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Analysis of organochlorine pesticides in human milk: preliminary results

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Abstract

In the face of evidence of human milk contamination by organochlorine pesticides, an analysis was performed on samples of milk obtained from healthy lactating women in the provinces of Granada and Almería in southern Spain. The samples were obtained by the Neonate Section of the Department of Pediatrics of Granada University Hospital (Neonatology Division) and by the Neonatal Service of Poniente Hospital in El Ejido, Almería. A liquid–liquid extraction procedure was performed. The cleaning of the sample before gas chromatography–mass spectrometry (GC–MS) used silica Sep-Pak. Among other pesticides, aldrin, dieldrin, DDT and its metabolites, lindane, methoxychlor and endosulfan were identified. The presence of these products was confirmed by mass spectrometry. The identification and quantification of these organochlorine molecules is important because they have estrogenic effects. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

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1. Introduction

Organochlorine pesticides, polychlorinated biphenyls (PCBs), dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) are widely distributed halogenated aromatic compounds which persistently contaminate the environment.

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Dieldrin, lindane, toxaphene, endosulfan, and metabolites of endosulfan were recently shown to have estrogenic hormonal activity. [1,2]. Humans, as part of the food chain, are constantly exposed to these products through the consumption of animals, fish and vegetables. Over 90% of human exposure is through food and liquid intake.

Lipophilic xenobiotics are habitually eliminated in the fatty fraction of milk. In women, there is a strong correlation between the concentrations of organochlorine pesticides in their adipose tissue at the end of the pregnancy and those in the fatty fraction of their milk [3]. The mean fat content of human milk is 3.5 g% [4], more than enough for milk to be considered a vehicle for toxins during breast-feeding. Many researchers have assessed the concentrations of organochlorine pesticides in this setting as an index of human contamination. In the USA, studies have shown rising concentrations of dieldrin, aldrin and lindane, but falling concentrations of the now restricted hexachlorobenzene and DDT [5]. Many different countries, such as Norway, France, Jordan, Russia, Kenya, and Sweden regularly report the presence of DDT, DDE and PCBs in the milk of breast-feeding mothers [6–12].

To summarize, organochlorine pesticides, PCBs, and dioxins are found in samples of human milk worldwide, in both rural and urban areas and in countries at all stages of development [13].

The present work describes the preliminary results of our analysis of the milk of lactating mothers who live in an area of intensive agriculture (El Ejido, Almería) or an urban zone (Granada), both in southern Spain.

2. Methods

(1) Gas chromatography. The chromatograph apparatus used was a Varian-3350 ECD Electron Capture Detector (^{63}Ni) (GC-ECD) with Millennium Chromatography Manager Software. The carrier and auxiliary gas was nitrogen, at a flow rate of 30 and 40 ml/min, respectively.

(2) Gas chromatography–mass spectrum. A Saturn 2000 ion trap mass spectrometer from Varian Instruments (Sunnyvale, CA, USA) was used. The integrated gas chromatograph was fitted with an autosampler (Model 8200), a split/split-less programmed temperature injector SPI/1078, operated in the split-less mode, and a DB5-MS (J&W Scientific, Folsom, CA, USA) chromatographic column (30 m \times 0.25 mm id. \times 0.25 μm film thickness). The ion trap mass spectrometer was operated in the electron ionization (EI) mode and the MS/MS option was used. The computer that controlled the system possessed an EI–MS/MS library specially created for the target analyses in our experiment. Further EI–MS libraries were also available. The carrier gas used was helium (purity 99.999%).

2.1. Chromatographic conditions

2.1.1. GC–ECD

A 1- μl aliquot of the extract was injected. The injector and detector temperatures were 250 and 300 $^{\circ}\text{C}$, respectively. The temperature column was programmed from 130 to 150

°C at 20 °C min⁻¹; 150 to 200 °C at 10 °C min⁻¹; 200 to 260 °C at 20 °C min⁻¹. The carrier gas was at a flow rate of 30 ml min⁻¹ at 190 °C oven temperature.

2.1.2. GC–MS/MS

Two-microliter volumes were injected by the autosampler at a flow rate of 1 µl s⁻¹. The GC employed a septum-equipped temperature-programmable injector that was initially maintained at 90 °C for 0.1 min before being increased to 280 °C at a rate of 200 °C min. The GC oven was initially maintained at 80 °C for 2.5 min and then increased at 50 °C min to 140 °C and was finally increased from 140 °C at 5 °C min to 260 °C and maintained at this temperature for 3 min.

2.1.3. GC–MS conditions

The solvent delay was 11 min; electron impact energy, 70 eV, scan rate, 0.6 scans/s, and scanned range *m/z*, 85–450. The transfer line and ion trap manifold were maintained at 260 and 200 °C, respectively. The automatic gain control (AGC) was switched on with a target fixed at 5000 counts. Helium (99.999%) at a flow rate of 1 ml min⁻¹ was used as the carrier and collision gas. The mass spectrometer was calibrated weekly [14,15].

Table 1
Comparison between Almeria (Al) and Granada (Gr) study populations (ng/ml)

Pesticides	Mean		SD		Maximum		MQ		% F	
	Al	Gr	Al	Gr	Al	Gr	Al	Gr	Al	Gr
	Colostrum									
Lindane	0.3	1.5	0.5	2.5	2.2	9.8	0.2	0.3	5.3	9.1
Aldrin	3.3	2.2	5.4	1.9	31.9	6.7	0.5	0.3	9.1	100.0
Dieldrin	0.6	0.8	0.9	0.7	5.1	2.5	0.6	0.7	6.9	8.1
Mirex	1.4	1.5	2.2	2.4	6.6	7.7	1.2	1.5	3.8	3.8
Chlordane	15.6	24.8	16.9	14.4	37.2	80.2	8.9	13.4	7.2	7.1
HCB	16.8	14.4	42.5	18.5	245.5	80.5	0.6	0.6	100.0	100.0
<i>p,p'</i> DDE	24.7	30.5	20.5	26.5	64.9	78.5	1.8	4.5	100.0	100.0
<i>p,p'</i> DDD	8.1	11.5	9.8	15.8	43.6	50.1	4.3	2.7	6.3	6.2
<i>o,p'</i> DDT	0.2	0.5	0.4	1.5	1.7	6.6	0.2	0.4	4.4	23.8
<i>p,p'</i> DDT	2.2	1.8	1.9	3.4	7.2	14.4	1.1	1.2	8.1	5.7
Methoxychlor	0.8	L.C.	3.2	–	18.1	0.6	0.9	0.6	28.1	19.0
Endosulfan α	L.C.	L.C.	–	–	0.6	0.5	0.2	0.2	4.7	6.7
Endosulfan β	2.4	2.2	5.5	6.1	22.8	24.8	2.0	3.1	25.0	28.5
E. Ether	3.7	0.4	5.9	0.5	23.0	2.3	0.1	0.1	9.7	10.0
E. lactone	L.C.	L.C.	–	–	1.5	–	1.5	L.C.	7.5	6.2
E. diol	L.C.	L.C.	–	–	0.4	0.2	0.2	0.2	4.7	5.2
E. sulfate	3.8	2.0	1.9	16.9	7.5	75.9	7.7	3.4	21.9	14.2

MQ=Minimum quantifiable; % F=percent frequency; E=endosulfan; L.C.=Limit quantifiable.

3. Sample selection

Selection of the study population: healthy breast-feeding volunteers aged from 17 to 35 years were selected at random from two distinct geographical settings: one an area of intensive agriculture, El Ejido in Almeria (recruitment site, Hospital del Poniente), and the other, a city, Granada (Clinico University Hospital). All of the women gave their written informed consent and the study was approved by the ethical committee of each hospital.

The milk samples were drawn during three periods: 1–7 days post-delivery (colostrum), 6–12 days (transition), and 13–35 days (mature milk) post-delivery transition. The samples were gathered between 11 and 12 am using the following protocol: 5–10 ml was gathered into a tube from the first breast, the baby then fed from this breast for 5–10 min and, finally, more milk was drawn from the same breast. The same procedure was then followed with the other breast. The milk taken at each sampling was mixed together, yielding a total of 10–20 ml. These samples were immediately stored at -70°C . At each sampling, the double-weighing method was used to determine the volume of the baby's milk intake.

4. Sample preparation and analysis

1. Four to eight tubes of milk sampled from each woman were mixed in a glass jar by shaking.

Table 2
Comparison between Almeria (Al) and Granada (Gr) study populations (ng/ml)

Pesticides	Mean		SD		Maximum		MQ		%F	
	Al	Gr	Al	Gr	Al	Gr	Al	Gr	Al	Gr
	Transition milk									
Lindane	0.27	1.83	0.48	3.61	2.30	8.29	0.18	0.21	40.6	80.0
Aldrin	1.96	0.95	2.10	0.98	9.67	4.77	0.56	1.69	93.8	100.0
Dieldrin	0.68	5.60	1.77	0.40	9.06	1.06	0.56	0.72	93.8	80.0
Mirex	1.66	Q.L.	2.26	–	7.92	1.86	1.15	1.27	37.5	60.0
Chlordane	22.99	23.93	46.58	13.20	234.60	47.05	8.33	15.52	7.8	10.0
HCB	11.71	21.38	0.08	37.96	41.12	47.05	0.68	0.75	93.8	100.0
<i>p,p'</i> DDE	36.79	30.30	28.77	31.97	115.92	76.51	3.39	3.07	100.0	100.0
<i>p,p'</i> DDD	6.42	14.10	9.46	12.53	31.64	32.16	2.67	4.97	40.6	80.0
<i>o,p'</i> DDT	0.62	0.22	1.37	0.33	6.18	0.75	0.32	0.30	31.3	40.0
<i>p,p'</i> DDT	4.69	1.07	7.17	1.32	26.39	3.01	1.05	1.80	81.3	60.0
Methoxichlor	0.24	0.20	0.62	0.44	2.49	0.98	0.95	0.90	12.5	20.0
Endosulfan α	0.40	Q.L.	1.32	–	6.55	0.37	0.16	0.20	46.9	40.0
Endosulfan β	3.10	3.30	5.50	7.10	15.50	15.90	5.20	15.9	21.9	40.0
E. ether	2.50	1.20	4.80	2.30	16.40	5.30	0.20	0.20	9.7	10.0
E. lactone	Q.L.	Q.L.	–	–	1.06	–	0.7	L.C.	9.0	8.0
E. diol	Q.L.	Q.L.	–	–	0.9	–	0.2	L.C.	18.6	20.0
E. sulphate	2.2	4.6	4.4	10.4	15.6	23.3	2.7	23.3	21.9	20.0

MQ = minimum quantifiable; %F = percent frequency; E = endosulfan; Q.L. = Quantifiable limit.

2. To this mixture of 4–8 ml of milk, half of the same volume of methanol was added and the solution was shaken for 5 min; 0.1 g of sodium oxalate was then added and shaken.
3. The extraction was performed using 10 ml ethyl ether/hexane (1:1 v/v).
4. The extract was centrifuged for 15 min at 3000 rpm.
5. The organic phase was obtained and the extraction procedure was repeated twice more.
6. The three organic residues were collected.
7. These residues were concentrated at low pressure in a vacuum concentrator to a volume of 1 ml.
8. To this residue, 0.5 ml of concentrated sulfuric acid was added and centrifuged for 10 min at 3000 rpm.
9. The acid residue was extracted twice more with the addition of 1 ml hexane.
10. The three organic phases were collected and dried in a flow of nitrogen.
11. The dry residue was redissolved in 1 ml of hexane and cleaned up.

5. Clean-up

The organic extract obtained from any of the extractions were purified with the use of silica Sep-Pak after the prior treatment of the cartridge with 2 ml hexane. The extract was

Table 3
Comparison between Almeria (Al) and Granada (Gr) study populations (ng/ml de leche)

Pesticides	Mean		SD		Maximum		MQ		%F	
	Al	Gr	Al	Gr	Al	Gr	Al	Gr	Al	Gr
Lindane	0.31	0.79	0.36	0.67	1.12	1.89	0.16	0.29	72.2	100.0
Aldrin	1.80	4.67	1.44	7.13	4.54	17.30	0.96	1.42	88.9	80.0
Dieldrin	L.C.	1.29	–	1.31	1.65	3.61	0.62	0.65	72.2	100.0
Mirex	1.43	L.C.	2.38	–	7.98	1.22	2.05	1.22	33.3	20.0
Chlordane	15.06	59.06	19.52	74.53	81.78	17.99	9.50	6.95	6.6	8.0
HCB	17.08	5.29	19.37	2.03	80.77	8.05	0.62	3.22	94.4	100.0
<i>p,p'</i> DDE	37.52	19.87	30.04	11.95	104.36	31.97	1.33	3.92	100.0	100.0
<i>p,p'</i> DDD	7.91	11.00	13.21	15.36	46.60	31.75	4.01	23.25	44.4	40.0
<i>o,p'</i> DDT	0.36	L.C.	0.44	–	1.65	0.32	0.23	0.32	55.6	20.0
<i>p,p'</i> DDT	2.62	4.75	3.88	8.24	16.23	19.44	1.19	1.02	66.7	100.0
Methoxichlor	L.C.	L.C.	–	–	1.01	1.11	1.01	1.11	11.1	40.0
Endosulfan α	0.17	0.16	0.24	0.19	0.87	0.48	0.23	0.17	61.1	80.0
Endosulfan β	5.32	0.36	8.62	0.55	26.89	1.24	2.78	1.24	44.4	40.0
E ether	7.67	0.19	16.11	0.24	57.58	0.62	0.22	0.08	10.0	9.0
E. lactona	L.C.	L.C.	–	–	1.63	–	0.61	QL.	9.4	8.0
E. diol	L.C.	L.C.	–	–	0.28	0.15	0.18	0.15	3.8	6.0
E. sulphate	1.23	L.C.	3.56	–	14.35	–	1.13	QL.	16.7	60.0

MQ = Minimum quantifiable; %F = percent frequency; E = endosulfan.

eluted with 10 ml hexane and then with 10 ml hexane: methanol: isopropanol (45:40:15; v/v). Both eluates were collected and dried in a stream of nitrogen.

The dry residue was dissolved in 1 ml hexane, labeled with the *p-p'* dichlorobenzophenone internal standard and analyzed with GC–ECD. The results were confirmed with GC–MS.

6. Results

The results for the two study populations are shown in Tables 1–3. The colostrum, transition milk and mature milk samples all contained significant amounts of organochlorine pesticides.

7. Discussion

Our study clearly shows that human milk is an elimination route for xenobiotics and a contamination source for the breast-feeding baby. Most of the samples contained *p,p'*DDD, *o,p'*DDT and *p,p'*DDT, while *p,p'*DDE was detected in 100% and hexachlorobenzene (HCB) in 94–100% of the samples. Other pesticides and their derivatives were also widely found. The concentration of organochlorine molecules in milk mainly depends on their accumulation in the maternal fatty tissue and their subsequent mobilization. Reports on the exposure of babies to environmental contaminants through maternal milk have called into question the safety of breast-feeding [16–18]. The risk that a sample may carry is assessed by establishing its toxic equivalents (TEQs), but while this method has been widely used for PCBs and TCDD/Fs, it has been little-used for the organochlorine pesticides, which are found at similar or greater concentrations compared with PCBs. There is just one TEQ parameter established for TCDD/Fs and PCBs and none for organochlorine pesticides. In order to express the organochlorine pesticides determined in milk in TEQs, the same method must be followed. The European Office of the World Health Organization [19] determined a Tolerable Daily Intake of 1–4 toxic equivalents of pg/kg/day for dioxins and dioxin-like PCBs. The daily intake per kilogram body weight of a breast-feeding child is estimated to be 50-fold that of an adult, so that in the first 6 months of breast-feeding, the baby accumulates as much TCDD/F as an adult does in 25 years. Thus, a burden of organochlorine molecules is passed by breast-feeding from one generation to another [20]. The present study found organochlorine pesticides in human milk at higher concentrations than the TEQ limit proposed by the WHO, consistent with the findings of other researchers [9,21]. The discrepancy between the limits imposed on organochlorine pesticides and those on TCDD/Fs must be corrected. Limits on exposure to these widespread organochlorine molecules must be established.

The risk is not confined to human lactation. Any living being can be similarly contaminated by pesticides. Bio-health controls must be established for women and for the animals whose milk provides infant formulas [22,23].

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