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Human blood and environmental media screening method for pesticides and polychlorinated biphenyl compounds using liquid extraction and gas chromatography–mass spectrometry analysis

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Abstract

Screening assessment methods have been developed for semi- and non-volatile persistent organic pollutants (POPs) for human blood and solid environmental media. The specific methodology is developed for measuring the presence of ''native'' compounds, specifically, a variety of organochlorine pesticides (OCPs), organophosphate pesticides (OPPs), and for polychlorinated biphenyls (PCBs). The method is demonstrated on anonymous Red Cross blood samples as well as two potential environmental sources, tracked in soil and dog hair. This work is based on previously developed methods for semi-volatile hydrocarbon exposure from fuels usage and similarly employs liquid solvent extraction, evaporative volume reduction, and subsequent specialized gas chromatography–mass spectrometry analysis (GC–MS). Standard curves, estimates of recovery efficiency, and specific GC–MS SIM quantification methods were developed for common pesticides including diazinon, aldrin, chlorpyrifos, malathion, dieldrin, DDT, permethrin, cyhalothrin, and cypermethrin, and for seven selected PCBs. Trace levels of certain PCBs and pesticides such as permethrin, dieldrin, malathion, lindane, diazinon, and chlorpyrifos were tentatively identified in anonymous blood samples as well as in two potential environmental sources, tracked in soil and dog hair. The method provides a simple screening procedure for various media and a variety of common organic pollutants without extensive sample preparation. It is meant to complement and augment data from more specific or complex methodology, to provide initial broad spectrum guidance for designing targeted experiments, and to provide confirmatory evidence for the usual metabolic biomarker measurements made to assess human exposure. Published by Elsevier Science B.V.

Keywords: Persistent organic pollutants; Organochlorine pesticides; Organophosphate pesticides; Polychlorinated biphenyls

1. Introduction

Certain pollutants are of concern because of their persistence in the environment and their proclivity for accumulation in the food chain and eventually in human tissues. Many such compounds have been shown to have adverse long-term health effects. The typical human exposure assessment approach is to measure metabolic biomarkers in blood or urine. In this methods development paper, we focus on measuring ''native'' compounds rather than metabolic markers; this provides additional information on circulating blood levels and exposure route that must

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otherwise be estimated from various model assumptions. The method described here is fairly simple requiring minimal sample preparation and is broad-ranging in its analyte spectrum. It is meant to provide exploratory and confirmatory data and, though focused on blood analysis, is also applicable to environmental media that contribute to exposure.

We have chosen some commonly used pesticides and polychlorinated biphenyl (PCBs) compounds for this work. Organochlorine pesticides (OCPs) and organophosphate pesticides (OPPs) are effective pest control agents and have been widely applied in agriculture all over the world. Pyrethroids are synthetic versions of the naturally occurring plant ''defense'' chemicals called pyrethrins, and are an important insecticide class used in agriculture, forestry, horticulture, public health (e.g. in hospitals and homes) [1–6]. The modified chemical and physical properties have improved their biological activity and thus pyrethroids become the most potent lipophilic insecticides [2]. Polychlorinated biphenyls (PCBs) are another group of chemicals which have found widespread use in a number of applications and are present in products such as heat-transfer fluids, hydraulic fluids, capacitors and transformers. All these groups of persistent organic pollutants (POPs) have shown to be ubiquitous environmental pollutants due to their great chemical stability and lipid solubility. In addition to causing direct human occupational exposure (pesticides application, manufacturing, etc.), these compounds have entered the ambient environment from their common large volume usage and are now commonly present in the food chain exposing animals and humans. POPs are routinely detected in fish, wild life, human adipose tissue, blood and breast milk [7,8].

In this study we established specific and simple methods to screen the basal level of a series of representative POPs. The particular pesticides were selected based on their occurrence, persistence, toxicity, and prevalence in human matrices [9–16] and the PCB congeners in this study are the marker PCBs commonly used to monitor overall occurrence and distribution [17,18]. The selected POPs are listed in Table 1 as four subgroups.

Liquid–liquid (or solid–liquid) extraction is one of the fundamental techniques for the separation of a chemical species from a medium or from other coexisting components [19]. The most widely used procedure is extraction from an aqueous medium into a properly chosen organic solvent [20], particularly for partial purification of a biological fluid such as blood containing toxic chemicals and has been used routinely in the pharmaceutical industry for drug analysis in human body fluids [21,22]. Other applications similarly use a solvent to soak and extract contaminants in solid media such as soils, dust, fibers, absorbents, carpets, hairs, etc. In previous work published in this journal, we have used this technique to extract and analyze JP-8, a kerosene-based jet fuel, in bovine plasma and human whole blood for assessment of human exposure [23,24]. In this current study we extend and apply this technology to establish a baseline method for assessing human exposure to a series of selected pesticides and PCBs and demonstrate its efficacy using randomly selected anonymous human blood samples from the American Red Cross (Research Blood specimens) and environmental samples of tracked in dirt and dog hair. Samples were extracted by using liquid–liquid extraction, centrifugation, volume reduction and subsequently analyzed on GC–MS with a large volume injection technique developed in this laboratory [24].

2. Experimental

2.1. Materials

Hexane, ethyl acetate, and acetone solvents were purchased from Burdick & Jackson (Muskegon, MI, USA) as GC–MS grade. The reagents 2,4-D-methyl, diazinon, malathion, methyl parathion, chlorpyrifos, DCPA, 2,4′-DDT, 4,4′-DDT, 4,4′-DDE, 2,4′-DDE, lindane, dieldrin, endosulfan I and II, endrin, pentachloronitrobenzene, pentachlorophenol (PCP), heptachlor, heptachlor epoxide, aldrin, fenpropathrin, L-cyhalothrin, cyfluthrin, permethrin, cypermethrin, fenvalerate and PCB congeners PCB 28, PCB 52, PCB 101, PCB 138, PCB 118, PCB 153, and PCB 180 were purchased from AccuStandard (New Haven, CT, USA) as analytical standards. Deuterated Endosulfan I (Endosulfan I-d_4), was purchased from Cambridge Isotope Laboratories (Andover, MA, USA). Reagent sodium chloride and anhydrous

Table 1
Selected POPs in this study

Subgroups	Compound name	CAS #	Molecular formula	Molecular weight
Organophosphates	Diazinon	333-41-5	$C_{12}H_{21}N_2O_3PS$	304.10
	Malathion	121-75-5	$C_{10}H_{19}O_6PS_2$	330.04
	Methyl parathion	298-00-0	$C_8H_{10}NO_5PS$	263.0
	Chlorpyrifos	2921-88-2	$C_9H_{11}NO_3PS$	348.93
Organochlorines	2,4-D-methyl ester	94-75-7	$C_9H_8Cl_2O_3$	221.04
	Lindane	58-89-9	$C_6H_6Cl_6$	290.85
	Pentachloronitrobenzene	82-68-8	$C_6Cl_5NO_2$	295.5
	Pentachlorophenol	87-86-5	C_6HCl_5O	266.4
	Heptachlor	76-44-8	$C_{10}H_5Cl_7$	373.34
	Heptachlor epoxide	1024-57-3	$C_{10}H_5Cl_7O$	385.82
	Aldrin	309-00-2	$C_{12}H_8Cl_6$	361.88
	DCPA	1861-32-1	$C_8H_2Cl_4O_4$	303.90
	o,p′-DDE	3424-82-6	$C_{14}H_8Cl_4$	315.93
	p,p′-DDE	72-55-9	$C_{14}H_8Cl_4$	315.93
	o,p′-DDT	789-02-6	$C_{14}H_9Cl_5$	354.51
	p,p′-DDT	50-29-3	$C_{14}H_9Cl_5$	354.51
	Endosulfan I	959-98-8	$C_9H_6Cl_6O_3S$	406.96
	Endosulfan II	33213-65-9	$C_9H_6Cl_6O_3S$	406.96
	Dieldrin	60-57-1	$C_{12}H_8Cl_6O$	377.87
	Endrin	72-20-8	$C_{12}H_8Cl_6O$	377.87
Pyrethroids	Fenpropathrin	39515-41-8	$C_{22}H_{23}NO_3$	349.46
	Permethrin	52645-53-1	$C_{21}H_{20}Cl_2O_3$	391.30
	Cyhalothrin	91465-08-6	$C_{23}H_{19}F_3ClNO_3$	449.9
	Cyfluthrin	68359-37-5	$C_{22}H_{18}FCl_2NO_3$	434.3
	Fenvalerate	66230-04-4	$C_{25}H_{22}FClNO_3$	419.9
PCBs	Biphenyl,			
PCB 28	2,4,4′-Trichloro-	7012-37-5	$C_{12}H_7Cl_3$	255.9613
PCB 30	2,4,6-Trichloro-	35693-92-6	$C_{12}H_7Cl_3$	255.9613
PCB 52	2,2′,5,5′-Tetrachloro-	35693-99-3	$C_{12}H_6Cl_4$	289.9224
PCB 101	2,2′,4,5,5′-Pentachloro-	37680-73-2	$C_{12}H_5Cl_5$	323.8834
PCB 118	2,3,4,4′,5-Pentachloro-	31508-00-6	$C_{12}H_5Cl_5$	323.8834
PCB 138	2,2′,3,4,4′,5-Hexachloro-	35694-06-5	$C_{12}H_4Cl_6$	357.8444
PCB 153	2,2′,4,4′,5,5′-Hexachloro-	35065-27-1	$C_{12}H_4Cl_6$	357.8444
PCB 180	2,2′,3,4,4′,5,5′-Heptachloro-	35065-29-3	$C_{12}H_3Cl_7$	391.8055

sodium sulfate was purchased from Fisher Scientific (Fair Lawn, NJ). Nitrogen and helium gases were from National Speciality Gases (Durham, NC, USA). Human whole blood (treated with heparin) was purchased from American Red Cross (Charlotte, NC, USA) and stored in a −20 °C freezer.

2.2. Instrumentation

2.2.1. GC–MS apparatus and conditions

Chromatographic analysis was achieved with an HP 5890 Series II gas chromatograph (Hewlett-Packard, Santa Clarita, CA, USA) directly connected to an HP 5971A mass selective detector. The system was controlled by an MS Chemstation (Windows 3.1; Hewlett-Packard). A deactivated pre-column (10 m×0.53 mm I.D., Hewlett-Packard) was used to accommodate the direct cold on-column injection of large volume samples (up to 25 μl); the separation was made with a fused-silica capillary column (Rtx-CL pesticides), 30 m long with an internal diameter of 0.25 mm and 0.25 μm thickness coating (Restek, Bellefonte, PA, USA). Helium was used as the carrier gas (inlet pressure: 100 kPa). The MS conditions were as follows: the source temperature was 250 °C; the instrument was operated in full-scan mode (44–350 amu) with electron impact ionization; parameter values were optimized for maximum

sensitivity. Samples were analyzed using full scan through which we acquired spectra, found background and confounding compounds, and gleaned characteristic ions for the selected compounds of interest. Quantification was performed using selected ion monitoring (SIM) mode.

2.2.2. Centrifugation

A centrifuge (Marathon 21K/BR; Fisher Scientific, Pittsburgh, PA, USA) was used to separate organic solution from biological media (blood) with an eight-place fixed-angle rotor. Samples were separated at 1600 *g* in 28-ml centrifuge vials.

2.3. Standard curves

Standard curves were made for standard compounds diazinon, aldrin, chlorpyrifos, malathion, dieldrin, *p,p′*-DDT, permethrin, cyhalothrin and cypermethrin. Endosulfan I-d_4 was used as internal analytical standard. Solutions of the standard compounds were prepared by dissolving the above compounds in acetone as 233 ng/μl and diluted into 14.97, 7.48, 3.74 and 1.50 ng/μl with hexane. Addition of only acetone (C = 0) was used as control. Internal analytical standard Endosulfan I-d_4 (310 ng/μl, 10 μl) was added to each standard solution (300 μl) before injecting it onto the GC system. This internal standard solution was used purely as an instrumental quantification standard to assure that any changes in performance were not misconstrued as a difference in recovery. Peak area ratio (PAR) of each compound monitored to the internal analytical standard was obtained from GC–MS analysis of each compound at different concentrations (ng/μl) and was used to construct the standard curves. Linear regression from Prizm (GraphPad Software, San Diego, CA, USA) was used to analyze the relationship between the two variables with 95% confidence intervals.

2.4. Extraction and analysis of selected pesticides spiked in human blood for extraction efficiency study

The initial spike solutions were prepared by dissolving all the chemicals including diazinon, aldrin, chlorpyrifos, malathion, dieldrin, *p,p′*-DDT, permethrin, cyhalothrin and cypermethrin in acetone to a series of specific concentrations; blank solvent was used as a control. Then 300 μl of each of the above prepared solutions were mixed in 6 ml human blood followed by the addition of deionized water (6 ml) to achieve synthetic sample concentrations of 0.0915, 0.367 and 0.915 ng/μl in a 12-ml volume. After adding 2 g of sodium chloride to each sample to saturate the blood solutions, the resulting solutions were extracted with hexane (12 ml) by vortexing for 1 min. After extraction, the mixture was then centrifuged at 1600 *g* for 30 min. The organic phase (top) was then separated from the aqueous phase. The aqueous phase was extracted again following the above procedure with 6 ml of solvent. The extracts were then combined and dried over anhydrous sodium sulfate. After separation from the drying agent, the remaining solutions were evaporated under N_2 flow. The evaporation process was stopped when 300 μl of solution was left. Internal standard Endosulfan I-d_4 (10 μl) was added before the analysis. The extract was analyzed by GC–MS. The oven temperature program for analysis was 80 °C × 10 min and then 2 °C/min to 250 °C and held at 250 °C for 20 min. One μl of sample was injected. Each sample analysis was duplicated for statistical purposes. Individual ions for each selected pesticide were monitored using SIM mode.

2.5. Extraction and detection of selected pesticides and PCBs from human blood

The procedure for extraction of human blood samples from American Red Cross was similar to the procedure described in Section 2.4. except no spiked pesticides were added. Each extraction and analysis was duplicated for statistical purposes. Water (6 ml) was used as control for blood surrogate and extracted following the same procedure. The analysis of the extracts were conducted following the procedure as in Section 2.4. except more compounds were monitored using the SIM mode.

2.6. Extraction and detection of selected pesticides and PCBs from dirt (and hair) samples

The dirt samples were collected by sweeping dust and soil from smooth (indoor) concrete surfaces that

were frequently traveled by pet dogs that spent appreciable time in residential backyards. Samples were stored in glass vials at room temperature until they were prepared by weighing out 1.0 g and placed in Teflon FEP centrifuge tubes. Hexane or ethyl acetate–hexane (1:4, v/v) (12 ml) was added. The mixture was vortexed for 1 min and then centrifuged at 1600 *g* for 10 min. The organic phase was removed from the centrifuge tube and transferred to a glass vial. The remaining dirt was mixed with 12 ml of extracting solvent again and left soaking at room temperature overnight. Following the same procedure, the organic solvent was separated from dirt and combined with previous fraction and evaporated under N_2 flow until 300 μl was left. The same analytical procedure was followed for the analysis of the extracts as that for blood extracts. The method was also applied to dog hair samples collected (with a brush) from two dogs, but only 0.2 g/sample was used. Each sample preparation, extraction, and analysis was duplicated for statistical purposes.

2.7. Calculation of recovery efficiency

The recovery efficiency was calculated using the following equation:

$$\text{Recovery } \% = \frac{\left(OC_{\text{extract}} \Big/ IS_{\text{extract}}\right)}{\left(OC_{\text{spike}} \Big/ IS_{\text{spike}}\right)} \times 100\% \qquad (1)$$

where OC_{extract} = peak area for the organic compound (OC) in the extract (300 μl), IS_{extract} = peak area for the internal analytical standard in the same extract, OC_{spike} = peak area for the OC in the spike solution (300 μl), and IS_{C} = peak area for the internal analytical standard in the same spike solution.

The same initial spike solutions prepared (Section 2.4.) by dissolving all the chemicals including diazinon, aldrin, chlorpyrifos, malathion, dieldrin, *p,p′*-DDT, permethrin, cyhalothrin and cypermethrin in acetone at a series of specific concentrations were analyzed by first mixing the each solution (300 μl) with 10 μl of internal standard endosulfan I-d_4 solution of 310 ng/μl. Then an aliquot of these samples was injected directly onto the GC column. Analysis using SIM mode was performed for each sample and each sample analysis was duplicated for statistical purposes. The relative peak area to internal analytical standard for each organic compound (OC) in the standard spike solution before spiking into the blood at the certain concentration was compared with the relative peak area to internal analytical standard for each OC in the extraction solution spiked at the same concentration.

3. Results and discussion

3.1. GC–MS separation and identification of 34 selected pesticides and PCBs

Thirty four representative pesticides and PCBs (Table 1) were chosen for this study. Separation of a series of chemically and structurally similar chemicals always presents a technical challenge. As a first attempt, we chose an analytical column from Restek (Rtx-CL pesticides), specifically designed for separating OC pesticides for this study. After optimizing the oven temperature profile, the mixture was fully separated except between malathion and DCPA (#14 and #15, Fig. 1), between o,p′-DDT and endrin (#22 and #23) and between cypermethrin and cyfluthrin (#32 and #33, Fig. 1). Each analyte was identified by comparing with its standard mass spectrum. The chromatogram is shown in Fig. 1 and the identification of each chemical separated is listed in Table 2. When large volumes of sample were injected, longer solvent delay time was required (10 min). To reduce the loss of more volatile compounds during the elution of the solvent, the initial oven temperature was lowered to 80 °C for 10 min and then increased at 2 °C/min to 250 °C, which caused slightly less efficient separation as shown on Fig. 2. However, the analytes in each of three coeluted groups do not share the major ion fragments used for ion extraction and thus could be identified during the analysis of real blood extract samples.

3.2. Standard curve

Standard curves were created for the quantification using standard solutions of the selected analytes in hexane. Fig. 3 shows the linearity of standard curves of each analyte analyzed. The linear regression equations for each selected pesticides are listed in

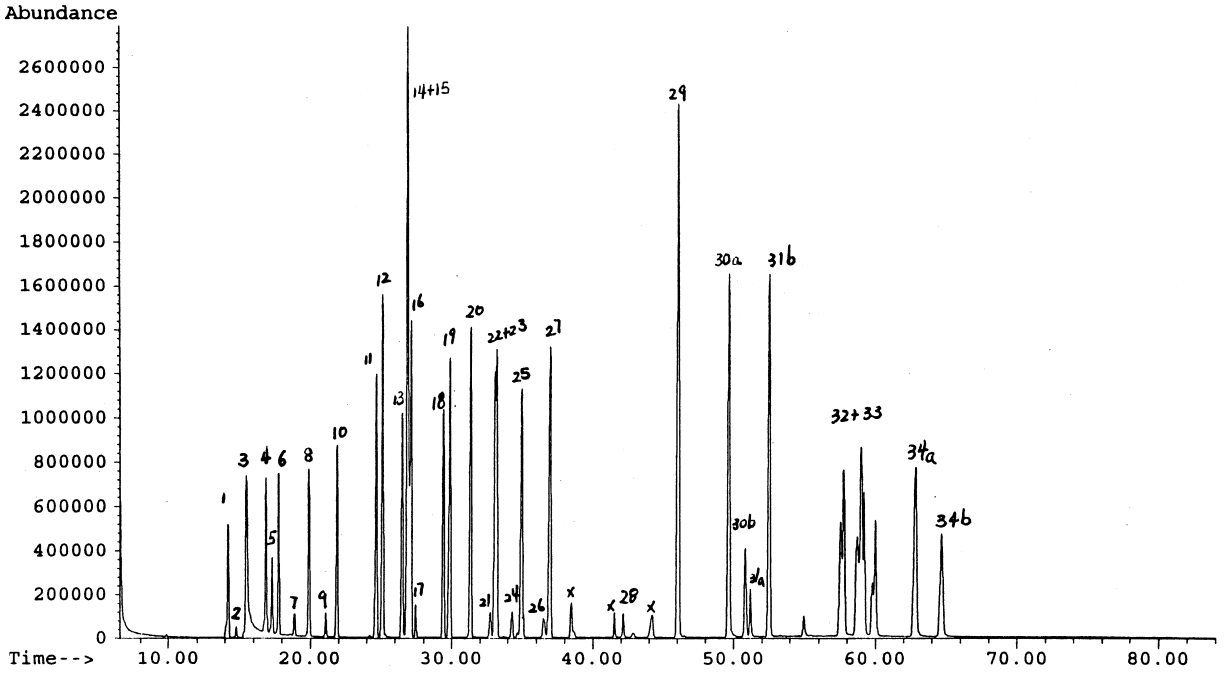

Fig. 1. Chromatogram of separation of 34 pesticides and PCBs listed in Table 2. Oven temperature profile I: initial temperature = 150 °C and then 2 °C/min to 250 °C and then at 250 °C for 30 min.

Table 3; these are used in concert with the extraction efficiencies for quantitative determination of the analytes in the extraction matrices. Based on these and similar results, we estimate an on-column level of quantification (LOQ) of 25–50 pg for these compounds.

3.3. Recovery efficiencies study

Recovery efficiency study was conducted for a series of selected pesticides including diazinon, aldrin, chlorpyrifos, malathion, dieldrin, *p,p′*-DDT, permethrin and cyhalothrin. Determination of recoveries was performed by extracting the human blood spiked with different amount of analytes and then comparing the peak area ratio of each analyte to that in standard solution at the same concentration. The calculation of the recovery efficiency was done according to Eq. (1) and the average recovery efficiencies for each compound spiked in blood at different concentrations are listed in Table 4. From this table, it can be seen that the recovery efficiency varies largely depending mainly on the compound extracted. For most organophosphate pesticides the recovery was close to 100%, with malathion as an outlier at 128±11.0%; we attribute this to the difficult chromatography for this compound and the peak tailing using the particular broad spectrum GC method. For organochlorine pesticides, recovery ranges from 53.4±16.9 to 98.2±30.5%; for pyrethroids, the recovery efficiency dropped to a range of 18.8±3.5 to 35.0±12.8%. These differences may be due to the difference of lipophilicity among each group of pesticides; more polar functional groups are involved in pyrethroids and thus these compounds are more difficult to isolate from the matrix.

3.4. Assessment of the selected pesticides and PCBs in randomly selected human blood

3.4.1. Qualitative blood results

Using the liquid–liquid extraction method described in Section 2.4, the qualitative assessment of selected POPs in human blood samples was carried out first by extracting randomly selected human blood specimen with both hexane and a mixture of

Table 2
Identification of the analytes in Fig. 1

#	Retention time (min)	Compounds identified	Ions selected for SIM
1	14.21	2,4-D-methyl	234, 199
2	14.78	PCB 30	256, 186
3	15.50	Pentachlorophenol	266, 165
4	16.89	Lindane	219, 181
5	17.32	Pentachloronitrobenzene	295, 237
6	17.79	Diazinon	304, 199
7	18.89	PCB 28	256, 186
8	19.90	Heptachlor	272, 372
9	21.07	PCB 52	290, 255
10	21.89	Aldrin	263
11	24.69	Chlorpyrifos	314, 197
12	25.16	Methyl parathion	263
13	26.53	Heptachloro epoxide	353
14	26.93	Malathion	173
15	26.93	DCPA	301, 332
16	27.16	*o,p′*-DDE	316, 246
17	27.46	PCB 101	326, 256
18	29.44	Endosulfan I	277, 195
19	29.91	*p,p′*-DDE	316, 246
20	31.38	Dieldrin	378, 263
21	32.73	PCB 118	324, 254
22	33.10	*o,p′*-DDT	235
23	33.22	Endrin	261
24	34.29	PCB 153	360, 288
25	35.01	Endosulfan II	277, 195
26	36.52	PCB 138	360, 288
27	37.00	*p,p′*-DDT	235, 165
28	42.16	PCB 180	394, 324
29	46.463	Fenpropathrin	349, 181
30	49.73, 50.64	Permethrin	183
31	51.20, 52.59	Cyhalothrin	209, 181
32	57–60.5	Cypermethrin	209, 181
33	57–60.5	Cyfluthrin	226
34	62.92, 64.72	Fenvalerate	225, 167

20% ethyl acetate in hexane. The extracts (duplicated from both hexane and ethyl acetate–hexane extraction) were concentrated and analyzed by GC–MS. Large volume direct on-column injection technique was used for the sample introduction. Up to 25 μl of samples were injected. Selected ions (Table 2) for each specific analyte were monitored using SIM mode. The analytes were identified and quantified by extracting the specific ions monitored at the specific retention time region defined by the chromatogram of the standard; some examples are shown in Fig. 4. Though most peak shapes looked fairly symmetric, we note that diazinon in Fig. 4a (blood) exhibits some tailing at low concentrations probably due to matrix effects from the blood lipids. When using hexane as extracting solvent, lindane, diazinon, permethrin and fenpropathrin were found in each sample extract; while 2,4-D-methyl ester, pentachloronitrobenzene, heptachlor, heptachlor epoxide, DCPA, endosulfan I and II, endrin, *o,p′*-DDT, *p,p′*-DDT, PCB 30, PCB 118 and PCB 180 were rarely detected; the remaining POPs were inconsistently distributed. When hexane–ethyl acetate (4:1, v/v) was used as the extracting solvent, we found higher background levels of a variety of non-target compounds indicating that this solvent combination is more aggressive. With the exception of lindane and permethrin, the additional background compounds caused a slight degradation of sensitivity for many of the analytes.

3.4.2. Quantitative blood estimates

From the standard curves, we had estimated a typical level of quantification (LOQ) sensitivity of 25–50 pg on column for the pesticides. Depending on initial blood volume, extraction (final) volume, GC-injection volume, and recovery efficiency results, we can reasonably expect to see about 2–5 ng/ml in blood for 1.0-μl injections and 6-ml blood samples sensitivity with confidence. We can also make more aggressive estimates below these levels by increasing injection volume, reducing extracts to lower final volumes, and manual peak shape interpretation, though with some peril of misidentification. Based on these cautions, we estimated the following ranges of pesticides in the demonstration blood samples: diazinon, 0–65 ng/ml, aldrin, 0–0.2 ng/ml, chlorpyrifos, 0.8–11 ng/ml, malathion, 0–2.3 ng/ml, dieldrin, 10–50 ng/ml, *p,p′*-DDT, 0.3–8 ng/ml, and permethrin, 0.04–0.3 ng/ml. Although most of the PCBs were detected in the blood, their variable presence in a subset of the control extracts indicates some (unknown) trace level laboratory background or contamination; as such we are not able to make any confident quantitative estimates.

3.5. Assessment of selected pesticides and PCBs in residential "tracked in" dirt

The application of liquid extraction on solid matrices has been used widely. In this study, we utilized this technique to extract and analyze dirt

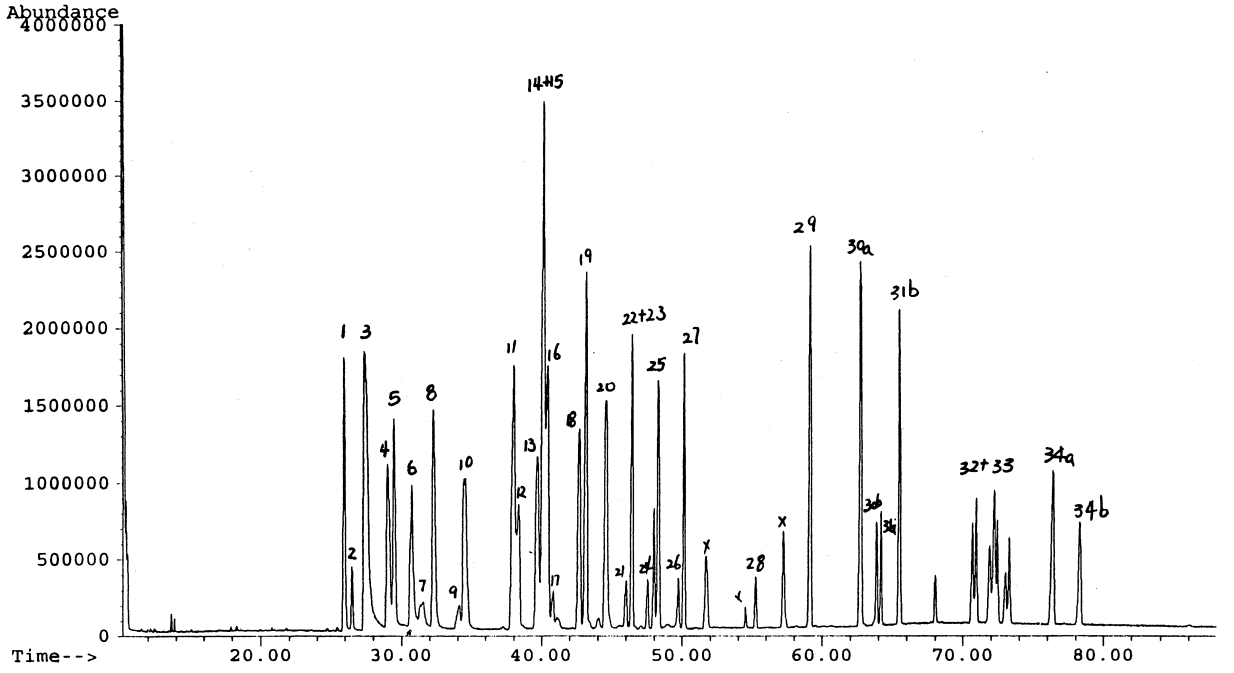

Fig. 2. Chromatogram of separation of 34 pesticides and PCBs listed in Table 2. Oven temperature profile II: initial temperature = 80 °C for 10 min and then 2 °C/min to 250 °C and then at 250 °C for 30 min.

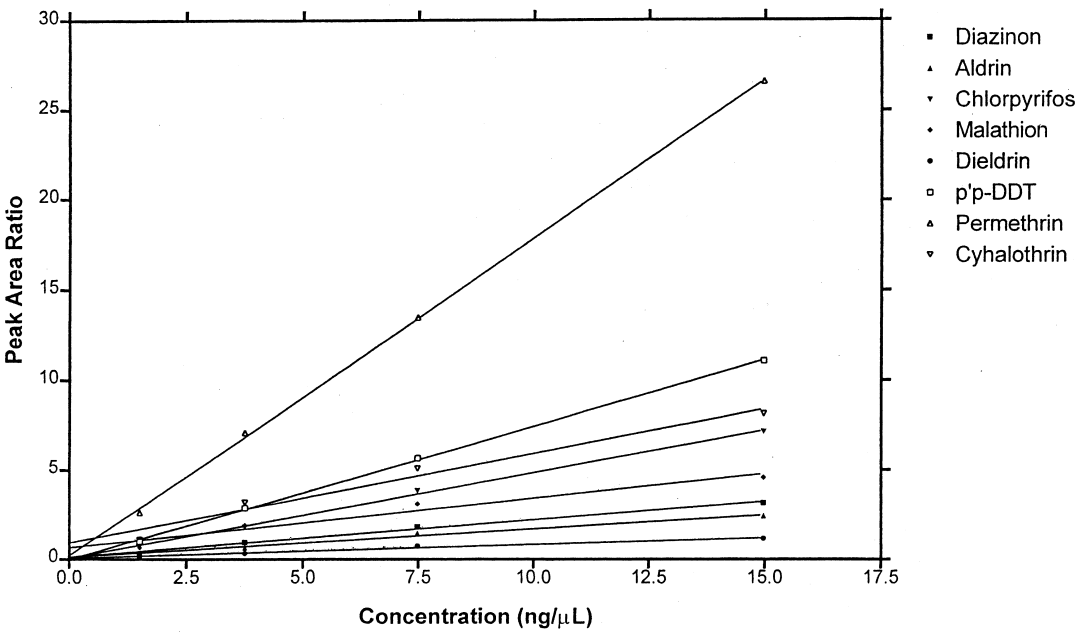

Fig. 3. Standard curves of the selected pesticides listed in Table 3.

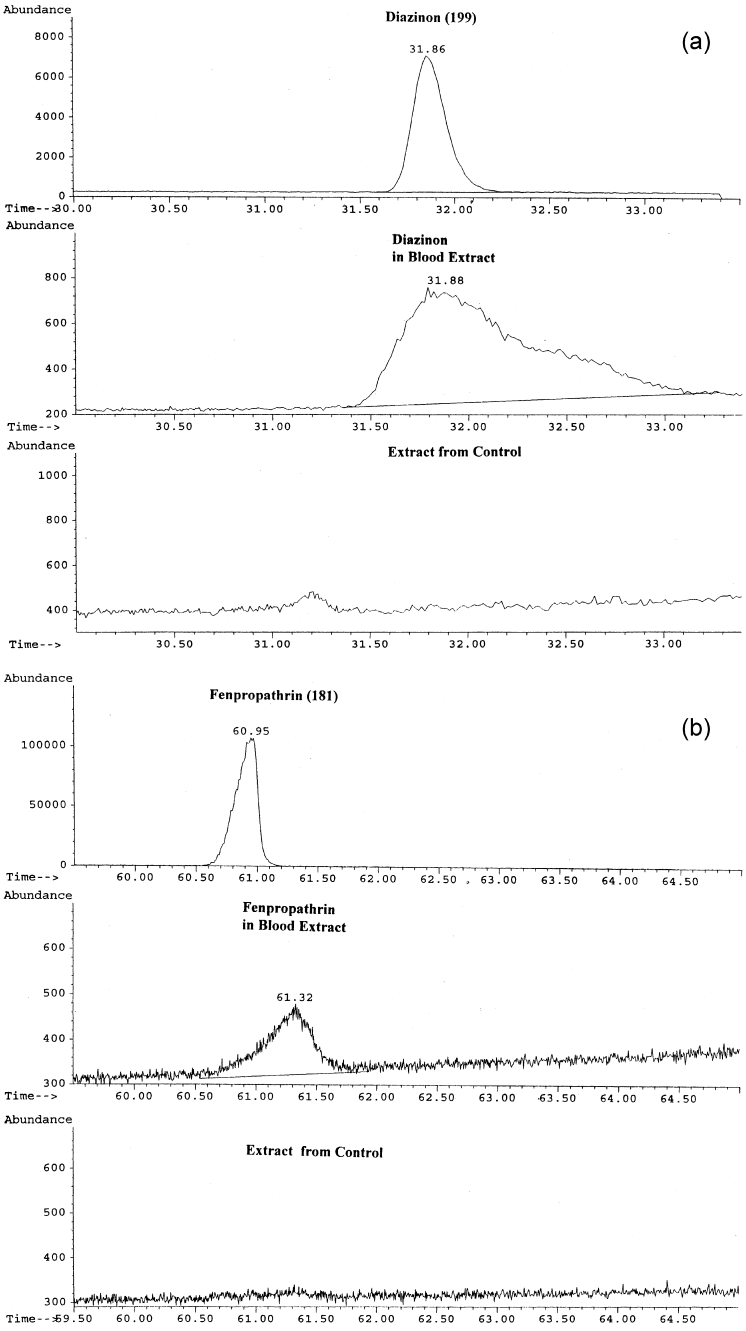

Fig. 4. (a) Chromatograms of diazinon in standard solution (4.0 ng/μl, 10 ml injection, SIM, top), in human blood extract (10 μl injection, estimated as 26.5 ng/ml in blood, SIM, middle) and in control extract (SIM, bottom) with selected ion of diazinon as 199. (b) Chromatograms of fenpropathrin in standard solution (40 ng/μl, 1 ml injection, full scan, top), in human blood extract (10 μl injection, estimated as 1.4 ng/ml in blood, SIM, middle) and in control extract (bottom) with selected ion of fenpropathrin as 181. (c) Chromatograms of permethrin in standard solution (40 ng/μl, full scan, top), in human blood extract (10 μl injection, estimated as 0.58 ng/ml in blood, SIM, middle) and in control extract (bottom) with selected ion of permethrin as 183.

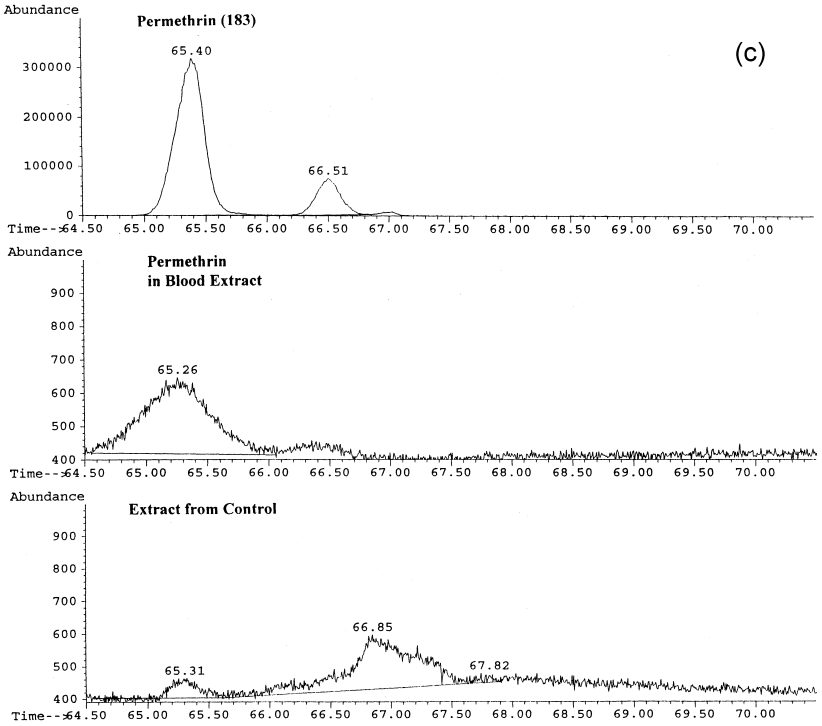

Fig. 4. (*continued*)

samples tracked into the living space from residential back-yards by pet dogs and samples of the dogs' hair. Both hexane and ethyl acetate–hexane were used as the extracting solvent system, duplicate dirt samples were processed and the extracts concentrated following the procedure described in Section 2. An analytical method similar to that of blood extract analysis was used. GC–MS analysis of the dirt extracts showed that pesticides diazinon, heptachlor epoxide, malathion and permethrin were found in all extracts from both hexane alone and hexane with ethyl acetate (4:1, v/v) as solvent; Fig. 5 gives some examples of chromatograms. Though we found some background contamination in the controls, the dirt samples had appreciably higher levels of certain analytes than did the blood samples; for example, we estimated diazinon up to 1.2 mg/g, chlorpyrifos from

Table 3
Linear regression equations for selected pesticides

Compound	Equations from standard curves	r^2
Diazinon	$y = 0.2062x + 0.1413$	0.9915
Aldrin	$y = 0.1568x + 0.1266$	0.9909
Chlorpyrifos	$y = 0.4778x + 0.0799$	0.9980
Malathion	$y = 0.2766x + 0.6522$	0.9531
Dieldrin	$y = 0.07426x + 0.07792$	0.9801
p,p'-DDT	$y = 0.7433x + 0.01026$	0.9994
Permethrin	$y = 1.768x + 0.215$	0.9996
Cyhalothrin	$y = 0.5003x + 0.9279$	0.9727

Table 4
Recovery efficiency of extraction and concentration of selected pesticides spiked in human blood

Selected pesticides	Recovery efficiency (%) (±SD)
Diazinon	105.2±29.6
Aldrin	53.4±16.9
Chlorpyrifos	103.5±12.1
Malathion	128.1±11.0
Dieldrin	98.2±30.5
p,p'-DDT	67.8±13.7
Permethrin	18.8±3.5
Cyhalothrin	30.3±18.3
Cypermethrin	35.0±12.8

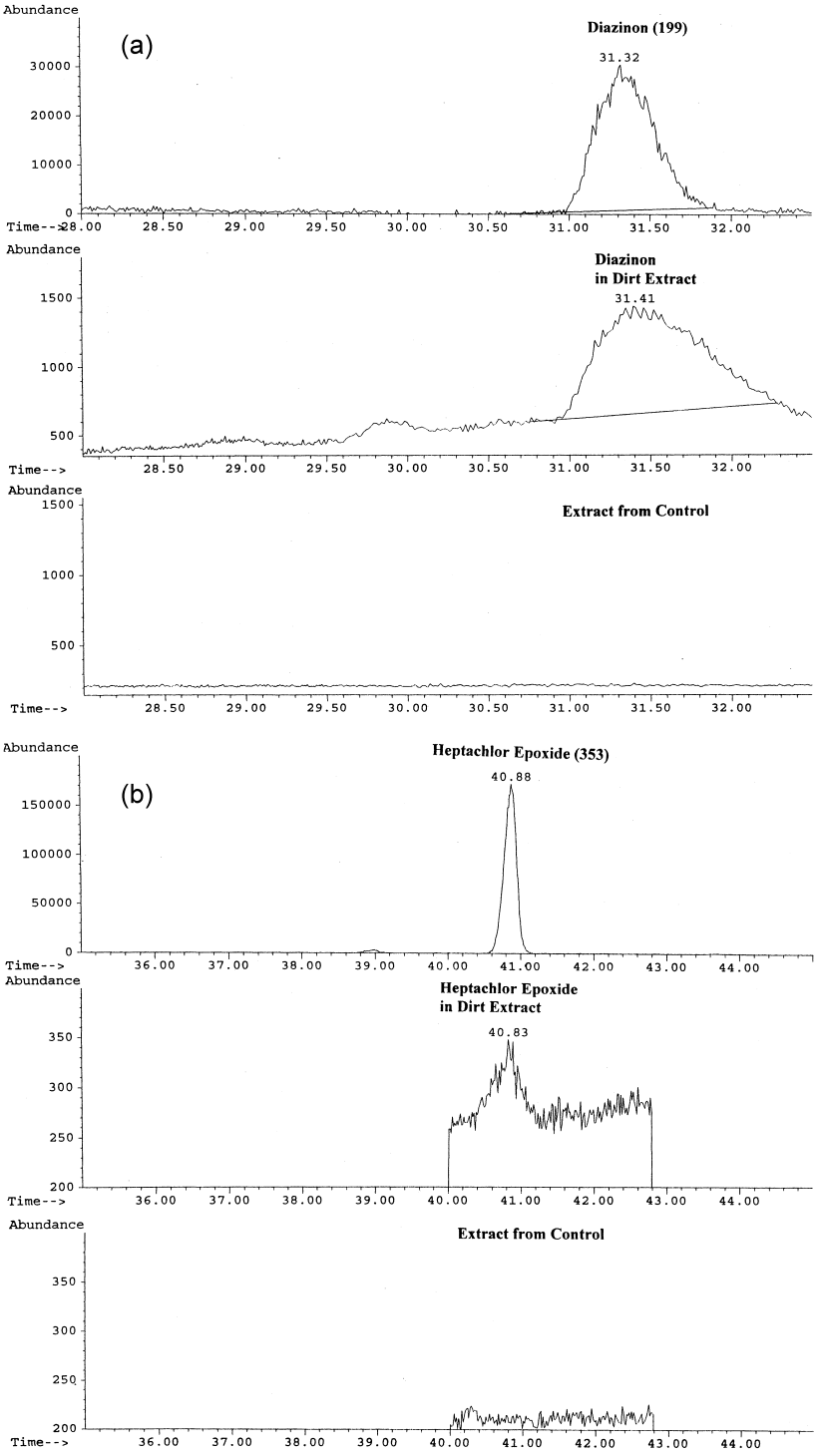

Fig. 5. (a) Chromatograms of diazinon in standard solution (40 ng/μl, full scan, top), in dirt extract (1 μl injection, estimated as 1.2 mg/g in dirt, SIM, middle) and in control extract (bottom) with selected ion of diazinon as 199. (b) Chromatograms of heptachlor epoxide in standard solution (40 ng/μl, full scan, top), in dirt extract (1 μl injection, estimated as 7.8 ng/g in Dirt, SIM, middle) and in control extract (bottom) with selected ion of heptachlor epoxide as 353.

70 to 700 ng/g, malathion at 7–24 ng/g, and permethrin up to 340 ng/g. The dog hair samples gave similar results; we estimated chlorpyrifos at 33–700 ng/g, diazinon at about 20 ng/g, and permethrin up to 100 ng/g.

4. Conclusion

Liquid–liquid (and solid–liquid) extraction methodology has been successfully developed as a screening tool for some common pesticides and PCBs and has been demonstrated using human blood samples and residential dirt/hair samples. From the extraction of blood spikes, it was found that most of the pesticides tested can be extracted with efficiency ranging from 18.8 to over 100%, depending upon the specific compound. Both hexane and hexane with ethyl acetate (4:1, v/v) as solvent were tested and the latter was found to be more aggressive for a variety of non-target compounds thus actually diminishing sensitivity slightly for compounds of interest. The large volume injection (LVI) technique as applied to these compounds was found to be useful in improving sensitivity but did not precisely scale with volume. Various common pesticides were found at the ng/ml (g) level in some demonstration ''real-world'' blood and dirt samples. In general, this simple methodology using standard analytical laboratory instrumentation has shown value in screening for a variety of pesticides and PCBs in complex matrices at typical ambient levels. The capability for detecting native pesticides and PCB compounds in biological (and environmental) media adds a valuable confirmatory dimension to the usual measurement of metabolic biomarkers.

We conclude that future work should focus on improvement of recovery in general by looking closely at matrix effects such as those imposed by variability in blood lipid content. More specifically, we will explore in detail pyrethroids recovery by testing some different solvent systems and investigating the sources of PCBs background in blanks. We also plan to expand the POPs target analytes list to include polyaromatic hydrocarbons (PAHs) from exhaust and fuels use, and ''chlortriazines'', such as the herbicide atrazine and related compounds.

We caution that this is a generalized screening method deliberately designed to cover a wide range of analytes in various media with minimal sample preparation and therefore is not optimized for any particular scenario. However, this methodology is accomplished with standard laboratory equipment and modest GC–MS instrumentation which makes it easily and quickly implemented in most laboratories and can serve as a flexible baseline for further specific development.

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