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Letter do Editors

DDT and Its Metabolites in the Tissues of Certain Fish Species from Podlasie Province

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Abstract

The content of DDT and its metabolites (p,p'-DDD and p,p'-DDE) was determined in the muscles, liver and brain of crucian carp (*Carassius carassius* L.), carp (*Cyprinus carpio* L.), pike (*Essox lucius* L.) and sunbleak (*Leucaspius delineatus* Haeckel) collected from breeding ponds in Knyszyn, Podlasie province. Two-stage column chromatography with Celit 545 and Florisil was used to extract DDT and its metabolites. Measurements were performed using a gas chromatograph, PYE Unicam 104 type, with an electron capture detector. Relatively low concentrations of polychlorine pesticides (including trace amounts) were found. The highest mean concentrations of DDTs were detected in the brain and liver (1.1650 and 0.5469 mg kg^{-1} of tissue, respectively). In the muscles, the concentration was substantially lower (0.0530 mg $kg^{-1}$of tissue).

Keywords: DDT and its metabolites, freshwater fish, *Carassius carassius* L., *Cyprinus carpio* L., *Esox lucius* L., *Leucaspius delineatus* Haeckel.

Introduction

The group of pesticide compounds includes chloroorganic insecticides used to eliminate human and animal parasites and fight agricultural pests. Among chemical substances frequently detected in animal organisms are DDT and its metabolites, differing in structure, which also affects their chemical and physical properties.

DDT and its metabolites are toxic compounds which, like other poisons, get to the living organisms via the respiratory tract, alimentary tract and skin [1]. Environmental monitoring confirms the high persistence of DDT in water, air, soil and food [2-5]. Despite the fact that DDT has not been used in Poland and in many countries in the world since 1973, its occurrence in an unaltered form or as metabolites still poses a real problem. This compound, subjected to slow degradation, remains in the environment and together with food and water gets to many organisms, including human, where it is deposited in an unchanged form or as metabolites [6-8].

The aim of this study was to determine the level of accumulation of DDT and its metabolites in chosen freshwater fish species in Podlasie province.

Material and Methods

Our study was conducted on crucian carp (*Carassius carassius* L.), carp (*Cyprinus carpio* L.), pike (*Esox lucius* L.) and sunbleak (*Leucaspius delineatus* Haeckel). Fish specimens for analyses were obtained from breeding ponds in Knyszyn, the largest fish farm in Podlasie province,

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consisting of 5 ponds (850 ha, 300 tons of carp production per year). The ponds are situated in the upper part of the River Jaskranka valley, 41 kilometers long, a right tributary of the River Narew. The analyses were repeated 9 times and each time a few fishes of each species, two years of age, were used. Muscle, liver and brain tissues were collected for analysis. DDT and its metabolites p,p'-DDD and p,p'DDE (Fig. 1) were determined by means of analytical method according to the generally accepted scheme: extraction, purification of extracts, and analysis. Two-stage column chromatography with Celit 545 and Florisil was used to extract the pesticide from the respective fish tissues. Quantitative and qualitative measurements of DDT were taken using a gas chromatograph, PYE Unicam 104 type, with an electron capture detector ECD, glass column aeropack 30, 100/120 mesh, liquid phase 1.5% OV-210, carrier gas argon [9, 10]. The chemical compounds were identified by comparing peak retention times between sample and standard chromatograms. The results concerning the content of polychlorine substances in fish were subjected to statistical analysis using t-Student test for two means and have been presented as means x and standard deviation SD [11].

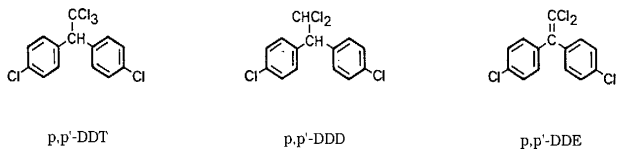

Fig. 1. Formula of DDT and its metabolites.

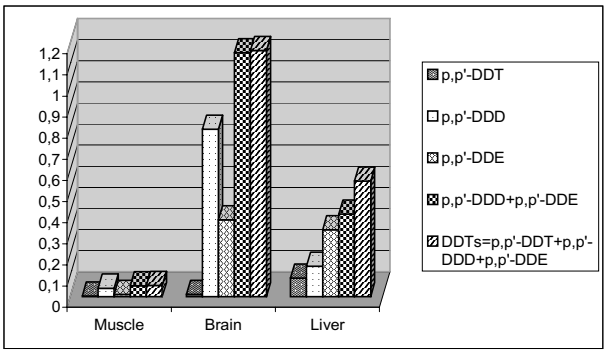

Fig. 2. The concentration of DDT and its metabolites in tissue of fish (mg kg^{-1})

Results

Chromatographic analysis has shown that DDT and its metabolites occur in the muscles, brain and liver of crucian carp, carp, pike and sun sunbleak (Table 1, Fig. 2). The p,p'-DDT and its metabolites such as p,p'-DDD and p,p'-DDE were detected in the examined fish specimens (Fig. 2). Differences were found between the fish species in the accumulation of this pesticide and its metabolites. The smallest amounts of DDT were noted in carp individuals (0.3962), the greatest in pike (0.6941 mg kg^{-1} of tissue). Significant differences referred also to the concentration of DDT and its metabolites in the organs subjected to analysis. The lowest concentration of DDTs (DDT summary) and metabolites was observed in the muscles (0.053), and substantially higher in the brain and liver (1.1650 and 0.5469 mg kg^{-1} of tissue, respectively). As for metabolites, higher levels of p,p'-DDD and p,p'-DDE were found, compared to p,p'-DDT.

Table 1. Concentration of DDT and its metabolites in chosen tissues of four fish species (mg kg^{-1})

Fish species	Tissue	p.p'-DDT		p.p'-DDD		p.p'-DDE		p.p'-DDD+ p.p'-DDE	DDTs (p.p'DDT+ p.p'-DDD+ p.p'-DDE)
		$\overline{X}$	SD	$\overline{X}$	SD	$\overline{X}$	SD	$\overline{X}$	$\overline{X}$
Carassius carassius	Muscle- wet mass	0.0001	0.0003	0.0047	0.01	0.0039	0.006	0.0086	0.0087
Cyprinus carpio		0.0005	0.0009	0.0009	0.0009	0.0013	0.001	0.0022	0.0027
Esox lucius		0.0061	0.0160	0.0181	0.002	0.0286	0.03	0.0464	0.0533
Leucapius delineatus		0.0000	0.0000	0.0162	0.02	0.0088	0.008	0.0250	0.0249
Carassius carassius	Brain- wet mass	0.0100	0.02	1.3817	2.70	0.0657	0.12	1.4474	1.4574
Cyprinus carpio		0.0000	0.00	0.0354	0.08	0.6838	0.10	0.7192	0.7192
Esox lucius		0.0150	0.03	1.2779	1.5	0.0296	0.05	1.3075	1.3225
Leucaspius delineatus		0.0047	0.008	0.4730	0.55	0.6731	0.66	1.1461	1.1931
Carassius carassius	Liver- wet mass	0.0143	0.03	0.0117	0.02	0.1439	0.25	0.1556	0.1697
Cyprinus carpio		0.0287	0.07	0.1617	0.33	0.2765	0.38	0.4382	0.4666
Esox lucius		0.0216	0.04	0.1182	0.21	0.5668	0.52	0.6850	0.7064
Leucaspius delineatus		0.0286	0.41	0.2817	0.41	0.2766	0.38	0.5583	0.5867
Carassius carassius	Mean for muscles, brain and liver	0.0008		0.4660		0.0712		0.5372	0.5453
Cyprinus carpio		0.0097		0.0660		0.3205		0.3865	0.3962
Esox lucius		0.0146		0.4714		0.2083		0.6796	0.6941
Leucaspius delineatus		0.0111		0.2570		0.3195		0.5765	0.6016

Discussion

The values obtained in the present study are lower than those found for fish in other water reservoirs of northeastern Poland, in Gdańsk Bay, Szczecin Bay [12-18] and in the northern part of the Baltic Sea [9-20]. On the coasts of Sweden, herring muscles contain approximately 5 mg kg^{-1} of mass. However, the content of DDTs in guilemo eggs reaches even 100 mg kg^{-1} [20]. In fish caught in the Atlantic Ocean the level of DDTs was higher (0.025 – 0.3 mg kg^{-1} of tissue) than in the present study [21], but still considerably lower compared to Baltic fish. Such high concentrations of DDTs in organisms from the Baltic, compared to the Atlantic Ocean, could be due to the fact that the Baltic Sea is a closed reservoir which accumulates large amounts of this pesticide flowing *via* rivers of countries situated in its basin [22]. DDT content in a few Arctic lake fish species was $0.7 \cdot 10^{-4}$ mg kg^{-1} [23]. In Spain, toxicological examinations of many chloroorganic pesticides performed on *Salmo trutta* revealed that the muscles of this fish species contained the highest levels of p.p'-DDT [21]. In our study, p,p'-DDT content in the fish muscles was 0.0025 mg kg^{-1} of tissue. In the muscles of river fish in Florida, small amounts of p.p'- DDE ($5.4 \cdot 10^{-4}$ mg kg^{-1}) were noted [25]. Low p.p'-DDE concentrations were also encountered in fish from the Baltic near Denmark [26]. A reduction even to trace amounts in the level of polychlorine compounds, including DDT, is undoubtedly related to the withdrawal of this pesticide in the majority of countries. However, its maintenance even at a low level in the tissues of the fish specimens subjected to analysis indicates that polychlorine peptides are still present in the natural environment. It should also be emphasized that the concentration of chloroorganic pesticides in the main rivers of Poland remains a fraction of 10^{-3} mg l^{-1} and rarely goes beyond the Polish norms [27]. The concentration of chloroorganic insecticides in surface waters only sporadically reaches the limit of toxicity and amounts to 10^{-3} mg l^{-1} for the most sensitive organisms. However, their content in bottom sediments is still quite high, indicating a still existing threat to the aquatic biocenosis [28]. Fish absorbs pesticides partly with water and mainly with food, and it is here that these pesticides are metabolized or accumulated [29-32]. DDT and its metabolites get to water reservoirs mainly with rainwater flowing down from cultivated fields, and only to a small degree as dry deposits from the atmosphere [22]. Thus, their content in a particular water reservoir depends on the size of the surrounding drainage area. As has already been mentioned, the ponds in Knyszyn lie in the upper part of the valley of the River Jaskranka, whose drainage area is small and brings in small amounts of pesticides compared to larger areas. This could explain why the amounts of DDT and its metabolites in the fish tissue examined are inconsiderable compared to other reservoirs. Of importance also is the use of smaller amounts of pesticides than in other regions of Poland. As mentioned before, differences were found in the total content of DDTs between the respective fish species. The lowest concentration in carp specimens is associated with additional food provided for this species. Crucian carp, which is given additional food in ponds, shows a lower content of DDTs compared to pike and sun bittern which feed naturally, but higher than carp. Sunbleak is a planktonphagous species which feeds on the second trophic chain link (zooplankton), while pike as a predacious species represents a higher link of the food chain; thus, according to pesticide biocumulation principles, it contained the greatest amounts of DDT and its metabolites of all four fish species. Chloroorganic compounds in animals cause reproductive disorders and disturb functioning of the alimentary system and its glands, especially of the liver. These pesticides also show a neurotoxic action [30-32]. Polychlorine compounds undergo biocumulation at the respective levels of the trophic chain. In the aquatic environment they get to fish mainly on phytoplankton and zooplankton, being the base of their food [7]. Although according to Polish norms fish specimens from the breeding ponds in Knyszyn, Podlasie province, showed low concentrations of DDTs [33], the fact is that DDT and its metabolites in edible parts of the fish body may be a threat to humans due to their bioconcentration in the respective trophic links.

Chloroorganic substances frequently cause diseases in fish. It has been shown that paraquat inhibits the activity of acetylcholinesterase [34]. Another pesticide, aldrin, exerts an unfavourable effect on the circulatory and nervous system of *Clarias batrachus* [35]. Histopathological changes were also found in the blood of *Carassius carassius* due to intoxication with DDT [36]. Histopathological and biochemical alterations due to pesticides were observed in *Oreochromis niloticus* [37]. Attempts to determine the effect of water chemistry on pesticide accumulation in brook trout *Salvelinus fontinalis* have revealed that such physicochemical parameters of water as pH, alkalinity, colour, content of calcium, magnesium, potassium and sodium have no great effect on the accumulation of these toxins in fish [38]. However, temperature exerts a negative effect. An increase in water temperature from 4 to 20°C was found to cause higher accumulation of paraquat Cu SO_4 in the brain, heart and kidneys of carp [39]. Chloroorganic compounds have frequently been detected in various organs and fluids of the human body where they get with contaminated food. The presence of pesticides in people can be caused by their accumulation in the trophic chain. In humans, the concentrations of pesticides have frequently been found higher than in the organisms at the lower levels of the food pyramid. Pesticides have been detected in such body fluids as blood, milk, urine, in tissues (e.g. fat tissue), and in the liver, kidneys, lungs, brain and skin. Most of them cause functional disorders of many organs, leading to various types of pathological changes, including neoplasms [32, 40, 41, 42]. Humans situated at the top of the trophic pyramid, are particularly exposed to the harmful effects of these pesticides. Orfanochlorine pesticides, including DDT, are an environmental hazard due to their persistent nature and potential health effects. DDT and its metabolites are

lipid-soluble pesticides, which accumulate in fatty tissue and are, therefore, more present in fat-containing foods such as meat, fish, milk, cheese and oil than in fruit, vegetables and grains. Scientists have for some time been concerned about human exposure to DDT and the potential risk of breast cancer due to its oestrogenic activity[43]. Persistent pesticides are still detected in the the nervous system of humans, showing unfavorable effects on the reproductive and hormonal systems [1, 32, 42].

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