

Assessment of organochlorine hydrocarbons transformation in contaminated agricultural products and foodstuffs under gamma-radiation

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Abstract. The problem of an estimation of organochlorinated pollutants transformation (particularly organochlorinated pesticides (OCP) and polychlorinated biphenyls (PCB)) under gamma-irradiation has become important in connection with radiation technologies application in the food industry. According to earlier researches, small doses of OCP lead to serious damages of an organism, comparable with damages from high doses. Among radiolysis products of OCP in model solutions various substances on a structure have been found out. Though of trace concentration of each of them, in sum with the initial pesticides residue they make up significant of mass contamination (as shown earlier up to 90% from initial OCP). In this work fish samples (bream) containing OCPs (15.20 ng/g of hexachlorocyclohexane isomers and 87.10 ng/g of DDT and its metabolites), as well as PCB (18.51 ng/g) were studied. The minced fish was irradiated at dose of 10 kGy with dose rate of 1.35 Gy/sec. Then, by methods of gas-liquid chromatography (GLC) and gas chromatography-mass spectrometry (GC-MS), it was found that the OCPs degradation varied from 3 up to 61% and the PCB degradation – 24-52%. Significant complication of chemical composition was shown comparing to the primary biological sample contamination. As a result of fish irradiation, secondary pollution appeared that included residues of primary organochlorine hydrocarbons and their radiation-induced metabolites. Among the investigated OCPs the most stable proved to be alfa-hexachlorocyclohexane (alfa-HCH), the least stable – DDT which corresponds to the previous findings about the radiation stability of OCPs in model solutions. Mass spectra of the irradiated samples of minced bream showed the presence of radiation metabolites of OCPs, that had also been found at irradiation of model solutions of 2,2-di(4-chlorophenyl)-1-chlorethylene (DDMU), DDD and 1a, 2e, 3e, 4e, 5e-pentahlorocyclohexane. There was revealed a decomposition product formed during the deep destruction of chlorinated xenobiotics molecules – 1,1,2-trichloro-4-methyl-1-pentene (C₆H₉Cl₃); this substance was absent in the original fish samples. In the samples of irradiated fish there was not defined any OCP oxidation products or any intermediates that may be due to the conditions of sample preparation. To estimate toxicity of the secondary pollution, integral bioassays methods are proposed to apply.

1. Introduction

Currently, in order to optimize processes of agro-food production, new technologies that involve exposure of agricultural raw materials and prepared food products have intensively being implemented. This, in particular, allows improving crop yields, increasing shelf life and reducing the



time during transportation. In our country these technologies have to meet requirements of special standard documentation [1–2].

The relevance of the study of composition and properties of irradiated foods is, first of all, determined by the need to substantiate their absolute safety. Based on the results of experiments carried out with biological objects [3–4], it is shown that the radiation treatment of food products, initially containing OCPs in residual concentrations, may initiate processes leading to their secondary contamination.

Studies carried out previously [5] showed that irradiation of the model solutions of OCPs (alpha-HCH, gamma-HCH, p,p-DDE and p,p-DDT) resulted in decreasing of organochlorine substances content in these solutions. This process of the OCPs degradation was dependent on chemical nature and initial concentration of pesticide, solvent properties (polarity and presence of dissolved oxygen) as well as irradiation dose (D) and dose rate (\dot{D}). Conditions for maximum degradation of OCP at low initial concentrations ($C = 0.01$ g/ml, $D = 10$ kGy, $\dot{D} = 0,43$ Gy/sec) were determined [5].

In subsequent studies, the OCP radiolysis products in model solutions were identified. It has been shown that irradiation leads to the OCPs radiochemical degradation according to mechanisms of dechlorination, dehydrochlorination, destruction and oxidation [6]. It was also found that the transformation occurs due to isomerization of both initial and dechlorinated molecules [6]. It is possible to suggest that the negative biological effect may be caused by the total action of initial pesticide, radiolysis products and addition products.

Based on the previous findings, it appeared interesting to identify the features of radiation transformations process of OCP in a natural matrix of some biological object. With this purpose, foodstuff containing trace concentrations of organochlorine hydrocarbons were irradiated under optimal conditions found in [5].

2. Materials and methods

For the study, samples of minced fish – bream imported from the Astrakhan region for municipal market – were used. This foodstuff was chosen for irradiation according to the following requirements: a) high coefficient of OCPs bioconcentration [7]; b) significant contribution in population consumption [8] - preparation technology for the consumer may include radiation treatment stage [9-10].

According to our earlier studies [11] the total OCPs content in different samples of fish varied from 12.59 up to 87.10 ng/g. As shown in [12], in recent years, concentrations of some persistent organic pollutants (of limited application) decreased in comparison with the period of the 1990s. However, as a result of coastal enterprises and marine fleet activities, some PCBs continue to come into the water, and pesticides from previous incomes accumulate in sediments. This is why the OCPs and their metabolites are occasionally detected in water and in hydrobionts.

The OCPs, PCBs as well as their products of radiation transformations were determined in bream before and after irradiation. Procedures for samples analysis during the sample preparation stage were developed earlier and described in [11,13-14].

Sample preparation included: homogenization of test sample; extraction of organochlorine hydrocarbons from the sample (with hexane-acetone) and drying (with anhydrous sodium sulfate); pre-concentration of the extract by distillation; acid cleaning and separation of the extract on an adsorption column; final concentration (with nitrogen stripping solvent) of some chromatographic fractions, followed by their analysis with gas-liquid chromatography (GLC). Removal of the lipids was controlled by multiplicity of sulfuric acid treatment of extracts, which was applied 3-5 times depending on the fat content in the sample.

Qualitative and quantitative analysis of the OCP stable transformation products was carried out using gas chromatograph Varian 3400 (Varian, Inc. (USA)) and chromatography-mass spectrometer Varian Saturn 4D (Varian, Inc. (USA)).

The OCPs stability was estimated on the basis of their degradation under irradiation (P , %):

$$P = 100\% - \frac{C_{after}}{C_{before}} 100\%, \quad (1)$$

where P – degradation extent, %, determined by liquid gas chromatography as ratio of their concentration before and after irradiation; C_{after} – concentration of pesticide after irradiation, ppm; C_{before} – concentration of pesticide before irradiation, ppm.

State standard samples of OCPs were used for the calibration of instrumental techniques. The identification of the components was carried out with the full mass spectra library (NIST/EPA/NIH Mass Spectral Library 2011 5th Edition).

Fish (bream) was irradiated on "Issledovatel" (^{60}Co) installation at dose of 10 kGy with dose rate of 1.35 Gy/sec. Doses were estimated with the ferro-sulfate method using the Fricke dosimeter. An uncertainty of this dosimetry method does not exceed 10%. LET was about 0.2 keV/m [16].

3. Results and discussion

The results of the chromatographic analysis of fish showed (Table 1) that the content of the individual OCPs meet the requirements for maximum permissible concentration (MPC). Samples containing the highest amount of pesticides [17] were selected for further analysis. This provided the most informative results of comparative studies of OCPs (before and after irradiation of samples) since there are usually some losses during procedure of biomaterial sample preparation.

Table 1. Concentrations of OCP in bream (in terms of fat)

Sample	Concentration, 10^{-3} ppm				Σ concentrations, ppm	
	alfa-HCH	gamma-HCH	DDT	DDE	HCH	DDT+DDE
№1	- ^a	- ^a	72,90±0,70	14,20±13,40	- ^a	87,10
№2	1,80 ± 1,10	- ^a	1,10±0,70	12,30±4,30	1,80	13,40
MPC ^b					200	200

^a Pesticide is not detected.

^b [17].

The chromatograms obtained from the preliminary analysis on a packed column showed different content of pesticides in non-irradiated and irradiated samples №1. DDE and DDT contents in irradiated sample significantly decreased (Figure 1, A and B, respectively). This indicates the radiation degradation of these substances. At the same time, the peaks (1, 2, 3, 4 in Figure 1) missing in the chromatogram A were detected in the chromatogram B. It indicated the appearance of new substances in the irradiated sample. The peaks could be identified as following chemical compounds: 1 – ϵ -3a,4e,5e,6e-tetrachlorocyclohexene, 2 – 1e,2a,3a,4a,5e-pentachlorocyclohexane, 3 – 1e,2a,3e,4e,5e-pentachlorocyclohexane and 4 – p,p-DDD.

Stereochemical formulas of the OCPs detected before and after irradiation as well as possible ways of derivation of their transformation products are shown in Figure 2.

Gas chromatography-mass spectrometry analysis on capillary column of the irradiated fish samples (№1 and №2), initially differing in content of the OCPs, gave an additional information about radiation metabolites generated in these samples.

On the chromatograms of extracts obtained before and after irradiation of minced fish (Figure 3, *a* and *b*, respectively), compounds were marked that were possible to determine quantitatively. The OCPs concentrations and the radiation degradation degree (P) are presented in Table 2. There are identified 6 pesticides in sample №2 (unlike sample №1); their concentrations after irradiation decreased as it was in the first experiment. It followed from comparing the degradation degrees that the same degradation degree of the OCPs appeared in fish and in model solutions [5-6]. The most

stable was alfa-HCH, the least stable – DDT. Organochlorine hydrocarbon p,p-DDD found in fish sample №2 had a stability comparable to the stability of p,p-DDT. The degradation degrees of the same OCPs were the same despite their different content in the samples. Apparently, the effect of the OCPs concentration in the fish on the degradation degree was impossible to reveal, using the specified method of sample preparation.

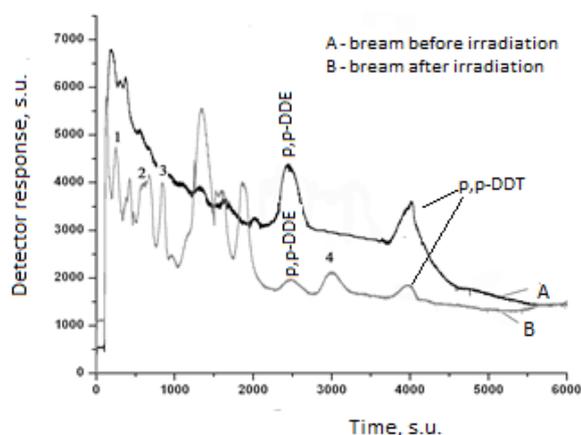


Figure 1. Chromatograms of breem extracts before and after irradiation (dose 10 kGy, dose rate 1.35 Gy/sec). Qualitative and quantitative analyses of the OCPs stable transformation products by gas chromatograph “Model 3700”: packed column (2 m), stationary phase SE-54; electron capture detector (^{63}Ni); carrier gas – nitrogen. Isothermal mode. Sample volume - 4 μl .

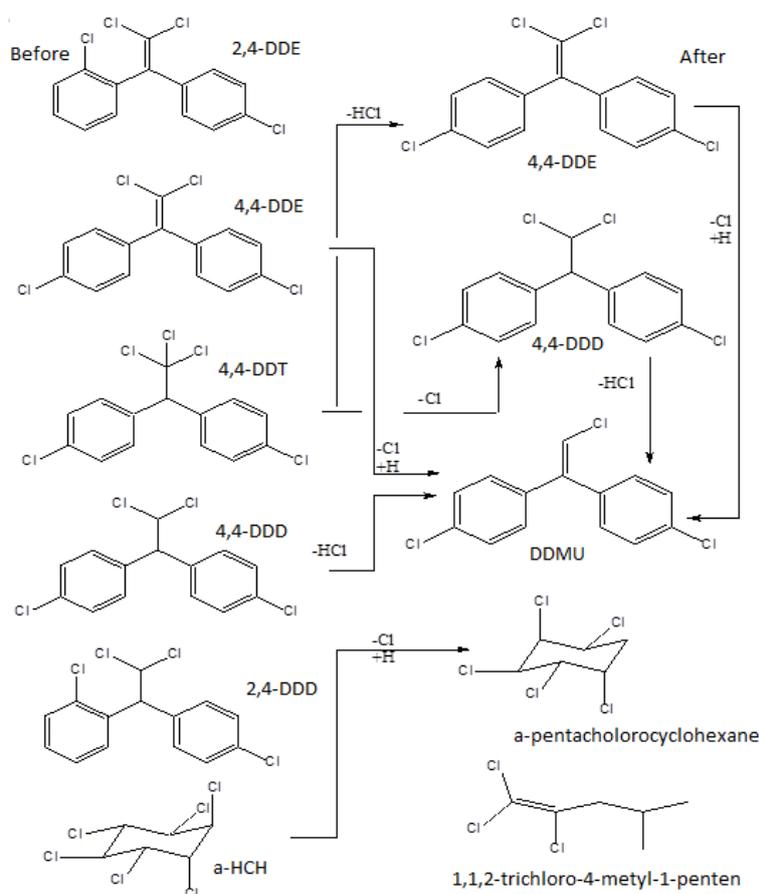


Figure 2. OCPs and products of radiation-chemical transformations.

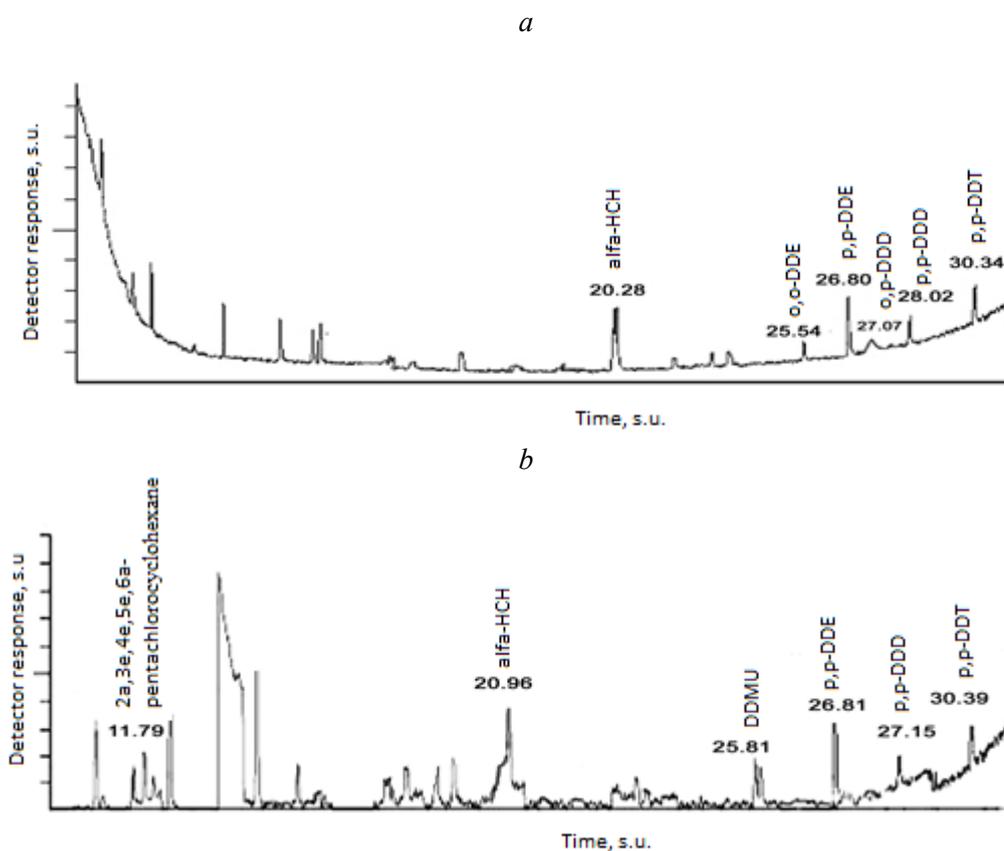


Figure 3. Chromatograms of breem extracts (sample № 2) before (a) and after (b) irradiation (dose 10 kGy, dose rate 1.35 Gy/sec). Analyses by gas chromatography-mass spectrometer Varian Saturn 4D.

Table 2. Concentrations and degradation degree of OCPs in breem (samples № 1, 2) before and after gamma-irradiation

Pesticide	Sample №1			Sample №2		
	Concentration, ng/g		<i>P</i> , %	Concentration, ng/g		<i>P</i> , %
before	after	before		after		
α -HCH	-	-	-	$1,80 \pm 1,10$	$1,70 \pm 1,21$	2,70
2,4-DDE	-	-	-	$0,035 \pm 0,020$	-	-
4,4-DDE	$72,90 \pm 0,70$	$44,15 \pm 3,55$	39,44	$12,32 \pm 4,11$	$7,72 \pm 3,00$	37,40
2,4-DDD	-	-	-	$0,32 \pm 0,18$	-	-
4,4-DDD	-	-	-	$13,64 \pm 5,03$	$5,30 \pm 1,71$	61,40
4,4-DDT	$14,20 \pm 13,40$	$5,79 \pm 1,52$	59,23	$1,64 \pm 0,73$	$0,68 \pm 0,29$	58,53
Σ DDT metabolites	87,10	49,94	-	29,31	15,40	-

In sample №2 before and after irradiation by chromatography-mass spectrometric analysis there were found not only initial dechlorination products of pesticides, but also products of deep degradation of their molecules.

For example, among the newly formed after fish irradiation substances was found 1,1,2-trichloro-4-methyl-1-pentene ($C_6H_9Cl_3$). This compound can be formed as a result of the radiation transformation of any of the OCPs contained in bream. However, this substance can also be formed in the radiolysis of other chlorinated xenobiotics present in fish. It appeared that sample №2 was contaminated not only by the OCPs but also by polychlorinated biphenyls (PCB). In an extract from fish sample №2 before irradiation there were found 15 PCBs, containing from 3 up to 7 atoms of chlorine (Table 3). After irradiation all PCB undergone to degradation. The degradation extent varied from 24 up to 52%.

Table 3. Concentrations and degradation degree of PCBs in bream (sample № 2) before and after gamma-irradiation

PCB isomers	Concentration, ng/g		P, %
	before	after	
№28/№31 [CL3]	0,43	0,50	-
№52 [CL4]	0,36	0,28	24,7
№101 [CL5]	1,18	0,75	36,86
№99 [CL5]	1,23	0,82	33,33
№110 [CL5]	1,76	0,88	50,00
№118 [CL5]	2,05	1,18	42,54
№153 [CL6]	3,81	1,84	51,71
№105 [CL5]	0,73	0,36	49,79
№138 [CL6]	2,88	1,41	50,96
№187 [CL7]	0,76	0,37	51,06
№183 [CL7]	0,40	0,19	52,32
№128 [CL6]	0,81	0,41	49,57
№156 [CL6]	0,33	0,21	36,54
№180 [CL7]	1,09	0,54	50,92
№170 [CL7]	0,72	0,36	49,79
ΣPCB	18,51	10,08	45,55

The OCPs oxidation products and intermediates (compounds containing residues of OCPs molecules and molecules of the organic matrix material (e.g., fish fat)) was not detected in the irradiated samples. Obviously, this is due to losses during sample preparation. Products of chemical transformation of complex organic compounds are usually more polar than the original compounds themselves [3]. Therefore, in the adsorption treatment of the organic extract some transformation products of the OCPs are not eluting.

This study showed that organochlorine hydrocarbons in the biological object under irradiation were not completely destroyed and transformed into compounds with similar properties (including toxicological ability). In addition, metabolites with unstudied toxicological properties appear. That is a new contamination of biological object appears. The danger of such pollution will be determined by synergistic or antagonistic effects or the combined effects due to the initial and newly formed contaminants.

At present to estimate the toxicity of secondary pollution, an integral method of bioassays is often proposed to apply. These methods by indicators of substances biological activity are able of detecting deviation in the system from the equilibrium state. We also plan to use such approach in the future to assess toxicity of radiation metabolites of organochlorine hydrocarbons contaminating food.

As shown in [18], the OCPs keep stable even under irradiation at high dose of 100 kGy but with low dose rates (0.30 - 0.50 Gy/sec). At the same time, a wide range of doses and dose rates could be applied in up-to-date radiation technologies for foodstuffs irradiation [19]. Doses may vary between 1 and 60 kGy and dose rate – from 0.003 up to 0.25 Gy/sec [20-21]. The effect of these variations on the

stability of the OCPs in biological objects has not been carefully studied so far. In our opinion, this issue also becomes very important in the case of food irradiation.

4. Conclusions

On the example of this study with irradiated fish it could be concluded that a detailed understanding of the composition and properties of radiation metabolites is necessary to optimize process of foodstuffs decontamination and avoid the potential deterioration of product quality due to the secondary contamination.

Acknowledgments

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