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Maria STUDNICKA, Antonina SOPIŃSKA, Jadwiga NIEZGODA

Toxicology

EFFECTS OF LINDANE, DDT, DDD, α-HCH, AND PCB ON CARP GONAD CELL CULTURE*

DZIAŁANIE LINDANU, DDT, DDD, α-HCH ORAZ PCB NA HODOWLĘ KOMÓRKOWĄ GONAD KARPIA

Inland Fisheries Institute, Olsztyn
Institute of Contagious and Invasive Diseases, Academy of Agriculture, Lublin

Toxicity of DDT, DDD, α HCH, lindane, and PCB was assessed from cytotoxic effects in a carp gonad primary cell culture exposed for 96 h to concentrations of 1; 0.5; 0.1; 0.05; and 0.01 mg l^{-1} of each compound. The 0.01 mg l^{-1} concentration of the compounds tested was found to bring forth no cytopathologic changes. The remaining concentrations differ in their cytotoxic effects, depending on a compound testes.

INTRODUCTION

Chlorinated hydrocarbons and polychlorinated biphenyls (PCB) penetrate to the natural environment mainly via industrial sewage and agricultural runoff. A reduction of

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or a total ban on production and application of DDT has not, however, resulted in a decrease of the compound and its metabolites contents in aquatic environments as these chemicals are retained in bottom sediments, posing a further threat of a contamination. Lindane is regarded as a low toxicity compound, for which reason it is widely used in agriculture. Our studies (Studnicka et al., 1981), however, show the lindane toxicity to increase in a fish organism with time.

The PCB content in tissues of fish caught in natural water bodies have been shown to increase over the recent years. A comparison of our 1974 studies on the PCB content in salmon fat (Studnicka et Niewiadomska, 1974) and the Swedish studies of 1968 (Jensen et al., 1969) demonstrated higher contents to be recorded in the first. Both the salmon from the Swedish waters and the trout caught in the Vistula mouth overlan in their distribution in the Baltic; thus the pollution of this sea can be regarded as increasing.

As a continuation of our previous studies on chloroorganic pesticides toxicity for fish, one of the most sensitive biological tests was applied to determine the toxicity of the compounds in question for living organisms.

The objective of the project was to determine the lowest concentrations of DDT, DDD, α HCH, lindane, and PCB that would affect a carp gonad cell culture. The criterion adopted for a toxicity assessment was the presence of a cytopathologic effect.

MATERIALS AND METHODS

The test was performed on a primary cell culture, the cells originating from gonads of 2-yr-old carp females weighing 600–800 g. When starting the culture, literature data (Fijan 1971; Gravell et Malsberger, 1965; Macura et al., 1973) as well as our own experience (Studnicka et al., 1977) were taken advantage of. The culture growth and the full cover were obtained froman 0.3% cell suspension incubated at 26°C for 96 h, after which time the original medium (Eagle fluid of pH 7.2–7.4 enriched with 10% calf serum, 100 i.u./l penicillin, and 1 g/500 ml streptomycin) was removed and substituted by appropriate concentrations of DDT, DDD, lindane, α HCH, and PCB. The following concentrations were used: 1; 0.5; 0.1; 0.05; 0.01 mg l^{-1}. The basic solutions of 100 mg l^{-1} were prepared by dissolving each compound in methyl alcohol. The solutions were brought to the appropriate concentrations by adding the nutritive medium. In order to eliminate the toxic effects of the solvent, two controls were used, one containing the nutritive medium only and the other involving a sufficient amount of methanol in the medium (1:100). The solutions to be used in the assays were prepared ex tempore. Each concentration was tested in 20 vials of the primary culture, the assays being run in triplicates.

The cytotoxic effect was assessed under a microscope, the extent of deterioration of the cellular culture being estimated after 24, 48, 72, and 96 h of exposing the culture to the compounds tested.

Table 1

Cytopathologic effects in carp (Cyprinus carpio) gonad cell culture exposed to DDT, DDD, α–HCH, lindane, and PCB

Compound tested	Exposure time (h)	Extent of cell culture deterioration at various concentrations (mg l^{-1})						
		1	0.5	0.1	0.05	0.01	Control I	Control II
DDT	24	++	+	–	–	–	–	–
	48	+++	+++	++	–	–	–	–
	72	+++	+++	+++	+	–	–	–
	96	+++	+++	+++	++	–	–	–
DDD	24	++	+	+	–	–	–	–
	48	++	+	+	–	–	–	–
	72	+++	++	+	+	–	–	–
	96	+++	++	++	+	–	–	–
α–HCH	24	+	–	–	–	–	–	–
	48	++	–	–	–	–	–	–
	72	++	++	+	+	–	–	–
	96	++	++	+	+	–	–	–
Lindane	24	+++	+	–	–	–	–	–
	48	+++	++	–	–	–	–	–
	72	+++	++	+	–	–	–	–
	96	+++	++	+	–	–	–	–
PCB	24	+++	+	+	–	–	–	–
	48	+++	+	+	–	–	–	–
	72	+++	++	+	+	–	–	–
	96	+++	++	+	+	–	–	–

Explanations:

Control I: nutritive medium; control II: nutritive medium + alcohol;

– no changes; + changes poorly visible (up to 25% of cells changed);

++changes heavuly marked (up to 50% of cells changed); +++ changes very heavily marked (up to 75% of cells changed)

RESULTS AND DISCUSSION

The results of studies on cytotoxic effects of the compounds tested on the carp gonad cell culture are presented in Table 1.

Of the compounds tested, the heaviest cytotoxic effects were produced by DDT and DDD (a DDT-derivative). Both compounds, when applied for 96 h at 1 mg l^{-1} concentrations, bring about similar deterioration of the culture as compared to the control (Fig. 1). The effects incolve a considerable destruction and disintegration of the cells. In some places fibroblasts retain their continuity and shapes, their internal structure being, however, markedly obliterated and containing granulations (Fig. 2). Similar changes were observed after a 96-h exposure of the culture to lower concentrations of DDT (0.5; 0.1; 0.05 mg l^{-1}), while DDD at those concentrations produced less intensive changes (Table 1).

The lindane and PCB concentrations of 1 mg l^{-1} resulted also in a marked destruction of the uniform cellular layer, the changes being, however, of a different nature than those caused by DDT and DDD. The cells occur separately and take on rounded shapes. The cytoplasm is observed to have vacuolised. granulations being present in the cells affected (Fig. 3). Lower concentrations of lindane produce weaker cytotoxic effects than those caused by PCB (Table 1).

The weakest cytotoxic effects of the concentrations used were observed after exposing the culture to α HCH. At 1 mg l^{-1}, the cells' continuity and shapes in some places are

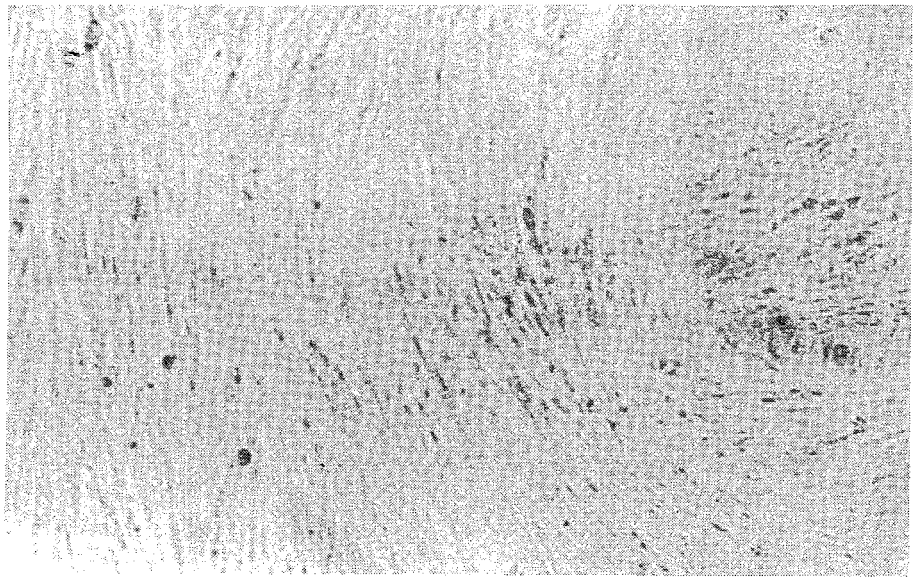

Fig. 1. Cell culture – control test

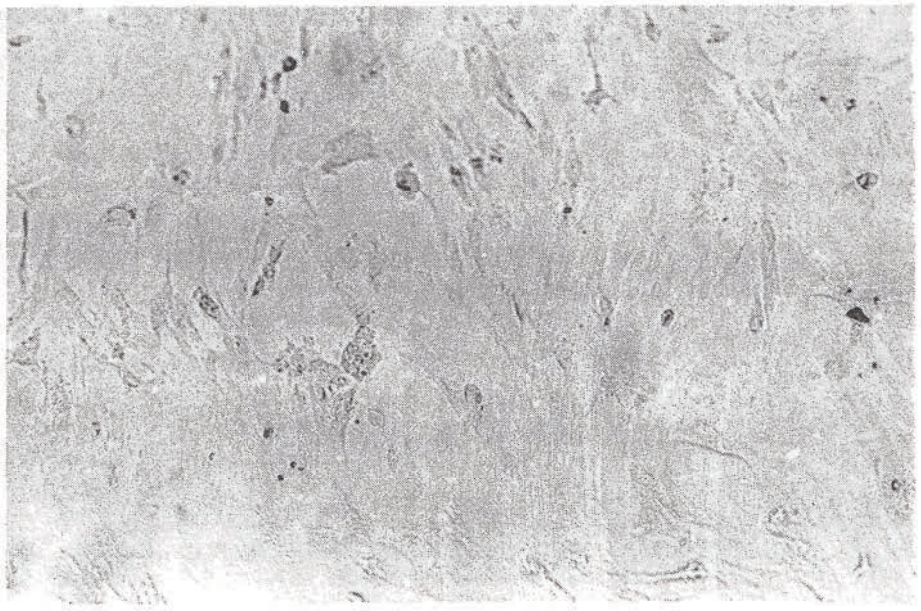

Fig. 2. Cell culture after 96 hours exposure to DDT in concentration of 1 mg l^{-1}

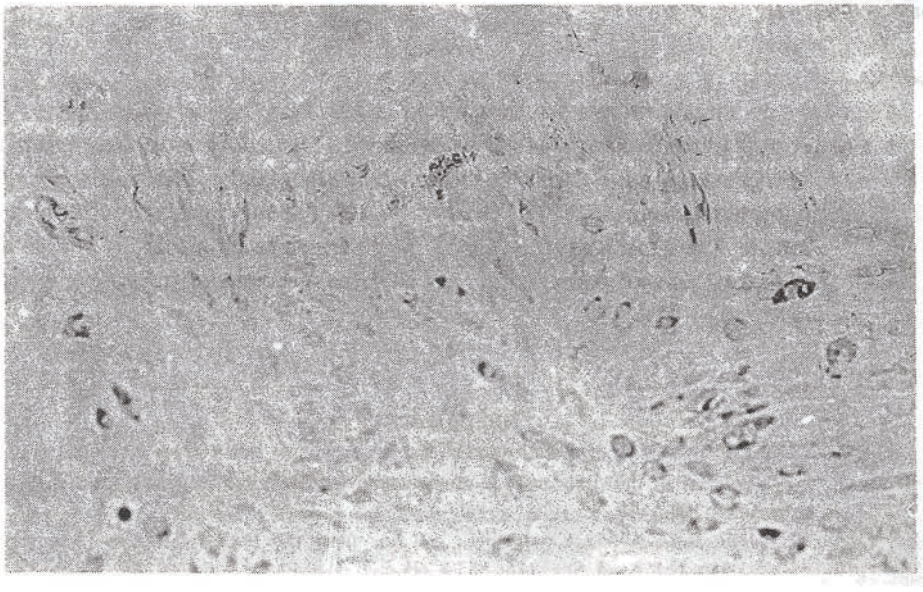

Fig. 3. Cell culture after 96 hours exposure to lindane in concentration of 1 mg l^{-1}

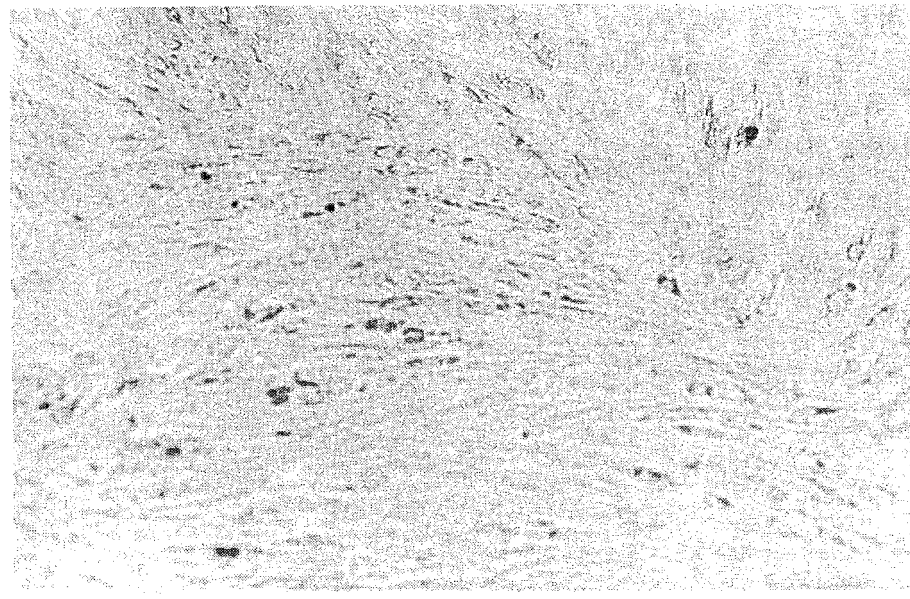

Fig. 4. Cell culture after 96 hours exsposure to α-HCH in concentration 1 mg l^{-1}

retained, the changed cells showing obliterated internal structures and vacuolisation (FIg. 4).

When applied at 0.01 mg l^{-1}, all the compounds tested produced no visible cytotoxic effects, the picture of the culture resembling that in the control (Fig. 1).

The assays performed showed lindane applied in lower concentrations to be less toxic than the remaining compounds. On the other hand, the nature of lindane-produced changes in the culture is more destructive than when using DDT, DDD, and α HCH. Of the compounds tested, only PCB was observed to bring about changes similar to those caused by lindane. The latter, therefore, cannot be described as a low toxicity compound. Its wide used in agriculture can pose a threat of sublethal intoxication in living organisms, fishes included.

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Translated: Dr. Teresa Radziejewska

M. Studnicka, A. Sopińska, J. Niezgoda

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STRESZCZENIE

Toksyczność badanych związków określano działając różnymi ich stężeniami przez okres 96 godzin na pierwotną hodowlę komórkową gonad karpia. Zastosowano następujące stężenia: 1 mg. l^{-1}; 0,5 mg.l^{-1}; 0,1 mg.l^{-1}; 0,05 mg.l^{-1}; 0,01 mg.l^{-1}. Za kryterium oceny toksyczności przyjęto wystąpienie efektu cytopatycznego. Stężenie 0,01 mg.l^{-1} badanych związków nie powoduje widocznych zmian cytotoksycznych w hodowli. Spośród testowanych związków największe działanie cytotoksyczne wykazuje DDT i DDD. Lindan i PCB powodują również znaczną destrukcję komórek hodowli, zmiany mają jednak inny charakter aniżeli po działaniu DDT i DDD. W niższych stężeniach lindan wykazuje mniejsze działanie cytoksyczne aniżeli PCB. α HCH w stosowanych stężeniach wywołało najsłabiej zaznaczone zmiany.

М. Студницка, А. Сопинска, И. Незгода

ДЕЙСТВИЕ ЛИНДАНА, DDT, DDD, HCH и PCB на КЛЕТОЧНУЮ КУЛЬТУРУ ГОНАД КАРПА

Р е з ю м е

Токсическое действие исследованных соединений определяли путем воздействия различных их концентрации в течение 96 часов на первичную клеточную культуру гонад карпа. Применяли следующие концентрации: 1мг /л , 0,5 мг/л, 0,1 мг/л, 0,05 мг/л, 0,01 мг/л. Критерием оценки токсического действия являлось проявление Цитопатического эффекта. Концентрация 0,01 мг/л исследованных соединений не вызывает видимых цитотоксических изменений в культуре. Среди тестируемых соединений самое большое цитотоксическое действие проявляют DDT и DDD . Линдан и PCB вызывают также значительную деструкцию

культивированных клеток, однако изменения имеют другой характер, нежели при действии DDT и DDD. При меньших концентрациях линдан проявляет низшее цитотоксическое действие чем PCB . HCH в применяемых концентрациях вызвало наиболее слабо выраженные изменения.

Перевод: Dr Józef Domagała

Authors address:
Instytut Rybactwa Śródlądowego
Zakład Rybactwa Stawowego
Zabieniec k. Warszawy
05-500 p. Piaseczno 1
Polska (Poland)

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