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Risk assessment of the herbicide Clomazone to aquatic life

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Introduction

Due to their widespread distribution and toxic nature, pesticides may have a serious impact on the aquatic environment. Clomazone (2-(2-chlorophenyl)methyl-4,4-dimethyl-3-isoxazolidinone) is a post emergence herbicide widely used in paddy rice fields in South Brazil, with activity against Gramineae like *Echinochloa crusgalli*. The recommended application rate (RAR) of the emulsible concentrate is 1.4 L/ha.

The high vapor pressure of Clomazone (1.44×10^{-4} mm Hg 25 °C) contributes to volatilization losses. Thus, the contamination of the aquatic environment by the herbicide may occur in aerial application. Other ways of the aquatic ecosystem contamination may occur by drainage from irrigated rice fields.

In this work, studies were conducted with two primary producers and with a primary consumer in order to evaluate the risk of Clomazone to aquatic life based on maximum application rates (MAR).

Material and methods

Chemical: Clomazone used in the toxicity tests was an emulsible concentrate containing 500 g per liter (GAMIT®, FMC Corporation). The nominal concentrations were based on the active ingredient.

Studies with algae: Stock cultures of the unicellular green algae *Selenastrum capricornutum* were grown in 250 ml flasks sealed with cotton bungs and containing 200 ml of an inorganic medium prepared as recommended by OECD (OECD 1981). Mature cells were exposed to 15.4, 27.5, 50.0, 90.0, 162.0 and 291.5 mg/L Clomazone in a total volume of 101 ml. Each system contained a nominal concentration of 2.10^4 cells/ml. The flasks were incubated in an orbital shaker at 20 ± 2 °C for 96 h under a continuos light of 3000 lux. Cell concentrations were determined microscopically at 0, 24, 48, 72 and 96 h using a haemocytometer. The 96 h EC50 value (concentration that inhibits 50 % of the growth rate) was estimated using a linear regression model with the variables probit of inhibition rate growth relative to the control vs. log doses. The 96 h EC50 for biomass loss was estimated in the same way as above using the variable probit of biomass reduction (cell number) relative to the control (OECD 1981).

Studies with macrophytes: The duckweed EC50 test was conducted with a clone taken from specimens collected originally on ponds located in South Brazil paddy rice fields. The duckweed was identified as *Lemna valdiviana*. The stock clone of *L. valdiviana* was maintained in axenic culture using OECD medium with agar (OECD 1981). Prior to fifteen days before test, plants were transferred aseptically to OECD liquid medium in order to grow exponentially, to attain sufficient biomass, and to be adapted to the experimental environment. Exposure was conducted in 250 ml Erlenmeyer flasks filled with 150 ml of OECD medium. Clomazone was added to final concentrations of 14.3, 28.6, 57.2, 114.3 and 228.3 mg/L. There were four replicates for each concentration and for the untreated control. Four plants (9–11 fronds) were inoculated in each flask and maintained during 96 h in a controlled environment growth chamber at 20 (±1) °C under illumination of 2500 lux and 16 L : 8 D photoperiod. The test solution was renewed once in 48 h. The numbers of fronds were counted at the beginning and after 96 h of exposure. The 96 h EC50 value was calculated from the numbers of fronds increments using the same procedure for the algal test.

Studies with invertebrates: The cladocerans *Daphnia similis* were reared 10 liter glass aquaria containing reconstituted water prepared as recommend by Hosokawa et al. (1991) and enriched with macronutrients, trace elements and vitamins (Elendt & Bias 1990). The stock experimental animals were fed with *S. capricornutum*. Physico-chemical data were pH = 7.8, conductivity = 327 µS/cm and total hardness = 111 mg/L. The acute test consisted of exposing groups of 10 neonates to six concentrations (5.0, 9.2, 16.6, 30.0, 54.0 and 97.2 mg/l) of Clomazone and a dilution water control. All treatments were replicated twice. The test beakers containing a total volume of 20 ml were maintained in a temperature controlled environmental chamber set a 20 °C ± 1 °C and light cycle of 16 L : 8 D. Immobilization of the test organisms was recorded after 48 h. The 48 h EC50 value and their corresponding 95 % confidence intervals were estimated using the probit analysis (Finney 1971).

The maximum application rates (MARs) for acute and chronic effects were estimated based on the EC50/3 and the EC50/10 (CETESB 1992), respectively, when a direct input of the herbicide attain a 10 cm water column.

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Table 1. EC50 values (in mg/L) for three aquatic organisms exposed to the herbicide Clomazone.

Organism	Exposure Time (h)	EC50	95 % Fiducial Limits	Parameter Evaluated
S. capricornutum	96	14.5	–[a]	biomass increment inhibition
	96	31.9	–[a]	growth rate inhibition
L. valdiviana	96	32.2	–[a]	biomass increment inhibition
D. similis	48	13.8	10.7 to 17.2	immobilization

[a] The 95 % fiducial limits were not estimated because usually methods for calculate them are not adequate for quantitative variables (WALSCH et al. 1987).

Table 2. Maximum application rate of Clomazone formulation (expressed in times of the RAR) for protection of aquatic organisms against toxic effects calculated on the basis of a direct input in a 10 cm water column.

Species	Effect	
	Acute	Chronic
S. capricornutum	6.9	2.0
L. valdiviana	15.3	4.6
D. similis	6.5	2.0

Results and discussion

The EC50 for Clomazone ranged from 14.5 to 32.2 mg/l (Table 1). The EC50 data show that among the tested species *D. similis* was the species that showed more susceptibility to Clomazone and the *L. valdiviana* was more resistant to the herbicide effects. We observed that the EC50 for growth rate was twice as high than for biomass increment in the *S. capricornutum* test. This suggests that it is important to define

Table 3. EC50 values of herbicides to aquatic organisms and the relative toxicity to Clomazone according our data.

Herbicide	Organism	Exposure Time (Days)	EC50 (mg/l)	Relative Toxicity	Reference
Glyphosate	*Chlorella fusca* (microalgae)	1	377	0.08	FAUST et al. (1994)
	Daphnia pulex (cladocera)	2	7.9	1.5	HARTMAN & MARTIN (1984)
	Lemna minor (macrophyte)	14	2.0	16.1	HARTMAN & MARTIN (1984)
Simazine	*Chlorella fusca* (microalgae)	1	0.073	430	FAUST et al. (1994)
2,4 D	*Chlorella fusca* (microalgae)	1	88.9	0.35	FAUST et al. (1994)
Alachlor	*Daphnia pulex* (cladocera)	2	10.4	1.3	HARTMAN & MARTIN (1985)
GARLON® (61 % triclopyrBEE)	*Daphnia pulex* (cladocera)	4	1.2	11.5	SERVIZI et al. (1987)
TREFLAN® (trifluralin)	*Alonella* sp. (cladocera)	2	0.06	230	NAQVI et al. (1985)
CUTRINE PLUS® (9 % copper alkalonamine)	*Alonella* sp. (cladocera)	2	11.3	1	NAQVI et al. (1985)
MSMA (monosodium methanear sonate)	*Alonella* sp. (cladocera)	2	39.3	0.35	NAQVI et al. (1985)
Triclopyr TEA	*Daphnia magna* (cladocera)	2	1170	0.01	GERSICH et al. (1985)
Picloram	*Daphnia magna* (cladocera)	2	68.3	0.2	GERSICH et al. (1985)

which parameter is more appropriate for toxicity evaluation. In this work, the lowest value observed, was used for safety reasons. The results indicate that the formulation tested is slightly toxic to the three species studied according to the EPA toxicity classification.

Table 2 shows the estimated maximum application rates (MAR) that would not cause acute and chronic effects to the test organisms. The MAR's observed for acute effects ranged between 6.5 and 15.3 times the recommended application rate (RAR). This indicates a low acute risk for the aquatic environment. On the other hand, the values for protection against chronic effect are close to the RAR.

For a comparison purpose with our results, toxicity data of other herbicides to aquatic organisms are shown in Table 3. Data suggest that Clomazone is as toxic as Glyphosate, Alachlor and Cutrine plus® to cladocerans, and it is 430 times less toxic than Simazine to microalgae. On the other way, it is about 100 times more toxic than Triclopyr TEA to cladocerans.

Although Clomazone impair the chlorophyll synthesis in target plants (GAMIT) the susceptibility of non-photosynthetic organisms, i.e. *D. similis*, seems to be in the same grade as primary producers, i.e. *S. capricornutum*.

Conclusions

According to this work:
1) The toxicity of Clomazone was highest to *D. similis* and *S. capricornutum*.
2) Clomazone was slightly toxic to the test species.
3) The use of Clomazone at the recommended application rate did not cause deleterious acute effects on the organisms studied.
4) The acute toxicity of Clomazone for cladocerans is similar to Glyphosate, Alachlor and Cutrine plus®.
5) Other mechanisms, despite photosynthesis inhibition, are important in the toxicity of Clomazone to non-target organisms.

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Authors' address:

C. M. JONSSON, A. H. N. MAIA, C. J. A. FERREIRA and E. O. RIBEIRO, EMBRAPA/National Research Center for Monitoring and Environmental Impact Assessment. P.O. Box 69, 13.820-000, Jaguariúna/SP, Brazil.