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Carbofuran Acute Toxicity to *Eisenia foetida* Savigny. Earthworms

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Carbamates are insecticides widely 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 which is normally used in agricultural crops (Worthing 1987). As other chemicals, and environmental pollutants as pesticides, the ecotoxicity studies related with the effects on soil organisms are needed in order to know them and to prevent the potential risk of these environmental pollutants on the terrestrial ecosystems (Anton et al 1990a).

Earthworms are more important organisms of the soil invertebrate fauna. Their beneficial actions in the agricultutral soils has been reported in several studies from many years ago (Bouche 1984; Van Gestel 1991). Only few scientists had studied the ecotoxicity of carbofuran on earthworms (Sternensen et al 1973; Gilman and Vardanis 1974; Fischer 1976; Tomlin 1974; Sternensen 1979; Cathey 1982; Roberts and Dorough 1984 and 1985).

In the present work it has determined the acute ecotoxicity of technical carbofuran (75%, a.i.) on Eisenia foetida Sav., selected earthworm to study the effects of chemicals on the soil invertebrate fauna of terrestrial ecosystems (Van Gestel 1991) and proposed earthworm for the ecotoxicity bioassays of chemicals by the International Official Organizations (OECD 1984; ECC 1985 and 1988).

MATERIALS AND METHODS

The earthworms species used, Eisenia foetida, was cultured at an average ambient temperature of 20°-22° C, using horse manure as the food source. The earthworms used in the ecotoxicity bioassays were adult with a well-developed clitellum, selected depend on their sexual maturity, each

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weighing 300-600 mg, and acclimated several days in a medium containing fermented horse manure at half-darkness (20±2°C), high humidity and pH near 7.0. Every 6-8 wk new nutritive medium was added.

The adult earthworms were exposed to the technical carbofuran (75%, i.a.), in three types of bioassay: A) "residual film" or by direct contact with pesticide; B) in immersion bioassays in a suspension of carbofuran; and C) in artificial soil. First is a bioassay that gives some idea about the range of ecotoxic response on the exposed earthworms. Several doses, control and solvent (acetone 1%) were assayed in the three types of bioassay.

In the "residual film", earthworms were placed in glass Petri plates with imbibed filter paper with a known diameter (to know the exact quantity of pesticide -mg/cm^2-) with 1 mL. of pesticide dilution. After solvent evaporation, 1-2 mL water was added and earthworms were placed. With a plastic film to permit inner aeration, plates were covered and holed. 5-10 replicates for each tested dose were kept in a big box at the darkness to ambient temperature for 48-72 hr. Every 24 hr, mortality and general aspect of all individuals were recorded.

In the immersion bioassay, adult earthworms were submerged in a suspension of know doses of pesticide for 30 minutes. 4-7 replicates for doses were prepared. Earthworms washed in distilled water and were put in glass Petri plates with filter paper imbibed in 1-2 mL of distilled water. Finally, they were covered similarly that former bioassay was.

In the artificial soil bioassay, was prepared a soil with fermented horse manure (10 %), fine quartz sand (70 %) and kaolin (20 %), with a pH of 6.7-7.2. 200 g of soil were placed in plastic boxes (15 x 10 x 3 cm), moistened with distilled water (40 mL/box) and then were sprayed with doses of carbofuran, in dilutions of solvent, homogenizing into the soil after solvent evaporation. 4-5 adults previously weighed were placed in each box, covered with parafilm and holed for aeration. 3-4 replicates (boxes) by dose were used, and other for control and solvent. It was also prepared a last box to control humidity. All boxes were kept into a large wooden box in the darkness to ambient temperature. Every 7 days were observed and weighed the earthworms; were also recorded the mortality, malformations, mobility and general aspect of individual health of earthworms, and also the hatched cocoon number.

Acetone was used as solvent in all bioassays. In the artificial soil bioassays, the assayed concentrations were put in mg of technical carbofuran (75 %) by kg (dry weight) of artificial soil prepared. The assayed concentrations technical carbofuran (75 %) on the three types of bioassays

were: From 0.1 to 50 mg/L (a.i.) on the "residual film" or direct contact and on the immersion bioassays. In the artificial soil bioassays the assayed concentrations of insecticide were: from 0.1 to 100 mg/kg (dry soil weight, a.i.).

When the bioassays were finished, it were calculated the percentage of mortality by each tested concentration and the LC50 (lethal mean concentration) for 48 and 72 hr in the "residual film" and in immersion bioassays, and for 7 and 14 d in the artificial soil bioassay. It were compared the "concentration-effect" values in a regression analysis "logarithmic-probit" with the aid of a modified computer program (Abou-Setta et al 1986), and finally, was calculated the exact value of LC50. Moreover it was also verified that the regression coefficients were in the confidence interval.

RESULTS AND DISCUSSION

LC50 (48 hr) or the mean lethal dose to Eisenia foetida S. was 58.54 mg/L of technical pesticide (43.9 mg/L, a.i.) and LC50 (72 hr) was 24.58 mg/L (18.43 mg/L, a.i.) in the "residual film" or acute ecotoxicity bioassay by direct contact. From 5 mg/L of technical carbofuran, anomalies appeared as the swelling of clitellum on the exposed earthworms, and after 48 hr, when they were exposed to the high dose (40 mg/L, or 30 mg/L, a.i.).

LC50 (48 hr) was 26.14 mg/L of technical carbofuran (19.61 mg/L, a.i.) and LC50 (72 hr) was 0.95 mg/L (6.79 mg/L, a.i.) on the exposed earthworms to immersion bioassays. There was a little effect after the immersion on 0.1 mg/L (0.075 mg/L, a.i.) of the carbofuran suspension at 48 and 72 hr. The earthworms appeared to be shrunken and with malformations on several individuals when they were exposed at doses higher than 0.1 mg/L of technical carbofuran (24 and 48 hr).

In the artificial soil bioassay (14 d), LC 50 was 4.125 mg/kg (or 3.09 mg/kg, a.i.). In control and solvent boxes, at the 7th and 14 th d of bioassay, the soil was mixed more than on the treated boxes with insecticide, especially at high doses than 0.5 mg/L of technical carbofuran. Moreover the loss of weight in exposed earthworms was higher than in controls, and principally at doses as high as 1 mg/kg and up to 7.5 mg/kg, after 14 days. The exposed earthworms never had hatched cocoons, and only few cocoons were hatched on the unexposed earthworms in control and solvent boxes.

The correlation between the three types of bioassay is not easy to understand, and as Van Gestel (1991) said studying other organic chemicals, the comparison of the obtained

results of the direct contact or "residual film" bioassay with those obtained in artificial soil, demonstrated that the first bioassay, and probably also the immersion bioassay, they have not predictive value for the chemical toxicity in soil. Moreover the pH and the organic matter content influence on the soil adsorption of these chemicals, having an influence on the soil ecotoxicity of chemicals to the earthworms (Van Gestel and Van Dis 1988)

However the LC50 values obtained from the acute ecotoxicity bioassays do not inform too much about the true potential risk to earthworms, and neither about their sublethal effects on the same organisms caused by chemicals, although can serve to clasify these chemicals according with their ecotoxicity (EEC 1988).

Many carbamates had demonstrated to be very toxic to the earthworms (Edwards and Lofty 1977) and to kill them rapidly (Edwards and Thompson 1973). Other studies of carbofuran ecotoxicity on earthworms had also shown that this insecticide is very toxic (Sternensen et al 1973; Gilman and Vardanis 1974; Fischer 1976; Sternensen 1979; Cathey 1982). The studies of Roberts and Dorough (1984 and 1985) also had showed that carbofuran is an insecticide very toxic to these soil organisms, more than other assayed insecticides. These data are in agreement with the data in our study where the carbofuran has been shown to be very toxic in three types of bioassay, more than other pesticides studied in our research group (Antón et al 1990b).

Perhaps the carbofuran contributes to diminish the biomass of earthworms in agricultural soils as Tomlin and Gore reported (1974). Nevertheless the related studies about the degradation of this insecticide into the agricultural soils by the microbian action (Read 1983 and 1986; Read and Gaul 1983; Goring et al 1974) has demonstrated that their persistence in soils it was not very prolonged. Despite of their high toxic levels to the earthworms, this insecticide can rapidly disappear from soils, and especially from the planted soils by hydrolithic processes (Brahmaprakash and Sethunathan 1985).

However it would be interesting to analyze the sublethal effects of carbofuran on earthworms knowing their degradation in soils to better understand their risk to the soil fauna and environment.

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