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Carbofuran Acute Toxicity to Freshwater Algae and Fish

F. A. Antón, E. Laborda, P. Laborda, and E. Ramos

Ecotoxicology Group. Environmental Sciences Center, Spanish Council for Scientific Research, Serrano 115-dpdo. St., Madrid 28006, Spain

Carbamates are insecticides used in crop protection for their low persistence, wide action spectra and ability to control pests. Carbofuran (2,3 dihydro-2,2 dimethyl benzofuran-7-yl-methyl carbamate) is an acaricide, insecticide and nematicide used in agriculture (Worthing 1987). As with other chemicals, ecotoxicity studies are needed in order to know its effects on the aquatic and terrestrial organisms and their potential risk as environmental pollutant (Butler,1977; Anton et al,1990).

Planktonic algae and fish are important organisms of the aquatic ecosystems, affected by chemicals, especially pesticides (Butler 1977). Only few scientists had studied the ecotoxicity of carbofuran on algae (Kar and Singh 1978; Padhy 1985; Megharaj et al 1989) and on fish (Gill 1980).

In the present work it have determined the acute ecotoxicity of carbofuran (75 %, a.i.) on planktonic algae Chlorella pyrenoidosa Chick., and on Carassius auratus L. (F. Cyprinidae). The algae and fish are two very important species in the trophic chains of the spanish freshwater ecosystems.

MATERIALS AND METHODS

According to the OECD (1984) and EEC (1988) protocols, Chlorophyta algae are suitable species to study the effects of chemicals on their growth population. They were cultured "in vitro" in a Kuhl and Lorenzen modified medium (1964). The initial inocule of algae was supplied to us by the Culture Collection of Algae and Protozoan of the Botany School, Cambridge University (U.K.).

The algae stock cultures were kept in a liquid medium at neutral pH with constant illumination and aeration. An aliquot of algae was inoculated once a wk into a new

Send reprint requests to Dr. FA Antón at the above address.

culture medium with salts. Every 8-10 wk the cultures were renewed from algal cells cultured in 4 % agar solid medium. All procedures, the inoculation of new media and the culture handling were made under sterile conditions.

Culture medium was prepared for the bioassay using an aliquot of the stock culture medium of 6 d old Chlorella pyrenoidosa in their growth exponential time, to give 10^4 cells/mL. In the bioassay several doses of technical carbofuran (75 %, a.i.) were assayed; their effects were analyzed on three replicates by dose. 250 mL. Erlenmeyer flasks were used for the algal bioassay. All doses of carbofuran were diluted with a suitable solvent (acetone 1 %). Their replicates were also diluted from a stock solution of the technical pesticide. Flasks were inoculated with the exact quantity of a dilution of technical carbofuran in solvent. Finally, they were filled to 100 mL. with the algal culture medium of salts to 10^6 cells/mL.

The flasks were placed in an Astrolab 60 (Madrid, Spain) climatic chamber. It were controlled the light (photoperiod 16 hr light, 8 hr darkness), temperature (20 ± 1 °C and humidity up to saturation. Everyday flasks were randomly changed in their places in the climatic chamber to give similar conditions of illumination. They were also stirred to make the aeration easier and to avoid the algal precipitation in the flasks.

The Optical density (O.D.) values of chlorophyll a were daily measured at 430 nm wavelength (Barrington,1983) using a Perkin-Elmer UV/VIS model Lambda 1A colorimeter (Madrid, Spain). When bioassay was finished, all O.D. data was converted to number of cells/mL. using a regression analysis program from a computer. From the stock algae culture (in the first day of bioassay), was made a calibration curve with a pool of dilutions from algal culture medium. In the following days, O.D. values of the samples (3 mL by flask or replicate) were measured in the colorimeter. The effects of several assayed doses of technical carbofuran, of the solvent and of the control on algal growth were analyzed when they were compared to the obtained values of O.D. in the calibration curve (O.D. against to the number of cells/mL).

Results are given in number of cells/mL for all samples after the calculation of the growth rate in all flasks with algae. When the bioassays were finished, the percentage of algal growth inhibition was calculated according to EEC (1988) method. With the data for 96 hr., the IC 50 (value which inhibit 50 % of algal growth populations exposed to technical carbofuran), and the NOEC (value of dose without effects on the algal growth) were calculated from the obtained data every 24 hr in algal flasks: control, solvent and flasks with exposed algae to carbofuran. Previously

this data were translated to cells/mL values, an the mean value for all three replicates were also calculated. The IC 50 values from previous bioassays were useful to know more exactly the range of pesticide doses that could cause a growth inhibition on the algal populations under observation.

The assayed doses of technical carbofuran (75 %,a.i.) on Chlorella pyrenoidosa C. were from 0.01 to 1,000 mg/l.

Carassius auratus L., from commercial fisheries (6 cm length and 4 to 8 g weight), with 7 d in acclimation before the bioassays, in 60 L aquaria were kept. They were suitable aeration and illumination in running tapwater that was previously dechlorinated. They were fed a commercial and standard food for cold freshwater fish. The temperature of the aquatic medium was 20±1°C.

Bioassay procedures were in agreement with the International and Official Organizations (EPA 1975; EEC 1984; OECD 1984) and a special "mobility index" was also used with the following scale of values: 0: fish without mobility; 1: fish with limited mobility; and 2: fish with normal mobility. These indexes and their mean value were recorded in all aquaria and bioassays.

Fish bioassays were made without removal of either carbofuran or the aquatic medium, for 96 hr. Fish were exposed to technical carbofuran (75%, a.i.) in dechlorinated running tapwater. Insecticide was added to water with a solvent (acetone 1 %), and then it was poured into the solvent and treated aquaria. A control aquarium had only received dechlorinated running tapwater, but without solvent and without carbofuran. Anomalies which arose and the fish mortality on the bioassay time were recorded for all aquaria every 24 hr and also when the bioassay was finished. Before the last bioassay, were made other prevoius bioassays that gave useful information about the range of effective doses of carbofuran on fish.

The experimental conditions for all bioassays were following: Six fish in 12 L. illuminated aquaria (photoperiod 16 hr light, 8 hr darkness), with aeration and 20 ± 1 °C of temperature, but without food. The fish were weighed when the bioassays began and finished. Every 24 hr were also taken interesting notes about their conditions and visible anomalies. Daily pH, temperature, salinity and dissolved oxygen in the aquatic medium were recorded in all aquaria.

When the bioassays were finished, it were calculated the percentage of mortality by dose and the LC 50 (lethal mean dose). The assayed doses of carbofuran were: 2, 5, 9, 11, 12, 15, and 20 mg/L. As in the algal bioassays were

compared the "dose-response" values in a regression analysis "logarithmic-probit" with a computer program (Abou-Setta et al 1986). Finally, was calculated the exact value of LC 50 (96 hr).

RESULTS AND DISCUSSION

IC 50 (96 hr), mean value of the growth inhibition on algal populations of Chlorella pyrenoidosa C. was 272.64 mg/L of technical carbofuran, or 204.48 mg/L of activ ingredient. The pH values of the culture media from 0 to 96 hr were near to neutrality (6.8-7.8) in all flasks with exposed to carbofuran and control algae. The results of the growth inhibition on algae by carbofuran can be seen in table 1.

For higher doses than 750 mg/L of technical pesticide (562.5 mg/L, a.i.), the algal growth was completely inhibited. Crystals or turbid suspension suspensions were developed by the reaction of carbofuran with the aquatic medium of algae.

From 0.01 mg/L of technical carbofuran, the algal growth seems to be enhanced by pesticide, and also with the used solvent. Their interaction with the toxic seems to stimulate the development of algal population, but in other bioassays with higher doses than 1 mg/L of technical pesticide, there was an inhibition on the algal population growth: from 2% to 8% between 1 and 50 mg/L of technical carbofuran. The effects of doses from 75 to 1,000 mg/L were more importants, reaching the 100 % of growth inhibition in several doses; sometimes there was not growth and it were completely null the growth rates in these cultures. In control and solvent flasks the growth rates were more high than 0.07%, and in the all samples of other bioassays the growth rates were similar.

When carbofuran doses had surpassed 5 mg/L, this pesticide seems to have caused a decrease on algal growth, especially for doses higher than 25 mg/L. The response to toxic action was greater when the dose had increased (25 % to 35 %, approximately for 75 to 100 mg/L of technical pesticide). For doses higher than 100 mg/L, algal growth inhibition was near 100%.

On Carassius auratus L., LC50 (96 hr) was 10.25 mg/L, of technical pesticide (7.9 mg/L, a.i.). When fish were exposed to 20 mg/L, it was a mortality level of 100 %. The NOEC value (dose without effects) was smaller than 2 mg/L (1.5 mg/L, a.i.). The solvent (DMSO, 1 %) changed the clear aquatic medium in turbid medium in the higher doses. All days, in most aquaria, pH was near neutral (6.8-8.0) and the temperature was between 15°C and 25°C. The dissolved oxygen concentration was always higher than 80 %; the salinity increased up to the end of bioassays, but only

slightly, especially in the higher doses of carbofuran without surpassing 130 mg/L (from 64 to 122 mg/L); it was only moderately increased when bioassay time was finished (96 hr).

In respect to the mobility index on exposed fish, their mobility generally decreased when the dose increased (from 10 to 20 mg/L) in all time periods (3, 24, 48, 72 and 96 hr) of the bioassay. Solvent and control aquaria contained the fish with the maximum value of this mobility index (2.00).

The unexposed fish to carbofuran did not suffer some impact when they were placed into the aquatic medium at the beginning of the bioassays. Conversely the exposed fish suffered a shock after 3 hr and kept some annihilates as if they were exhausted. They are showing lethargic movements, although in the following days they survived. This effect probably could be due to the fast degradation of pesticide in the aquatic medium, although mortality had continued after the initial shock in the aquarium with the higher assayed dose (20 mg/L).

When the "logarithmic-probit" regression analysis to know the "dose-response" relation in all bioassays was made, it was verified that the regression coefficients were in the confidence interval.

In a review (from 1946 to 1975), Butler (1977) concluded that planktonic algae were affected by pesticides, especially carbamates, on their population growth, oxygen yield, dry weight, metabolism and on other aspects, always depending on the level of absorption of these toxic products. Other scientists have studied the carbamate incidence on cyanobacteria and on Chlorella pyrenoidosa (Kar and Singh 1978; Padhy 1985). These authors analyzed the pH changes of the media and the influence of several light intensities. In this work only it have studied the ecotoxicity of carbofuran "in vitro" bioassays. Some authors have also reported the algal growth stimulation by low doses of carbofuran (Standynk et al 1971; Padhy 1985), that it´s in agreement with our data and probably depend on the carbon assimilation of algal cells from the degradation of insecticide on natural ecosystems. In respect to the toxic levels on algae, for doses higher than 700 mg/L, it was not easy to study the "in vitro" carbofuran ecotoxicity on algae, because the maximum level of solubility is close to this concentration in the aquatic medium (Edwards 1976). According to these results, carbofuran prevents the growth of Chlorella pyrenoidosa at doses higher than 1 mg/L, and more strongly at higher doses than 25 mg/l. At 100 mg/L in the aquatic medium, it could cause the disappearance of the algal population. It is important to study the bioaccumulation processes of this insecticide on algae to

Table 1. Growth rate and percentage of the growth inhibition on algal population of Chlorella pyrenoidosa Chick., exposed to concentrations of technical carbofuran (75 %, a.i.).

Bioassay	Dose	Growth Rates	% Growth Inhib.
I	Control	16.12 ± 0.05	
	Acetone	18.17 ± 0.60	+ 12.72 ± 3.46
	0.01 mg/L	17.55 ± 0.41	+ 8.88 ± 2.52
	0.05 mg/L	17.94 ± 0.45	+ 11.27 ± 3.12
	0.10 mg/L	18.03 ± 0.67	+ 11.83 ± 3.92
	0.50 mg/L	17.32 ± 0.43	+ 7.45 ± 2.45
	1.00 mg/L	17.56 ± 0.60	+ 12.72 ± 3.46
II	Control	82.80 ± 0.02	
	Acetone	81.20 ± 0.02	1.13 ± 2.54
	1.00 mg/L	77.80 ± 0.04	5.26 ± 3.30
	5.00 mg/L	82.10 ± 0.04	0.04 ± 2.11
	10.00 mg/L	81.70 ± 0.06	0.51 ± 3.18
	25.00 mg/L	80.10 ± 0.06	2.53 ± 3.52
	50.00 mg/L	77.80 ± 0.04	5.26 ± 2.53
III	Control	35.12 ± 0.04	
	Acetone	33.37 ± 0.08	4.97 ± 2.37
	75.00 mg/L	24.05 ± 0.20	31.45 ± 6.63
	100.00 mg/L	24.21 ± 0.06	31.94 ± 3.33
	500.00 mg/L	25.34 ± 2.41	27.82 ± 4.49
	1,000 mg/L	Turbid medium	Turbid medium
IV	Control	78.13 ± 0.05	
	Acetone	75.23 ± 0.14	7.41 ± 1.59
	100.00 mg/L	0.00 ± 0.00	100.00 ± 0.00
	250.00 mg/L	0.00 ± 0.00	100.00 ± 0.00
	500.00 mg/L	15.70 ± 0.90	80.03 ± 28.20
	750.00 mg/L	Turbid medium	Turbid medium
	1,000 mg/L	Turbid medium	Turbid medium

Growth rates. Mean values for 3 replicates (flasks). The data are given in 10^6 cells/µL/day.

% Growth Inhibition. All data are mean values for 3 replicates or flasks. In the first bioassay (I), there was a growth on algal population, but not growth inhibition.

know if low chronical doses could affect to algal growth like Christie said (1969)

Several papers about carbofuran and carbamates effects on fish, other aquatic organisms and risks to this freshwater fauna were pointed out (Gill 1980; Woodward and Mauck 1980). With the results of this work could be thought that more additional studies about their toxicity and metabolism on fish are needed to better understand their true risk to environment.

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