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ASSESSING THE HEALTH RISK OF TOXAPHENE CONTAMINATED SOILS USING ELISA AND GAS CHROMATOGRAPHY

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Introduction

Technical toxaphene (TTX), a broad spectrum organochlorine insecticide and a persistent organic pollutant (POP) of international concern[1], was produced in bulk by Hercules Inc. at their Brunswick, Georgia (USA) facility for more than 30 years[2]. Because several public access areas are in close proximity to the facility, concerns over increased human health risks due to toxaphene contamination in soils have been raised, particularly for children[3]. Previous studies have disagreed on the levels and extent of local toxaphene contamination, with some reporting levels as high as 64 ppm[4], while others conclude that TTX is nondetectable[5]. Variable definitions and analytical difficulties in distinguishing toxaphene residues from other related POPs are two main reasons for this discrepancy.

Because GC-based techniques for trace organic contamination are costly and time-consuming, enzyme linked immunosorbent assays (ELISA) present a lower cost analytical alternative, particularly where semiquantitative results are satisfactory. Results of "screening" studies for various combinations of organic contaminants and environmental matrices using commercially available ELISA kits are in good agreement with GC-based concentrations in some[6], but not all cases[7]. The objective of this study was to determine if toxaphene contamination in topsoils from public access areas poses a potential human health risk by comparing the screening results of a commercially available, ELISA-based "toxaphene in soils test kit" with concentrations based on multidimensional gas chromatographic techniques.

Materials and Methods

Sample Collection. Based on the suggestion of elevated toxaphene[4], we collected a total of 94 topsoil samples in May-June 2002 from 4 public access areas that lie within 2 km of the Hercules Inc. chemical plant. Three schools -- Goodyear Elementary (GYES), Burroughs-Molette Elementary (BMES) and Risley Middle School (RMS), and one public recreational area – the Edo Miller/Lanier Field Recreational Area (EM/LF), were sampled. Each site was divided into square (930 m^2) or rectangular (30.5 m x 71 m) grids and five topsoil (0-7.5 cm depth) grabs per grid were collected with a steel bulb planter, mixed in a methanol-rinsed aluminum pan, and composited in a 250 ml glass I-Chem jar. Composites were kept cool (~4^{o}C) and out of direct sunlight for $\leq$ 3 d prior to testing.

ELISA. All samples were prepared and analyzed using the Toxaphene in Soil Test Kit (part no. 7420000) after extraction of soil using the (methanolic) Soil Extraction Kit (Part no. 7420000EA) from Strategic Diagnostics Inc. (Newark, DE, USA) in accordance with vendor procedures[8]. Absorbance (λ=450 nm) was measured using a Cary dual beam UV-VIS spectrophotometer.

Results were semiquantitatively classified as <0.5; 0.5<x<2; 2<x<10; and x>10 ppm. Interfering levels of related cyclodiene pesticides were in general much lower than the 0.5 ppm minimum detection level for toxaphene (Table 1). Toxaphene concentrations ("ELISA-TOX") were also estimated using a nonlinear, 3-point absorbance calibration curve.

Table 1. ELISA method detection limits (MDLs) and concentrations that inhibit 50% of negative control color intensity (IC50) for toxaphene and interfering compounds[8].

Compound	MDL	IC50
Toxaphene	***0.5ppm***	***2.8ppm***
Endrin	3.9ppb	22ppb
endosulfan I/II	6.4/5.0ppb	36/28ppb
Dieldrin	7.5ppb	42ppb
Heptachlor	6.1ppb	34ppb
Chlordane	***17.9ppb***	***100ppb***
γ-BHC (lindane)	0.8ppm	4.6ppm

GC-ECD and GC-MS. Toxaphene, chlordane, PAH and PCB concentrations for 36 of the 94 soil samples, including all with >2 ppm according to ELISA, were quantified using a complementary GC approach[9]. Eight g soil was extracted with 90% CH_2Cl_2/10% methanol (v/v) using a Dionex Automated Solvent Extraction (ASE) system at 100^{o}C and 1500 psi. Extracts were exchanged to hexane, separated into 2 fractions by Florisil column chromatography and analyzed on a Varian 3400CX GC with electron capture detection (GC-ECD) and a HP6890 Series 2 Plus GC coupled to a 5973 MSD operating in the electron and negative chemical ionization modes. Fused silica DB-XLB columns (30m x 0.25mm x 0.25μm) were used to separate target analytes. Quantitation was based on mean response factors from 3-point calibration curves of authentic standards. Total toxaphene (ΣTOX) was estimated by summing peaks eluting in a pre-specified ECD retention time window.

Quality Assurance/Quality Control. ELISA negative controls and GC procedural blanks were within vendor specifications and/or showed no indication of target analyte contamination. TTX-spiked methanol gave higher than expected ELISA results (Table 2). GC-based recovery of TTX spiked into reference soil (~5 μg/g) was 95%. Mean recovery of DBOFB and α-HCH added prior to ASE extraction in GC extracts was 66±22%.

Table 2. ELISA concentration ranges and estimates ("ELISA-ΣTOX") for reference soil amended with 3 levels of technical toxaphene were consistently higher than spiked values.

Sample ID	Absorption	Spike [ppm]	Conc Range [ppm]	ELISA-ΣTOX[1] [ppm]
MS#1	**0.7016**	**0.1**	**0.5 < x < 2**	**1.28**
MS#2	**0.5355**	**1.0**	**2 < x < 10**	**2.59**
MS#3	**0.2538**	**10.0**	**x > 10**	**10.2**
MS#4	**0.6582**	**0.1**	**0.5 < x < 2**	**1.57**
MS#5	**0.5015**	**1.0**	**2 < x < 10**	**2.95**
MS#6	**0.2312**	**10.0**	**x > 10**	**17.9**

[1] total toxaphene estimated from 3-point nonlinear absorbance calibration curve

Results and Discussion

By ELISA, the majority of topsoils (72%) contained undetectable or low levels of toxaphene (i.e. <2 ppm); 27% were classified as moderately contaminated (2<x<10 ppm) and a single sample from GYES was classified as highly contaminated (>10 ppm) (**Table 3**). Fifty six and 25% of GYES and RMS soils were moderately to highly contaminated (2<x<10 ppm), respectively. In contrast, most soils from BMES and EM/LF contained low or undetectable levels of toxaphene (<2 ppm).

Table 3. Percentage distribution of soil samples with undetectable (<0.5 ppm); 0.5<x<2 ppm; 2<x<10 ppm; and >10 ppm levels of toxaphene as measured by ELISA.

Study Site	x < 0.5 [ppm]	0.5 < x < 2 [ppm]	2 < x < 10 [ppm]	x > 10 [ppm]
Burrough-Molette ES	**15%**	**85%**	**0%**	**0%**
Goodyear ES	**22%**	**18%**	**56%**	**4%**
Risley Middle School	**21%**	**54%**	**25%**	**0%**
Edo Miller/Lanier Field	**17%**	**83%**	**0%**	**0%**
All	**38%**	**34%**	**27%**	**1%**

In contrast to ELISA, GC-based ΣTOX was <0.01 to 0.38 µg/g, with 28% (10 of 36) of the soils analyzed containing detectable levels of toxaphene. Nearly all samples contained detectable levels of PAH, PCB and chlordanes with maximum concentrations of 22, 0.064 and 0.79 µg/g, respectively. GC-MS clearly confirmed the presence of chlordanes – not toxaphene -- in most samples, including the sole sample classified by ELISA as highly contaminated (>10 ppm) (GYES16) (Figure 1).

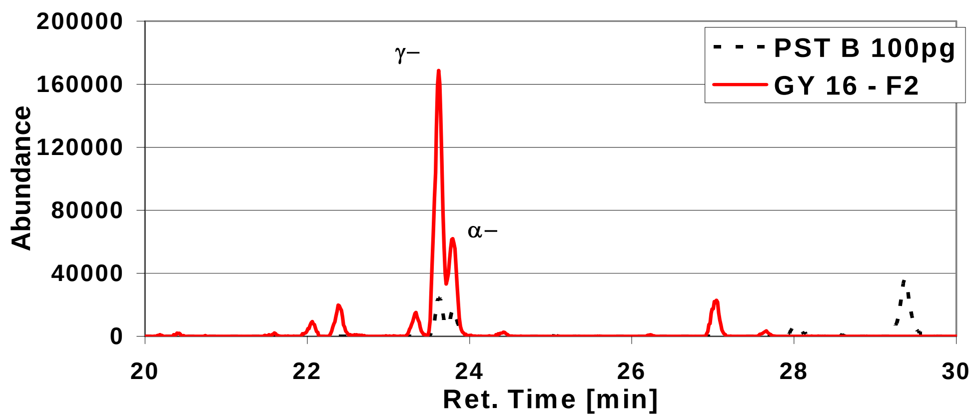

Figure 1. γ– and α–Chlordanes dominate the GC-ECNI-MS chromatogram of soil extract from Goodyear Elementary School, grid 16 (GY 16). γ-Chlordane was 795 ng/g, a level 44-fold higher than the ELISA test kit MDL (Table 1).

Linear regression indicated that GC-based Σchlordane was strongly correlated with ELISA-TOX (Table 4). A weaker association between GC-based and ELISA-ΣTOX was also statistically significant. Neither ΣPAH nor ΣPCB were significantly correlated with ELISA-ΣTOX.

Table 4. Strength of correlation between ELISA-ΣTOX and GC-estimated ΣTOX, Σchlordane, ΣPAH and ΣPCB.

Relationship	N	R^2	*P* value
ELISA-ΣTOX. vs. ΣChlordane	35	0.574	8.7E-08
ELISA-ΣTOX. vs. ΣToxaphene (GC-based)	10	0.472	0.020
ELISA-ΣTOX. vs. ΣPCB	35	0.0903	0.075
ELISA-ΣTOX. vs. ΣPAH	36	0.0323	0.287

Because cyclodiene pesticides are similar in chemical structure to bicyclic monoterpenes (toxaphene), ELISA kit was clearly interfered in part by chlordanes in our test soils. The presence of chlordanes at or above ELISA kit thresholds (Table 1) coupled with low or undetectable levels of toxaphene by GC indicates that toxaphene was unreliably quantified and in most cases, overestimated by ELISA. Furthermore, toxaphene levels as determined by GC were well below soil thresholds (~1 ppm) that trigger State of Georgia regulatory action. Thus, it is likely that the risk to children by toxaphene in these contaminated topsoils at these public access areas is negligible.

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