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Acute Toxicity of Malathion and the New Surfactant "Genapol OXD 080" on Species of Rice Basins

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The lower Guadalquivir River marshes (Seville, Spain) have approximately 35.000 ha dedicated to rice farming. When planting farmers usually apply Malathion to aid in controlling Chironomid larvae (bloodworm). These larvae feed on the seed endosperm and the developing roots of young seedling, resulting in poor crop establishment (Stevens and Warren 1994). The crayfish, *Procambarus clarkii* also adversely effects seedling establishment by removing the soil with their movement (Grigarick and Way 1982; Hill et al. 1982).

Apart from damaging rice fields, *Procambarus clarkii* is a commercialized species for human consumption. In fact, the lower Guadalquivir River marshes have become the main european producer and exporter of this crustacean (Gaudé 1984; Cano and Ocete 1994). These factors lead to a project (Integrated Management of Red Swamp Crayfish Populations in Rice Fields- An Application of Cleaner Technologies and Ecotechnology) subsidized by the EU and having as its main objective of controlling crayfish without decreasing either their production or the rice yield. To this end, a new surfactant, genapol OXD 080, developed by the Hoescht Company has been tested. This surfactant, which is applied at the same time as malathion has as its function reducing crayfish mobility. It does this by decreasing the breathing rate. Consequently the crayfishes stay immobile.

The present study was undertaken in order to determine in the laboratory the susceptibility of different species of the rice basins to the new surfactant as well as malathion.

MATERIALS AND METHODS

The species selected for this study (Table 1) were chosen because their high presence during the initial phases of rice production. They were obtained from the lower Guadalquivir River marshes (UTM 29SQB534246) throughout the rice growing season in 1996. Crayfish were obtained with a passive trap (fish trap); mosquito&h were collected using a pond net (mesh size 3 mm), while the remaining species were collected using a small sain (mesh size 250 (m). The

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collected specimens were taken to the laboratory. Crayfish and mosquitofish were adapted for 48 hours. The other species, except for *Daphnia magna*, were acclimated for 4 hours. From *D. magna*, only the ones that had ephippia were selected and separated in order to test the ones born in the laboratory.

Laboratory conditions were: temperatures of 25 ± 1° centigrade (for crayfish and mosquitofish, 20 ± 1° centigrade and 30 ± 1° centigrade) and photoperiod of 16:8 (Light:Dark). The water used for the tests was dechlorinated by aireation for 48 hours. During the testing individuals were not fed. Dissolved oxygen and pH were recorded at the begining and at the end of each test. Total hardness and conductivity were recorded at the begining of each test.

Tests with crayfish and mosquitofish were made in 44 L aquariums, during 96 hours. Aireation was provided for these experiments at the rate of 85 mL/min with an aquarium aerator. Tests for the remaining species were 24 hours in durations without aireation, and made in round recipients of 1 liter of capacity. The number of individuals used in teasted varied with species (Table 1). In each case 5 controls and 5 replicates were made with each test condition.

Table 1. Tested animal characteristics

Species	Common Name	Family	Size/Age	N
Procambarus clarkii	Red Swamp Crayfish	Cambaridae	7 cm	120
Gambusia affinis	Mosquitofish	Poeciliidae	27 mm	240
Chironomus sp.	Bloodworm	Chironomidae	3rd and 4th larval instar	750
Daphnia magna	Water flea	Daphniidae	≤ 24 hr	450
Cloeon sp.	Mayfly	Ephemeridae	3rd and 4th larval instar	300
Culiseta longieroelata	Mosquito	Culisidae	3rd and 4th larval instar	300

Mortality data were pooled and analysed using a computer program (Finney 1971) incorporating probit analysis. The toxicity of malathion was compared to that of genapol OXD 080 by calculating a ratio of the mean lethal concentrations estimated for each product. A ratio > 1.0 suggested that genapol OXD was more toxic than malathion. A ratio < 1.0 suggested that genapol was less toxic (Mayer and Ellersieck 1986). Survival was analysed by ANOVA (differences were considered statistically significant at ≤ 0.05).

RESULTS AND DISCUSSION

Environmental physical-chemical factors (Table 2) are something very important to be analysed, because Beyers and Myers (1996) according to Chapman and Cole (1982) establish that the degradation period for malathion may be predicted by the

values of pH and temperature (in our case it lasts approximately 90 hours). The toxicity of a product increases along with temperature (Sogorb et al. 1986) which is something valid also for genapol OXD 080, because the LC_{50} values obtained in the same species with different temperatures decrease while temperature increases.

Malathion is a chemical that exerts its toxic effects by inhibiting the enzyme acetylcholinesterase (AchE), causing disruption of the central nervous system (McEwen et al. 1991). This product and triclorfon are recommended for controlling Chironomids during the initial phases of rice production because they provide good initial mortality, moderate persistence and relatively low toxicity (Stevens 1991). According to preliminary tests of Genapol OXD 080 (20 ° centigrade), its total biodegradation happens after 20 days, and the recommended dose is 0.005 %.

The LC_{50} values obtained for the different species with malathion at 25°centigrade and genapol OXD 080 at different temperatures are shown on Table 3. LC_{50} value for each species using genapol OXD 080 is different depending on the tested temperature, it is lower at low temperatures and viceversa. LC_{50} value for *Chironomus* sp using malathion was impossible to obtain, because there was a mortality superior to 90 % for unknown reasons. LC_{50} value for *Culiseta longiareolata* using genapol OXD 080 had a very high standard error because of to low mortality during the test,

Table 2. Water characteristics by animal and chemical

Species		Disolved oxygen (mg/L)		pH		Total hardness (ppm $CaCo_3$)	Conductivity (μ siemens)
		0 hr	End test	0 hr	End test		
Procambarus clarkii	**Malathion**	5.5	6.3	7.7	7.8	194.9	287
	Genapol	5.5	5.9	7.7	7.7	142.8	322
Gambusia affinis	**Malathion**	5.6	5.1	7.8	8.1	194.9	327
	Genapol	5.8	5.5	7.9	7.8	142.8	383
Chironomus sp.	**Malathion**	5.9	5.9	6.7	7.9	107.1	298
	Genapol	6.2	5.8	7.7	7.9	107.1	312
Daphnia magna	**Malathion**	5.1	5.5	7.9	8.1	142.8	296
	Genapol	4.3	4.5	7.9	8.1	194.9	299
Cloeon sp.	**Malathion**	5.8	5.3	7.7	7.9	107.1	325
	Genapol	5.7	5.3	7.7	7.8	142.8	295
Culiseta longiareolata	**Malathion**	5.3	5.2	7.8	8	142.8	362
	Genapol	5.0	5.1	7.7	7.9	107.1	356

Table 3. Toxicity of malathion and genapol OXD 080

Species	Malathion (mg/L) $LC_{50} \pm SD$	Genapol OXD 080 (mg/L) $LC_{50} \pm SD$	Time Test (hr)	Temperature (°centigrade)	Ratio
Procambarus clarkii		10.7 ± 1	96	20	
	1.34 ± 0.2	6.95 ± 1.5	96	25	0.19
Gambusia affinis		0.22 ± 0.015	96	20	
	0.3 ± 0.075	0.19 ± 0.01	96	25	1.57
		0.12 ± 0.008	96	30	
Chironomus sp.	*	0.1 ± 0.029	24	25	< 1*
Daphnia magna		0.72 ± 0.12	24	20	
	0.00235± 1.85	0.33± 0.14	24	25	0.007
Cloeon sp.	0.0055 ± 0.012	0.15 ± 0.038	24	25	0.036
Culiseta longiareolata	0.017 ± 0.037	6.83 ± 166.4	24	25	0.0024

* Although the LC_{50} for Malathion could not be calculated, Stevens (1992) obtained a value of 8.5 ppb for *Chironomus tepperi*.

In the lower Guadalquivir River marshes malathion is usually used at a rate of 1.5 kg/ha, causing a massive mortality of all the species in the rice basin. This fact is specially important for Procambarus clarkii, because its production is strongly decreased (Cano and Ocete 1994). LC_{50} values (Table 3) obtained for the different species using malathion were very low. These results confirmed that malathion is very noxious.

In general, ours LC_{50} results are similar to other reports with the exception for Procambarus clarkii , LC_{50} value is 1.3 mg/L while for other authors is 50 mg/L (Cheah et al. 1979-80). This difference may be due to the crayfish stress condition, because of the recent drought in Iberian Peninsula Southern and also because of the before expositions to pesticides in the field. Compared to another freshwater species, the value for *Gambusia affinis* almost the same as for *Ptychocheilus lucius* (Beyers and Sikoski 1994) and very different to the one for Gila elegans (Beyers *et al* 1994). The differences for *Chironomus* (Stevens 1992) are due to the fact that there are different species. LC_{50} value cited in literature is $1.2x10^{-9}$µM/L(Vighi et al. 1991).

Genapol is applied at the same time as malathion, for it is important to know its effect on Chironomids. The LC50 values (Table 3) obtained for *Chironomus* sp (25° centigrade) with genapol are lower than the recommended application dose and consequently may be used for controlling pest species from the *Chironomus* genera.

The ratio calculated (Table 3) for the estimated mean lethal concentration of malathion and genapol OXD 080 (25° centigrade) to each species turned to be lower than 1 for *Procambarus clarkii, Chironomus* sp., *Daphnia magna, Cloeon* sp. and *Culiseta longiareolata,* and superior to 1 for *Gambusia affinis.* We

conclude that malathion is more toxic than genapol OXD 080, except for *Gambusia affinis.*

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