DISTRIBUTION OF ORGANOCHLORINE PESTICIDES IN VARIOUS PORK TISSUES

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Introduction

Polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) are among the most prevalent environmental pollutants, being detected in almost all living organisms. One common feature is that they concentrate in fatty tissues and bioaccumulate in the food chain. According to previous studies, PCBs and OCPs are stored in muscle and fat of animals and humans, but they also can reach other compartments such as brain, liver and lungs. Farmed animals from Romania have been shown to contain relatively high concentrations of OCPs (1) and thus, can be used for studying their distribution in different tissues.

Methods

Samples

Pork tissues were obtained from 4 different specimens from 4 different farms located in Romania (Table 1). All samples were kept at temperatures < 4°C until analysis.

Analysis

The following compounds were included: hexachlorobenzene (HCB), α-, ß- and γ-HCH hexachlorocyclohexane isomers (the sum expressed as HCHs), o,p'-DDE, p,p'-DDE, o,p'-DDD, p,p'-DDD, o,p'-DDT and p,p'-DDT (the sum expressed as DDTs) and 7 PCB congeners (IUPAC no: 28, 52, 101, 118, 138, 153, 180). The method used for the determination of selected POPs in animal tissues has been previously described and validated (2) and briefly presented below. Between 5 and 10 g of tissue were homogenised with anhydrous Na_2SO_4, spiked with internal standards (e-HCH, PCB 46 and 143) and extracted for 2h by hot Soxhlet with 100 ml hexane:acetone (3:1). After lipid determination, the extract was purified on acidified silica. After elution with 15 ml hexane and 10 ml dichloromethane (DCM), the cleaned extract was concentrated to approximately 80 µl. One µl was injected in splitless mode into a GC-µECD, equipped with a 50 m x 0.22 mm x 0.25 µm HT-8 capillary column.

For chiral analysis, extracts were fractionated on a silica SPE cartridge. The 1st fraction, containing all PCBs, p,p'-DDE and p,p'-DDT, was eluted with 4 ml hexane, and the 2nd fraction containing all HCH isomers and p,p'-DDD, was eluted with 3ml DCM. After concentration, the 2nd fraction was analysed by GC/NCI-MS using a 30m x 0.25mm Chirasil-Dex column (Chrompack). A volume of 5 x 5 µl of the extract was injected in large volume injection mode. Three ions from the molecular cluster (253, 255 and 257) were monitored for α-HCH. For the validation of the chiral analytical procedure, enantiomeric ratio (ER) of a-HCH was determined on NCI by 5 successive injection of α-HCH standard (100 pg/ml) with a mean ± SD of 0.99 ± 0.02.

Results and discussion

Concentrations of HCB were lower than 3 ng/g lipid in all samples and therefore, were not further taken into consideration. Concentrations of individual HCH isomers, DDT metabolites and PCBs are given in Table 1. It can be observed that organochlorine pesticides (HCHs and DDTs) are the principal

contaminants in all samples. The concentrations found are rather high, even if they are below the EU norms for DDT and HCH in animal fat (1000 ng/g fat). Moreover, similar or higher concentrations were already measured in Romanian farmed animals (1), showing that pesticide contamination of the food chain is still an acute problem. This extends also to milk and diary products imported from Romania for which the EU norms (100 and 75 ng/g fat for α-HCH and ß-HCH, respectively, in products with >2 % fat) were regularly exceeded (3).

ß-HCH was the most persistent HCH isomer in all tissues, accounting for 40-97 % of sum HCHs (Table 1). The highest concentrations of ß-HCH and HCHs were found in liver, while the lowest HCH concentrations were measured in brain and spinal marrow.

The highest concentrations of DDTs were measured in muscle and fat, with p,p'-DDE being the principal contributor and with a variable contribution of p,p'-DDD and p,p'-DDT. In liver, p,p'-DDD has a higher contribution to the sum DDTs, while in all 4 livers analysed the concentration of p,p'-DDT was very low. This may be due to the fact that at high concentrations of DDT in the body, there is an induction of P450 1A and 2B enzymes and thus a lower concentration of p,p'-DDT in the liver (4). P,p'-DDT could be measured in brain, lung and heart.

PCB concentrations were low (< 30 ng/g fat), in accordance with previously reported concentrations from Romanian animal farms (1). These values are lower than PCB values reported from Western European countries such as Belgium (5) and Sweden (6). The highest PCB concentrations were found in liver and lung, while the lowest values were measured in fat.

The brain and spinal marrow contained the lowest concentrations of pesticides (expressed in ng/g lipid), due to higher lipid contents (up to 20-25 %) and to a different lipid composition from that of fat, muscle and other organs. While in fat and muscle, triglycerides comprise more than 90 % of the total lipid content, in brain, the main lipidic constituents are cholesterol and phospholipids (a relatively equal distribution of both). The distribution of DDT and HCH isomers in tissues is very dependent of their distribution in the animal feed, age and weight of animal and probably, other factors. Moreover, HCH, DDT and PCB levels in the tissues were not correlated.

Enantiomeric ratios (defined as as the proportion of peak area of the first to the second eluting peak for the optical isomers) were calculated for α-HCH. For the column used in this work (Chirasil-Dex, Chrompack), we considered the elution order reported by Wiberg et al. (7), with the (+) α-HCH isomer eluting before (-) α-HCH.

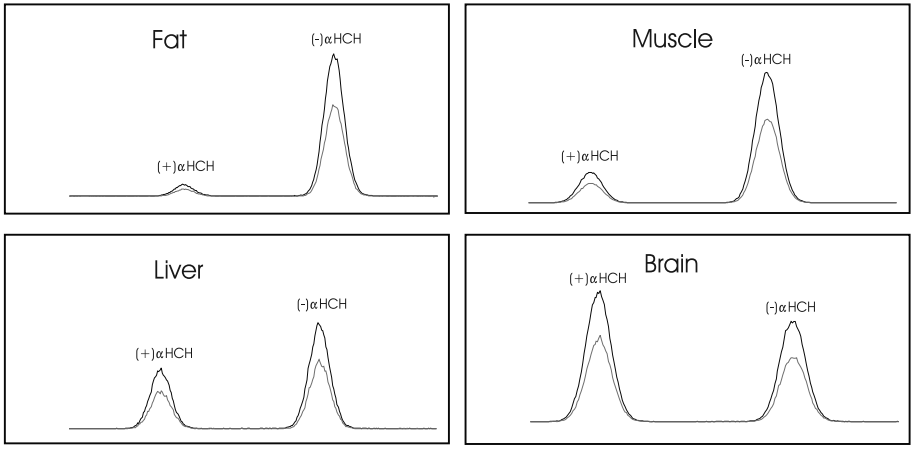

Figure 1. Enantiomeric separation of a-HCH in fat, muscle, liver and brain from animal no.4.

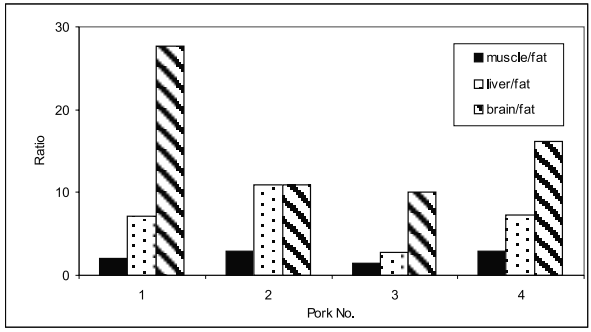

Figure 2. Ratios between ERs in different tissues and ER in fat for each animal

In all samples (except 2 brain samples), (+) α-HCH was depleted and (-) α-HCH was enantioenriched (Figure 1 and Table 1). For these samples, ERs < 1 (or EFs < 0.5) were obtained for all animals. Remarkably low ERs (0.03-0.4) were measured in livers of roe deers from two remote regions in Germany (8). Similar results (ER <1) in fat and liver of seven samples from sheep were obtained by Möller et al (9). Moreover, the sheep brain showed a higher level of (+) α-HCH with ER of 1.4-3.8 (7), which is very similar with results obtained in our study where ER in brain was the highest measured values between all organs (Table 1). Another interesting result is the fact that for animals no.1 and 2 (females), ER in brain was < 1, while for animals no. 3 and 4 (males), ER was >1. Although the number of samples is too low to draw conclusions, it might be that, at least for some species, there is a sex-related dependency of ER. Ratios between ER in different organs and ER in fat (the lowest value) show that ERs increase in the order fat < muscle < liver < brain for all studied animals (Figure 2).

In marine ecosystems and especially in marine mammals, ERs > 1 for α-HCH were measured, showing an enantioenrichment of (+) α-HCH in these animals (10). Furthermore, in some species, such as harbour seals from Iceland (11), very high ERs were found in brain and in some cases, (-) α-HCH isomer was not present. This finding is interesting, because it sustains the hypothesis that the blood-brain barrier is partially selective towards certain fat-soluble organochlorines, such as α-HCH. An enantioenrichment of (+) α-HCH was observed in both terrestrial (8, 9 and this study) and marine mammals (10), suggesting that the (+) isomer is able to penetrate the barrier, while the (-) isomer is largely held back by it.

References

1. Covaci, Hura C, Schepens P (2001) Sci Total Environ 280, 143-152.
2. Covaci A, Ryan JJ, Schepens P (2002) Chemosphere 47(2), 207-217.
3. Pesticides Safety Directorate, Food Alerts, http://www.pesticides.gov.uk
4. Nims RW, Lubet RA, Fox SD, Jones CR, Thomas PE, Reddy AB, Kocarek TA (1998) J Toxicol Environ Health A, 53, 455-477.
5. Schepens P, Covaci A, Jorens PG, Hens L, Scharpe S, van Larebeke N (2001) 109, 101-103.
6. Glynn AW, Wernroth L, Atuma S, Linder CE, Aune M, Nilsson I, Darnerud PO (2000) Sci Total Environ 246, 195-206.
7. Wiberg K, Brorström-Lunden, Wangberg I, Bidleman TF, Haglund P (2001) Environ Sci Technol 35, 4739-4746
8. Pfaffenberg B, Hardt I, Hühnerfuss H, König WA, Rimkus G, Glausch A, Schurig V, Hahn J (1994) Chemosphere, 29, 1543-1554
9. Möller K, Huhnerfuss H, Rimkus G (1993) J High Resolut Chromatogr, 16, 672-673.
10. Tanabe S, Kumaran P, Iwata H, Tatsukawa R, Miyazaki N (1996) Mar Pollut Bull 32, 27-31.
11. Kallenborn R, Hühnerfuss H. Chiral Environmental Pollutants, Springer, Berlin, 2001.

Table 1. Lipid percent, concentrations (ng/g lipid) of organochlorine pollutants and enantiomeric ratios for a-HCH in pork tissues.

Pork No.	Sex	Tissue	Lipid (%)	α-HCH	γ-HCH	ß-HCH	**Sum HCHs**	**ER** (+/-)	**EF**	p,p'-DDE	p,p'-DDD	p,p'-DDT	**Sum DDTs**	**Sum PCB***
1	F	muscle	9.6	4.9	1.4	29.1	**35.3**	0.05	0.05	92.7	27.7	86.0	**206.3**	**8.9**
1	F	fat		6.3	1.1	20.2	**27.7**	0.02	0.02	93.9	27.3	101.6	**222.8**	**2.9**
1	F	liver	3.1	5.7	3.4	303.5	**312.6**	0.17	0.14	83.7	77.6	7.9	**169.2**	**22.3**
1	F	brain	11.8	1.1	0.6	15.5	**17.2**	0.65	0.39	6.0	1.5	5.2	**12.8**	**3.5**
1	F	lung	2.1	4.1	9.0	32.6	**45.6**	0.41	0.29	31.6	12.5	13.5	**57.6**	**32.8**
1	F	sp marrow	29.6	0.8	0.4	4.4	**5.6**	0.45	0.31	5.2	1.1	5.6	**11.9**	**0.8**
1	F	kidney	3.5	3.1	3.2	52.9	**59.2**	0.19	0.16	26.9	19.6	6.6	**53.0**	**10.2**
1	F	spleen	2.4	1.9	4.3	20.8	**27.0**	0.30	0.23	12.2	8.4		**20.6**	**10.8**
1	F	heart	3.4	2.2	2.6	164.8	**169.6**	0.19	0.16	17.3	7.8	8.1	**33.2**	**9.5**
2	F	muscle	5.4	5.4	14.6	8.7	**28.6**	0.11	0.11	30.8	4.3	17.1	**52.3**	**10.9**
2	F	fat		6.2	8.4	6.7	**21.3**	0.04	0.04	38.4	5.6	21.9	**65.9**	**3.8**
2	F	liver	4.5	6.0	8.7	104.2	**118.9**	0.42	0.30	27.8	14.5	2.2	**44.5**	**15.1**
2	F	brain	14.9	1.3	2.5	4.2	**8.1**	0.42	0.30	2.6	1.2	1.9	**5.7**	**6.9**
3	M	muscle	5.1	4.5	6.2	13.5	**24.2**	0.21	0.17	183.1	68.4	75.7	**327.2**	**17.5**
3	M	fat		3.9	2.9	9.2	**16.0**	0.18	0.12	176.3	75.1	83.1	**334.5**	**6.3**
3	M	liver	5.4	3.4	6.2	51.8	**61.4**	0.38	0.28	145.9	111.4	2.6	**260.0**	**27.6**
3	M	brain	24.0	1.4	3.9	2.9	**8.2**	1.37	0.58	7.0	5.2	2.6	**14.8**	**3.2**
4	M	muscle	14.5	4.2	6.4	11.2	**21.9**	0.23	0.19	55.9	6.2	16.7	**78.8**	**8.6**
4	M	fat		3.9	2.5	10.8	**17.2**	0.08	0.07	52.0	3.9	12.6	**68.5**	**2.6**
4	M	liver	5.5	5.0	12.8	99.9	**117.7**	0.57	0.36	35.6	12.3	7.2	**55.2**	**12.7**
4	M	brain	17.1	1.7	4.0	4.3	**10.1**	1.27	0.56	12.7	8.4	23.2	**44.2**	**4.6**
4	M	lung	4.3	8.8	23.4	16.4	**44.6**	0.61	0.38	32.5	17.1	11.3	**72.8**	**11.7**

*IUPAC no. 28, 52, 101, 118, 138, 153, 180; $ER = \frac{(+)\alpha - HCH}{A(-)\alpha - HCH}$; $EF = \frac{ER}{1+ER}$