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Toxicology

EFFECT OF LETHAL CONCENTRATIONS OF SELECTED PHOSPHORO-ORGANIC INSECTICIDES UPON RAINBOW TROUT *SALMO GAIRDNERI* RICH.

WPŁYW LETALNYCH STĘŻEŃ WYBRANYCH INSEKTYCYDÓW FOSFOROORGANICZNYCH NA PSTRĄGA TĘCZOWEGO *SALMO GAIRDNERI* RICH.

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Tests of acute toxicity for rainbow trout fry of chlorfenviphos and bromfenviphos were undertaken in order to establish the LC_{50} values. Studies were also made on the effect of lethal concentrations, corresponding to 24 h LC_{50}, upon the activity of selected enzymes and hematological indices. Activity of brain acethylcholinesterase, and blood peroxidase and catalase, was assessed. Hematological studies embraced: content of hemoglobin, hematocrit, number of red and white corpuscles, percentage share of lymphocytes, red/white cell ratio, and MCV, MCH, and MCHC indices.

INTRODUCTION

Use of insecticides is necessary for effective agriculture. On the other hand, it results in undesirable changes in natural environment. Insecticides reach water bodies through various sources. Due to high biological activity of these compounds, they constitute an immediate danger to fish and other water organisms. (Livingston 1977). The most frequently used insecticides embrace, among others, phosphoro-organic compunds. Although their toxicity is lower than of chlorinated hydrocarbons, they may be highly toxic to fish (Weiss 1959, Mawdesley-Thomas 1971, Post and Leasure 1974, Coppage and Matthews 1974). Apart from studies on toxicodynamics of insecticides (tests of acute toxicity), many works were undertaken on the effect of these substances upon physiological processes. Attention was especially paid to the inhibition of cholinesterase activity in brain and other fish tissues, i.e. to the essential mechanisms of the effect of these compounds on fish organism (Weiss 1958, 1959, 1961, Williams and Sova 1966, Holland et al. 1967, Gibson et al. 1969, Coppage and Matthews 1974, Cook et al. 1976, Metelev and Trostina 1969, Metelev 1972, Sakaguhi 1972). Hematological studies constitute successive step in the assessment of physiopathological effects. So far, these studies are rather rare and fragmentary (Metelev 1972, Metelev et al. 1977, Svobodová 1975, Lone and Javaid 1976, Kanaev et al. 1977).

MATERIAL AND METHODS

Studies were carried out in laboratory conditions on rainbow trout (*Salmo gairdneri* Rich.) at the age of 1+. Toxic effect of two physphoro-organic insecticides was studied: chlorfenviphos (0,0-diethyl-0-1-(2,4-dichlorophenyl/2-chlorvinylphosphate) (CVP), and bromfenviphos (0,0-diethyl-0-1-(2,4-dichlorophenyl/2-bromvinylphosphate) (BVP).

The following experiments ware made for each insecticide: acute toxicity tests in order to estimate LC_{50} (experiment I), and studies on the effect of lethal concentrations as 24 h LC_{50} upon the activity of selected enzymes and hematological indices (experiment II).

Total length of trout used in the experiments amounted to 14 cm, and body mass – to about 30g.

Technical concentrates of the insecticides were used. They contained about 95% of active substance (a.s.). Toxic solutione were prepared from acetone solutions of the compounds.

Experiments were carried out in aquaria, with the volume of 250 dm^3, supplied with lake water. Constant aeration was used, and toxic solutions were changed every day. Oxygen content, pH value, and temperature (Tab. 1) were measured daily. For every experiment full water analyses were made once (Tab. 2).

Acute toxicity tests were performed according to standard procedures (Sprague 1969, 1972, Alabaster 1970). LC_{50} values were calculated with the probit method of Litchfield and Wilcoxon (1949).

In the second experiment activity of brain acetylcholinesterase (AChE), catalase, and blood peroxidase, as also hematological indices (Tab. 3), were studied in trout which survived 24 h exposition to insecticide concentration corresponding to 24 h LC_{50}. Blood was taken from heart with a syringe (Klontz and Smith 1968). Activity of AChE was estimated with Hestrin (1949) method, as modified by Weiss (1958), of peroxidase – with guaiacol method, according to Bach and Zubkova (Metelev 1971), and of catalase – with manganese metric method. Standard procedures were used for hematological studies (Klontz and Smith 1968, Blaxhall and Daisley 1973). The results were analysed statistically, with variance analysis of Fisher (test F).

Table 1

Oxygen content, temperature, and reaction of water

Number of experiment and season	Oxygen content ($mg\ O_2/dm^3$)		Water temperature (°C)		Water reaction (pH)	
	range	mean	range	mean	range	mean
Experiment I May	7.5–10.4	8.9	13.5–15.5	14.5	7.4–7.8	7.6
Experiment II June	7.8–10.5	8.6	16.5–18.0	17.0	7.5–7.7	7.6

Table 2

Physico–chemical composition of water

Parameters	Number of experiment and month	
	I May	II June
Total hardness mval	3.34	2.99
Oxydability ($KMnO_4$/mg O_2/dm^3)	10.2	9.6
Conductivity uS/cm	340	310
Nitrogen as $N\text{-}NH_4$ mg/dm^3	0.09	0.10
$N\text{-}NO_2$ mg/dm^3	0.003	0.019
$N\text{-}NO_3$ mg/dm^3	0.22	0.12
Total nitrogen N mg/dm^3	0.67	0.69
Phosphates as $P\text{-}PO_4$ mg/dm^3	0.052	0.030
Total phosphorus P mg/dm^3	0.089	0.084
Chlorides Cl mg/dm^3	13.2	12.1
Sulphates SO_4 mg/dm^3	–	13.6
Silicates SiO_2 mg/dm^3	8.2	4.8
Total iron Fe mg/dm^3	0.27	0.20

RESULTS AND DISCUSSION

The following values of LC_{50} (in parentheses 95% confidence limits) for 24,48 and 96 h expositions were obtained in acute toxicity tests: for CVP 1.15 mg/dm^3 (1.06–1.25), 0.77 mg/dm^3 (0.71–0.83), and 0.54 mg/dm^3 (0.50–0.58), for BVP respectively 2.25 mg/dm^3 (1.97–2.56), 1.95 mg/dm^3 (1.72–2.20), and 1.65 mg/dm^3 (1.50–1.82). According to the criteria by an International) Group of Experts (ref. after

Sprague 1972), based on 96 h LC_{50}, CVP can be classified as highly toxic for fishes (I class of toxicity, LC_{50} below 1 mg/dm³), and BVP as toxic (II class of toxicity, LC_{50} in the range of 1–100 mg/dm³). Statistical analysis performed with the method of Litchfield and Wilcoxon (1949) supported highly significant differences in the toxic effects of CVP and BVP on rainbow trout for 96 h exposition.

Table 3

Activity of enzymes and hematological indices of rainbow trout (*S. gairdneri* Rich.) after 24 h exposition to 24 h LC_{50} of insecticides

Parameters under study	Control group K n = 50	Experimental group CVP n = 50	Experimental group BVP n = 50	Differences highly significant ($P < 0.01$)
	$\bar{x} \pm S_{\bar{x}}$	$\bar{x} \pm S_{\bar{x}}$	$\bar{x} \pm S_{\bar{x}}$	
Brain AChE µm ACh/mg brain/h	1.52 ± 0.11	0.12 ± 0.06	0.49 ± 0.13	K-CVP, K-BVP, CVP-BVP
Blood peroxidase guaiacol units	1.61 ± 0.25	1.21 ± 0.29	1.02 ± 0.26	K-CVP, K-BVP, CVP-BVP
Blood catalase activity units	4.1 ± 0.6	3.4 ± 0.8	3.0 ± 0.8	K-CVP, K-BVP, CVP-BVP
Hemoglobin g%	9.8 ± 1.2	7.3 ± 1.2	6. 6 ± 1.5	K-CVP, K-BVP, CVP-BVP
Hematocrit % (PCV)	41.0 ± 4.2	32.0 ± 4.5	28.5 ± 5.5	K-CVP, K-BVP, CVP-BVP
Erythrocytes mil./ /mm³	1.22 ± 0.14	0.95 ± 0.15	0.86 ± 0.18	K-CVP, K-BVP, CVP-BVP
Leukocytes thousand/mm³	14.7 ± 4.6	4.4 ± 1.6	3.6 ± 1.6	K-CVP, K-BVP, "–"
Lymphocytes %	95.1 ± 2.8	74.7 ± 15.7	80.6 ± 14.8	K-CVP, K-BVP, "–"
Red/white cell ratio	91 ± 28	241 ± 90	286 ± 136	K-CVP, K-BVP, "–"
MCV µM³	339 ± 40	341 ± 54	338 ± 62	"–" "–" "–"
MCH pg	80 ± 12	78 ± 13	77 ± 15	"–" "–" "–"
MCHC %	23.9 ± 3.0	23.1 ± 2.8	23.1 ± 4.2	"–" "–" "–"

Second experiment was made in order to compare physiopathological effects of insecticide concentrations of 24 h LC_{50}. Enzymatic and hematological tests (Tab. 3) were performed. It might have been expected that with respect to survival both concentrations should be similar. However, it was noted during LC_{50} assessment that concentrations of CVP, corresponding to 24 h LC_{50}, had a more negative effect on fishes. Rainbow trout which survived 24 h exposition in CVP solution showed symptoms of strong poisoning (no fish movement was observed; all fishes lied at the side of their bodies). On the other hand, in BVP solution, most individuals retained swimming ability, although their balance was disturbed in a varying degree. Results of enzymatic and hematological studies (Tab. 3) explain these differences of physiological effect of insecticides. It appeared that the main role was probably played by brain AChE activity. In fishes exposed to lethal CVP concentration this activity decreased to a level corresponding to only 8% of average level for control fishes, whereas in case of BVP, respective value amounted to 32%. Metelev and Osetrov (1968) are of the opinion that inhibition of activity by more than 80% reflects strong poisoning. It should be underlined that my results, obtained for AChE activity, agree with those by Coppage and Matthews (1974) obtained in similar experimental conditions (24 h exposition of 4 fish species in concentrations of phosphoro-organic insecticides – malathion, naled, guthion and parathion – resulting in 40–60% mortality). These authors conclude that in concentrations close to 24 h LC_{50}, reduction of activity by about 80% (average for particular experiments 70–96%) is characteristic for surviving fish. In view of the observed drops of AChE activity, it should be considered whether it would be possible to reactivate AChE after fish transfer to clean water. In case of the insecticides under study it can be expected that after the exposition, further decrease of AChE may occur, as described by Weiss (1958, 1961). This statement is supported by observations of enzyme activity, undertaken during 30 days after 24 h exposition to a half lower concentrations (50% 24 h LC_{50}) (Piotrowska, 1978). Such exposition to CVP resulted in a decrease of activity in the period after exposition to values amounting to 35% of control fish value on the average. Respective level after exposition to BVP amounted to 76%. In case of CVP, significant drop of enzyme activity (Tab. 3) can hardly be overcome, and increase to normal levels should not be expected. It should be underlined that studies of AChE activity after exposure to phosphoro-organic compounds give more detail picture of physiological effects and of real danger to fish life, and thus are of great value.

Further informations reflecting deep negative changes, taking place in fish organism under the effect of toxic concentrations, were provided by studies on blood catalase and peroxidase, as well as on hematological indices (Tab. 3).

Both oxyreductases showed highly significant decrease of activity. Activity of peroxidase in fishes exposed to CVP amounted to 75% of the control value, and for BVP – to 63%. Respective values for catalase were slightly higher, amounting to 82 and 73%. These results point to significant disturbances of cell respiration. Changes of blood peroxidase under the effect of phosphoroorganic insecticides were studied by Metelev

(1972). Alekseev and Lesnikov (1977) stated a decrease of activity of many enzymes (also of peroxidase) under the effect of compounds belonging to this group. They also discussed possibilities of reverse effect for catalase.

Lethal concentrations of insecticides under study caused several changes in blood indices, reflecting some anaemia. In both cases there was statistically significant decrease of hemoglobin content, hematocrit, and number of erythrocytes (Tab. 3). In fishes exposed to CVP and BVP solutions hemoglobin content decreased – compared to the control – to a level of about 75% and 67% respectively, hematocrite – to 78% and 69%, and number of erythrocytes – to 78% and 70% respectively.

Kanaev et al. (1977) stated in their work on toxic effects of ftalophos (imidan, prolat) on carp, that hemoglobin content decreased by 17.0% number of red corpuscles – by 55.3%, and hematocrit – by 13.1%, at simultaneous occurance of qualitative changes in erythrocytes.

In a similar experiment (24 h exposition of carp to phenitrothion, dichlorphos and imidan, corresponding to 24 h LC_{50} Svobodová (1975) noted totally different changes in blood picture. These changes were similar in character for various compounds. They consisted, among others, of an increase of hematocrit, wheras number of erythrocytes, and hemoglobin content did not differ from control fishes.

Visible leucopenia also reflected negative changes in trout organism under the effect of CVP and BVP. Number of white cells decreased sharply down to 30 and 25% of control value for CVP and BVP respectively. At the same time percentage share of white cells changed. In control fishes average share of lymphocytes amounted to 95%, whereas in fishes exposed to lethal concentrations of CVP and BVP – to 75 and 81% respectively. Increase of relative share (in %) of heterophilic granulocytes corresponded to the above drop.

Also the red/white cell ratio (Tab. 3) reflected pronounced changes in trout blood. In fishes exposed to CVP it increased by 2.5 times, and in case of BVP – by 3 times. These values support the statement on the occurance of leucopenia, which predominated the results despite the fact that some erythropenia also occured.

Red cell indices did not point to any differences between particular fish groups. On the other hand, Svobodová (1975) found in her studies that pohosphoro-organic insecticides result in a statistically significant increase of the average red corpuscle volume (MCV), and a decrease of the average hemoglobin concentration in red cells (MCHC).

Direction of changes noted in the enzymes and hematological indices after fish exposure to lethal concentrations of CVP and BVP was similar. On the other hand, however, mechanisms of their effect upon trout blood were different, as supported by statistically highly significant differences between the value of the parameters under study (Tab. 3). It appeared that BVP is characterized by a more negative effect upon the parameters under study. Fish exposure to this compound resulted in a higher decrease of peroxidase and catalase activity, hemoglobin content, hematocrit, and number of erythrocytes.

CONCLUSIONS

1. Studies on rainbow trout (*Salmo gairdneri* Rich.) as a test organism showed that chlorfenviphos should be classified as the I class poison, and bromfenviphos – as the II class poison to fish, as based on 96 h LC_{50}.

2. Chlorfenviphis is characterized by significant antycholinesterase effect, much stronger than bromfenviphos.

3. Lethal concentrations of the enolphosphates under study resulted in pronounced changes in blood, of the anaemia character. In this case bromfenviphos resulted in more negative effects.

4. Results of experiments support the significance of differences in the structure of chemically related compounds (different halogen aubstituent) in their toxico-dynamics and mechanisms of their effect upon fish organism.

Translated: Maria Bnińska

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WPŁYW LETALNYCH STĘŻEŃ WYBRANYCH INSEKTYCYDÓW FOSFOROORGANICZNYCH NA PSTRĄGA TĘCZOWEGO (*SALMO GAIRDNERI* RICH.)

Streszczenie

Przedstawiono porównawcze badania toksycznego działania dwóch insektycydów z grupy enolofosforanów – chlorfenwinfosu i bromfenwinfosu – na narybek pstrąga tęczowego (*Salmo gairdneri* Rich.). Podstawę do oceny stanowiły wyniki testów przeprowadzonych w zakresie stężeń letalnych. Oznaczono wartości CL_{50}, co pozwoliło zaliczyć analizowane związki do odpowiednich klas toksyczności. Badania enzymatyczne i hematologiczne wykonane u ryb, które przeżyły 24 h

CL_{50}, dostarczyły informacji odnośnie różnic w mechanizmie oddziaływania obu insektycydów na organizmy testowe.

Uzyskane wyniki wykazały znaczną toksyczność badanych enolofosforanów dla pstrąga tęczowego. Chlorfenwinfos wyróżnił się większą toksykodynamiką działania, która uwarunkowana była głównie silniejszymi właściwościami antycholinoesterazowymi. Bromfenwinfos wywołał bardziej niekorzystne zmiany we krwi. Potwierdzono istotny wpływ różnic w budowie pokrewnych chemicznie związków na stopień toksyczności i sposób oddziaływania na organizm ryb.

Е. Пиотровска

ВЛИЯНИЕ НА РАДУЖНУЮ ФОРЕЛЬ (SALMO GAIRDNERI RICH.) ЛЕТАЛЬНЫХ КОНЦЕНТРАЦИИ НЕКОТОРЫХ ФОСФОРООРГАНИЧЕСКИХ ИНСЕКТИЦИДОВ

Резюме

Даются сравнительные исследования токсического действия двух инсектицидов из группы энолофосфатов - хлорвенвинфоса и бромфенвинфоса - на мальков радужной форели (Salmo gairdneri Rich.). Основанием для оценки были результаты тестов проведенных в диапозоне летальных концентрации. На основании определения значения Cl_{50} засчитали анализированные соединения к соответствующим классам токсичности. Результаты энзиматических и гематологических исследований проведенных на рыбах проживших 24 чася в растворе Cl_{50} были основой для получения отличий в механизме действия обеих инсектицидов на тестируемые организмы. Полученные результаты показали довольно большую токсичность исследованных энолофосфатов для радужной форели. Хлорфенвинфос отличался большей динамикой токсического действия, которая была обусловлена прежде всего более сильными антихолинэстеразными свойствами. Бромфенвинфос вызывал более отрицательные изменения в крови. Подтверждено существенное влияние различной в строении химически родственных соединений на степень токсичности и способ действия на организм рыб.

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