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Fish toxicology

BIOACCUMULATION OF ORGANOCHLORINE PESTICIDES IN THE TROPHIC CHAIN ALGA-FRESHWATER FISH

BIOKUMULACJA PESTYCYDÓW CHLOROORGANICZNYCH W ŁAŃCUCHU TROFICZNYM GLON-RYBA SŁODKOWODNA

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The study was carried out on the alga *Chlorella vulgaris* and young specimens of crucian carp *Carassius carassius*. The algae and fish were exposed to a pesticide gamametox powder containing 0.6% of lindane and 3% of methoxychlor. Gas chromatography method was used to determine the content of these two active substances in the material. Based on the mean lindane and methoxychlor contents it was revealed that they were bioaccumulated at different levels of the trophic chain. Their accumulation was higher in fish than in algae, which were the source of their food. Their concentration in the muscles of crucian carp was higher when absorbed from food (i.e. *Chlorella vulgaris* cells), than directly from water. A similar situation was observed in relation to lindane content in the liver. In the brain, however, the concentration of both pesticides was diversioned or similar. The highest concentrations were revealed in the brain of crucian carp, slightly lower in the liver and the lowest in the muscles, i.e. edible parts of fish, irrespective of the way of absorption.

INTRODUCTION

Environmental pollution is caused by a long-term application of toxic chemical compounds in different ecosystems, including pesticides which due to their high efficacy and easy use have eliminated some biological methods of pest control. Pesticides have been applied to fight against pests of plants, animals, and humans. However, the introduction of pesticides to the natural environment has also some negative effects, including unintentional intoxication of useful insects, fish, birds, mammals, and other inhabitants of aquatic and terrestrial biocenoses (Łuczak et al. 1982; Senthilkumar et al. 2001). Some pesticides, particularly those which show high persistence undergo bioconcentration in the respective links of trophic chains. Accumulation of poisons in plants and animals poses a threat to man, considered the top level of the trophic pyramid. The humans accumulate considerable amounts of various toxins, including organochlorine pesticides (Mazur et al. 1987; Góralczyk and Struciński 1996).

Therefore, the aim of this study was to determine the bioaccumulation of lindane and methoxychlor in algae and fish which absorbed the pesticides via the absorption from the lower link of the trophic chain, i.e. algae and from water.

MATERIAL AND METHODS

Toxicological studies were conducted on *Chlorella vulgaris* Beijer and on young specimens of crucian carp *Carassius carassius* L. The initial breeding material of pure cultures of *Chlorella vulgaris* was obtained from the Laboratory of Radiation Genetics, the Institute of Biophysics in Moscow. Fish specimens were collected from breeding ponds in Knyszyn. The study used gamametox powder, containing active substances: 0.6% of lindane, (gamma isomer 1,2,3,4,5,6 hexachlorcyclohexane) and 3% of methoxychlor DMDT (1,1,1 of trichloro-2 bis 4 methoxyfenyloetan). Algae were cultured on Knop's medium. Gamametox powder was introduced to pure alga cultures, in a dose of 10^{-2} (lindane 6×10^{-5} mg·dm^{-3}, methoxychlor 3×10^{-4} mg·dm^{-3}), 10^{-4} (lindane 6×10^{-7} mg·dm^{-3}, methoxychlor 3×10^{-6} mg·dm^{-3}), 10^{-6} mg·dm^{-3} (lindane 6×10^{-9} mg·dm^{-3}, methoxychlor 3×10^{-8} mg·dm^{-3}). After 10 days, the algae were collected, filtered and then divided into parts, of which one (pesticide-intoxicated algae) was given as food to fish. The remaining algae were subjected to chemical analysis for the pesticide content.

Toxicological analysis using fish was repeated eight times (n = 8), with several specimens of the species in each of the following groups:

Control group I – crucian carp kept in pesticide-free water and given non-intoxicated algae as food.

Group II – crucian carp in the water containing gamametox powder in a dose of 0.5 mg·dm^{-3} (lindane 3×10^{-3} mg·dm^{-3}, methoxychlor $1.5 - 10^{-2}$ mg·dm^{-3}).

Group III – crucian carp which received the food of *Chlorella vulgaris* contaminated with gamametox powder in a dose of 10^{-2} mg·dm^{-3} (lindane 6×10^{-5} mg·dm^{-3}, methoxychlor 3×10^{-4} mg·dm^{-3}).

The fish were kept in aquaria for 30 days and then underwent pesticide estimation with the method of gas chromatography. Two-stage column chromatography with Celit 545 and Florisil was used for the extraction of lindane and methoxychlor. Quantitative and qualitative measurements 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. The chemical compounds were identified by comparing peak retention times between sample and standard chromatograms (Stec and Juszkiewicz 1972; Amarowicz et al. 1986). The results were subjected to statistical analysis using *t*-test and mediana test. *T*-test was used to determine the significance of differences in mean concentrations of the parameters in the respective groups (Łomnicki 1995). Dry weight of algae and wet weight of fish were chosen as the criterium of randomness. The samples met the criterium at $\alpha = 0.05$. In view of this, results of the analyses can be transferred to the population of the organisms examined (algae, fish) with error probability < 0.05. All the results concerning the pesticide content have been presented as means $\bar{x}$ and standard deviation SD. Pesticide determinations in algae were performed in a similar way. Basic differences referred to the phase of extraction, using acetonitril, 5% sodium chloride solution and n-hexane (Vavrova et al.1976; Zadrozińska et al. 1983).

RESULTS

Table 1

Lindane and metoxychlorine contents in *Chlorella vulgaris* cells following intoxication with gamametox powder (mg·kg^{-1}, dry weight n = 8)

Dose of pesticide (mg·dm^{-3})	Statistical parameters	Lindane	Methoxy-chlor
10^{-2}	$\bar{x}$	0.0674*	0.4900*
	SD	0.04	0.09
	Me	0.0670	0.4900
10^{-4}	$\bar{x}$	0.0291	0.2014*
	SD	0.04	0.09
	Me	0.0670	0.1900
10^{-6}	$\bar{x}$	0.0200*	0.0863
	SD	0.002	0.01
	Me	0.0190	0.0810

Significance of differences in statistical analysis.
*p < 0.05.

Tables 1, 2, and 3 show the contents of lindane and methoxychlor in the algae and in the muscles, liver, and brain of crucian carp which absorbed pesticides directly from water and algal food. All the tables show mean values, standard deviations, medians, and statistically significant differences. It can be stated, based on the numerical data that there are significant differences in the mean contents of the respective toxic substances (lindane and methoxychlor). Dry weight of *Chlorella vulgaris* following intoxication with gamametox powder showed the highest concentration of lindane and methoxychlor due to the 10^{-2} mg·dm^{-3} dose, being 0.0674 and 0.4900 mg·kg^{-1}, respectively. In algal cells, the content of methoxychlor was seven times higher than of lindane. At lower concentrations of the pesticide, lindane and metoxychlorine exhibited slightly lower values (Table 1). The mean lindane and methoxychlor contents in the muscles of crucian carp reached higher levels when the fish absorbed the pesticides from food, i.e. algae after intoxication and not directly from

Table 2

Contents of lindane and methoxychlor absorbed by crucian carp from food—*Chlorella vulgaris* cells—intoxicated with gamametox powder (mg·kg^{-1} wet weight, n = 8)

Fish tissue	Statistical parameters	Lindane	Methoxychlor
Muscle	$\bar{x}$	0.0326*	0.1102*
	SD	0.03	0.04
	Me	0.0150	0.0950
Liver	$\bar{x}$	0.3500*	1.0083*
	SD	0.11	0.26
	Me	0.3600	1.0850
Brain	$\bar{x}$	0.6943*	1.7050*
	SD	0.32	0.87
	Me	0.5900	1.7350

Significance of differences in statistical analysis.
$^{*}p < 0.05$.

Table 3

Contents of lindane and methoxychlor absorbed by crucian carp directly from water contaminated with gamametox powder in a dose of 0.5 mg·dm^{-3} (mg·kg^{-1}, wet weight, n = 8)

Fish tissue	Statistical parameters	Lindane	Methoxychlor
Muscle	$\bar{x}$	0.0175*	0.0746*
	SD	0.02	0.04
	Me	0.0060	0.0840
Liver	$\bar{x}$	0.2200*	1.0014*
	SD	0.10	0.29
	Me	0.1900	1.0700
Brain	$\bar{x}$	1.2629*	1.5571*
	SD	0.67	0.48
	Me	1.1700	1.6400

Significance of differences in statistical analysis.
$^{*}p < 0.05$.

water. A similar situation was observed in the case of lindane in the liver. In the brain, however, it was the diversion or similar (Tables 2 and 3). Considerably higher pesticide concentrations were found in the brain and liver than in the muscles, irrespective of the way of absorption (food, water). In the muscles of fish fed on *Chlorella vulgaris*, lindane level was 0.0326 mg·kg^{-1}, being twice as high as that in water (0.0175 mg·kg^{-1}). The content of methoxychlor in crucian carp which absorbed the pesticide from algal food and water reached similar values in the muscles (0.1102 and 0.0746 mg·kg^{-1}, respectively). Lindane absorbed from food and water showed slight differences in the brain (0.6943 and 1.2629), just like methoxychlor (1.7050 and 1.5571 mg·kg^{-1}, respectively). In the liver, the contents of lindane absorbed from food were approximately 1.5 times higher compared to water values (lindane 0.3500 and 0.2200 mg·kg^{-1}; but the concentrations of methoxychlor the same (1.0083 and 1.0014 mg·kg^{-1}, respectively).

DISCUSSION

The present toxicological experiment showed the accumulation of lindane and methoxychlor in algae and fish, due to their intoxication with gamametox powder. After a 10-day exposure of *Chlorella vulgaris* to gamametox powder, its active substances—lindane and methoxychlor were found to accumulate in the alga cells. Another pesticide—

DDT was also found to accumulate in *Chlorella vulgaris* due to similar intoxication doses (Subba-Rao and Alexander 1980). Similar results were obtained for gamma-HCH and DMDT when studying edible mushrooms (0.1–1 $mg \cdot kg^{-1}$) (Chmielnicka and Piątkowska 1972). Metoxychlor reached the level of 0.1 $mg \cdot kg^{-1}$ in potatoes (Novickij 1986). The concentrations of such organochlorine pesticides as HCB, alpha HCH, gamma HCH, beta HCH, DDT, and metabolites of DDD and DDE were not high in citrus fruit (Zadrozińska et al. 1983). In rice grains, lindane content was considerably higher (20.2 and 37.5 $mg \cdot kg^{-1}$) (Mostafa 1987). DDT and lindane reached a slightly higher level compared to other organochlorine pesticides in such crops as spinach, potatoes, oranges, salad, and clover (Dogheim et al. 1996). The study of the effect of DDT on the amino acid composition of cyanobacteria and green algae revealed that this pesticide affected the quality and quantity of the amino acid composition in the concentration-dependent manner (Czeczuga and Gierasimow 1977). DDT caused a considerable decrease in the level of certain carotenoids in the algae *Chlamydomonas viridis*, *Chlorella vulgaris*, and *Scenedesmus quadricauda*, but stimulated such pigments as lutein, β-carotene and neoxanthin (Czeczuga and Bobiatyńska 1977). Lindane and methoxychlor accumulation was higher in fish than in algae being the fish food. The experiment referring to the trophic chain following lindane intoxication revealed its considerably lower content in *Chlorella vulgaris cells*, compared to the tissues of stickleback, *Gasterosterus aculeatus* (80% and 92%, respectively) (Hansen 1980). It was also noted that the content of pesticides in fish was higher when they were absorbed from algal food and not directly from water. The study on DDT bioaccumulation showed that it was absorbed by fish in 35% from food, while only in 3.55% from water (Macek and Korn 1970). Using paraquat for *Cyprinus carpio* intoxication it was found that its accumulation was considerably higher in the specimens which absorbed it from food (Nemcsòk et al. 1984). Similarly higher lindane accumulation was observed in cyprinid fish than in food (Sabharval and Belsare 1986; Salanki et al. 1982). Our own experiments and the studies of other authors indicate that the concentration of organochlorine pesticides in fish tissues was a few to a few hundred times higher than in algae, the enrichment coefficient in the case of non-decomposed poison being 10 on average for each level of the trophic chain. Thus, fish may contain even a few thousand times more insecticides than found in their habitat (Zakrzewski 1997). Toxic compounds dissolved in water are mainly absorbed via the gill and the alimentary tract. Irrespective of the absorption route, pesticides are transported with blood throughout the organism. In the initial phase, the concentration of these chemical substances depends on blood supply of the organs; hence, higher concentrations in the liver and brain (Nimno 1982; Ben-Jonathan 1999; Struciński et al. 2000). Experiments have demonstrated that lindane and methoxychlor accumulate mainly in tissues showing high fat content. Then, they are transported with body fluids to various parts where they exhibit toxic action. The present experiment revealed a similar regularity. Lindane and methoxychlor were found to accumulate in significantly greater amounts in the liver and brain than in the muscles. Significantly smaller quantities are found in the muscles—edible parts of fish, compared to the other tissues. Irrespective of the way of absorption (skin, airways, alimentary tract), pesticides are absorbed to the blood and in this way are transported to the respective tissues. The concentration of these substances in the organs may depend on their blood supply. Therefore, higher concentrations can be found in the brain and liver, compared to other organs (Felly and Jordan 1998; Seńczuk 1999). Moreover, the liver is a storage site for various compounds absorbed by the organism, including toxins to which pesticides belong. Such storage effectively removes these toxins from the circulatory system and lysosomal enzymes metabolise them, which is very important in detoxification (Novickij 1986; Schenk et al. 1997). High concentrations of pesticides found in the fish liver may not be of great significance in their bioaccumulation, as part of them is neutralised in the liver and then excreted via kidneys. However, lypophilic pesticides accumulated and stored in fatty tissue have a capacity for accumulation in the food chain (Zakrzewski 1997). Thus, the presence of lindane and metoxychlor even in small amounts in the fish muscles can lead to their multiplication in the organisms at the higher level of the food chain. Organochlorine pesticides easily overcome the blood-brain barrier and accumulate in the brain. The brain is known to have a substantial blood supply, like the liver, and contains considerable amounts of fatty compounds (sphyngomyeline) (Seńczuk 1999). The highest concentration in the brain of the fish examined may not be harmful especially for the health of fish and then people. Organochlorine pesticides are known to exert a neurotoxic effect and can cause many diseases (Seńczuk 1999; Struciński et al. 2000). They circulate in aquatic organisms within food chains. Pesticide accumulation in algae may be a serious threat to aquatic biocenoses. Residual pesticides in natural habitat, e.g. water, are included in the biological food chain. Algae constitute phytoplankton, being a basic food for small animals, mainly invertebrates, which serve as food for vertebrates. In trophic chains associated with water, toxin concentrations are considerably higher in fish, being the food for higher vertebrates, such as piscivorous birds and mammals (Falandysz et al.1999; Falandysz et al. 2000; Senthilkumar et al. 2001). Lindane and methoxychlor have been detected in phyto- and zooplankton, and in fish. The studies confirmed the bioaccumulation of organochlorine pesticides in the aquatic food chain (Kiziewicz et al. 1991; Kiziewicz 1993; Ridal et al. 2001). Due to pesticide absorption the food chain is enriched with poisons. Its initial links, including algae, accumulate small amounts of toxic compounds. The subsequent stages of consumption show an increase in the concentration of these substances, which may be biologically multiplied. The degree of biological multiplication depends on the length of the food chain and poison persistence.

Lindane is a persistent pesticide, with disintegration time of 3–10 years (Dojlido 1995). The present study showed pesticide bioaccumulation in *Chlorella vulgaris* and *Carassius carassius*. During the experiment, relatively low levels of lindane and methoxychlor were found in the muscles—edible parts of fish. Accumulation of organochlorine pesticides has been examined in sea fish at various age. The experiments have revealed that the levels of pesticides in the gonads and liver are similar in adult and young specimens. This seems to indicate that the age of fish is not a significant factor in pesticide accumulation (Albaiges et al. 1987). Organochlorine substances frequently cause diseases in fish. It has been shown that paraquat inhibits the activity of acetylcholinesterase (Nemcsòk et al. 1984). Another pesticide aldrin exerts an unfavourable effect on the circulatory and nervous system of *Clarias batrachus* (Verma et al. 1987). Histopathological changes were also found in the blood of *Carassius carassius* due to intoxication with DDT (Barrufaldi and Cucchi 1989). Histopathological and biochemical alterations due to pesticides were observed in *Oreochromis niloticus* (Zapata et al. 2000). Attempts to determine the effect of water chemism 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 had no great effect on the accumulation of these toxins in fish (Haines 1983). However, temperature exerts a negative effect. The increase in water temperature from 4 to 20°C was found to cause higher accumulation of paraquat $CuS0_4$ in the brain, heart, and kidneys of carp (Nemcsòk et al. 1987). Organochlorine 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. The concentrations of pesticides in humans 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 (Struciński et al. 2000; Pietrzak-Fiećko and Smoczyński 2001; Schinas et al. 2002).

CONCLUSIONS

1. In algal cells, the content of methoxychlor was seven times higher than of lindane.
2. The mean lindane and methoxychlor contents in the muscles of crucian carp reached higher levels when the fish absorbed the pesticides from food, i.e. algae after intoxication and not directly from water.
3. Considerably higher pesticide concentrations were found in the brain and liver than in the muscles, irrespective of the way of absorption (food, water).

4. During the experiment, relatively low levels of lindane and methoxychlor were found in the muscles – edible parts of fish.
5. Lindane and methoxychlor have been detected in phytoplankton—*Chlorella vulgaris*, and in fish—*Carassius carassius*. The studies confirmed the bioaccumulation of organochlorine pesticides in the aquatic food chain.

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BIOKUMULACJA PESTYCYDÓW CHLOROORGANICZNYCH W ŁAŃCUCHU TROFICZNYM GLON – RYBA SŁODKOWODNA

STRESZCZENIE

Badania zostały wykonane na glonie, zielenicy *Chlorella vulgaris* oraz na osobnikach młodocianych karasia *Carassius carassius*. Glony i ryby poddano ekspozycji pestycydem gamametoxem pylistym zawierającym 0.6% lindanu i 3% metoksychloru. Metodą chromatografii gazowej oznaczono w badanym materiale zawartość obu substancji czynnych wchodzących w skład tego pestycydu. Na podstawie średniej zawartości lindanu i metoksychloru w badanych organizmach stwierdzono, że pestycydy te uległy biokumulacji na różnych poziomach łańcucha troficznego. Kumulacja pestycydów – lindanu i metoksychloru była wyższa w rybach niż w glonach, które stanowiły ich źródło pokarmu. Zwrócono uwagę również na to, iż stężenie pestycydów: lindanu i metoxchloru w mięśniach karasia było wyższe wtedy, gdy ryby pobrały je razem z pokarmem w postaci komórek *Chlorella vulgaris* niż bezpośrednio z wody. Podobnie było z zawartością lindanu w wątrobie. Zauważono, że w przypadku koncentracji obu pestycydów w mózgu było odwrotnie lub poziom był podobny. Najwyższe stężenia pestycydów stwierdzone zostały w mózgu karasia, nieco niższe w wątrobie, najniższe natomiast w mięśniach – jadalnych częściach ryb niezależnie od sposobu wchłaniania.

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