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# Environmental Impact of Two Molluscicides: Niclosamide and Metaldehyde in a Rice Paddy Ecosystem

S. M. F. Calumpang,[1] M. J. B. Medina,[1] A. W. Tejada,[1] J. R. Medina[2]

[1]National Crop Protection Center and [2]Entomology Department, College of Agriculture, University of the Philippines Los Baños, College, Laguna 4031, Philippines

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The golden apple snail, *Pomacea canaliculata*, is a major pest of rice. It was first introduced in the Philippines as an alternative protein source but due to its rapid reproductive rate and voracious feeding on rice seedlings, it soon became a pest. Pest management strategies include cultural, mechanical, biological and chemical methods. The two chemicals recently approved by the Philippine Fertilizer and Pesticide Authority for use as molluscicides in rice, include niclosamide (2',5-dichloro-4'-nitrosalicylanilide) and metaldehyde (2,4,6,8 tetramethyl- 1,3,5,7- tetroxocane). Niclosamide is a chemical recommended for the control of golden apple snail in transplanted and direct seeded rice (Palis et al., 1993). Proper timing of application as well as water management were critical for maximum efficacy (Dupo, et al.,1994). Metaldehyde has been found to be effective in controlling the golden apple snail in transplanted rice at the rate of 0.5 kg ai/ha (*Litsinger and Estano, unpublished data*).

Field trials were therefore conducted to determine the fate of niclosamide and metaldehyde in a rice paddy ecosystem as well as to assess the toxicity of paddy water effluent to fish.

## MATERIALS AND METHODS

Supervised residue trials were conducted to determine the fate of niclosamide and metaldehyde in a rice paddy ecosystem at the Central Experiment Station, University of the Philippines, Los Baños between March 27, 1991 to December 31, 1991. Niclosamide (Bayluscide 250 EC) was sprayed at the rate of 0.3 kg a.i./ha while 6% metaldehyde pellets were broadcast at the rate of 4 kg product/ha one day after transplanting (DAT) of rice seedlings. Paddy water, maintained at 13 in. depth, was allowed to overflow into canals where cages containing five tilapia and five carp fishes were positioned at 0, 1, 5, 20 and 100 m away from the treated paddy. Fish mortality was recorded after 24 hr exposure at 0, 1, 3 and 7 days after initial exposure .

Random soil and water samples were collected at 0 (<3 hr), 1, 2, 3, 7, 14 and

*Correspondence to:* A. W. Tejada

23 days after application. At harvest, (127 DAT) leaves, stalk, and rice grain samples were also collected. All samples were weighed and frozen prior to analysis. Three replicates of each substrate were used.

Paddy soil and water were analyzed for niclosamide residues using the procedure of Luhning et al. (1979) with some modifications. In this case, 200 ml of water sample was used and 50 ml of 0.5 N $H_2SO_4$ was added prior to partitioning with 10 ml of chloroform. Likewise, 50 g of soil was extracted three times with 250, 125 and 125 ml of acidic acetone. The pooled filtrate was concentrated to 50 ml prior to partitioning with 30 ml of hexanes after the addition of 400 ml of 0.1 N sulfuric acid, and 5 ml of saturated NaCl solution.

The plant sample was extracted using the procedure of Muir and Grift (1980) with some modifications. The niclosamide residues were extracted by blending with 20% water in methanol (3 x 150 ml) for 1 min/extraction instead of shaking and was filtered.

Niclosamide was determined as the alkali derivative, 2-chloro- 4-nitroaniline (CNA) using the hydrolysis method of Luhning et al.(1979) and by gas liquid chromatography. Water samples were analyzed using a Hewlett Packard 5840 gas chromatograph equipped with a nitrogen-phosphorus flame ionization detector (NP-FID), glass column 6 ft x 2 mm i.d. packed with a mixture of 1.5% OV-17+1.95% OV 210 on 80-100 mesh Chromosorb W-HP; temperatures (°C): injection port, 250; column, 190; detector, 300; gas flow (ml/min): 50, 3, 30, for air, hydrogen and nitrogen, respectively.

The soil and plant samples were analyzed using a Tracor 560 gas chromatograph equipped with an electron capture detector (ECD), glass column 6 ft x 2 mm i.d. packed with a mixture of 2.5% DC-200+2.5% QF-1 on 100-120 mesh Aeropak 30; temperatures (°C): injection port, 250; column, 200; detector, 300; nitrogen gas flow of 30 ml/min. The concentration of niclosamide was calculated using the following equation,

$$Concentration, ug/g = [A \times (327.1 + 73.7)] + B$$

Where A = amount of CNA (ug); B = weight of sample (g).

Water samples (1L) were analyzed for metaldehyde residues by extraction with dichloromethane (3 x 100 ml). The combined organic extracts was extracted with sodium hydrogen sulfite solution (20 ml) for 5 min, washed with distilled water (2 x 50 ml), evaporated to dryness under slight vacuum and taken up in 20 ml toluol. Paddy soil samples (100 g) were extracted using the same procedure for water with slight modifications. Extraction using dichloromethane (2 x 100 ml) was by successive shaking for 1 hr followed by another shaking for 30 min. Stalk and grain (100 g) or leaves (50 g) were extracted by shaking with 200 of ml dichloromethane for 1 hr. The same steps, after extraction of water as indicated above, were followed.

Depolymerization and derivatization of the samples was done by adding 5 ml of 2,4-dinitrophenylhydrazine solution to the toluol solution and allowing it to stand to 2 hr (Selim and Seiber,1973). The organic phase was washed with distilled water (2 x 20 ml), dried over anhydrous sodium sulfate and rinsed with 10 ml toluol. The filtrate was evaporated to dryness under slight vacuum and dissolved in n-hexane.

Quantitative estimation of the metaldehyde-derivative was made using a Hewlett Packard 5840A gas chromatograph equipped with an N-P flame ionization detector (NP-FID). The parameters used were as follows: Glass column 3.5 ft x 2 mm i.d. packed with 2% of OV-101 on 80-100 mesh Chromosorb W-HP. Temperatures (°C): Injector 250, column oven 180, detector 300. Gas flow rate (ml/min): nitrogen 30, air 50, hydrogen 3.

## RESULTS AND DISCUSSION

A recovery of 86% was obtained for niclosamide in both spiked control samples of paddy water and paddy soil. The minimum detection limit in paddy water and soil, obtained at the 5:1 signal:noise ratio, were 0.002 mg/L and 0.03 mg/kg, respectively.

There was a rapid decline in niclosamide residues in paddy water from an initial level of 0.96 mg/L to 0.02 mg/L at two days after application (Figure 1). Degradation followed pseudo-first order kinetics showing a half-life of 0.3 day. Residues found three days after spraying (DAS) were below the detection limit of 0.002 mg/L. Niclosamide residues found two days after application and thereafter were below the $LC_{50}$ (24 hr) of 0.25 mg/L for golden snail (*Bajet, C.M.,personal communication*).

Niclosamide in paddy soil was below the detection limit of 0.03 mg/kg on the day of application (Figure 1). Maximum concentration of 0.1 mg/kg was attained at 1 DAS. However residues were rapidly degraded to levels below the minimum detection limit of 0.03 mg/kg at 2 to 28 DAS. At harvest, niclosamide residues were below the detection limit of 0.03 mg/kg in rice leaves, stalk and grain indicating that the use of niclosamide as a molluscicide in rice production does not lead to persistent residues in the various components of a rice paddy ecosystem.

Recoveries of the method for metaldehyde at a spiking level of 0.1 mg/kg were 95.5% for soil, 110% for water, and 96% for plant materials. Paddy water taken within 3 hr after application did not contain residues above the minimum detection limit of 0.03 mg/L in water. The dissolution of the pellets gave rise to residues which remained fairly constant at 1.58 to 1.47 mg/L for 1 to 3 days after application. The formulation used constantly released the active ingredient thus maintaining the metaldehyde level for snail control. Residues could be detected only up to 9 days after application. Its degradation pattern followed pseudo first-order kinetics showing a half-life of 0.27 day. Metaldehyde residues detected up to nine days after application were above the $LC_{50}$ (24 hr) for golden snail which is 0.04 mg/L (*Bajet, C.M.,personal communication*).

Release of paddy water into irrigation canals may therefore result to mortality of snail species used for human consumption and, although it may not be common practice, should therefore be avoided.

The deposition of metaldehyde to paddy soil was only apparent three days after application. Residue levels ranged from 0.053 to 0.127 mg/kg at 3 to 14 days after application, reaching a peak at 0.127 mg/kg at 14 days after application. At harvest, metaldehyde residues in rice leaves and grain were below the minimum detection limit of 0.016 mg/kg while 0.075 $\pm$ 0.024 mg/kg were detected in stalk. The use of metaldehyde as a molluscicide in rice production does not lead to persistent residues in the various components of a rice paddy ecosystem.

The toxicity of niclosamide and metaldehyde to two fresh water fish species, commonly reared for human consumption, was also assessed. Niclosamide was toxic to carp at the rate of 0.250 and 0.375 kg a.i./ha. at the time of application where 100% mortality was observed from 0 to 100 m away from the treated paddy (Table 1). The practice of draining the paddy water takes place 60 days after transplanting and therefore the problem of fish toxicity is not a matter of concern. The dilution effect from unexpected torrential rains accompanying typhoons would be enough to lower the concentration to those below toxic levels. Carp mortalities were not observed at 1, 3 and 7 days after application of niclosamide under overflowing conditions, showing the rapid decline of residues in paddy water to non-toxic levels.

Niclosamide was slightly toxic to tilapia as only 20% and 33% mortality was observed inside treated paddies on the day of application. A corrected percent mortality of 26% was observed for tilapia at 1 m from the treated paddy using 0.375 kg a.i./ha, at one day after application. This effect could be due to the desorption of niclosamide from the soil matrix which was previously adsorbed when the initial overflowing was done. Mortalities observed in paddies treated at 0.250 kg a.i./ha were no different from the natural mortalities observed in untreated control paddies. In areas where rice-fish culture is practiced, introduction of fish in niclosamide treated paddies have to be done at least three days after molluscicide application.

The difference in fish sensitivity to niclosamide is due to differences in metabolism. It has been reported that the metabolic rate of smaller fish is greater than that of large fish because of differences in body surface. The greater

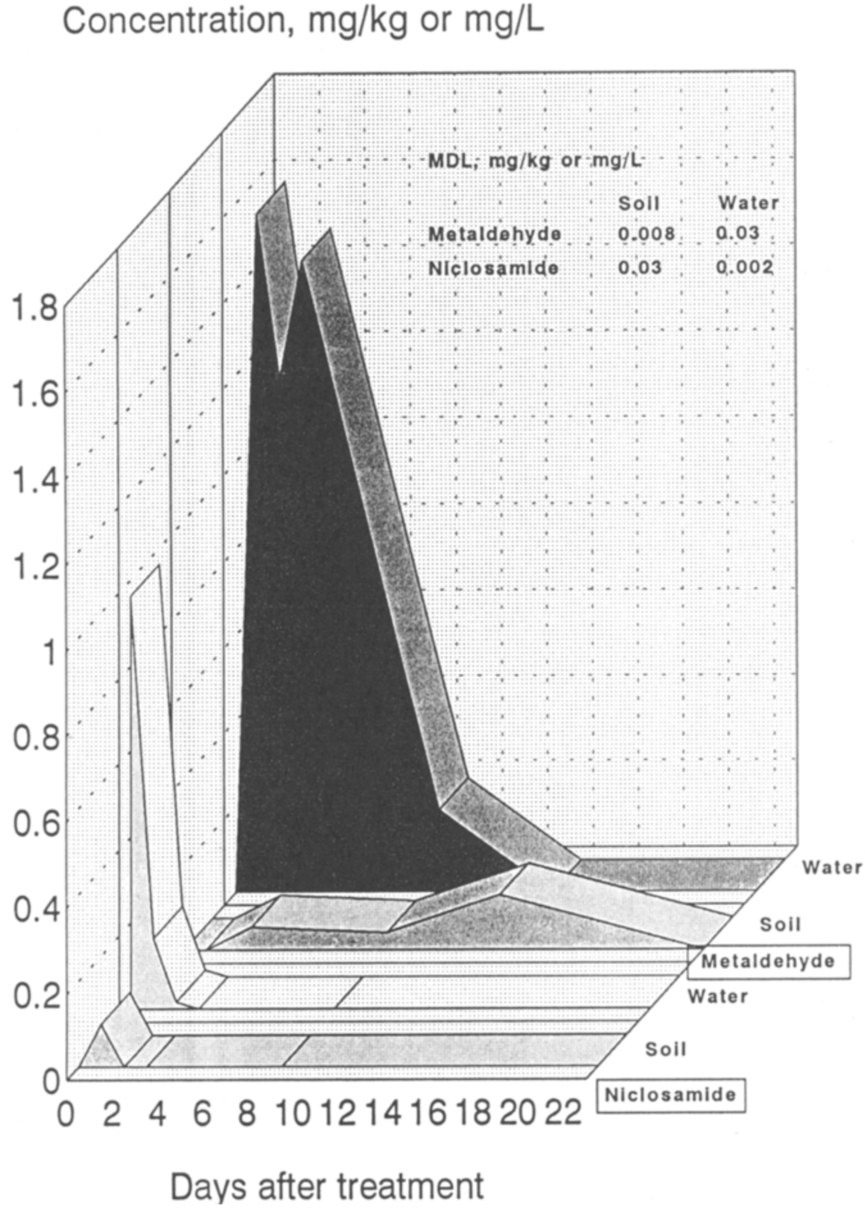


**Figure 1. Metaldehyde and niclosamide residues in paddy soil and water.**

Table 1. Fish toxicity assessment (% mortality) of rice paddy water treated with niclosamide.

| Distance | CARP | | | | TILAPIA | | | |
|---|---|---|---|---|---|---|---|---|
| | *Days after exposure* | | | | *Days after exposure* | | | |
| | *0* | *1* | *3* | *7* | *0* | *1* | *3* | *7* |
| Treatment 1: 0.375 kg a.i./ha | | | | | | | | |
| 0m | 100 | 0 | 0 | 0 | 20 | 7 | 0 | 0 |
| 1m | 80 | 0 | 0 | 0 | 0 | 33 | 0 | 0 |
| 5m | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 20m | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 100m | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Treatment 2: 0.250 kg a.i./ha | | | | | | | | |
| 0m | 100 | 0 | 0 | 0 | 33 | 7 | 0 | 0 |
| 1m | 100 | 0 | 0 | 0 | 0 | 7 | 0 | 0 |
| 5m | 92 | 0 | 0 | 0 | 0 | 7 | 0 | 0 |
| 20m | 100 | 0 | 0 | 0 | 0 | 7 | 0 | 0 |
| 100m | 100 | 0 | 0 | 0 | 0 | 7 | 0 | 0 |
| Treatment 3: Control | | | | | | | | |
| 0m | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1m | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 0 |
| 5m | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 20m | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 0 |
| 100m | 0 | 0 | 0 | 0 | 15 | 20 | 0 | 0 |

Table 2. Fish toxicity assessment (% mortality) of paddy water treated with metaldehyde.

| Distance | CARP | | | | TILAPIA | | | |
|---|---|---|---|---|---|---|---|---|
| | *Days after exposure* | | | | *Days after exposure* | | | |
| | *0* | *1* | *3* | *7* | *0* | *1* | *3* | *7* |
| Treatment 1: 1 kg a.i./ha - Wettable Powder | | | | | | | | |
| 0 m | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1m | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 5m | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 20m | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 100m | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 0 |
| Treatment 2: 0.5 kg a.i./ha - Pellets | | | | | | | | |
| 0m | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1m | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 5m | 33 | 0 | 0 | 0 | 13 | 0 | 0 | 0 |
| 20m | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 100m | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Treatment 3: Control | | | | | | | | |
| 0m | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1m | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 5m | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 20m | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 100m | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

oxygen consumption of the smaller fish/unit weight was evidence of the relatively greater caloric need of smaller fish (Schaeperclaus, 1933). Uptake of niclosamide residues by several fish species vary depending on the species. Niclosamide residues could be detected in tilapia, ten-pounder and goby while residues could not be detected in milkfish and tarpon (Calumpang, 1994).

Metaldehyde was relatively non-toxic to fish (Table 2). Fish mortalities were not observed in the treated paddies at zero day. However, 13 and 33% mortality (carp) and 13% (tilapia) were observed at a distance of 1, 5 and 20 meters, respectively. It appears that these mortalities may be only due to physical factors from the oral intake of the pellets itself which floated into the test fish cages inasmuch as the rate used (0.5 kg a.i./ha) is lower than that used for the wettable powder (1.0 kg a.i./ha). From this observation, the wettable powder formulation would be a better formulation.

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