Use of Microbiotests to Compare the Toxicity of Water Samples Fortified with Active Ingredients and Formulated Pesticides

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ABSTRACT: In Portugal rice occupies a relevant position among other irrigated crops and, since treated water is frequently discharged to the surrounding water bodies, field studies to determine the influence of pesticides used in rice treatments to aquatic biota are being performed. In order to better understand the toxicity results obtained in field conditions, particularly the influence of the pesticide formulations in the toxic effects, laboratory studies were performed using water samples fortified with the active ingredient and the respective formulated product (commercial product) of some pesticides (molinate, MCPA, propanil, endosulfan, chlorfenvinphos) usually applied in Portuguese paddies. The range of concentrations tested reflects the rates used by the farmers in real field conditions. The toxicity values observed in the laboratory during 1998 with the microbiotests Daphtoxkit *magna* F^{TM}, Thamnotoxkit F^{TM}, Artoxkit M^{TM}, and Algaltoxkit F^{TM} are presented and discussed in this study. The results obtained until now suggest that organic solvents and surfactants present in some of the tested formulations may affect the toxicity of the sample. In fact, water samples fortified with formulated pesticides seemed to be, in the majority of the cases, more toxic than the respective active ingredients solutions, particularly in the case of the three tested crustaceans. The microbiotests used seem to be a useful tool to evaluate toxicity with relative low cost, rapidity, and simplicity, compared with the traditional tests. Environ Toxicol 15: 401–405, 2000

Keywords: microbiotests; paddy rice; aquatic organisms; toxicity; pesticides

INTRODUCTION

Rice, along with wheat and corn, is one of the three crops on which the human specie largely subsists. In fact, almost two billion people depend primarily on rice (Ronald, 1997). It is also an important crop in terms of pesticide consumption, particularly herbicides, since weeds are the main enemy of this cereal (Sattin et al., 1995; Woodburn, 1990). Since rice is an irrigated crop, the use of pesticides during cereal growth may affect the quality of the surrounding aquatic environment. Studies with the aim of clarifying the potential toxic effect of the pesticides used in the paddies to the aquatic biota are therefore important and should be performed.

In Portugal, some field and laboratory studies concerning the toxicity of surface waters from rice fields treated with pesticides to aquatic biota, are being performed (Cerejeira et al., 1998; Cerejeira et al., 1999; Pereira et al., 2000). In this study some results from laboratory experiments are presented. Microbiotests based on immobilized forms of the crustaceans *Daphnia magna, Thamnocephalus platyurus, Artemia salina*,

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and on the algae *Raphidocelis subcapitata* were used to determine the acute toxicity of water samples fortified with the most frequently applied pesticides in the Portuguese paddies (i.e., molinate, propanil, MCPA, chlorfenvinphos, endosulfan). A comparison between the toxicity obtained with water samples fortified with active ingredients and with commercial products, at different levels of concentration, was performed and the results obtained are presented.

MATERIAL AND METHODS

Pesticides

For this study five products (the herbicides molinate, propanil, MCPA, and the insecticides chlorfenvinphos and endosulfan) were selected, since they are frequently applied in the Portuguese paddies. Water samples were fortified with each of the above active ingredient (a.i.) and with the respective most used commercial product (c.p.).

Preparation of the Tested Solutions

In order to cover the range of the different pesticide concentrations used in real field conditions, different dosage levels (L/ha or kg/ha) were defined for the fortification of water samples of each commercial product (Table I). The lowest, the most used, and the highest dosages used by farmers as well as the recommended dosages, were considered. Since the exact amount of the insecticides used were unknown, for these products, residue levels measured in water samples from rice fields were also taken into account. The different dosages defined for each compound are represented as "C_x." After setting up the dosage levels to be tested, concentrations in water of c.p. and a.i. were calculated. For this purpose, concentrations of c.p. (μL/L or mg/L) were determined based on the defined dosages and on the depth of the water level in the rice field, either at the moment of its application, in the case of molinate and the two insecticides, or shortly afterwards, in the case of MCPA and propanil. Based on the c.p. concentration and on the a.i. content of each commercial product, the a.i. concentrations in water were calculated (mg/L). To obtain the required a.i. concentration, it was necessary to prepare "stock solutions" for each a.i. in acetone (acetone for residue analysis from Merck), since the amounts of a.i. used were not soluble in water. One-hundred mg molinate, 100 mg propanil, 20 mg MCPA, 20 mg chlorfenvinphos (*cis* and *trans*), and 20 mg endosulfan (α and β) were

TABLE I. Active ingredients, formulations, field water level, dosages, and concentrations tested

Active Ingredient (a.i.)	Commercial Product (c.p.) Formulation	a.i. Content	Field Water Level (cm)	Ref. (1)	Dosage (c.p. L/ha)	c.p. Concentration (μL/L)	a.i. Concentration (mg/L)
Molinate	Granule (GR)	7.5 g a.i./ 100 g c.p.	7.5	c1	20*	26.9*	2.00
				c2	40*	53.0**	3.98
				c3	60*	80.0**	6.00
				c4	80*	107.0**	7.95
MCPA	Emulsifiable concentrate (EC)	420 g/L	17.5	c1	0.10	0.06	0.24
				c2	0.50	0.29	0.12
				c3	1.50	0.86	0.36
				c4	2.75	1.60	0.67
Propanil	Emulsifiable concentrate (EC)	360 g/L	17.5	c1	8	4.5	1.62
				c2	13	7.4	2.67
				c3	18	10.2	3.67
				c4	25	14.2	5.11
Endosulfan	Emulsifiable Concentrate (EC)	380 g/L	10	c1	0.05	0.03	0.01
				c2	0.75	0.40	0.15
				c3	1.43	0.75	0.30
Chlorfenvinphos	Emulsifiable concentrate (EC)	240 g/L	10	c1	0.07	0.07	0.02
				c2	0.30	0.30	0.07
				c3	0.50	0.50	0.12

(1) Reference for the dosage level tested
* kg/ha.
** mg/L.

diluted in 100 mL of acetone. To evaluate the possible effects of this solvent to the tested organisms, toxicity tests were performed with a range of acetone concentrations from the lowest to the highest level used (i.e., 39.5 mg/L–3950 mg/L). The calculated concentration of the pesticides in water, are presented in Table I.

Toxicity Tests and Data Treatment

Toxicity testing was performed following the microbiotests methodology described in the respective standard operational procedure (SOP, 1998 a, b, c, d) included in the Toxkits package, from Creasel (i.e., Daphtoxkit F™ *magna*, Thamnotoxkit F™, Artoxkit M™, Algaltoxkit F™).

In the *Daphnia* test, immobility after 24 h and 48 h exposure was registered, while for *Thamnocephalus* and *Artemia* mortality was recorded after 24 h exposure. For the algal test, absorbance values obtained with a spectrophotometer (670 nm) (Hitachi U 2000) were registered at 0, 24, 48, and 72 h.

For *D. magna*, immobility percentages were considered as final values. For *T. platyurus* and *A. salina*, mortality percentages were considered. In the case of the algae *R. subcapitata* the percentage of growth inhibition was calculated. In order to carry out these calculations it was necessary to determine "multiplication factors" (MF) for the control and for each of the tested products. MFs were determined calculating the ratio between absorbance values at 72 and 0 h. Algal growth inhibition percentage was determined by the following expression: $[1 - (\text{MFproduct}/\text{MFcontrol})] \times 100$.

Since *A. salina* is a marine organism it was necessary to prepare a saline test solution of 15 ppt. Therefore, to each fortified solution crystalline NaCl was added in order to achieve that concentration.

Toxicity results presented in Figs. 1 to 5 are the average of the results obtained in three independent tests for each organism (in each of the three independent tests, three to four replicates were carried out, as is foreseen in the test methodology). To compare these values analysis of variance (ANOVA) was used ($p < 0.05$; $p < 0.01$; $p < 0.001$).

RESULTS AND DISCUSSION

In Fig. 1 the toxicity effects caused by water samples fortified with molinate a.i. and its c.p. to the aquatic species tested are presented.

From Fig. 1 it is possible to observe that molinate caused low toxicity (always less than 20% effect), except in the case of the algae *R. subcapitata*, in spite of molinate being considered as a very toxic product [e.g.,

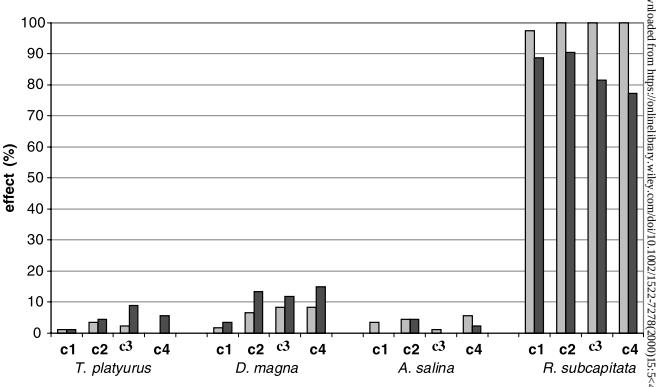

Fig. 1. Toxicity (% effect) to aquatic organisms caused by water samples fortified with active ingredient (▭) and commercial product (▬) based on molinate. (See Table I for c1–c4.)

to *Daphnia* 50% effective concentration (EC_{50}) 0.6 mg/L (Verschueren, 1983)]. The crustacean *T. platyurus* was the only organism where toxic effects caused by water samples fortified with c.p. were significantly higher ($p < 0.05$) than those caused by the respective a.i. solutions. For the algae, the opposite situation occurred, the difference being highly significant ($p < 0.001$). Concerning the other two crustaceans (*D. magna* and *A. salina*), significant differences were not observed. However, it is important to note that in all these crustacean tests, the test is considered valid if the immobility percentage in the controls does not exceed 10%. Therefore, in spite of using the statistical program to determine whether the difference is significant, in fact it is questionable if the mortality percentage close to this limit value can be interpreted as "significant."

Results obtained with water samples fortified with a.i. and c.p. based on the herbicide propanil (Fig. 2), showed that, except for *Thamnocephalus*, water samples fortified with a.i. caused higher toxicity than the respective c.p. solutions. *D. magna* and *R. subcapitata* are, in this case, the most affected organisms, particularly by the tested a.i. solutions (the differences between the acute toxicity registered with water samples fortified with a.i. and c.p. are significant ($p < 0.05$) or even highly significant ($p < 0.001$). The use of acetone may have influenced the results observed, since other solvents, like ethanol, have been reported as being highly toxic; e.g., to the algae *R. subcapitata* (El Jay, 1996). However, results obtained from the toxicity tests with the different acetone concentrations (39.5–3950 mg/L) revealed that it was not possible to establish a correlation between acetone concentrations and toxicity effects (r^2 values always lower than 0.4). On the other hand, studies performed by Leblanc and Sur-

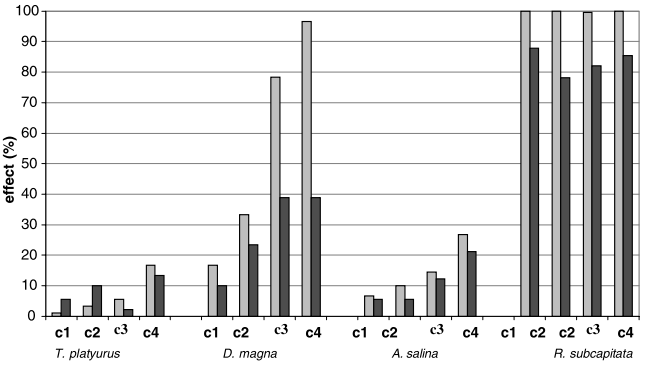

Fig. 2. Toxicity (% effect) to aquatic organisms caused by water samples fortified with active ingredient (▭) and commercial product (▬) based on propanil. (See Table I for c1–c4.)

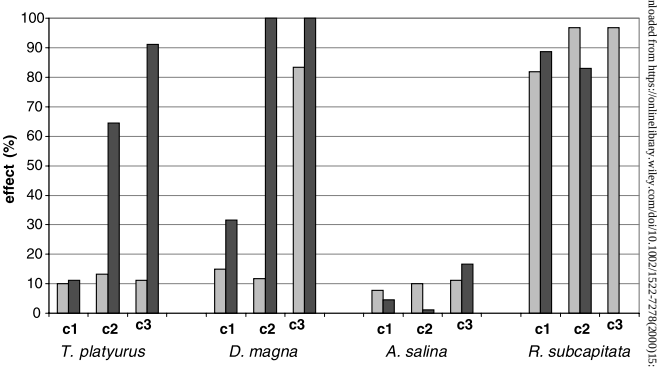

Fig. 4. Toxicity (% effect) to aquatic organisms caused by water samples fortified with active ingredient (▭) and commercial product (▬) based on chlorfenvinphos. (See Table I for c1–c4.)

prenant (1983) with acetone gave an EC_{50} (48 h) of 39,000 mg/L for *D. magna*, justifying, therefore, the low toxicity of this solvent, even in the highest concentration tested.

In Fig. 3 the percentages of toxic effect caused by water samples fortified with a.i. and c.p. based on MCPA are presented.

From the statistical data treatment it was possible to observe that *D. magna* was the only organism in which water samples fortified with MCPA c.p. were highly more toxic than a.i. solutions. (Fig. 3). On the contrary, in the *Thamnocephalus* test, a.i. solutions were always more toxic. Although, in most of the tested concentrations of algal test c.p. were more toxic than a.i., as was already noticed by Caux et al. (1996), a.i. solution C4 caused unexpectedly higher toxicity than c.p. In spite of the fact that *A. salina* showed lower toxicity than the other aquatic organisms, c.p. solutions caused, in general, higher toxic effects.

From the statistical comparison between the effects caused by water samples fortified with a.i. and c.p. based on the insecticide chlorfenvinphos, it is possible to conclude that there are significant differences between the toxicity of these solutions for *Daphnia* and *Thamnocephalus*, c.p. being more toxic than the respective a.i. solutions (Fig. 4). In two of the tested concentrations with algae and *Artemia*, a.i. solutions caused higher toxic effects than c.p. On the other hand, there was no explanation for the absence of toxicity in the highest concentration of the *R. subcapitata*.

The results obtained with the endosulfan solutions (Fig. 5) show that, in general, high toxicity was observed, except in *A. salina*, which was the least affected organism. For all the crustaceans, significant or highly significant differences (e.g., in *Thamnocephalus*) between c.p. and the respective a.i. solutions, were observed, the former being always more toxic. The algae was the organism most affected by the tested a.i. solutions.

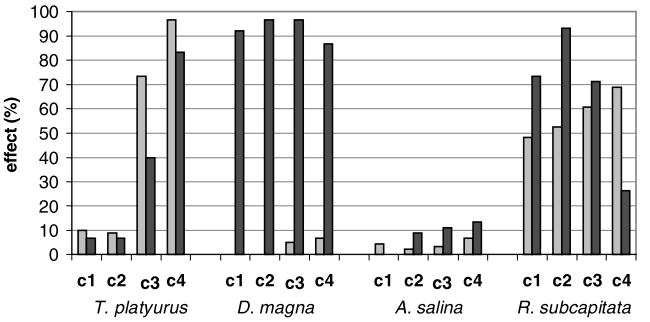

Fig. 3. Toxicity (% effect) to aquatic organisms caused by water samples fortified with active ingredient (▭) and commercial product (▬) based on MCPA. (See Table I for c1–c4.)

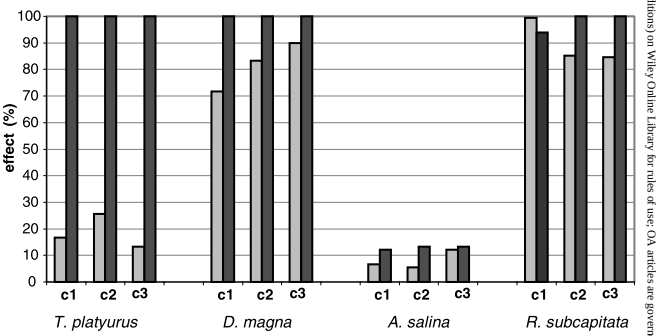

Fig. 5. Toxicity (% effect) to aquatic organisms caused by water samples fortified with active ingredient (▭) and commercial product (▬) based on endosulfan. (See Table I for c1–c4.)

CONCLUSIONS

From the comparison of results obtained with water samples fortified with active ingredients (a.i.) and commercial products (c.p.) using microbiotests it is possible to conclude that, except for propanil, commercial product solutions were significantly ($p < 0.05$) more toxic to *Daphnia* and *Thamnocephalus* than those prepared with the respective active ingredients. In Artemia, although commercial products caused, in general, higher effects the difference was not significant. The algae *Raphidocelis* was the only organism to which water samples fortified with active ingredients seemed to cause higher toxic effects, the difference being highly significant ($p < 0.001$) in the cases of molinate and propanil. Water samples fortified with molinate (c.p. and a.i.) were those that caused less toxic effects to the studied crustaceans. The other two tested herbicides seemed to be more toxic. Their formulation in emulsifiable concentrate may have contributed to this fact, due to the presence of organic solvents and surfactants, that could increase absorption of the a.i. by the organism.

The insecticides chlorfenvinphos and endosulfan seemed to cause higher toxic effects to the tested aquatic species than herbicides, particularly in the case of water samples fortified with commercial products. Water samples fortified with endosulfan (c.p.) were the most toxic to all the studied aquatic species.

The results obtained in this study indicate that microbiotests may be considered as being a promising tool, due to their rapidity, simplicity, and relative low cost, for an initial toxicity screening, namely to compare pesticide toxicity.

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