Electronic Supplementary Material (ESI) for Journal of Environmental Monitoring

A Pilot Study of Pesticides and PCBs in the breast milk of women residing in urban and agricultural communities of California

Rosana Hernandez Weldon[1], Dana Boyd Barr[2], Celina Trujillo[1], Asa Bradman[1], Nina Holland[1], and Brenda Eskenazi[1]

[1] Center for Environmental Research and Children's Health, School of Public Health, University of California, Berkeley, CA

[2] Emory University, Rollins School of Public Health, Atlanta, GA 30322

Electronic Supplementary Information

Breast Milk Analysis Methodology

Sample preparation consisted of solid phase dispersement of the sample over Hydromatrix ®, pressurized fluid extraction and extract purification using a solid phase extraction (SPE) column prepared in-house. Breast milk samples were stored at -70^{o}C until used. They were thawed and vortex mixed before an aliquot was taken. The 1-mL breast milk sample was pipetted into a tared 10-mL conical centrifuge tube and the weight was recorded. The sample was spiked with labeled internal standard to give a concentration of 200 pg/g in the milk and mixed allowing enough time for the internal standards to reach equilibrium with the milk components. The extraction portion of the sample preparation was achieved with pressurized fluid extraction utilizing an Accelerated Solvent Extraction (ASE) 200 extraction system (Dionex Corporation, Sunnyvale CA, USA). An extraction tube was prepared by capping one end of the 22-mL ASE cell, adding two cellulose filters to the bottom and filling the entire volume of the cell with Hydromatrix®. The breast milk sample was dispersed over the Hydromatrix® by transferring it to

the top of the sorbent with along with tube rinsates (~ 2mL) consisting of deionized water. An additional cellulose filter was placed on top of the sorbent, and the cell was capped. The top of the cell was kept constant through the process to take advantage of the unidirectional flow of the solvent through the cell. The cells were extracted on the ASE 200 with dichloromethane:hexane (1:1 v/v). The temperature of the extraction solvent was kept at 70^{o}C and the internal pressure of the cells was kept at 1000 psi. The solvent volume used was approximately 15 mL. Once the solvent in the cell temperature stabilized at 70^{o}C, the static extraction was held for five minutes after which time the solvent is purged into the centrifuge tube. This cycle was repeated two more times for each cell resulting in approximately 30 mL of extract. The cell was flushed with additional solvent (10 mL) and the hydromatrix was blown dry with nitrogen. The extraction process produced approximately 40 mL of solvent that was concentrated using a Rapid Vap (Labconoco, Olathe, KS, USA) solvent evaporation system. The Rapid Vap was maintained at 40^{o}C and the pressure was slowly decreased to produce a vacuum. The extract was concentrated to approximately 100 µL and solvent exchanged twice with 1 mL of acetonitrile. Care was taken to ensure the extract did not go to dryness. The extract volume is adjusted to 500 µL with acetonitrile for the next step. The extract purification was achieved using a Rapid Trace (Caliper Life Sciences, Hopkinton, MA, USA) automated SPE system. Columns were prepared in-house in empty 3mL SPE tubes by adding the following in order: a frit to the bottom, 750mg PSA sorbent, another frit, 1g of neutral alumina sorbent and a final frit to pack the material. A modified plunger was used on the Rapid Trace to accommodate the larger bed size of the column. With the Rapid Trace system, the columns were cleaned with 3 mL acetonitrile, and the 500 µL extract was added to the column followed by another 500 µL acetonitrile rinse of the extract tube. The load volume was allowed to pass through to waste. The column was eluted with and the next 2.5mL of acetonitrile. The purified extract was concentrated with the Rapid Vap to approximately 100 µL and transferred, with rinses, to a GC insert. The extract was spiked with 20 µL of recovery standard in toluene and the acetonitrile

was allowed to evaporated leaving the residue in the toluene recovery standard (a final 20µL volume) for analysis. This constituted a 50-fold concentration of the original breast milk sample.

Extracts were analyzed using gas chromatography-high resolution mass spectrometry (GC-HRMS). Chromatographic separation was achieved using an Agilent 6890GC (Waters Corporation, Milford, MA). A J&W Scientific DB-5MS chromatography column was used (30m, 0.25mm ID, 0.25µm film) with a GC temperature program to optimize the separation. A 1µL injection was used with an injector temperature of 270^{o}C and a single gooseneck split/splitless liner. The temperature began at 100^{o}C for two minutes, was increased at 3.4^{o}C/min to 130^{o}C, increased at 13^{o}C/min to 240^{o}C which is held for 5 min, , increased at 30^{o}C/min to 320^{o}C which was held for 6 min. The linear flow of the helium through the column was 1mL/minute. The transfer line to the mass spectrometer was held constant at 290^{o}C. The total run time was 32 minutes.

Analyte detection was achieved using a ThermoFinnigan MAT95XL high resolution mass spectrometer. The HRMS was operated at 10,000 mass resolution (10% valley definition). Electron impact ionization was used and was optimized at 45eV. FC53 gas was fed into the ion source by molecular bleed and was used for the lock and calibration masses necessary to compensate for the mass drift of the instrument. The instrument was operated in multiple ion detection (MID) mode with 11 timed segments over the chromatographic run. For each analyte, two abundant masses were chosen for analysis. The most stable ion was used for quantitation and the secondary ion was used for confirmation. Table 1 lists the ions selected for analysis for each analyte. We determined the composition of each fragment ion used then calculated the exact mass for that fragment using software provided with the instrument.

Identification of each analyte was based on the retention time, the exact mass and the ratio of the quantitation and confirmation ions. For compounds with halogenated atoms, theoretical ion ratios were used and the observed ratio had to fall within ± 20% of the theoretical ratios to be considered a positive detection. For those compounds without halogenated atoms the confirmation ion was usually several times less abundant than the quantitation ion and could only be seen at higher concentrations; thus, the ion ratio was determined from repeated measurements of the analyte in solvent standards. Regardless, both the quantification and confirmation ions had to be present and present within limits of a predetermined ratio to be considered a positive detection.

Quantitation of each analyte was achieved using isotope dilution. Isotopically labeled internal standards were available for most analytes. If the analyte did not have its own internal labeled standard, a surrogate was chosen which eluted close to the compound and was in the same compound class. Table 1 lists the quantitation and internal standard ion used for each analyte. A ten-point calibration plot was analyzed in solvent with a range of 1-1000 pg/µL except for p,p'-DDE which had an upper range of 5000 pg/µL. From the calibration standards, a relative response factor (i.e., area of the analyte divided by the area of the internal standard) was generated for each analyte and a plot of the relative response factors versus the standard concentrations was used to quantitate unknown concentrations. This concentration represented the concentration of the analyte in the final extract, thus the concentrations were then multiplied by 50 to obtain the concentrations in 1 mL of breast milk.

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Table 1. Target analytes, the monoisotopic mass used for analysis, retention time and window of acquisitions and lock and calibration masses for analysis. The labeled compound used for the internal standard in quantification is listed. Isotopically labeled analogues of all target compounds were used except for disulfoton, vinclozolin, chlorpyrifos methyl, and methoxychlor. Confirmation ions are not listed because of limited space but are available upon request.

Window	Ratio	Lock, Calibration		Analyte	RT	Quan Ion	Labeled Compound	RT	Quan Ion
1	1.29	130.991467,	168.98827						
				propoxur	16.58	152.0832	propoxur-isopropyl d7	16.53	159.1271
				bendiocarb	17.33	151.0390	bendiocarb-13C6	17.34	153.0457
2	1.04	280.981887,	292.9818						
				HCB	17.70	283.8102	HCB-13C6	17.7	289.8303
3	1.59	218.985080,	318.97923						
				atrazine	18.17	200.0823	atrazine-ethylamine d5	18.14	205.1011
				fonofos	18.37	246.0296	fonofos-dyfonate-ring 13C6	18.51	252.0496
				diazinon	18.55	304.1005	diazinon-diethyl d10	18.48	314.1633
				b-HCCH	18.16	216.9145	b-HCCH-13C6	18.16	222.9347
				disulfoton	18.76	274.0279	fonofos-dyfonate-ring 13C6	18.51	252.0496
4	1.04	280.981887,	292.9818						
				chlorpyrifos-methyl	19.41	285.9256	chlorpyrifos-diethyl ester d10	20.16	324.0196
5	1.13	292.981887,	330.978693						
				chlorpyrifos	20.22	313.9569	chlorpyrifos-diethyl ester d10	20.16	324.0196
				dacthal	20.30	300.8801	chlorthal-dimethyl - dimethyl d6	20.26	303.899
6	1.26	242.985080,	304.981887						
				o,p'-DDE	21.47	246.0003	*o,p'*-DDE - ring 13C6	21.47	258.0406
				p,p'-DDE	22.14	246.0003	*p,p'*-DDE - ring 13C6	22.13	258.0406
7	1.48	230.985080,	342.97869						
				o,p'-DDT	23.25	235.0081	*o,p'*-DDT - ring 13C12	23.24	247.0484
				PCB 118	22.94	323.8834	PCB 118-13C12	22.94	335.9237
				p,p'-DDT	24.31	235.0081	*p,p'*- DDT - ring 13C12	24.30	247.0484
8	1.65	192.988274,	318.978693						
				PCB 153	23.51	298.9037	PCB 153-13C12	23.51	301.944
				PCB 138	24.37	289.9037	PCB-138-13C12	24.37	292.944
9	1.08	318.978693,	342.978693						
				PCB 180	26.57	323.8834	PCB 180-13C12	26.56	335.905
10	1.37	168.988274,	230.98508						
				cis-permethrin	28.42	183.0804	*cis*- permethrin-phenoxy 13C6	27.47	189.1006
				trans-permethrin	28.62	183.0804	*trans*- permethrin-phenoxy 13C6	28.65	189.1006
11	1.44	180.988274,	242.98508						
				cyfluthrin *	29.24 - 29.57	226.0663	cyfluthrin-phenoxy 13C6	29.25	232.0864

				cypermethrin*	29.75 - 30.10	181.0648	cypermethrin-phenoxy 13C6	29.77	259.9368
12	1.11	242.985080,	268.981887						
				deltamethrin	32.11 - 32.64	252.9045	cypermethrin-phenoxy 13C6	29.77	259.9368

* Theses pesticides had four isomeric peaks with the same monoisotopic mass but chromatographically separated. All four peaks had to be found to be considered detected. Single isomeric peaks were used for labeled internal standards.

Quan = quantification. Lock and calibration refer to the masses used to establish these parameters on the high resolution mass spectrometer (HRMS). Mass measurements made within this mass range are accurate to at least 4 decimals places. Units are m/z. Quan ion units are m/z and the mass is the monoisotopic mass which is accurate at 10,000 resolution. RT = retention time in minutes. Window refers to a timed segment in which the ions within that window are the only ions monitored. Ratio is the ratio of the high mass to the low mass in a given timed window segment. To retain good lock and calibration on the HRMS, the ratio is typically <1.5. As the ratio increases, the mass accuracy decreases slightly

Table 2. Relative recoveries (accuracy) and precision (relative standard deviation) of the analytes measured using this method at three concentration points.

	Low: 400 pg/g			Medium: 2000 pg/g			High: 20,000 pg/g		
	Mean (pg/g)	RSD (%)	Relative Recovery (%)	Mean (pg/g)	RSD (%)	Relative Recovery (%)	Mean (pg/g)	RSD (%)	Relative Recovery (%)
Organophosphates									
Chlorpyrifos	484	31	105	2371	8	115	21149	9	105
Chlorpyrifos-methyl	516	22	123	2422	15	120	23422	7	117
Diazinon	498	41	125	2534	11	127	22903	12	115
Disulfoton	375	44	94	2433	10	122	19367	7	97
Fonofos	449	24	107	2308	8	115	22422	4	112
Carbamates									
Bendiocarb	421	38	105	1447	17	69	16322	28	81
Propoxur	702	31	175	3469	12	174	30515	23	153
Pyrethroids									
cis-Permethrin [a]	259	26	65	2184	24	98	19687	29	97
trans-Permethrin [a]	232	29	58	2107	22	93	19179	26	95
Cyfluthrin	<LOD	---	---	2262	21	113	20600	19	103
Cypermethrin	<LOD	---	---	2131	25	107	19659	25	98
Deltamethrin	354	22	89	2055	21	103	18621	30	93
Other pesticides									
Atrazine [c]	661	33	165	3073	22	154	31776	31	159
Organochlorines									
Dacthal	456	20	107	2376	8	117	17367	5	87
o,p'-DDE	422	6	106	1995	1	100	18851	4	94
p,p'-DDE [a]	507	5	130	2125	4	106	18153	8	91
o,p'-DDT [a]	368	4	92	1973	3	99	17005	4	85
p,p'-DDT [a]	319	21	80	2473	7	105	19803	12	97
HCB [a]	376	25	94	2772	7	117	21261	11	104
β–HCCH [a]	503	27	126	2947	22	132	24507	4	121
PCBs									
118 [a]	363	7.7	91	1943	5	97	17999	10	90
138 [a]	266	22	67	2568	6	99	19806	12	96
153 [a]	211	21	53	2754	9	97	19392	16	93
180 [a]	235	22	59	2616	16	102	20058	21	97