HAIR ANALYSIS: ANOTHER APPROACH FOR THE ASSESSMENT OF HUMAN EXPOSURE TO PERSISTENT ORGANOCHLORINE POLLUTANTS (POPs)

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Introduction

Human and environmental monitoring of organic chemicals has become increasingly important for exposure and risk assessment. Body fluids and tissues are commonly used to evaluate human exposure[1,2]. Adipose tissue analysis gives an estimation of the total exposure (used in body burden assessment), while serum analysis illustrates the present status of exposure to organochlorines. Thus, hair can be a suitable alternative and an overall bioindicator for short- and long-term exposure to persistent organochlorine pollutants (POPs). Hair analysis is very much used in drug testing, especially for assessing past exposure[3].
Although, it is a matrix easy to obtain by non-invasive methods (repeated sampling of the same individual), only few papers[4,5] describe analysis methods for organochlorines in hair. Moreover, there are no consistent studies to compare different extraction and clean-up techniques.
Few is also known about the elimination kinetics in different body compartments. Available studies[6,7] suggest a similar distribution of xenobiotics in different compartments, when normalized on lipid content. Thus, hair is a good matrix for non-invasive monitoring purposes for pollutants.

Methods and Materials

- *Sampling*: Hair samples from Crete, Greece were obtained from women with reported pregnancy or other gynaecological problems and with known pesticide exposure. These samples were pulverised. Hair samples from Belgium and Romania were obtained from apparently healthy individuals. They were washed with hot water and acetone, dried and cut in small pieces.
- *Incubation*: 30-100 mg of hair were accurately weighted, spiked with 5 ng of internals standards (PCB 46 and PCB 143) and incubated with different agents: 2 ml HCl 3M (overnight, 40°C), 2 ml thioglycolic acid (TGA) 0.2 M in 8M urea[5] (overnight, 40°C), 1.5 ml H_2SO_4 7M[8] (1h; 70°C), 2 ml methanol (MeOH) (overnight, 40°C), 2 ml dichloromethane (DCM) (overnight, 40°C), 2 ml MeOH : DCM (overnight, 40°C), 2 ml hexane (overnight, 40°C).
- *Extraction*: For samples incubated with acids, SPE on C18 disk extraction cartridge (3M) was evaluated. After conditioning of the cartridge with MeOH and water, the acid hydrolysates were loaded on the cartridge. After rinsing the cartridge with water, 1.5 ml hexane was used for the elution of POPs. For all incubated samples, liquid-liquid extraction (LLE) with 2 x 3 ml hexane:DCM (4:1) was evaluated as an alternative extraction method.
- *Clean-up*: All hexane eluates (from SPE or LLE) were purified on a cartridge filled with acid silica (concentrated sulphuric acid:silica=1:1) and anhydrous Na_2SO_4. It was shown that 1 g acid silica was efficient for complete clean-up of 200 mg hair. Acid silica cartridges were eluted with 4

ml hexane, the final eluate was concentrated to approximately 50 µl under a gentle nitrogen stream and spiked with 5 ng recovery standard (1,2,3,4-tetrachloronaphthalene -TCN).

➢ *Analysis*: A Hewlett Packard 6890 gas chromatograph with µ-ECD, equipped with a HT-8 (SGE, Australia) capillary column (30m x 0.22mm x 0.25µm) was used. Injector and detector temperatures were 270°C and 320°C, respectively. Helium was used as carrier gas at a flow rate of 1 ml/min. Argon:CH_4 was used as make-up gas at a flow rate of 40 ml/min. Two µl were injected in pulsed splitless mode (pressure pulse of 25 psi for 1 min) with the split outlet opened after 1.5 min. The temperature program started from 90°C, kept for 1 min and then increasing the temperature with 15°C/min to 180°C, kept for 1 min, further by 3°C/min to 250°C and further by 15°C/min to 290°C, kept for 6 min.

For confirmatory purposes, a Hewlett Packard 6890 gas chromatograph was connected to a Hewlett Packard 5793 mass spectrometer (MS) and equipped with a DB-5ms (J&W Scientific, Folsom, USA) capillary column (30m x 0.25mm x 0.25µm). Injector and transfer line temperatures were 265°C and 280°C, respectively. Helium was used as carrier gas at a flow rate of 1 ml/min. One µl was manually injected in pulsed splitless mode (pressure pulse of 20 psi for 1 min) with the split outlet opened after 1.25 min. The temperature program started from 90°C, kept for 1 min and then increasing the temperature with 15°C/min to 275°C, kept for 10 min.

Peak identification was based on relative retention time (to TCN) and for MS on specific group ions. The compounds under investigation were HCHs, DDTs, HCB and PCBs (IUPAC no. 99, 118, 138, 149, 153, 170, 180, 187). Multi-level calibration curves ($r^2 > 0.99$) were created for the quantification using the above mentioned analytical conditions.

Results and Discussion

Acid hydrolysis was shown to be an efficient method for destruction of the keratin matrix. Basic hydrolysis was excluded, due to the lability of some pesticides in alkaline media.

Recoveries of internal standards (spiked before incubation) together with the concentration of p,p-DDE obtained by different treatments from a pulverised pooled hair sample are presented in Table1.

➢ SPE gave bad recoveries due to the low solubility of organochlorines in aqueous media and because the compounds were not removed from their keratinic binding sites.

➢ MeOH:DCM was a good combination for the extraction of organochlorines from pulverised hair, but solvent extractions are not efficient from cut hair. MeOH extracts lead to more interfering peaks, including late eluting peaks.

➢ Sulphuric acid gave slightly lower recoveries than TGA or HCl hydrolysis, probably due to some degradation at high temperature. Another disadvantage of this method was that sulphuric acid forms easily emulsion when extracted with hexane, making the two phases difficult to separate. TGA and HCl incubations were found to be the most acceptable, providing milder conditions. HCl was further used, because it is easy to work with.

➢ Hexane : DCM should be used as solvent mixture for LLE extraction of acid hydrolisates in order to have higher recoveries for DDTs. Recoveries of the studied organochlorines (selected PCBs, DDTs and HCHs) ranged between 65 and 80%.

Table 1. Recoveries of internal standards and p,p-DDE from a pooled hair sample.

Treatment	Extraction	CB 46	CB 143	C_{DDE} (pg/mg hair)
H_2SO_4 7M	LLE	71	84	450
	LLE	71	78	390
	LLE	71	78	390
TGA 0.2M	SPE	6	8	250
	LLE	77	86	410
HCl	SPE	8	10	330
	LLE	64	70	410
	LLE	75	86	420
	LLE	74	77	400
	LLE	85	86	420
MeOH+DCM	LLE	81	97	360
MEOH	LLE	75	82	420
DCM	LLE	74	74	380

LLE- liquid-liquid extraction, SPE-solid phase disk extraction

Since hair has not been used in monitoring programs, no certified reference material is available and the spiking is not easy to perform, especially when hair is not pulverised. It is very difficult to obtain homogeneous distribution of the spiking material on the hair.

The method chosen for further analyses was: incubation overnight at 40°C with HCl 3M, extraction with 2 x 3 ml hexane:DCM (4:1) and clean-up on acid silica. This method was used for the determination of organochlorines in different hair samples from Crete, Romania and Belgium. (Table 2).

Table 2. Concentrations of POPs in individual hair samples from Greece, Romania and Belgium.

No. sample	age	country	HCHs (pg/mg)	DDTs (pg/mg)	PCBs* (pg/mg)	Pesticide exposure (years)
1	28	Romania	24.5	31.8	5.5	-
2	29	Romania	39.1	66.6	10.0	-
3	42	Belgium	13.4	22.4	10.5	-
4	65	Belgium	17.3	45.9	40.4	-
5	68	Belgium	19.8	299.8	44.7	-
6	26	Belgium	8.8	10.7	5.1	-
7	66	Greece	95.2	754.2	< 2	10
8	41	Greece	58.7	191.5	4.4	5
9	53	Greece	53.1	101.2	< 2	10
10	67	Greece	59.8	159.3	< 2	30
11	49	Greece	81.5	169.8	17.2	10
12	42	Greece	49.7	180.6	17.6	3
13	57	Greece	53.9	136	10.4	10
14	53	Greece	79.5	116.5	12.9	10

*- sum of congeners 99, 149, 118, 153, 138, 180 and 170 (IUPAC numbering).

Patterns of organochlorines in hair differ from country to country. In Cretan samples, the presence of DDTs (including o,p-DDE) and HCHs (predominantly γ-HCH) is remarkably high. Cretan levels of PCBs are the lowest. Samples from Romania contain moderate levels of PCBs as well as p,p-DDE and p,p-DDT (as predominant DDTs) and β-and γ-HCH. Hair samples from Belgium contain the highest levels of PCBs together with p,p-DDE as the predominant pesticide. However the concentration is closely related with age.

Further investigations will focus on the effect of hair color, the need of a desulphuration step (which might become necessary when using MS), other extraction procedures (e.g. Soxhlet), large volume injection for determination of minor compounds and correlation of accumulation patterns between hair and other body tissues.

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