ECOCHEMISTRY OF BHC IN CZECHOSLOVAKIA

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ABSTRACT

The 'ecochemistry' of BHC and other chlorinated insecticides in soil, surface waters, in selected foodstuffs of plant and animal origin, in human milk and other human materials (fat and the uterus content after an artificial abortion), has been studied. The investigations were accomplished in three regions in Slovakia: two regions with an intensive predominating agricultural production and one submountain country with a prevalence of meadows and pastures. Each year 50–60 samples of 13 kinds of materials were examined on the basis of a special questionary.

In all the investigated samples α-, β- and γ-isomers of BHC were found in addition to lindane. Relations between the cumulation of γ- and β-BHC in different media show the effect of a retarded cumulation, and elimination of β-BHC in animal foodstuffs. This effect was expressed in human materials, where the content of the β-isomer clearly exceeds the content of the γ-isomer.

The results also show a gradual shift of the DDT-contamination on lindane.

The authors finally present a survey of the connection between the embryonic exposure and post mortem levels of pesticides, and the degree of the use of pesticides in different regions.

New scientific knowledge has become available during recent years, particularly in respect to the health effects of chemical agents in various environmental media, to their pathways from the environment to man, to methods of detection of these hazards in the environment, and to the priorities of research. Biomedical research on the effects of pesticides in man has never been undertaken as extensively from an ecochemic, toxicologic and epidemiologic viewpoint as has been done in the last few years. The experimental designs and methodologic approaches used recently reflect a sophistication that has offered promising leads for exploration and has provided results of significant importance.

In the report given by the Director-General of WHO Mahler[1] to the 27th World Health Assembly we can read the following statement: 'While the approach of the past, i.e. to deal separately with problems of air, water, food and the home and work environment is still valid as regards legislation, and planning and enforcement of programmes of environmental control, it is now recognized that only a comprehensive view of ecology and the effects on man of adverse conditions in the total environment will provide an adequate basis for the assessment and prevention of long-term environmental risks to which man is exposed.'

With respect to the wide use of BHC in Czechoslovakia a complex *eco-chemical and epidemiological study of BHC* and its isomers in comparison with DDT has been initiated. Results of this study obtained hitherto are presented in this paper.

All the samples were taken from three regions of Slovakia, one of them representing a lowland type geographical region, another a mountainous region and the third one representing a mixed region with lowlands-like agriculture.

PRINCIPLES OF THE ANALYTICAL METHODS

The petroleum-ether extract of the sample was pre-refined, if necessary, with acetonitrile and then purified on a florisil or celite column with oleum and then used for the identification and quantitative determination by thin-layer and gas chromatography.

For thin-layer chromatography a method of one-step chromatography of chlorinated insecticides was applied using commercial standard thin-layer plates with multiple development on standardized 'Silufol' plates (0.13 mm silica gel). By this method thirteen chlorinated insecticides could be separated.

For most of the insecticides the limit of detection lies between 0.01 and 0.05 μg^2. For gas chromatography we used a column containing the mixed filling: 1.5% OV-17 with 2.0% QF-1 on Chromosorb W. In this column the above-mentioned isomers of BHC as well as DDT and its metabolites can be separated[3,4]. The accuracy of this method expressed by standard deviation ranges from $\pm$ 0.017 to $\pm$ 0.004 mg/kg, the coefficient of variation ranges between 3.00 and 10.42 per cent.

The recovery of the method varied from 85.6 to 98.5 per cent.

In the 'sixties DDT was the most important environmental contaminant among the organochlorine pesticides in Czechoslovakia.

The amounts of *Lindane and DDT* used *in agriculture* in Slovakia in 1968–1972 (kg/ha) are shown (*Table 1*). Since 1970 the decreasing use of organochlorine insecticides has led to a decrease in soil contamination with Lindane. Lindane is also used for disinfection of warehouses, for wood protection as well as in households. The exposure to BHC isomers can also be influenced by imported fodder containing BHC residues.

The mean values of *BHC and DDT residues in soil* and potatoes (*Table 2*) show a prevalence of the BHCγ-isomer with smaller amounts of the β-isomer. As can be seen, the DDT content in soil is markedly higher than that of the

Table 1. The amounts of Lindane and DDT used in agriculture in Slovakia in 1968–1972 (kg/ha)

Year	Lindane	DDT
1968	0.093	0.130
1969	0.069	0.102
1970	0.094	0.083
1971	0.061	0.055
1972	0.069	0.041

Table 2. Content of BHC and DDT residues in soil and potatoes in Slovakia in 1971–1973 (mg/kg)

Residues	Soil (183 samples)			Potatoes (142 samples)		
	Med.	Min.	Max.	Med.	Min.	Max.
γ-BHC	0.007	0	0.191	0.012	0	0.171
β-BHC	0	0	0.036	0.003	0	0.038
α + δ BHC + HCB*	0.011	0	0.108	0.010	0	0.090
p p'-DDE	0.032	0	0.765	0.019	0	0.120
p p'-DDT	0.082	0	6.415	0.018	0	0.093

* HCB is hexachlorbenzene

BHC isomers. No similar finding could be stated for potatoes. The amount of residues in surface and drinking waters was determined from the samples withdrawn from surface water and urban water supplies as well as from wells. Results are given in *Table 3.* In the case of *surface water* both the *DDT* and *DDE levels* were found to be lower in the majority of seven rivers than the total content of BHC. Only in one river was this ratio found to be reversed. In ground water the BHC content was lower than that of surface waters; this also applies for the sum of DDT. The γ:β isomer ratio appeared to be similar to that found in soil, i.e. γ-BHC did surpass the β-BHC content.

The highest concentrations found in the water supply of the city of Ferrara was 0.1 μg/1; the corresponding values for DDT and DDE were 1.58 and 0.36 μg/1 respectively[5].

In relation to foodstuffs of animal origin, our interest was concentrated on eggs and butter. Eggs were sampled either at poultry breeding farms (with

Table 3. Content of BHC and DDT residues in surface and ground water in Slovakia in 1971–1973 (μg/1)

Residues	Groundwater (92 samples) (drinking water)			Surface water (176 samples)		
	Med.	Min.	Max.	Med.	Min.	Max.
γ-BHC	0.09	0.01	0.34	0.08	0.00	3.60
β-BHC	0.02	0.00	0.22	0.03	0.00	2.55
α + δ-BHC + HCB	0.05	0.00	0.30	0.05	0.01	16.35
p p'-DDE	0.02	0.00	0.16	0.04	0.00	0.50
p p'-DDT	0.04	0.00	0.32	0.07	0.00	0.83

several thousands of hens fed with industrially prepared feeding mixtures). Feeding mixtures were also sampled in order to determine the relation between the residue contents in them and in eggs, respectively.

Residues in both egg matter and dried eggs from egg processing enterprises as well as in fresh eggs were determined.

Residues in eggs and feeding mixtures showed different features (*Table 4*). In the feeding mixtures the BHC γ:β isomer ratio was shifted in favour of the β-isomer and/or the DDE metabolite. Similar observations were also made on the movement of residues of the given insecticides in the samples

Table 4. Content of BHC and DDT residues in egg yolk and feed for laying hens in 1971–1973 (mg/kg)

Residues	Egg yolk (57 samples)			Feed (50 samples)		
	Med.	Min.	Max.	Med.	Min.	Max.
γ-BHC	0.025	0	0.267	0.018	0	0.182
β-BHC	0.010	0	0.084	0.002	0	0.096
α + δ-BHC + HCB	0.011	0	0.256	0.017	0	0.294
p p'-DDE	0.041	0	0.265	0.005	0	0.650
p p'-DDT	0.092	0	1.760	0.040	0	0.124

from egg processing enterprises, where no data on the content of these compounds in the feed were available.

Butter samples were taken twice a month from four selected milk processing enterprises from geographical regions with various degrees of application of chemicals in the agriculture. 193 samples were examined representing more than 10^6 hl of milk.

In the case of *butter (milk fat) the total BHC* content reached the *values of DDT (Table 5)*. The maximum values for BHC were even higher than those for DDT. The HBC content was included in the value for the total amount

Table 5. Content of BHC and DDT residues in butter (milk fat) and daily diet in Slovakia in 1971–1972 (mg/kg)

Residues	Butter (193 samples)			Daily diet (183 samples)		
	Med.	Min.	Max.	Med.	Min.	Max.
γ-BHC	0.041	0.006	0.157	0.004	0.001	0.009
β-BHC	0.044	0.003	0.117	0.003	0.000	0.009
α + δ-BHC + HCB	0.102	0.020	0.663	0.017	0.004	0.078
p p'-DDE	0.130	0.004	0.420	0.015	0.002	0.090
p p'-DDT	0.065	0.010	0.310	0.030	0.001	0.058

of BHC. In butter, the β-BHC isomer content reached the γ-isomer content, while in soil, potatoes and food mixtures as well as in eggs the γ-isomer prevailed.

The daily diet was sampled in six selected boarding houses twice a year (February and September) on five successive days by homogenizing the samples of breakfast, dinner and lunch together.

The *daily diet examination* showed (*Table 5*) a slight difference in *the γ:β isomer ratio* in favour of the γ-isomer, probably because the whole-day food contamination was largely due to the fat content of the food. Hence, residues are getting into organisms via fat-rich food products in such amounts that compared to water and other foods in the daily diet they cannot even change the γ:β BHC isomer ratio.

Human milk was taken twice a month from three observed regions (average of 20 mothers). The *residue levels in human milk* (*Table 6*) are of

Table 6. Content of BHC and DDT residues in human milk in Slovakia during 1972–73

Residues	Content in mg/l			Content in mg/kg fat		
	Med.	Min.	Max.	Med.	Min.	Max.
γ-BHC	0.010	0	0.068	0.333	0	2.267
β-BHC	0.014	0	0.085	0.467	0	2.833
p p'-DDE	0.080	0	0.410	2.666	0	13.667
p p'-DDT	0.061	0	0.229	2.033	0	7.633

great interest. The results showed higher BHC β-isomer than γ-isomer contents. The sum of DDT appeared to surpass the sum of both the β and the γ isomers. When the β-isomer levels in the cow milk fat and in the human milk fat were compared, they were found to be considerably higher in the latter. This ratio may be influenced by the degree of cumulation of BHC β-isomer in human fat tissue. The results reported in the previous studies which describe the levels of organochlorine pesticides in human milk vary rather widely[6–10]. We do not know whether the levels of DDT, its metabolite DDE and isomers of BHC have changed in human milk during the last ten years or whether differences between various geographic areas exist. Of great concern is our ignorance of the health implications of their presence in human milk.

It has been estimated that the average breast-fed child ingests daily 0.02 mg DDT-derived materials per kilogram of body weight[11]. This is four times the 'conditional' acceptable daily intake (ADI) of DDT-derived material recommended by the WHO. The difficulty in interpreting this level in terms of risk to infants is reflected in the fact that no scientific community in the world has recommended abandoning breast feeding in favour of other methods of infant nutrition.

Apart from the occurrence of acute poisoning, the only long-term effect which can be unequivocally attributed to the exposure to organochlorine pesticides is the acquisition of a tissue residue. No causal association of these levels with disease has as yet been demonstrated. The last 15 years have brought a substantial growth of information on both the qualitative and quantitative nature of the organochlorine pesticide spectrum in man and the concentrations of these compounds in blood and adipose tissue are used as indices to total body burden, and of exposure to these compounds. The information on these levels in adequately stratified groups within the general population is seen as a very important step towards a better understanding and surveillance of total exposure from all sources of pesticides. The first results of an epidemiological study on the long-term effects of pesticides on human health has been given by Paccagnella *et al*[12].

Different population surveys have demonstrated traces of DDT and its metabolites, BHC and its isomers, dieldrin and heptachlor epoxide in fat and more recently also in blood. From these pesticides, greatest emphasis has been placed from secular and geographical viewponts on the levels of DDT and its metabolites, BHC and dieldrin. In recent years residues of polychlorinated biphenyls and hexachlorobenzene have complicated the residue interpretation in human tissues.

From the epidemiological point of view it is worth noting that levels of organochlorine pesticides have been described in several chronic diseases. An association of these compounds with pathological changes is still contradictory. The epidemiological approach highlights the urgent need for an understanding of the significance of the levels of pesticides in the sick and normal individual.

As could already be seen from the first part of our study, BHC and its stereochemical isomers play an important role in Czechoslovakia, mainly for their ability to contaminate some of the foodstuffs of animal origin. Therefore, our attention was turned to the problem of the ecochemical characterization of this family of compounds, and an attempt was made to analyse the exposure of selected groups of the population to BHC isomers. Methodological aspects of this study will be published elsewhere[16].

As has been found in experimental studies, organochlorine pesticides are chemicals which are transferred to placenta and foetal life begins in utero under the influence of chlorinated hydrocarbon insecticides. Factors such as the concentration of drugs in maternal blood, passive diffusion, and active transport are of importance in the diffusion processes of various drugs across the placental barrier. Prenatal toxicology is interested in these problems of great concern from the point of view of mutageny and teratogeny.

Table 7. Residues of pesticides in the biological material (lowland and submountainous regions) in the year 1972

R	P	Residues of pesticides, mg/kg					
		In utero		Autopsy (fat)			
				F		Pa	
		L	S	L	S	L	S
		$n = 12$	$n = 20$	$n = 12$	$n = 27$	$n = 19$	$n = 18$
ΣDDT	med.	0.128	0.028	14.145	2.006	6.500	2.358
	min.	0.023	0.000	3.200	0.052	1.680	0.137
	max.	0.400	0.126	33.900	5.262	24.500	9.760
DDE	med.	0.046	0.006	10.185	1.170	3.200	1.560
	min.	0.012	0.000	2.400	0.000	1.240	0.057
	max.	0.180	0.050	27.400	3.600	18,000	7.200
DDT	med.	0.064	0.012	3.700	0.620	1.560	0.665
	min.	0.000	0.000	0.800	0.019	0.360	0.077
	max.	0.225	0.062	7.300	1.774	8.000	2.560
ΣBHC	med.	0.090	0.013	1.970	0,109	0.700	0.101
	min.	0.018	0.003	0.900	0,010	0.000	0.036
	max.	0.791	0.035	6.340	0,594	3.681	1.054
βBHC	med.	0.000	0.000	0.925	0,077	0.500	0.088
	min.	0.000	0.000	0.000	0.000	0.000	0.008
	max.	0.443	0.000	1.900	0.512	3.585	0.550
γBHC	med.	0.028	0.006	0.805	0.014	0.129	0.013
	min.	0.000	0.002	0.210	0.000	0.000	0.007
	max.	0.562	0.018	4.740	0.152	0.560	0.153

R = residues; P = parameter; L = lowland; S = submountainous region; F = forensic; Pa = pathology.

This part of our study is concerned with the very important problem of *exposure of human embryos* to the pesticides. The importance of this problem lies in the fact that already in the fourth week of gravidity the placenta is developed to such an extent that chemical substances can be transmitted from the circulatory system of the mother into foetal circulation.

For this reason we decided to find out the quantities of *DDT* and its metabolite *DDE* and the quantities of *BHC β and γ isomers in the uterine contents* in the second and third month of gravidity. In the literature there was no such material published up to the present. We are aware of the fact that the matter in question is a mixture of biological material containing in addition to embryos also other components. Here, too, we have proceeded in a way enabling us to compare regions with high and low pesticide use in agriculture.

From the results (*Table 7*) a predominance of total DDT emerges as compared to total BHC. Also higher concentrations of total DDT and β and γ isomers of BHC in lowland regions were observed compared to those of submountainous regions. DDT content is higher than the DDE content which is indicative of a path of residues different from the case of mother's milk when considering the constancy of the relationship DDT:DDE in human fat and the possibility of the placenta playing the role of an active metabolic organ. The predominance of DDT over DDE indicates both a short duration of exposure and also a lower lipid content of the material. There is a significant difference in the BHC γ-isomer content between different regions. It is not possible to arrive at definitive conclusions from the above discussed examinations.

Further attention must be devoted to *vernix caseosa* (an unctuous substance composed of sebum and desquamated epithelial cells which covers the skin of the foetus) whose role in the cumulation has not been elucidated up to the present. It was found (six samples examined) that, in contrast to the total uterine content, there is more DDE than DDT and also more *total DDT* than *total BHC*. This preliminary finding confirms our aforesaid explanations.

Our results from 1972 obtained from autopsy material have been summarized in *Table 7*. Results of the cumulation of *DDT and BHC in fat tissue* of persons up to 45 years of age dying accidentally have been obtained from the Department of Forensic Medicine. As in food products of animal origin the overall content of DDT and BHC in human fat tissue was found to be significantly higher in the areas of high pesticide use than in the submountainous areas. Concentrations of these chemicals were in this relatively small sample higher than was shown by Durham[13] in various countries. Moreover, in the areas of intensive pesticide use the lindane content in fat tissue was also found to be higher than that found in seven countries where the lindane fat levels ranged from 0.015 to 1.09 mg/kg[14].

Comparing the above results with the levels of these chemicals in the fat tissue of persons over 45 years dying of various diseases, their concentrations are found to be lower in the latter group. These problems require further analysis, since all over the world meaningful understanding of human pesticide residues has been severely restricted by small sample sizes and by the absence of stratification. While there is no question that we are surrounded

by pesticides, an extreme difficulty arises in demonstrating the adverse biological consequences of chronic exposure to low levels of these chemicals in the environment.

In the last part of our study, based on the average consumption of milk products, *the DDT and BHC burden of the human organism* in Czechoslovakia was calculated from median values. Results showed values of 0.0042 mg and 0.0037 mg of BHC and DDT per person per day, respectively. Similar DDT burdens due to the same sources were observed in the UK and in the USA.

The daily intake of DDT and BHC also was calculated from the levels of these chemical substances found in total diets. The daily intake represented 0.053 and 0.099 mg per day of BHC and DDT, respectively. Comparing our values with those obtained by Cummings[15] (mean values), the γ-BHC and DDT burden in our population was approximately half the burden found in the population of the USA. However, when comparing the contamination of the population with all the BHC isomers together nearly 2.5 times higher values could be observed in our population in comparison with the results of Cummings[15].

Based upon the above finding we evaluated the intake of these substances in our population with regard to the proposed acceptable daily intake values. The average DDT intake via milk products was found to be 100 times lower, but the DDT intake via the whole-day food only three times lower than the WHO ADI values. In the case of students, however, the maximum intake with the whole-day food surpassed the ADI values.

The World Health Organization established in 1968 the *ADI* for *DDT* (0.01 mg/kg) and for *lindane* (0.0125 mg/kg·day). The value of the ADI was then changed in 1970 for DDT to 0.005 mg/kg, while the ADI for lindane has been maintained at the original level. No ADI or overall value has been established for the BHC isomers (sum or individual). ADI has not been established for HCB, either. These values were based (in 1968) upon the interpretation of the s.c. 'no effect level' for both DDT and the BHC γ-isomer.

After the interest of specialists and of the public at large had been concentrated upon the problem of the residues of DDT and its metabolites, the original 'no effect level' for DDT has become doubtful from the point of view of the well known persistence and cumulative effect of this substance. Thus, in 1970, the ADI value for DDT was lowered to half the original value, i.e. by 2.5 times lower than the same for lindane.

However, in applying these values in calculating the 'permissible levels' and/or maximum limits for various foodstuffs (mainly for those of animal origin) many problems can arise. In most European countries the lowering of the DDT level was accompanied by a simultaneous rise in BHC levels, mainly of its β-isomer, as well as in HCB. Even if the problem of HCB is not taken into account, our study shows the importance of ADI to be established for both the sum of BHC and its β-isomer.

The present ecological study provides support to the view that in animal products the level of contamination by the β-isomer has reached the level of contamination by both DDT and its metabolites, and it has surpassed the BHC γ-isomer contamination. The β:γ isomer ratio, as well as their absolute concentrations in human materials gives support to the necessity of establishing ADI for BHC. Our conclusions are also based upon an analysis of the

contamination source showing Lindane to be the main compound applied and BHC β-isomer the main cumulator in animals.

In calculating permissible levels for the individual BHC isomers and/or for total BHC content we can refer only to the ADI values for Lindane. The ADI value for lindane is 15 and 250 times higher than the total level of BHC isomers in the daily food and milk fat, respectively. It cannot be acceptable for animal products, where contamination with BHC reaches or even surpasses the contamination with DDT.

Based upon these results we suggest the ADI for lindane should be lowered (as was done for DDT). It would be worthwhile to express this value as a sum of both the β and the γ-isomer constituents. Another way of solving this problem may be the expression of the ADI value for each BHC isomer separately, first of all for the β-isomer with parallel lowering of this value for lindane.

From the ecochemical and epidemiological viewpoint it is important that careful clinical and pharmacological observations along with measurements of body burdens with pesticides shall be made in exposed workers, in cases of poisoning. The pesticide levels might be determined in human tissues from samples selected to represent the general population. Populations to be investigated should not only include workers with high occupational exposure, but also persons, who have experienced acute toxicity episodes and persons with high tissue pesticide levels from other than occupational exposure. The continued study of pesticide levels in food should become an integral part of these studies. Such data are essential to a meaningful evaluation of the levels of these compounds found in the various media of the biosphere and in biological materials from the general population.

Such an attitude is in harmony with the complex long-term WHO programme directed to the establishment of health criteria (or exposure–effect relationships) and resultant primary protection standards, which is one of the essential elements for the design and implementation of practical programmes in environmental health and particularly for the control of the quality of air, water, food and the working environment. This has been recognized in a number of WHO Resolutions, as well as in the recommendations of the UN Conference on the Human Environment held in Stockholm (1972).

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