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Behavioral Response in Toxicity Assessment of the Insecticide Decis® 2.5 EC toward Freshwater Zooplankton

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Abstract: Chemical crop protection agents are widely applied in modern agricultural practice. As a result of surface runoff, these insecticides penetrate into rivers, ponds, and lakes, where they become a serious threat to aquatic organisms. The aim of the study was to determine the toxicity of increasing concentrations of the insecticide Decis® 2.5 EC to *Daphnia magna* and *Heterocypris incongruens*, which are components of freshwater zooplankton. The observed effect was immobilization of organisms, which were not able to swim after gentle agitation of the liquid for 15 sec. It was found that up to 135 min, increasing Decis® 2.5 EC concentrations inhibited the swimming of tested organisms. Initially, up to 135 min, *Heterocypris incongruens* was more sensitive to the tested insecticide. After 135 min of the experiment, ostracods acclimatized to the tested xenobiotic. However, after 360 min, the immobilization of organisms increased proportionally to the concentrations of Decis® 2.5 EC. The most toxic concentrations were 15 × 10^{-3}% and 30 × 10^{-3}%. The lowest observed effect concentration of Decis® 2.5 EC that reduced the swimming of daphnia and ostracod by more than 20% was >0.91 × 10^{-3}% (0.23 mg L^{-1} of deltamethrin). This experiment demonstrated that *Daphnia magna* and *Heterocypris incongruens* are good bioindicators of freshwaters polluted with Decis® 2.5 EC.

Keywords: water; insecticide; daphnia; ostracod; Decis; deltamethrin; Daphtoxkit; Ostracodatoxkit

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1. Introduction

The increase in the population of the Earth makes it necessary to meet their nutritional needs. Therefore, chemical methods have been developed, with higher crop yields obtained [1]. Fertilizers enrich the soil macro and micronutrients necessary for the proper development of the crop, and pesticides are used to combat harmful pests and protect against their negative impact on agricultural crops [1]. A characteristic feature is their toxicity to the pests for which they are recommended. They are deliberately introduced by humans into the environment to kill living organisms [2]. Chemical crop protection agents are widely applied in modern agricultural practice. These substances, including insecticides, are not a natural part of the environment. Insecticides are used to control pests of arable lands, orchards, gardens, and many others [3]. In order to increase the yields, their production and use increase each year. Many researchers have shown a correlation between frequent and long-term use of pesticides in farmland and contamination of groundwater [4] and water reservoirs [5]. Freshwater is contaminated with various chemical elements and compounds from industrial, agricultural, and municipal waste as well as from pesticides. As a result of surface runoff, pesticides penetrate into rivers, ponds, and lakes, where they become a serious threat to aquatic organisms [6]. Therefore, xenobiotics such as pesticides in the waters should be monitored. The European Environment Agency (EEA) publishes annual reports on environmental pollution by pesticides, including standards for surface water and groundwater. In 2006, in 6.49% of the samples, the concentration of pesticides in surface waters were above the environmental quality standards (EQSs) of 0.1 µg dm^{-1}. To determine the toxic effects of xenobiotics on aquatic ecosystems and the organisms living in

them, biotests are used. Research into crustaceans plays an important role in the evaluation of water pollution with pesticides. *Daphnia magna* (daphnia) and *Heterocypris incongruens* (ostracod) are widely used as indicator organisms in environmental risk assessments.

Therefore, in our work, we estimated the toxicity of the insecticide Decis® 2.5 EC in relation to freshwater invertebrates *D. magna* and *H. incongruens*. The selection of test organisms should characterize the ecosystem [7]. To avoid underestimation of the toxicity of the substance, the test is extended to a larger part of the ecosystem, including organisms inhabiting there [8]. Such ecotoxicological analysis enables the comparison of the sensitivity of organisms with different organizational levels [7]. The test organisms used in our research are an element of the freshwater zooplankton.

The aim of this study was to determine by biological Daphtoxkit F magna and Ostracodtoxkit F methods the effect of Decis® 2.5 EC concentrations of 0 to 30 $\times$ 10^{-3}% on freshwater crustaceans *D. magna* and *H. incongruens*, an important link in the food chain.

2. Materials and Methods

2.1. Characteristics of the Tested Insecticide Decis® 2.5 EC

Decis® 2.5 EC is an insecticide of which the active substance is deltamethrin ($C_{12}H_{19}Br_2NO_3$; [(S)-cyano-(3-phenoxyphenyl)methyl] (1R,3R)-3-(2,2-dibromoethenyl)-2,2-dimethylcyclopropane-1-carboxylate [9]. It is recommended for pest control of potato, garden strawberry, vegetables, fruit trees, ornamental shrubs, and crops, including winter oilseed rape. The insecticide is used in order to eliminate: Colorado potato beetle, tarnished plant bug, common pollen beetle, apple blossom weevil, flea beetle, cabbage aphid, rose aphid, and rose leafhopper [10]. The pesticide penetrates the harmful insects through the following route: stomach, respiratory, or contact. After penetrating nerve cells, it results in the inhibition of acetylcholinesterase (AChE). It causes death within a short time after application. Decis® 2.5 EC is available in the form of a concentrate (EC) for preparation of a water emulsion [10].

2.2. Test Daphtoxkit F^{TM} and Ostracodatoxkit F^{TM}

The toxicity of the insecticide was evaluated using two microbiotests used for testing environmental samples and solutions of various chemicals—Daphtoxkit F and Ostracodtoxkit F. The study was based on the biological responses of freshwater crustaceans: daphnia (*D. magna*) and ostracod (*H. incongruens*).

Standard medium solution (prepared with distilled water, pH usually 7.2–7.5, and dissolved oxygen usually 7.0–7.9 mg L^{-1}) was used to prepare Decis® 2.5 EC concentrations. Low concentrations of the insecticide Decis® 2.5 EC were prepared: 0; 0.23 $\times$ 10^{-3}%; 0.47 $\times$ 10^{-3}%; 0.94 $\times$ 10^{-3}%; 1.88 $\times$ 10^{-3}%; 3.75 $\times$ 10^{-3}%; 7.5 $\times$ 10^{-3}%; 15 $\times$ 10^{-3}%; and 30 $\times$ 10^{-3}% (Decis/solution, v/v), and a control sample was prepared.

2.3. Daphtoxkit F magna

D. magna organisms were obtained commercially from Daphtoxkit F magna (MicroBioTests Inc., Ghent, Belgium) by the hatching of the ephippia in a growth chamber (ALL-Round-Al 185-4) under continuous illumination (6000 LUX) with a temperature of 20–22 °C for 72 h.

The toxicity of Decis® 2.5 EC (Bayer SAS) to *D. magna* was tested according to the OECD Guideline 202 "*Daphnia* sp. Acute Immobilisation Test" [11] and ISO 6341 "Water quality—Determination of the inhibition of the mobility of Daphnia magna Straus (Cladocera, Crustacea)—Acute toxicity test" [12]. *D. magna* organisms (5 actively swimming neonates, not older than 24 h) were fed and transferred to plastic plate wells containing 25 mL of standard freshwater with increasing Decis® 2.5 EC concentrations. The responses of *D. magna* to Decis® 2.5 EC concentrations of 0; 0.23 $\times$ 10^{-3}; 0.47 $\times$ 10^{-3}; 0.94 $\times$ 10^{-3}; 1.88 $\times$ 10^{-3}; 3.75 $\times$ 10^{-3}; 7.5 $\times$ 10^{-3}%; 15 $\times$ 10^{-3}; and 30 $\times$ 10^{-3}% (v/v) were determined based on the immobilization (which was defined as daphnias which were not able to swim after gentle agitation of the liquid for 15 s, even if they could still move their antennae).

The standard exposure time for Daphtoxkit F magna was shortened. The immobilization was determined after 15, 45, 90, 135, 180, 225, 270, 315, and 360 min of the experiment.

2.4. Ostracodatoxkit F

H. incongruens organisms were obtained commercially from Ostracodatoxkit F (MicroBioTests Inc., Ghent, Belgium) by the hatching of the cysts in a growth chamber (ALL-Round-Al 185-4) under continuous illumination (3500 LUX) with a temperature of 25 °C for 52 h.

The toxicity of Decis® 2.5 EC (Bayer SAS) to *H. incongruens* was tested according to ISO 14371 "Water quality—Determination of fresh water sediment toxicity to *H. incongruens* (Crustacea, Ostracoda)" [13]. *H. incongruens* organisms (5 actively swimming ostracods) were fed and transferred to plastic plate wells containing 25 mL of standard freshwater with increasing Decis® 2.5 EC concentrations. The responses of *H. incongruens* to Decis® 2.5 EC concentrations of 0; 0.23×10^{-3}; 0.47×10^{-3}; 0.94×10^{-3}; 1.88×10^{-3}; 3.75×10^{-3}; 7.5×10^{-3}%; 15×10^{-3}; and 30×10^{-3}% (v/v) were determined based on the immobilization (which was defined as ostracods which were not able to swim after gentle agitation of the liquid for 15 s, even if they could still move their antennae). The standard exposure time for the Ostracodatoxkit F has been shortened. The immobilization was determined after 15, 45, 90, 135, 180, 225, 270, 315, and 360 min of the experiment.

2.5. Statistical Analysis

The experiment was conducted in 6 replicates. Results are expressed as mean ± standard deviation (SD). The results were statistically evaluated using analysis of variance (ANOVA) (F test) for two factors (to immobilization). The factors of the experiment were: time and concentration used. Significant differences were determined by Tukey's test at the level $p < 0.01$. Research results: immobilization of daphnia (*D. magna*) and ostracod (*H. incongruens*) exposed to Decis® 2.5 EC was determined using the STATISTICA 13.3 statistical package (TIBCO Software Inc., Palo Alto, CA, USA, 2018). Effective concentration (ECx) data were analyzed separately for each replicate using a plot and equation of the dependence of the immobilization of organisms (% of control) on the logarithm of the test substance's concentration to calculate the concentrations at 20%, 50%, and 90% response levels. For the values, the mean (m) and 95% confidence intervals (*CIs*) using Student-t distribution ($\alpha = 0.05$) were determined.

3. Results

The toxicity of the insecticide was evaluated using two microbiotests—Daphtoxkit F and Ostracodtoxkit F. The effect of low concentrations of Decis® 2.5 EC: 0%, 0.23×10^{-3}%; 0.47×10^{-3}%; 0.94×10^{-3}%; 1.88×10^{-3}%; 3.75×10^{-3}%; 7.5×10^{-3}%; 15×10^{-3}%; and 30×10^{-3}% (0, 0.06, 0.12, 0.23, 0.47, 0.94, 1.88, 3.75, and 7.50 mg L^{-1} of deltamethrin, respectively) was investigated for daphnia and ostracod manifested by immobilization of organisms.

3.1. Daphnia Magna

The ANOVA demonstrated that immobilization of organisms was changed by the Decis® 2.5 EC concentration and duration of the test (Table 1). Exposure of daphnia (*D. magna*) to Decis® 2.5 EC resulted in an increased number of immobilized organisms. It was observed that a concentration of 7.5×10^{-3}% to 30×10^{-3}% significantly immobilized all organisms after 15 min from the start of the test. This effect was observed up to 360 min. These concentrations were the most toxic to daphnia. The lower concentrations of the insecticide were less toxic to daphnia (Figure 1, Table 2). The concentration of 3.75×10^{-3}% caused immobilization of all organisms after 135 min. At a concentration of 1.87×10^{-3}% Decis® 2.5 EC, it was observed that the exposure time significantly increased the number of immobilized organisms, so that five immobilized daphnias were recorded in 360 min. In 360 min of the test, 4.33 organisms were immobilized at the concentrations of the insecticide

0.94 × 10^{-3}% and 1.88 × 10^{-3}% in 315 min. The lowest concentration—0.23 × 10^{-3}%—did not immobilize all daphnias, even after 360 min of the experiment, at which there were only three immobilized organisms.

Table 1. Analysis of variance (ANOVA) for immobilization of *Daphnia magna* exposed to Decis® 2.5 EC.

Source of Variation	*Daphnia magna*
	Immobilization
	F-Value
Intercept	15,976.96 *
Concentration of Decis (C)	1161.34 *
Time (T)	114.28 *
C × T	22.44 *

C—concentration, T—time, C × T– interactions between the factors, * significant at $p < 0.01$.

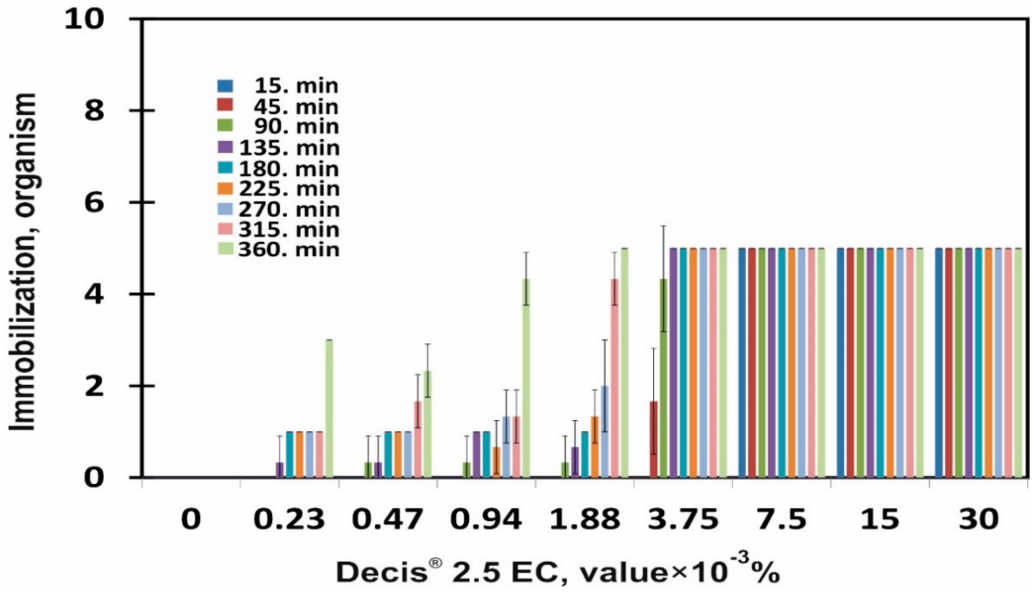

Figure 1. Immobilization of daphnia (*Daphnia magna*) exposed to Decis® 2.5 EC concentrations (0%; 0.23 × 10^{-3}%; 0.47 × 10^{-3}%; 0.94 × 10^{-3}%; 1.88 × 10^{-3}%; 3.75 × 10^{-3}%; 7.5 × 10^{-3}%; 15 × 10^{-3}%; and 30 × 10^{-3}%). Data points represent the mean ± SD, $n = 6$.

Table 2. The immobilization of *Daphnia magna* exposed to Decis® 2.5 EC. The table contains the mean of the examined features and in superscript the level of significance (a–g).

Parameter		Decis® 2.5 EC, %								
		0	0.23 × 10^{-3}	0.47 × 10^{-3}	0.94 × 10^{-3}	1.88 × 10^{-3}	3.75 × 10^{-3}	7.5 × 10^{-3}	15 × 10^{-3}%	30 × 10^{-3}%
Immobilization, organism	15 min	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	5.00 ^{g}	5.00 ^{g}	5.00 ^{g}
	45 min	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	1.67 ^{cde}	5.00 ^{g}	5.00 ^{g}	5.00 ^{g}
	90 min	0.00 ^{a}	0.00 ^{a}	0.33 ^{ab}	0.33 ^{ab}	0.33 ^{ab}	4.33 ^{g}	5.00 ^{g}	5.00 ^{g}	5.00 ^{g}
	135 min	0.00 ^{a}	0.33 ^{ab}	0.33 ^{ab}	1.00 ^{abcd}	0.67 ^{abc}	5.00 ^{g}	5.00 ^{g}	5.00 ^{g}	5.00 ^{g}
	180 min	0.00 ^{a}	1.00 ^{abcd}	1.00 ^{abcd}	1.00 ^{abcd}	1.00 ^{abcd}	5.00 ^{g}	5.00 ^{g}	5.00 ^{g}	5.00 ^{g}
	225 min	0.00 ^{a}	1.00 ^{abcd}	1.00 ^{abcd}	0.67 ^{abc}	1.33 ^{bcde}	5.00 ^{g}	5.00 ^{g}	5.00 ^{g}	5.00 ^{g}
	270 min	0.00 ^{a}	1.00 ^{abcd}	1.00 ^{abcd}	1.33 ^{bcde}	2.00 ^{def}	5.00 ^{g}	5.00 ^{g}	5.00 ^{g}	5.00 ^{g}
	315 min	0.00 ^{a}	1.00 ^{abcd}	1.67 ^{cde}	1.33 ^{bcde}	4.33 ^{g}	5.00 ^{g}	5.00 ^{g}	5.00 ^{g}	5.00 ^{g}
	360 min	0.00 ^{a}	3.00 ^{g}	2.33 ^{ef}	4.33 ^{g}	5.00 ^{g}	5.00 ^{g}	5.00 ^{g}	5.00 ^{g}	5.00 ^{g}

No changes in daphnia behavior were found in the control sample (Figure 1, Table 2). The Decis® 2.5 EC concentration of 4.31 × 10^{-3}% (1.08 mg L^{-1} of deltamethrin) and 0.08 × 10^{-3}% (0.02 mg L^{-1} of deltamethrin) immobilized daphnias by 20% (EC_{20}), respectively, in 15 and 360 min. Similarly, the concentration of the tested insecticide reducing the swimming of organisms by 50% (EC_{50}) decreased from 5.30 × 10^{-3}% (1.33 mg L^{-1} of

deltamethrin) in 15 min to 0.20 × 10^{-3}% (0.05 mg L^{-1} of deltamethrin) at the end of biotest (360 min) (Table 3).

Table 3. Effect of Decis® 2.5 EC on immobilization of *Daphnia magna.* The table contains the mean (m) and 95% confidence intervals (*CIs*) for mean.

Parameter		Decis® 2.5 EC, %		
		EC_{20}	EC_{50}	EC_{90}
Immobilization	15 min	4.31×10^{-3}	5.30×10^{-3}	7.00×10^{-3}
	CI	$4.31 \times 10^{-3} < m < 4.31 \times 10^{-3}$	$5.30 \times 10^{-3} < m < 5.30 \times 10^{-3}$	$7.00 \times 10^{-3} < m < 7.00 \times 10^{-3}$
	45 min	3.29×10^{-3}	4.36×10^{-3}	6.69×10^{-3}
	CI	$2.78 \times 10^{-3} < m < 3.79 \times 10^{-3}$	$3.80 \times 10^{-3} < m < 4.91 \times 10^{-3}$	$6.48 \times 10^{-3} < m < 6.89 \times 10^{-3}$
	90 min	2.15×10^{-3}	2.82×10^{-3}	4.43×10^{-3}
	CI	$1.19 \times 10^{-3} < m < 3.12 \times 10^{-3}$	$2.64 \times 10^{-3} < m < 3.00 \times 10^{-3}$	$3.41 \times 10^{-3} < m < 5.46 \times 10^{-3}$
	135 min	0.70×10^{-3}	2.50×10^{-3}	3.46×10^{-3}
	CI	$0.45 \times 10^{-3} < m < 0.96 \times 10^{-3}$	$2.42 \times 10^{-3} < m < 2.59 \times 10^{-3}$	$3.44 \times 10^{-3} < m < 3.48 \times 10^{-3}$
	180 min	0.23×10^{-3}	2.43×10^{-3}	3.44×10^{-3}
	CI	$0.23 \times 10^{-3} < m < 0.23 \times 10^{-3}$	$2.43 \times 10^{-3} < m < 2.43 \times 10^{-3}$	$3.44 \times 10^{-3} < m < 3.44 \times 10^{-3}$
	225 min	0.23×10^{-3}	2.32×10^{-3}	3.41×10^{-3}
	CI	$0.23 \times 10^{-3} < m < 0.23 \times 10^{-3}$	$2.20 \times 10^{-3} < m < 2.44 \times 10^{-3}$	$3.37 \times 10^{-3} < m < 3.44 \times 10^{-3}$
	270 min	0.23×10^{-3}	2.04×10^{-3}	3.31×10^{-3}
	CI	$0.23 \times 10^{-3} < m < 0.23 \times 10^{-3}$	$1.77 \times 10^{-3} < m < 2.31 \times 10^{-3}$	$3.22 \times 10^{-3} < m < 3.40 \times 10^{-3}$
	315 min	0.23×10^{-3}	1.23×10^{-3}	2.32×10^{-3}
	CI	$0.23 \times 10^{-3} < m < 0.23 \times 10^{-3}$	$1.14 \times 10^{-3} < m < 1.33 \times 10^{-3}$	$1.97 \times 10^{-3} < m < 2.68 \times 10^{-3}$
	360 min	0.08×10^{-3}	0.20×10^{-3}	1.16×10^{-3}
	CI	$0.08 \times 10^{-3} < m < 0.08 \times 10^{-3}$	$0.20 \times 10^{-3} < m < 0.20 \times 10^{-3}$	$0.98 \times 10^{-3} < m < 1.34 \times 10^{-3}$

3.2. *Heterocypris Incongruens*

The ANOVA demonstrated that immobilization of organisms was changed by the Decis® 2.5 EC concentration and duration of the test (Table 4). Ostracods (*H. incongruens*) after 135 min of the experiment were more sensitive than daphnias to the same concentration of the insecticide Decis® 2.5 EC. After 15 min, all organisms exposed to Decis® 2.5 EC at a concentration of $\geq 3.75 \times 10^{-3}$% were significantly immobilized (Table 5). At a concentration of 1.88 × 10^{-3} of the insecticide, almost the same test effect was found after 90 min of the experiment. The lowest concentration of the tested insecticide (0.23 × 10^{-3}%) insignificantly immobilized an average of 1.33 organisms after 15 min of the test (Table 5). In the control group, there were no changes in ostracod behavior. After 135–225 min of exposure, the organisms reacted less in Decis® 2.5 EC concentrations $\leq 3.75 \times 10^{-3}$%. After 225 min, the number of immobilized crustaceans did not exceed two organisms. However, after 360 min, the organisms lost their immunity proportionally to the concentrations of the tested insecticide (Figures 2 and 3).

Table 4. Analysis of variance (ANOVA) for immobilization of *Heterocypris incongruens* exposed to Decis® 2.5 EC.

Source of Variation	*Daphnia magna*
	Immobilization
	F-Value
Intercept	3451.90 *
Concentration of Decis (C)	175.17 *
Time (T)	27.34 *
C × T	6.85 *

C—concentration, T—time, C × T– interactions between the factors, * significant at $p < 0.01$.

Table 5. The immobilization of *Heterocypris incongruens* exposed to Decis® 2.5 EC. The table contains the mean of the examined features and in superscript the level of significance (a–h).

Parameter		Decis® 2.5 EC, %								
		0	0.23×10^{-3}	0.47×10^{-3}	0.94×10^{-3}	1.88×10^{-3}	3.75×10^{-3}	7.5×10^{-3}	15×10^{-3}%	30×10^{-3}%
Immobilization, organism	15 min	0.00 ^{a}	1.33 ^{abcde}	1.33 ^{abcde}	1.67 ^{abcdef}	2.67 ^{cdefg}	5.00 ^{h}	5.00 ^{h}	5.00 ^{h}	5.00 ^{h}
	45 min	0.00 ^{a}	2.33 ^{bcdef}	1.67 ^{abcdef}	2.67 ^{cdefg}	3.33 ^{efgh}	5.00 ^{h}	5.00 ^{h}	5.00 ^{h}	5.00 ^{h}
	90 min	0.00 ^{a}	2.00 ^{abcdef}	2.33 ^{bcdef}	3.33 ^{efgh}	4.67 ^{gh}	5.00 ^{h}	5.00 ^{h}	5.00 ^{h}	5.00 ^{h}
	135 min	0.00 ^{a}	1.00 ^{abcd}	0.33 ^{ab}	1.67 ^{abcdef}	2.33 ^{bcdef}	3.67 ^{fgh}	5.00 ^{h}	5.00 ^{h}	5.00 ^{h}
	180 min	0.00 ^{a}	0.33 ^{ab}	0.33 ^{ab}	0.67 ^{abc}	1.00 ^{abcd}	1.33 ^{abcde}	5.00 ^{h}	5.00 ^{h}	5.00 ^{h}
	225 min	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.33 ^{ab}	1.33 ^{abcde}	1.33 ^{abcde}	5.00 ^{h}	5.00 ^{h}	5.00 ^{h}
	270 min	0.00 ^{a}	0.33 ^{ab}	0.33 ^{ab}	1.33 ^{abcde}	1.33 ^{abcde}	1.33 ^{abcde}	5.00 ^{h}	5.00 ^{h}	5.00 ^{h}
	315 min	0.00 ^{a}	1.33 ^{abcde}	1.33 ^{abcde}	1.67 ^{abcdef}	1.67 ^{abcdef}	2.00 ^{abcdef}	5.00 ^{h}	5.00 ^{h}	5.00 ^{h}
	360 min	0.00 ^{a}	1.67 ^{abcdef}	2.33 ^{bcdef}	2.67 ^{cdefg}	3.00 ^{defgh}	5.00 ^{h}	5.00 ^{h}	5.00 ^{h}	5.00 ^{h}

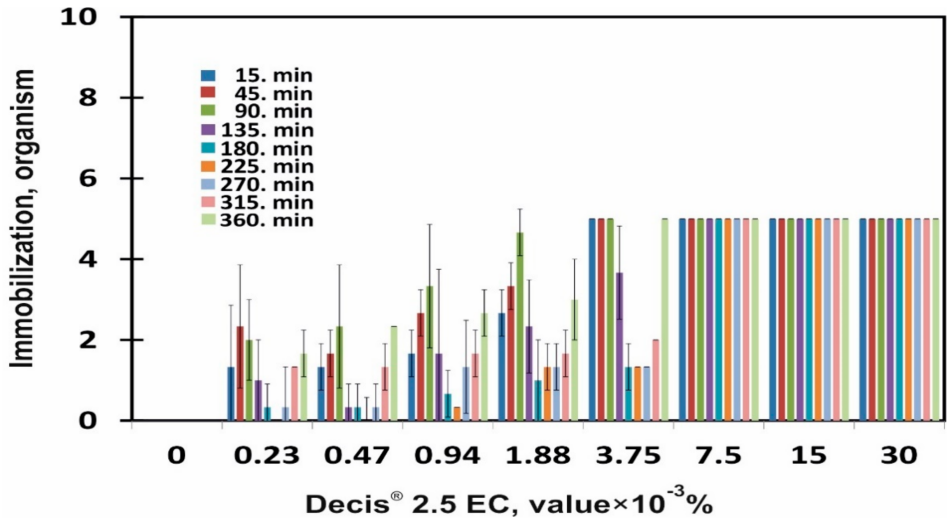

Figure 2. Immobilization of ostracod (*Heterocypris incongruens*) exposed to Decis® 2.5 EC concentrations (0%; 0.23×10^{-3}%; 0.47×10^{-3}%; 0.94×10^{-3}%; 1.88×10^{-3}%; 3.75×10^{-3}%; 7.5×10^{-3}%; 15×10^{-3}%; and 30×10^{-3}%). Data points represent the mean ± SD, $n = 6$.

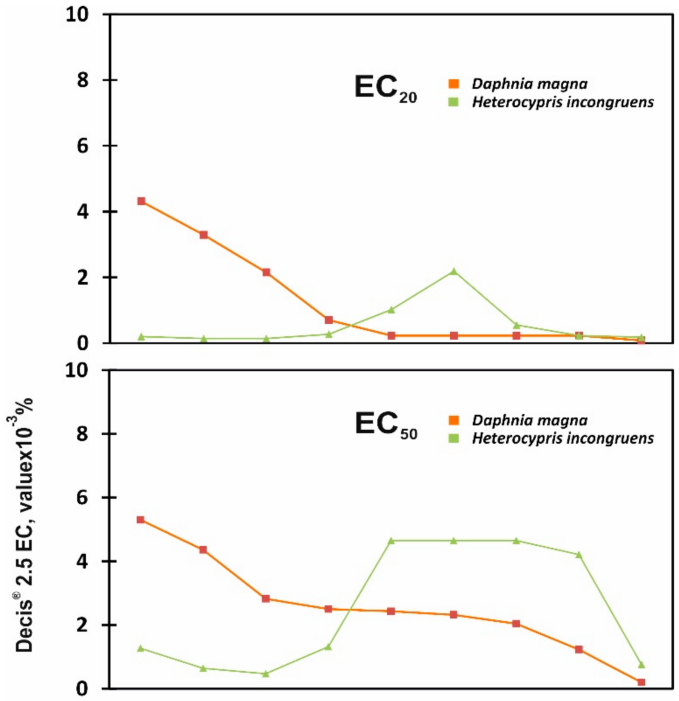

Figure 3. *Cont.*

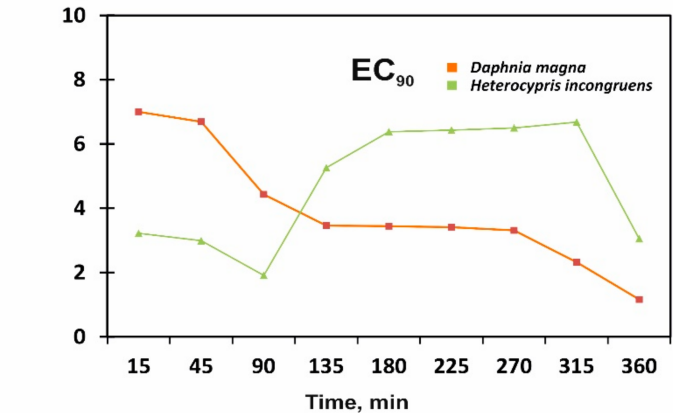

Figure 3. Effective concentration changing through exposure (15–350 min) of daphnia (*Daphnia magna*) and ostracod (*Heterocypris incongruens*) to Decis® 2.5 EC.

The effective concentration responsible for the 20% immobilization of ostracods decreased from 0.20 × 10^{-3}% (0.05 mg L^{-1} of deltamethrin) in 15 min to 0.14 × 10^{-3}% (0.04 mg L^{-1} of deltamethrin) of Decis® 2.5 EC after 90 min of exposure (Table 6). After 135 min of the experiment, ostracods acclimatized to the tested insecticide. The highest EC_{20} = 2.19 × 10^{-3}% (0.55 mg L^{-1} of deltamethrin) of Decis® 2.5 EC was noted after 225 min. A similar tendency of changes was observed in the EC_{50}. The EC_{50} after 15, 90, and 225 was 1.27 × 10^{-3}%, 0.47 × 10^{-3}%, and 4.65 × 10^{-3}%, respectively (0.32, 0.12, and 1.16 mg L^{-1} of deltamethrin, respectively) (Table 6, Figure 3).

Table 6. Effect of Decis® 2.5 EC on immobilization of *Heterocypris incongruens*. The table contains the mean (m) and 95% confidence intervals (*CIs*) for mean.

Parameter		Decis® 2.5 EC, %		
		EC_{20}	EC_{50}	EC_{90}
Immobilization	15 min	0.20×10^{-3}	1.27×10^{-3}	3.22×10^{-3}
	CI	$0.15 \times 10^{-3} < m < 0.24 \times 10^{-3}$	$0.94 \times 10^{-3} < m < 1.61 \times 10^{-3}$	$3.15 \times 10^{-3} < m < 3.28 \times 10^{-3}$
	45 min	0.14×10^{-3}	0.64×10^{-3}	2.99×10^{-3}
	CI	$0.08 \times 10^{-3} < m < 0.19 \times 10^{-3}$	$0.34 \times 10^{-3} < m < 0.95 \times 10^{-3}$	$2.80 \times 10^{-3} < m < 3.17 \times 10^{-3}$
	90 min	0.14×10^{-3}	0.47×10^{-3}	1.91×10^{-3}
	CI	$0.09 \times 10^{-3} < m < 0.19 \times 10^{-3}$	$0.15 \times 10^{-3} < m < 0.79 \times 10^{-3}$	$1.50 \times 10^{-3} < m < 2.31 \times 10^{-3}$
	135 min	0.27×10^{-3}	1.32×10^{-3}	5.26×10^{-3}
	CI	$0.16 \times 10^{-3} < m < 0.39 \times 10^{-3}$	$0.99 \times 10^{-3} < m < 1.63 \times 10^{-3}$	$4.11 \times 10^{-3} < m < 6.41 \times 10^{-3}$
	180 min	1.02×10^{-3}	4.65×10^{-3}	6.38×10^{-3}
	CI	$0.50 \times 10^{-3} < m < 1.54 \times 10^{-3}$	$4.41 \times 10^{-3} < m < 4.88 \times 10^{-3}$	$6.08 \times 10^{-3} < m < 6.67 \times 10^{-3}$
	225 min	2.19×10^{-3}	4.65×10^{-3}	6.43×10^{-3}
	CI	$1.28 \times 10^{-3} < m < 3.09 \times 10^{-3}$	$4.41 \times 10^{-3} < m < 4.88 \times 10^{-3}$	$6.29 \times 10^{-3} < m < 6.57 \times 10^{-3}$
	270 min	0.55×10^{-3}	4.65×10^{-3}	6.57×10^{-3}
	CI	$0.32 \times 10^{-3} < m < 0.77 \times 10^{-3}$	$4.41 \times 10^{-3} < m < 4.88 \times 10^{-3}$	$6.23 \times 10^{-3} < m < 6.91 \times 10^{-3}$
	315 min	0.23×10^{-3}	4.21×10^{-3}	6.68×10^{-3}
	CI	$0.11 \times 10^{-3} < m < 0.36 \times 10^{-3}$	$4.21 \times 10^{-3} < m < 4.21 \times 10^{-3}$	$6.68 \times 10^{-3} < m < 6.68 \times 10^{-3}$
	360 min	0.18×10^{-3}	0.76×10^{-3}	3.05×10^{-3}
	CI	$0.13 \times 10^{-3} < m < 0.24 \times 10^{-3}$	$0.43 \times 10^{-3} < m < 1.09 \times 10^{-3}$	$2.82 \times 10^{-3} < m < 3.27 \times 10^{-3}$

4. Discussion

Organisms used in the test were selected taking into account their availability, ease of testing, and compliance with available standards [11–13]. Exposure of daphnia (*D. magna*) on Decis® 2.5 EC resulted in an increased number of immobilized organisms. It was observed that the concentration of 7.5 × 10^{-3}% to 30 × 10^{-3}% immobilized all organisms after 15 min from the start of the test. This effect was observed up to 360 min. These concentrations were the most toxic for daphnia. The lower concentrations of the insecticide were

less toxic to daphnia. At the concentration of 1.88×10^{-3}% Decis® 2.5 EC, it was observed that the exposure time increased the number of immobilized organisms; however, five immobilized *D. magna* were observed after 360 min (Figure 1). The applied concentrations of the insecticide were also quite toxic to the ostracod. In the Ostracodtoxkit F test, the growth is determined as a standard. Meanwhile, Decis® 2.5 EC also affects the behavior of ostracod. For these crustaceans, at a concentration of 1.88×10^{-3}% of the insecticide, the test effect in the form of immobilization of all organisms was observed after 105 min of the experiment. In the lowest tested insecticide concentration (0.23×10^{-3}%) after 15 min, an average of 1.33 organisms were immobilized (Figure 2). The Decis® 2.5 EC concentration of 4.31×10^{-3}% (1.08 mg L^{-1} of deltamethrin) and 0.08×10^{-3}% (0.02 mg L^{-1} of deltamethrin) immobilized daphnias by 20% (EC_{20}), respectively, in 15 and 360 min (Table 3). The calculation of EC_{20} is useful in determining the lowest observed effect concentration (LOEC). The LOEC is defined as the lowest applied concentration of a chemical compound which reduces the measured response by more than 20% (>EC_{20}) [14]. For ostracods, the effective concentration responsible for the 20% immobilization of ostracods decreased from 0.20×10^{-3}% (0.05 mg L^{-1} of deltamethrin) in 15 min to 0.14×10^{-3}% (0.04 mg L^{-1} of deltamethrin) of Decis® 2.5 EC after 90 min of exposure. After 135 min of the experiment, ostracods acclimatized to the tested insecticide. The highest $EC_{20} = 2.19 \times 10^{-3}$% (0.55 mg L^{-1} of deltamethrin) of Decis® 2.5 EC was noted after 225 min (Table 6, Figure 3). However, after 360 min, the organisms lost their immunity proportionally to the concentrations of the tested insecticide (Figure 2). All organisms were immobilized. As demonstrated in the study about effects of sodium chloride on *D. magna*, non−swimming organisms still exhibit a heartbeat [15]. However, considering that the maximum acceptable concentration ($MAC_{eco,water}$—the concentration protecting aquatic ecosystems from effects due to short-term exposure or concentration peaks for freshwater) for deltamethrin is only 0.031 ng L^{-1}, it indicates that immobilized organisms (daphnia and ostracod) are probably dead [16]. Additionally, other aquatic organisms are sensitive to the tested insecticide. The freshwater Indian catfish, *Heteropneustes fossilis*, exhibits behavioral changes associated with exposure to Decis. The main behavioral changes observed during the experiments were: erratic swimming, restlessness, operculum beats, profuse mucous secretion, loss of equilibrium, and death of exposed animals [17]. Not only deltamethrin—the active substance in Decis—but also other chemicals may be the cause of toxicity. Magdalan et al. [18] reported a patient's death as a result of Decis poisoning because of a hydrocarbon base (solvent naphtha), which is a component of the commercial form of the insecticide. In the described case, the detected aromatic hydrocarbons in the blood and lung tissue and their metabolites in urine confirmed the absorption of these substances from the gastrointestinal tract into systemic circulation. The main component of the pesticide—deltamethrin—was rapidly biotransformed by hepatic enzymes. Pesticides are a factor that protects the crop yield, but on the other hand, their use in agricultural practice for many years contributes to a significant decrease in the number and diversity of wild plants and animals [19].

However, global legislation allows the use of chemical compounds in agricultural, fruit, and vegetable production, the purpose of which is to kill organisms harmful to crops (pests, pathogenic fungi, weeds, and insects) [20]. As demonstrated here, the insecticide Decis® 2.5 EC had an impact on freshwater invertebrates *D. magna* and *H. incongruens*—freshwater zooplankton. These organisms are the foundation of the food chain in aquatic ecosystems, and if Decis® 2.5 EC in water (LOEC—at an average concentration >0.91×10^{-3}% (>0.23 mg L^{-1} of deltamethrin)) (Tables 3 and 6) has an adverse effect on their behavioral and physiological reactions, not only does it affect these organisms but also the entire ecosystem. In assessing the quality of aquatic ecosystems, not only laboratory tests but also in situ research are used. The assessment of the abundance and composition of phyto- and zooplankton made in this way allows not only to determine the toxicity of substances and pollution but also the characteristics of the aquatic environment, such as: hydrography, eutrophication, or seasonal variation [21,22]. Meanwhile, the results obtained in our experiment not only indicate specific changes in the tested organisms but

also prove that the tests Daphtoxkit F magna and Ostracodtoxkit F can be successfully used to detect toxic residues in water in areas treated with deltamethrin and other highly toxic pyrethroid pesticides.

5. Conclusions

Water pollution is a major global problem that requires continuous assessment and control of water resources at all levels. Water pollution affects the entire biosphere, including animals and plants living in these reservoirs. In almost all cases, the effect of exposure is detrimental not only to individual species and populations but also to entire ecosystems. The environmental risk assessment for the insecticide included direct effects on non-target aquatic invertebrates.

It was found that increasing Decis® 2.5 EC concentrations inhibited the swimming of the tested organisms. The most toxic concentrations were 15×10^{-3}% and 30×10^{-3}%. Initially, up to 135 min, the daphnia immobilization was less modified by Decis® 2.5 EC than the immobilization of ostracod. Then, *H. incongruens* (ostracod) was less sensitive to the tested insecticide's concentrations. However, after 360 min, the immobilization of daphnia and ostracod increased proportionally to the concentrations of Decis® 2.5 EC. The lowest observed effect concentration of Decis® 2.5 EC reducing the swimming of daphnia and ostracod by more than 20% was $>0.91 \times 10^{-3}$% (0.23 mg L^{-1} of deltamethrin).

This experiment demonstrated that *D. magna* and *H. incongruens* are good bioindicators of freshwater pollution with Decis® 2.5 EC. The results of this study also confirm that Daphtoxkit F magna and Ostracodtoxkit F are useful analytical tools for predicting the consequences of the insecticide's contamination of freshwater bodies. Furthermore, accurate data on the impact of insecticides on aquatic crustaceans may accelerate the progress of ecotoxicological research and allow assessment of the degree of environmental exposure to pesticides.

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