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Acute Toxicity of Technical Captan to Algae and Fish

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Many research studies have revealed the effects of environmental pollutants, especially pesticides, on nontarget freshwater organisms such as algae and fish (Soeder et al 1969; Hermann 1975; Butler 1977; Wright 1978). Recently, the International Organizations have recommended the undertaking of several bioassays to assess the ecotoxic risks to these nontarget organisms and their environment (EEC 1988; EPA 1975; OECD 1984; ISO 1984).

Captan (N-trichloromethylthio cyclohex-4-n-1,2-dicarboximide) is a fungicide used on foliage and seeds for plant protection (Worthing 1987). Several scientific papers have described their toxicological aspects, but only few have described their ecotoxicological effects (Antón et al 1990). The aim of the present study is to assess in vitro the ecotoxic effects of technical captan (60.2 %, a.i., Aragonesas Co., Madrid, Spain) on algae and on spanish freshwater fish. They are nontarget organisms commonly used in the ecotoxicogical studies of chemicals. Chlorella pyrenoidosa Chick., unicellular microalgae and Carassius auratus L., spanish goldfish are the freshwater organisms that it were used in these bioassays.

MATERIALS AND METHODS

The experimental procedures used were according to the OECD and EEC guidelines for testing chemicals (EEC 1984; Hermann 1975; OECD 1984). EPA (1978) and EEC (1988) had published the protocols for bioassays with the planktonic algae Chlorophyta. It were used the growth inhibition on algal populations as parameter to assess the acute ecotoxicity of pesticides and other chemicals. Algae were cultured "in vitro" in a Kuhl and Lorenzen modified medium (1964). The initial inocule of Chlorella pyrenoidosa C., was supplied to us by the Culture Collection of Algae and Protozoan of the Botany School, Cambridge University (U.K.).

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The stock cultures of algae 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 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 for bioassays were prepared using an aliquot of the stock culture medium of 6 d old of Chlorella pyrenoidosa in their growth exponential time, to give 10^4 cells/mL. In the bioassay several doses of technical captan (60.2 %, a.i.) were assayed and their effects were analyzed on three replicates by dose. 250 mL Erlenmeyer flasks were used for the algal bioassay. All doses of technical captan were diluted with a suitable solvent (acetone 1 % or DMSO 1 %, dimethylsulfoxide). Their replicates were also diluted from a stock solution of the technical fungicide. Flasks were inoculated with the exact quantity of a dilution of technical captan 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 all samples (3 mL by flask or replicate) were measured in the colorimeter. The effects of several assayed doses of technical captan, of solvent and of 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 fungicide), and the NOEC (value of dose without effects on the algal growth) were calculated every 24 hr in algal flasks: control, solvent and flasks with exposed algae to captan. 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 fungicide doses that could cause a growth inhibition on the algal populations under observation.

The assayed doses of technical captan (60.2 %, a.i.) on Chlorella pyrenoidosa C. were from 0.05 to 50 mg/l, or 0.0301 to 30.1 mg/L of pure fungicide (100 %, a.i.).

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 kept with 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). Bioassays were made without removal of either captan or the aquatic medium, for 96 hr. Fish were exposed to technical captan (60.2 %, a.i.) in dechlorinated running tapwater. Fungicide 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 fungicide. 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 previous that gave useful information about the range of effective doses of captan on fish.

The experimental conditions for all bioassays were the 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 bioassays were finished, it were calculated the percentage of mortality by dose and the LC 50 (lethal mean dose). The assayed doses of technical captan on fish were from 0.25 to 10 mg/L, equivalent to doses from 0.1505 to 6.02 mg/L of pure fungicide (100 %, a.i.).

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

In the algal bioassays to study the effects of technical captan (60.2 %), IC 50 (96 hr) was 73.89 mg/L (or 44.5 mg/L of pure fungicide). This value was calculated with a regression analysis "logarithmic-probit", and the correlation coefficient was 0.94, with a significance level of 99.99 %. The NOEC value (96 hr), or the high dose of captan without effects on algal growth was 10 mg/L of technical fungicide (or 6.02 mg/L of pure captan). This dose in the aquatic medium of algae caused an inhibitory response lower than 10 % of inhibition of the algal growth of Chlorella pyrenoidosa C.

In the static bioassay of fish, LC 50 (96 hr) was 1.34 mg/L of technical captan (or 0.89 mg/L of pure fungicide). Also, at the 96th hr of the bioassay, for doses higher than 3 mg/L (or 1.86 mg/L, a.i.), was obtained the LC 100 value (total mortality on exposed fish in the aquaria with fungicide). During bioassays with fish, pH and salinity of the media with captan were slightly increased with the addition of fungicide to the aquatic media. Conversely, the dissolved oxygen concentration decreased in these media. When bioassays were finished (96 hr), control aquarium showed a very clean aquatic medium, but the aquaria with fungicide showed an aquatic media very turbid. This turbidity was probably caused by the fish residues or by the suspended captan or their degradation products on water (this fungicide was not quite soluble in the aquatic medium).

A limited amount of information is available on the ecotoxicological aspects of captan and on their metabolism processes which occur in the microalgae. It also occur about their ecotoxicity on the natural or in vitro cultured populations of phytoplanktonic algae. Freshwater algae that were cultured on enriched cultures showed a growth stimulation both with 1 mg/L of nabam or of captan in its medium; although later, its growth probably occurred due to the selection of a resistance form (Lazaroff 1967).

Butler (1977) reported that for doses lower than 1 mg/L, captan inhibited the algal development but not its population growth on axenic cultures of Nostoc muscorum and on other species of green and blue-green algae: in these algae, growth was inhibited by low doses of captan (Moore and Doward 1968). Captan also inhibited the autotrophic growth of 37 varieties of Chlorella spp., and only 3 of these varieties showed resistance to this fungicide. Two

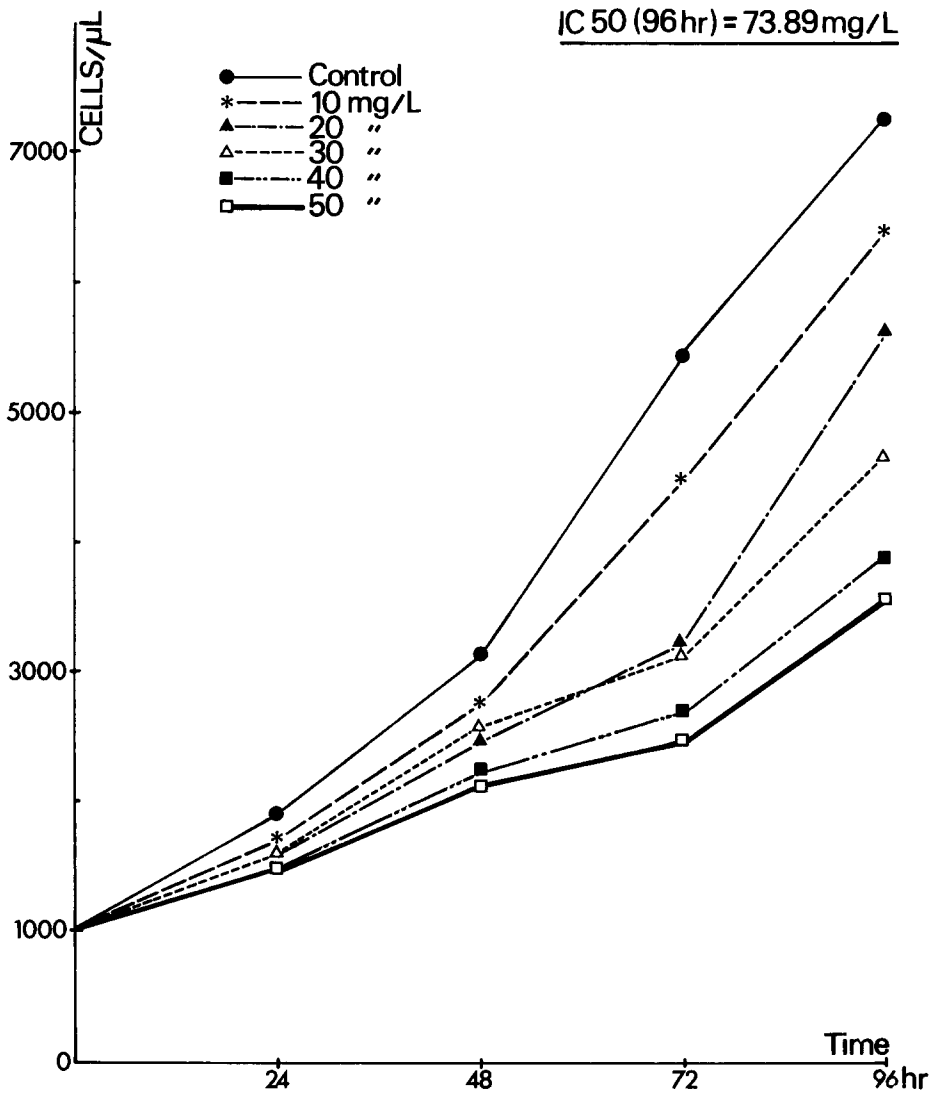

Figure 1. Effect of technical captan (60.2 %, a.i.) at several doses on Chlorella pyrenoidosa Chick. growth.

species of the green microalgae Scenedesmus spp. showed tolerance to captan up to concentrations of 50 mg/L and they did not show growth inhibition that normally was induced by this fungicide at low doses (Soeder et al 1969). In Chlorella, resistance to captan did not seem to be specific for different species. Butler et al (1975) reported that concentrations of captan in the aquatic medium from 0.01 to 0.08 mg/L decreased the growth of the

freshwater phytoplankton.

However, to know the effects of captan on algae and its reaction against this or other chemicals, only axenic and very controlled cultures can be used. Always must be used careful experimental conditions (Wright 1978).

Moreover, algae are considered very useful freshwater organisms to study the bioaccumulation processes of pesticides (Vance and Drumond 1969; Geyer et al 1984). It has been reported that microalgae may be very important organisms in the degradation processes of pesticides in the natural freshwater ecosystems, at least for the aromatic chemicals that Wright (1978) has pointed out.

After this study it can only say that the acute ecotoxicity of technical captan (60.2 %,a.i.) that was measured by its IC 50 (96 hr) value, was 73.89 mg/L (or 44.5 mg/L of pure captan). This value only describes the inhibition on algal growth in the exposed populations to this fungicide, but it can not describe other effects that could appear in the natural ecosystems where this phytoplanktonic specie lives.

An important problem in the ecotoxicity studies is the lack of solubility of pesticides, such as captan, in the aquatic medium of algae and fish. On the culture medium of algae the maximun dose that can be added is 50 mg/L of technical captan with DMSO or acetone as solvents. Higher doses up to 80 mg/L, it were not possible to dissolve in the aquatic medium. For this reason it is not easy to assess the acute ecotoxicity of captan on algae and fish to higher doses from their solubility level in water.

The IC 50 was calculated with the aid of a computer programm of regression analysis "probit-logarithmic" using the dose and the response data, but it were never reached the 50 % of growth inhibition of the algal population in the bioassay. Moreover, captan is an unstable fungicide that at alkaline pH can be degraded, and as the algal medium had a pH near 8.0, it was likely that the quantities of this technical fungicide would be degraded in the bioassay time. Figure 1 shows the algal growth inhibition curve due to captan with the assayed doses in these algal bioassays. (96 hr).

The acute ecotoxicity on freshwater fish bioassays of Carassius auratus L., had been noted before. Probably the degradation of captan (or perhaps its volatilization, precipitation or its absorption into the walls of aquaria) must be fast as we verified after the daily observations in the exposed aquaria with low and high assayed doses of this fungicide. There were 0.26 mg/L of pure captan in the beginning of bioassay in the exposed aquaria to the initial dose of 1 mg/l, varying to 0.03 mg/L of the pure pesticide

at the 48 hr of bioassay time. These measurements were made after determination of captan residues in the water of aquaria by chromatographic methods.

In the beginning of bioassays, captan caused an important shock on the exposed fish when it was poured into the aquatic media. At the 3rd hr of bioassay time, living fish acted more normal in the polluted aquatic medium, but when bioassay finished the acute ecotoxicity of captan to fish was smaller at low doses of technical fungicide.

It can be seen that captan was very toxic for Carassius auratus L., but more studies about the toxic aspects, chronic toxicities on these fish and their degradation in the freshwater aquatic medium are needed. It only can to conclude that this fungicide is highly ecotoxic on Carassius auratus L., but their risks in the freshwater ecosystems would not be very important because it can be degraded and so would decrease fastly their concentrations into the aquatic media.

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