC loads. In contrast, Sullivan et al. (1994), in Milli-Q water based studies, did not recover additional contaminant from SPEC disks with further solvent extractions. Our results suggest that the presence of lake water DOC on disks reduces the effectiveness of the ethyl ether-pentane extraction. It is conceivable that DOC bound to the SPEC disks, particularly larger molecular weight humic acid material, may physico-chemically prevent solvent penetration to C_{18} -bound analytes. Table 4 also shows total extractable recoveries obtained from both

extractions. Total OCs extracted ranged from 55-71% for 750-mL distilled water samples with 46-69%, 22-59%, 24.5-59%, and 22-51% recovery for respective 750, 1500, 2000, and 3000 mL lake water samples.

We measured contaminants passing through the SPEC disks (breakthrough) by sampling the effluent of selected samples. As shown in Figure 3, low levels of analytes were found in the effluent of distilled water samples; however, 6-38% of target compounds were found in the effluent of the lake water samples. The measurements of breakthrough in Figure 3 indicate that: (1) percent breakthrough did not increase for lake water samples when the total DOC load was increased; (2) increasing sample volume did not increase loss of analytes from the disks.

We also measured the amounts of analytes recovered from rinsing the containers holding the distilled and lake water solutions upon completion of the experiments. An additional 2-9% of the OCs were obtained from rinses of the spiked distilled water solution container, somewhat less than the 6-11% recovered from rinses of the lake water container.

Average total recoveries of compounds with log K_{ow} <6, obtained by adding results from all sources, were 74 ± 8% by LLE, 66 ± 4% for SPE DW, and 54 ± 11% for SPE lake water samples. Higher total recoveries were found for compounds with log K_{ow} >6: 85±11% by LLE, 72±4% for SPE DW, and 77±11% for SPE lake water samples. Unexplained losses could occur from solvent evaporation steps, losses to tubing and surfaces in the AutoTrace instruments, losses to glassware during sample transfers, and losses during Florisil cleanup of LLE samples. Lower overall recoveries of low log K_{ow} compounds may be the result of evaporative losses during the sample concentration step.

EXPERIMENT 2.

In this experiment, disks were loaded with increasing amounts of DOC followed by a standard 500 mL aliquot of distilled water fortified with OCs. Increasing amounts of DOC

were loaded on disks by processing varying volumes of lake water. These volumes ranged from 0 (no lake water) to 3000 mL of lake water resulting in DOC loads of approximately 3, 9, 15, 18, 24 and 36 mg. Immediately following each treatment, all sample lines were connected to the OC-spiked distilled water and this solution was pumped through all disks. Disks were extracted by both ethyl-ether/pentane and DCM extraction procedures.

Figure 4 shows recoveries of contaminants from the 500 mL of spiked distilled water from disks previously treated with increasing volumes of lake water compared to the SPE recoveries found by analysing the distilled water sample directly (DW; mean = $62 \pm 6\%$, 52 - 70% of OCs recovered). Recoveries by SPE for low K_{ow} analytes (PeCB - heptachlor) from DOC treated disks averaged 50±8% and ranged from 35-72%. No clear effect of DOC on recovery was observed for these low K_{ow} compounds. On the other hand, results for the compounds with K_{ow} ≥ 6 do show decreased recoveries when disks were pre-treated with lake water. Mean sample recoveries from lake water treated disks range from $52 \pm 7\%$ to $32 \pm 3\%$; all lower than recoveries ($62 \pm 5\%$) for the distilled water sample analysed directly.

Liquid-liquid extraction of the sample effluent recovered negligible (< 0.5%) amounts of the target compounds from untreated disks, while $\sim 0 - 8\%$ of analytes averaging $4.2 \pm 2.5\%$ were recovered for DOC-treated disks. These breakthrough values are 3-4 times lower-than those found in Experiment 1 for OCs spiked into lake water. Therefore, while breakthrough attributable to competition for sorption sites by lake water DOC does appear to occur, the higher values found when lake water was spiked directly suggest that the OC-DOC complexes formed in the lake water solution account for the majority of the observed breakthrough.

Using this data, indicative of losses from the disks other than OC-DOC binding, to correct the breakthrough data from Experiment 1, we calculated the OC-DOC partition coefficients

where:

K_{poc} = % Breakthrough/ (% Extracted x [DOC, g/mL])

The range of log K_{Doc} values falls between 3.83 and 4.64 and, with the exception of HCH, values increase with increasing K_{ow} . Excluding the HCH value gives the linear relationship ($r^2 = 0.96$) shown in Figure 5, which is in good agreement with the relationship:

 $Log K_{DOC} = 0.24 Log K_{ow} + 2.78$ (r = 0.61)

found by Eadie et al. (1990) for selected radio-labelled hydrophobic organic contaminants and DOC from Great Lakes waters. The HCH outlier in Figure 5 probably indicates that additional breakthrough of this low K_{ow} compound for the truly dissolved species, perhaps due to a lowering of affinity for the C₁₈ matrix in the presence of humic material. Disks employing C₈ reverse phase adsorbents has been found to provide greater recoveries of HCH isomers from Great Lakes water (T. Bidleman, personal communication).

CONCLUSIONS

DOC significantly influences the analysis of organochlorine pesticides using the Zymark AutoTrace® fitted with SPEC® disks. The following effects, attributable to the presence of lake water DOC, were noted:

1) Decreased efficiency of extraction with ethyl-ether pentane. A second extraction solvent is recommended;

2) Increased breakthrough compared with distilled water;

3) Increased losses of analytes to container and other surfaces.

It is recommended that surrogate or matrix spikes of compounds similar to the target

(1)

(2)

analytes be used to determine possible differences in extraction efficiencies between samples from natural waters. The use of appropriate internal standards following extraction is also indicated from this work to control for losses during sample concentration steps. Further investigation should be directed toward whether the design of the disks holders is optimal. The use of C_{18} cartridge type adsorbents, with much higher analyte capacities, may be required for water samples with significant amounts of DOC.

ACKNOWLEDGEMENTS:

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Table 1

Chemical characteristics of Lac Cromwe	ell water	
DOC (mg C/L)	12.1	•
Calcium (mg/L)	3.7	
Total P (µg/L)	9	,
pH	6.7	
DOC (mg C/L) of distilled water	2	

Table 2

List of analytes, their log octanol-water partition coefficients, and the concentrations in water samples after spikes were added.

Analyte	log K _{ow}	Target Concentration (ng/L)
α-Hexachlorocyclo-hexane (HCH)	3.8	87.6
Methoxychlor	4.3	100
tetra-Chloro-benzene (TeCB)	4.5	85.2
penta-Chloro-benzene (PeCB)	4.9	81.6
hexa-Chloro-benzene (HCB)	5.4	80.7
Heptachlor	5.4	96.2
g-Chlordane	6	221
a-Chlordane	6	78.4
trans-Nonachlor	6.1	41.8
cis-Nonachlor	6.4	44.0
DDT	6.4	80.4
Mirex	6.9	117

Table 3

Recoveries of organochlorine contaminants after direct spikes of solution mixes 1 & 2 onto disks and into dichloromethane.

Analyte	Percent Recovery from DCM (solution 1)	Percent Recovery from disk (solution 1)	Percent Recovery from disk (solutions 1 & 2)
НСН			63 ± 11
Methoxychlor			100 ± 7.5
tetra-CB	105 ± 3.1	75 ± 19	87 ± 7.5
penta-CB	109 ± 1.9	82 ± 12	89 ± 6.5
hexa-CB	105 ± 0.5	89 ± 3.5	91 ± 4.3
Heptachlor	·	,	82 ± 11
g-Chlordane	106 ± 3.1	97 ± 8.3	87 ± 3.5
a-Chlordane			86 ± 11
t-Nonachior		· ·	98 ± 11
c-Nonachlor			101 ± 16
DDT	88 ± 0.6	112 ± 9.2	100 ± 4.5
Mirex	103 ± 0.6	95 ± 9.7	83 ± 1.8

Table 4

Total amounts extracted for Experiment 1 with amounts recovered from additional DCM extractions shown in brackets.

Analyte	Percent Recovery DW	Percent Recovery +9 mg	Percent Recovery +18 mg	Percent Recovery +24 mg	Percent Recover y +36 mg
НСН	66 (2.1)	44 (n.d.)	22 (n.d.)	28 (n.d.)	22 (n.d.)
Methoxychlor	68 (1.8)	67 (1.8)	60 (4.1)	55 (6.5)	45 (6.4)
tetra-CB	71 (3.4)	69 (1.6)	25 (7.9)	25 (6.2)	31 (12)
penta-CB	68 (2,8)	61 (1.8)	26 (7.6)	29 (9.5)	33 (1 3)
hexa-CB	61 (2.6)	58 (2.5)	30 (7.7)	44 (1 2)	33 (11)
Heptachlor	57 (2.1)	55 (2.4)	34 (6.3)	45 (11)	35 (8.8)
g-Chlordane	62 (n.d.)	52 (n.d.)	41 (n.d.)	42 (n.d.)	50 (7.5)
a-Chlordane	70 (2.2)	54 (2.9)	46 (5.7)	51 (11)	49 (8.5)
t-Nonachlor	67 (n.d.)	58 (4.6)	46 (4.8)	40 (n.d.)	46 (7.9)
c-Nonachlor	61 (2.8)	55 (2.5)	52 (4.9)	55 (10)	51 (7.0)
DDT	68 (3.0)	69 (2.2)	59 (n.d.)	59 (10)	46 (7.5)
Mirex	55 (2.5)	49 (2.8)	46 (5.9)	38 (8.2)	37 (7.3)

List of Figures:

1/ Recoveries of target analytes by liquid-liquid (LLE) and solid phase extraction of distilled water (DW), and SPE of spiked lac Cromwell water (LCW) samples. LLE values shown are averages of the distilled and lake water values as very similar results (+/- 4%) were obtained for all analytes.

2/ Recoveries of target analytes from different volumes of spiked lake water. Indicated are the levels of DOC loaded onto disks based on the amount of lac Cromwell water analysed and a DOC content of 12.1 mg C/L.

3/ Recoveries of analytes (percent breakthrough) from liquid-liquid extractions of sample effluent.

4/ Comparison of analyte recoveries from spiked distilled water after disks were pretreated with various volumes of lake water. Indicated are total amounts of DOC to which disks were subjected prior to analysis of spiked distilled water.

5/ Plot of log K_{DOC} versus Log K_{ow} . Relationship calculated using data for +9 mg DOC analyses from Experiment 1 corrected with data from Experiment 2 as discussed in text.

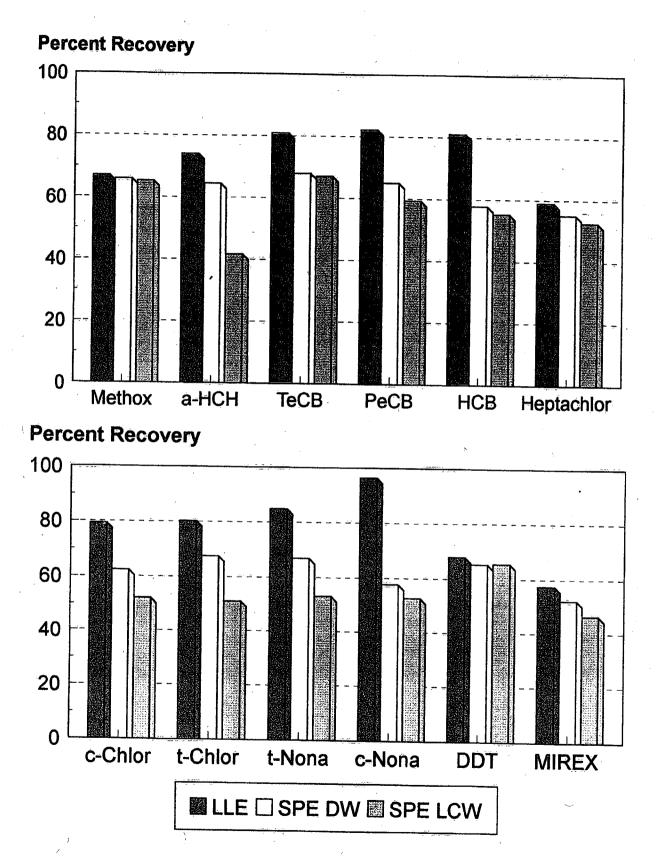
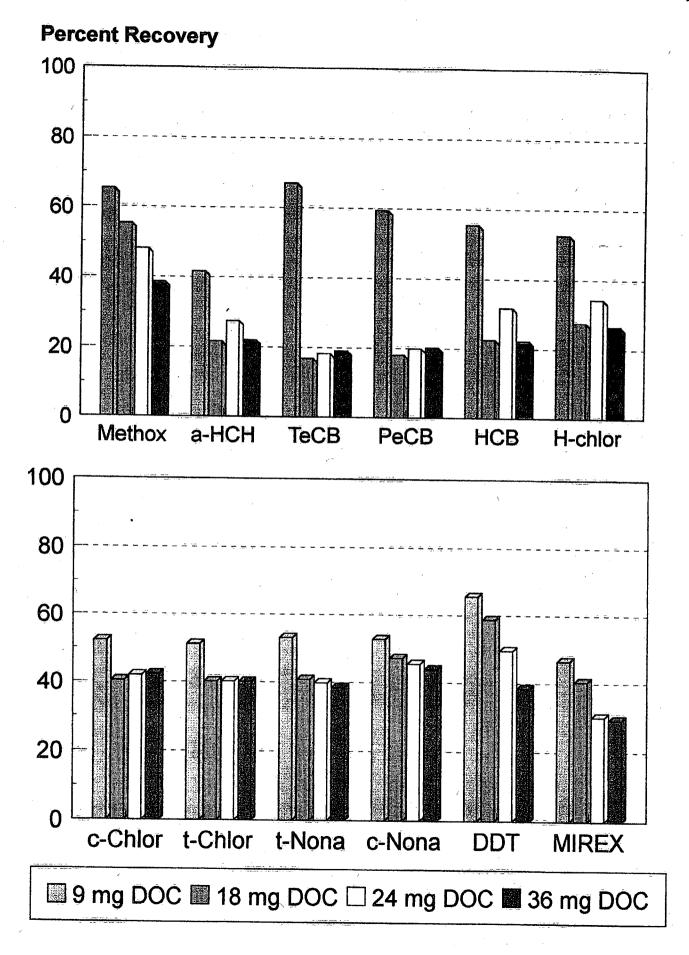
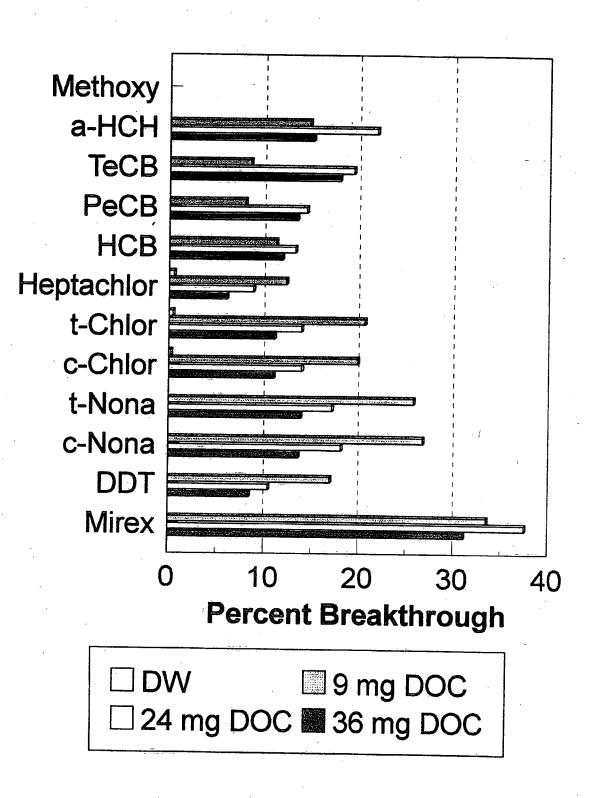


Fig.1



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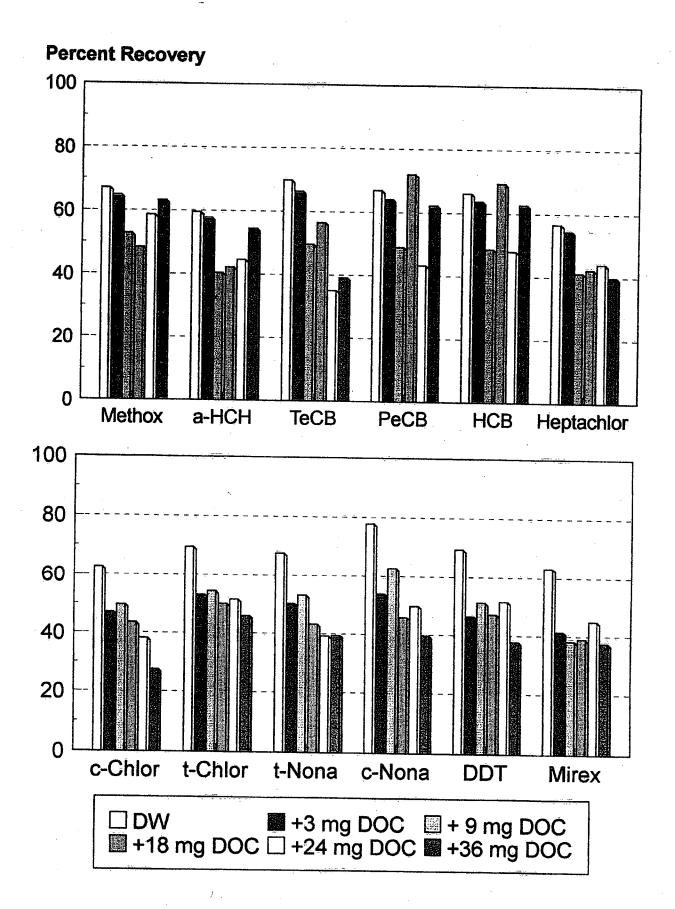
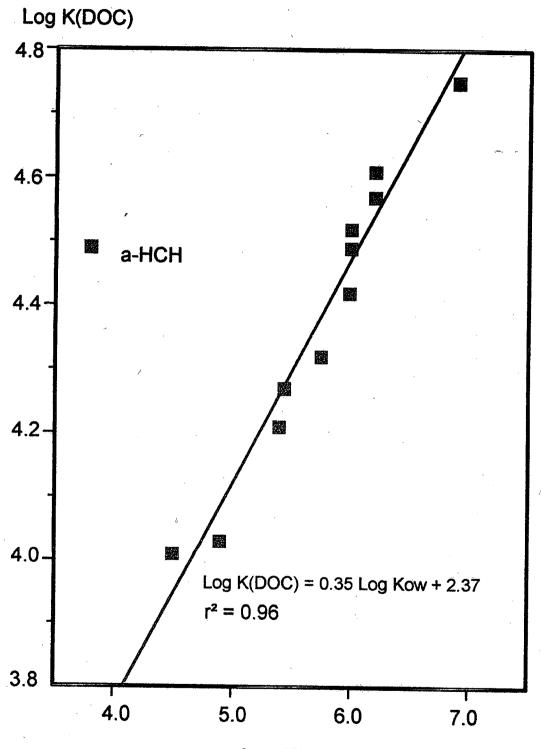


Fig 4





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