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Acute Toxicity of the Fungicide Captan to the Earthworm *Eisenia foetida* (Savigny)

F. Anton, E. Laborda,* and P. Laborda

Soil Science and Plant Biology Institute of CSIC,
C/ Serrano 115-Dpdo, Madrid 28006, Spain

Many research studies have investigated the effects of pesticides, on earthworms, both in laboratory biotests (*Anton et al, unpublished work* ; Cathey, 1982; Inglesfield, 1984; Karnak and Hamelink, 1982; Lebrun et al, 1981). and under field conditions (Edwards and Lofty, 1972; Lofs-Holmin, 1981 and 1982; Tomlin and Gore, 1976). Captan was evaluated by Edwards and Lofty (1972) and Cook and Swait (1975) under field conditions, but not under laboratory conditions. The purpose of the present study was to assess the laboratory toxicity of the technical 60.2 % (Aragonesas S.A.) fungicide Captan N-(trichloromethylthio) cyclohex-4-ene-1,2-dicarboximide) to the earthworm Eisenia foetida Savigny, according to the OCDE and ECC guidelines for testing chemicals (OCDE, and 1984; CEE, 1988).

MATERIALS AND METHODS

Earthworms used were selected on the basis of sexual maturity, each weigthing between 300-600 mg, and acclimated for several days in a medium containing fermented horse manure, with pH near to neutrality, high relative humidity and 20º + 1ºC in the darkness.

Three biotests were used: Contact or "Residual Film", "Inmersion" and "Artificial soil". The tests and their conditions were perfomed according to the Guidelines of International Organizations, EEC and OCDE specially.

In the first test, earthworms was placed in glass Petri plates with imbibed filter paper with a known diameter (to know 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 plastic film, to permit inner aireation, plates were covered and holed. 5-10 replies for each tested dose were kept in a big box at the darkness to ambient temperature for 48-72 h. Every 24h. mortality and general aspect of all individuals were noted.

In the inmersion test, adults earthworms were submerged in a suspension of know doses of pesticide for 30 minutes. 4-7 replies for doses were prepared. Earthworms washed in distilled water and in glass Petri plates with filter paper imbibed in 1-2 ml. distilled water were placed . Finally, they were covered similarly that forward bioassay was.

* Send reprint requests to E. LABORDA at the above adress

In artificial soil biotests, a soil with fermented horse manure (10%), fine quartz sand (70%) and kaolin (20%), pH near 7.0, was employed. 200 g. 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 captan dilutions and homogenizing into the soil after solvent evaporation. 4-5 adults previously weighed were placed in each box, covered with parafilm and holes for aireation. 3-4 replies (boxes) by dose, and another for control and solvent were used; a last box to control humidity was also prepared. The boxes were kept in another big one in the darkness to ambient temperature. Every 7 days was observed,weighed and mortality, malformations, mobility and general aspect of individual health earthworms and cocoon number formed, were noted.

Acetone was used as the solvent in the soil and "Residual film" biotests (1%), dimethylsulfoxide (DMSO, 2 ppm) in the inmersion tests. In soil tests, the dosages were expressed in mg of technical Captan (60.2%) per Kg (dry weight) of artificial soil prepared.

The dosages was used as follows:
a) "Acute toxicity by contact or "Residual film": 1, 10, 100, 200, 400, 600, 800, 1000 and 10000 ppm, similar to 0.602, 6.02, 60.2, 120.4, 240.8, 361.2, 481.6, 602 and 620 actual ppm.
b) "Inmersion biotests: 1, 100, 1000, 10000, 11000, 12000, 13000, 14000 and 15000 ppm, similar to 0.602, 60.2, 602, 6020, 6622, 7724, 7286 and 8426 actual ppm.
c) "Artificial soil biotests: 1000, 2000, 3000, 4000 and 5000 ppm, similar to 602, 1204, 1806, 2408 and 3010 actual ppm of captan.

In contact and inmersion biotests, ten replicates were prepared for each dose and for water and solvent controls. In soil biotests three boxes by five individuals were the replicates used.

In a) and b), at 24, 48 and 72 hours, the number of live earthworms and their anomalies appearing in each Petri dishes was recorded. In c), at 7 and 14 days, the mortality, anomalies appearing, weight of individuals and loss of humidity also was recorded.

Calculation of LC_{50} for each biotest was made by "logaritmic-probit" analysis with a computer program (Abbou-Setta et al, 1986).

RESULTS AND DISCUSSION

Results of three biotests are shown in the tables 1-4: Tables 1 and 2 shown the curve "dose-response" of technical captan for 48 and 72 hours respectively in the contact or "residual film" biotests. Tables 3 and 4 shown results of the inmersion (48 hr. and 72 hr.) biotests.

LC_{50} (48 hours) was 121.16 to 132.14 ppm of technical captan (60.2 %), similar to 73.2 - 79.54 actual ppm of pesticide, but there is mortality on doses higher than 100 ppm (60.2 ppm) of technical pesticide.

Affected earthworms died bursting by the clitellum and expelling their internal fluides and many individuals, not dead, malformations various suffered.

LC_{50} (72 hours) was 79.31-88.64 ppm of technical captan used (60.2 %), similar to 45.93-53.36 actual ppm of captan, having mortalities also from 100 ppm (60.2 % actual ppm).

Table 1. Dose-Response data in the residual film or acute toxicity by contact biotest (48 hours).Log-Probit curve regression and correlation

Dose ppm (Tech. captan)	% Death (Y)	Log. X	Probit Y	Expected Probit	Expected Y
10	0.01	2.3026	1.2810	1.1722	0.000
100	20.00	4.6042	4.1584	4.5857	33.929
200	90.90	5.2983	6.3356	5.6133	72.986
400	91.60	5.9915	6.3796	6.6409	94.955
600	91.60	6.3969	6.3796	7.2420	98.751
1000	99.99	6.9078	8.7190	7.9992	99.864

Probit Y = -2.2413 + 1.4824 log X; r-squared = 0.936;
LC_{50} (48 hr.) = 121.16 - 132.14 ppm of technical captan, similar to 73.2 - 79.54 actual ppm.

Table 2. Dose-Response data in Residual Film or Acute Toxicity by contac biotests (72 hours).Log-Probit curve regression and correlation

Dose ppm (Tech. captan)	% Death (Y)	Log. X	Probit Y	Expected Probit	Expected Y
10	0.01	2.3026	1.2810	0.8376	0.000
100	20.00	4.6052	4.1584	5.2299	59.057
200	90.00	5.2983	6.3356	6.5521	93.961
400	99.99	5.9915	8.7190	7.8743	99.798

Probit Y =-3.5546 + 1.9075 log X; r-squared = 0.930;
LC_{50} (72 hr.) = 79.31 - 88.64 ppm of technical captan, similar to 45.93-53.36 actual ppm.

After inmersion biotests (30 min.) in a suspension of technical captan, mortalities to 48 and 72 hours were observed. LC_{50} (48 hrs.) was 12,120.89 ppm (7,296.24 ppm of actual ppm), and LC_{50} (72 hrs.) was 6,814.67 ppm (4,102.43 ppm of actual captan).

Table 3. Dose-Response data in the inmersion biotests (48 hrs.). Log-Probit curve regression and correlation

Dose ppm (Tech. captan)	% Death (Y)	Log. X	Probit Y	Expected Probit	Expected Y
100	0.01	4.6052	1.2810	1.7323	0.054
1000	20.00	6.9078	4.1584	3.3006	4.454
10000	40.00	9.2103	4.7467	4.8690	44.775
12000	50.00	9.3927	5.0000	4.9932	49.707
13000	50.00	9.4727	5.0000	5.0477	51.874
14000	50.00	9.5468	5.0000	5.0982	53.903

Probit Y = -1.4044 + 0.6811 Log. Y; r-squared = 0.912;
LC_{50} (72 hr.) = 12,120.89 ppm of technical captan, similar to 7,296.24 actual ppm of pesticide.

Table 4. Dose-Response data in the inmersion biotests (72 hrs.) Log-Probit curve regression and correlation .

Dose ppm (tech. captan)	% Death (Y)	Log. X	Probit Y	Expected Probit	Expected Y
100	0.01	4.6052	1.2810	1.6297	0.037
1000	20.00	6.9078	4.1584	3.4679	6.266
12000	50.00	6.3927	5.0000	5.4517	67.413
13000	66.66	9.4727	5.4313	5.5156	69.685
14000	66.66	9.5468	5.4313	5.5748	71.715
15000	83.33	9.6158	5.9676	5.6299	73.546

Probit Y =-2.0468 + 0.7983 Log. X; r-squared = 0.935;
LC_{50} (72 hr.) = 6,814.67 ppm of technical captan, similar to 4,102.43 actual ppm of pesticide.

LC_{50} (7 and 14 days) in artificial soil biotests were higher than 5000 ppm of technical pesticide (60.2%) 3010 ppm of actual captan ,superior evaluated dose, but there was mortality at 7 days at 1000 and 3000 ppm of technical pesticide (602-1086 ppm of actual captan). There was not signs of malformations in the tested earthworms, with exception of a shorthening in some treated individual with 5000 ppm of technical pesticide. Earthworms weight loss at 7 days was least than 10%, and 10-20% at 14 days, and 14.7% in the controls without pesticide. The weight loss increased with the captan concentration and it was higher in earthworms treated with 4000 and 5000 ppm of technical pesticide. There was no production of new cocoons in treated earthworms until the fourteen day of test and there was in the controls.

The results of "in vitro" bioassays summarized in the tables 1-4 showing the LC_{50} values for different biotests, suggets that technical captan only proved to be non-toxic to the earthworm Eisenia foetida Sav. in the soil test because doses evaluated were higher than recommended for the control of pests in agricultural uses of this pesticide. Their

lack of toxicity probably was caused by their rapid degradation in the soil or by their binding or adsorption to the organic matter or soil clays that renders part of doses unavailable to the earthworms. Edwards and Lofty (1972) reported that Captan was toxic to earthworms when it was applied to 9 Kg/Ha. in the soil. Cook and Swait (1975) in a field treatment against apple scab and mildiu, found that captan sometimes diminished earthworms activity in the soil. Captan quantities applied in the foliage spray for plant diseases control are normally smaller than evaluated dose in this study and probably, they wouldn't have adverse effects on natural populations of earthworms when they are used under normal comercial conditions. In a similar way concerning to the inmersion biotests, the evaluated doses of pesticide were higher than the normal applied dose which can influence in the earthworms in the natural environment. Once again we state that the results reached with the acute toxicity by contact biotests, where is it showed that this formulated pesticide (Captan 60.2%) is toxic near to 100 ppm at 48 and 72 hours after their application to the earthworms.

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