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DETERMINATION OF HEXACHLOROBENZENE (HCB) IN THE PERIRENAL AND DORSAL FATTY TISSUES OF PIGS

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The aim of this study was to assess the suitability of the perirenal fatty tissue for the determination of an organochlorine pesticide. Fatty tissue samples were prepared by the matrix solid phase dispersion (MSPD) method, and pesticide levels were determined by gas chromatography on capillary column using an electron capture detector. Results were confirmed by gas chromatography/mass spectrometry (GC/MS) system. The results showed that the perirenal fatty tissue contained significantly higher levels of hexachlorobenzene (HCB) than the dorsal fatty tissue ($P < 0.01$). All the levels were below the criteria for maximum residue limits established by Croatia and the EU.

Key words: Hexachlorobenzene, fatty tissue, gas chromatography

Because of the stability of various chlorinated pesticides in the environment, their tendency to accumulate in animal and human fat tissues, and their harmful effects on the ecosystem and public health in general (Curley et al., 1969; Bloomer et al., 1977; Bažulić et al., 1984*a, b;* Najdek and Bažulić, 1988; Falandysz and Kannan, 1992; Waliszewski et al., 1996; Frković et al., 1996; Paton and Petterson, 1997; Harris et al., 2001), their extensive application has been limited since 1972. However, hexachlorobenzene (HCB) has remained in use as a fungicide for seed treatment. Regular monitoring of the levels of HCB contamination in fatty tissues of animals is mandatory for public health reasons related to the domestic consumption and export of foods.

Within the framework of the Croatian Residue Monitoring Programme in Meat and Foodstuffs of Animal Origin (Guidelines of the Ministry of Agriculture and Forestry, Veterinary Directorate, U. No. 525-06-93-1, Zagreb Croatia 1993), among other organochlorinated contaminants, HCB is routinely included in the determination of residues in animal dorsal fatty tissue.

By comparing the levels of HCB residues in the perirenal and dorsal fatty tissues, this work was aimed at assessing the suitability of the perirenal fatty tissue for the determination of this chlorinated pesticide.

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Materials and methods

A total of 40 samples of perirenal and dorsal fatty tissues were randomly collected at pig slaughterhouses. All samples were collected from domestic, regularly fed pigs exposed via the natural ways. The samples were prepared in parallel with the blank test and with the application of Supelco standard for HCB (LA 79393). A multiresidue extraction technique, i.e. matrix solid phase dispersion (MSPD) was applied for the extraction of organochlorinated pesticides. After the grinding of fat tissue (0.5 g) with octadecylsilyl derivatised silica (40 µm particle size), the C_{18}/fat matrix blend was transferred into a previously prepared 10 mL syringe barrel that contained 2 g activated Florisil. The hexachlorobenzene was then eluted from the column with 8 mL acetonitrile, and a 2 µL portion of the acetonitrile eluate was then directly analysed by gas chromatography (Long et al., 1991; Bažulić et al., 1998). All chemicals were of pesticide grade. Analytical quality assurance included periodical participation in interlaboratory control with the Croatian Institute of Public Health and recovery tests. For the determination of HCB an ATI Unicam type 610 gas chromatograph equipped with electron capture detector (ECD) and capillary column DB-608 (30 m × 0.32 mm I.D.; film thickness: 0.5 µm) was used. For the confirmation, an Automass System type 2 mass spectrometer with quadruple mass analyser was used.

Working conditions for the gas chromatograph

t (detector) = 330 °C; t (injector) = 250 °C; $t_{INITIAL}$ (column) = 120 °C (1 min) with Δt = 5 °C/min; t_{FINAL} (column) = 270 °C (12 min); splitless time – $T_{DISCONN.}$ = 1 min; carrier gas: He (70000 Pa).

Working conditions for GC/MS system

t (injector) = 250 °C; $t_{INITIAL}$ (column) = 120 °C (1 min); with Δt = 5 °C/min t_{FINAL} (column) = 270 °C (12 min) splitless time – $T_{DISCONN.}$ = 1 min; carrier gas: He (50,000 Pa); ionisation energy = 70 eV; filament = 800 µA, photomultiplier = 750 V; vacuum = 1.1066 Pa; t (source) = 130 °C; t (interface) = 250 °C.

Results

The results of HCB analysis in 20 samples of dorsal (a) and perirenal (b) fatty tissues from the same pigs are presented in Table 1. The pesticide concentrations, expressed in µg/kg, have been adjusted taking into account the recovery rate. The average recovery rate, at 10 µg/kg, was 67% (n = 12; CV = 25%). The background interference for the fat extract resulted in a limit of detection at < 1 µg/kg. By applying the GC/MS system for the confirmation of the prelimi-

nary GC results the presence and the levels of HCB obtained by GC method were proved. The differences between perirenal and dorsal fatty tissue levels of HCB were determined using the *t*-test at $p < 0.01$ (Vranić, 1971).

Table 1

Levels of HCB in dorsal (a) and perirenal (b) fatty tissues of pigs

Sample	HCB (µg/kg)	
	a	b
1	< 1	< 1
2	4	< 1
3	< 1	< 1
4	< 1	< 1
5	19	21
6	12	10
7	5	23
8	15	10
9	< 1	64
10	< 1	47
11	< 1	< 1
12	< 1	< 1
13	5	10
14	< 1	1
15	5	24
16	< 1	6
17	< 1	79
18	< 1	< 1
19	4	23
20	38	27

Discussion

The dorsal fatty tissue is regularly used for the assessment of exposure to chlorinated pesticides. However, in order to examine the pesticide levels in the perirenal fatty tissue and to assess the possibility of using this particular fatty tissue as an adequate analytical matrix, the determination of HCB was performed in both the perirenal and dorsal tissues of the same animals. Although Herrera et al. (1994) found HCB in all samples of Spanish meat and meat products, our research shows that the concentration of HCB was below the detection level (< 1 µg/kg) in 55% of dorsal and 35% of perirenal fatty tissues analysed. The results also prove that the differences between the HCB levels of the dorsal (a) and perirenal fatty tissues (b) are significant ($P < 0.01$). Namely, in samples originating from the same animals, the HCB levels determined in the dorsal fatty tis-

sue were lower than those found in the perirenal one. Specifically, the HCB levels ranged from < 1 μg/kg to 38 μg/kg in the dorsal fatty tissue (mean value: 5 μg/kg) and from < 1 μg/kg to 79 μg/kg in the perirenal fatty tissue (mean value: 17 μg/kg).

The HCB levels found in the dorsal fatty tissue appear to be in accordance with data reported for pigs in Croatia (Kipčić et al., 1996) and with our unpublished data. The observed mean concentration of HCB in the perirenal fatty tissue was almost twice as high as that reported by Iossifidou et al. (1999) in Greece. This difference must be related to the inadequate information concerning the age and sex of animals, the type or origin of feeds and the levels of feed contamination. However, the maximum HCB concentrations detected in our samples (38 μg/kg for dorsal and 79 μg/kg for perirenal fatty tissue) were below the Maximum Residue Limits set by the EU (Council Directive 93/57/EU, No. L. 211/1-5, 1993) and being in force in Croatia (100 μg/kg) (Official Gazette No. 46, Zagreb, Croatia, 1994, p. 1582).

The ultimate goal of the monitoring is to assess the load of pesticide residues in the farm animal and human general population. Considering its nutritional importance and frequency of consumption, the dorsal fatty tissue should be primarily used in the pesticide residue monitoring. The perirenal fatty tissue should be recommended for use only when the dorsal fatty tissue is not available for determining the presence and levels of chlorinated pesticide compounds in foods of animal origin.

References

Bažulić, D., Bauer, K., Vukušić, J., Kipčić, D. and Britvec, B. (1984*a*): Investigation of translocation, degradation and translocation of some pesticides in relation to residues in foodstuffs. Arh. hig. rada toksikol. **35,** 173–180.

Bažulić, D., Štampar-Plasaj, B., Bujanović, V., Stojanovski, N., Nastev, B., Rudelić, I., Sišul, N. and Žužek, A. (1984*b*): Organochlorine pesticide residues in the serum of mothers and their newborns from three Yugoslav towns. Bull. Environ. Contam. Toxicol. **32,** 265–268.

Bažulić, D., Sapunar-Postružnik, J. and Bilić, S. (1998): Significance of the quality of Florisil® in organochlorine pesticide analysis. Arh. hig. rada toksikol. **49,** 319–325.

Bloomer, A. W., Nash, S. I. and Price, H. A. (1977): A study of pesticide residues in Michigan's general population. Pesticid. Monit. J. **11,** 111–115.

Curley, A., Copeland, M. F. and Kimbrough, R. D. (1969): Chlorinated hydrocarbon insecticides in organs of stillborn and blood of newborn babies. Arch. Environ. Health **19,** 628–632.

Falandysz, J. and Kannan, K. (1992): Organochlorine pesticide and polychlorinated biphenyl residues in slaughtered and game animal fats from the northern part of Poland. Z. Lebensm. Unters. Forsch. **195,** 17–21.

Frković, A., Živković, A. and Alebić-Juretić, A. (1996): Organochlorine pesticide residues and polychlorinated biphenyls in human milk from northern Adriatic region (Croatia). Fresenius Environ. Bull. **5,** 474–481.

Harris, C. A., Woolridge, M. W. and Hay, A. W. M. (2001): Factors affecting the transfer of organochlorine pesticide residues to breastmilk. Chemosphere **43,** 243–256.

Herrera, A., Arino, A. A., Conchello, M. P., Lazaro, R., Bayarri, S. and Perez, C. (1994): Organochlorine pesticide residues in Spanish meat products and meat of different species. J. Food. Protect. **57,** 441–444.

Iossifidou, E. G., Kamarianos, A., Psomas, I. E. and Kilikidos, S. (1999): Organochlorine pesticide and polychlorinated biphenyl residues in perirenal animal fat from different species in northern Greece. Fresenius Envir. Bull. **8,** 78–85.

Kipčić, D., Vukušić, J. and Bešić, J. (1996): Environmental Exposure Assessment and Health Effect Studies in the Republic of Croatia 1980–1995. Croatian Academy of Sciences and Arts, Zagreb, pp. 100–104.

Long, A. R., Soliman, M. M. and Barker, S. A. (1991): Matrix solid phase dispersion (MSPD) extraction and gas chromatographic screening of nine chlorinated pesticides in beef fat. J. Assoc. Off. Anal. Chem. **74,** 493–596.

Najdek, M. and Bažulić, D. (1988): Chlorinated hydrocarbons in mussels and some benthic organisms from the northern Adriatic Sea. Mar. Pollut. Bull. **19,** 37–38.

Paton, M. W. and Petterson, D. S. (1997): Absorption by sheep of dieldrin from contaminated soil. Austr. Vet. J. **75,** 441–445.

Vranić, V. (1971): Probability and Statistics (in Croatian). Tehnička knjiga, Zagreb, pp. 118–145.

Waliszewski, S. M., Pardio, V. T., Waliszewski, K. N., Chantiri, J. N. and Infanzon, R. M. (1996): Levels of organochlorine pesticides in Mexican butter. J. AOAC International **79,** 784–786.