The Toxicity of Benomyl, Thiophanate-methyl, and BCM to Four Freshwater Organisms

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Fungicides are used on a large scale, i.e. in agricultural and horticultural practice. As a result of this use, these chemicals can contaminate the surface-water directly or indirectly. From the literature little information is available about harmful effects of these fungicides upon water-organisms. In this study, therefore, the short-term toxicity of 3 related fungicides to 4 freshwater organisms was examined. These fungicides belong to the group of the benzimidazoles: benomyl, thiophanate-methyl and BCM. These benzimidazole derevatives are used i.e. on apples and pears against Gloësporium and cancer of fruit-trees, on strawberries, gherkins, melons, tomatoes and on onions against Botrytis.

Since BCM is not only an active fungicide itself, but also a metabolite of benomyl and thiophanate-methyl (KILGORE and WHITE, 1970; PETERSON and EDGINGTON, 1969; SELLING et al, 1970), a long-term study was carried out with this substance too. From earlier studies (CANTON et al., 1975) it was known that the reproductive capacity of Daphnia magna is a sensitive criterium.

Materials and methods

The following is a list of the chemicals tested, along with their source of supply and stated purity:

Benomyl	: methyl-1-(butylcarbamoyl)2-benzimidazole carbamate (manufactured by AAgrunol) purity ca. 98%
Thiophanate-methyl	: 1,2-bis(3-methoxycarbonyl-2-thio-ureido)benzene (manufactured by Asepta) purity > 95%
BCM (MBC)	: methyl benzimidazole-2-yl carbamate (manufactured by BASF) purity 97.4%

The short-term toxicity of these fungicides was determined on freshwater organisms of different trophical levels, namely an alga (Chlorella pyrenoidosa), a crustacean

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(Daphnia magna) and 2 fish species: Lebistes reticulatus, and Salmo gairdneri. After a single administration of different amounts of each of the test chemicals to the water, the organisms were placed into the medium for 2 days. The test solutions were minimally aerated during the experiments, in order to have just enough oxygen available for the organisms. It was impossible to determine the exact concentration of each compound during the study because of the lack of suitable analytical methods.

Table 1 summarizes the experimental conditions; all experiments were carried out at least in duplicate.

TABLE 1
Experimental conditions

Species	Age	Number of organisms per group	Test volume per group in litres	Temp. in °C ± 1	Medium
Chlorella pyrenoidosa	taken from a culture in log-phase	ca. 10^7 cells	0.4	24	according to WANKA (1965)
Daphnia magna	< 1 day	20	1	20	according to FREEMAN and FOWLER (1953)
Lebistes reticulatus	ca. 3 wks.	10	1	24	according to ALABASTER and ABRAM (1965)
Salmo gairdneri	ca. 3 months	10	10	15	tap-water

The Chlorella test was carried out in tubes, according to the method of KOCH (1953); after 2 days the number of cells was determined by measuring the absorbance at 540 nm in a Beckman spectrophotometer model B. From the number of cells it was possible to estimate the EC_{50}: that concentration of the fungicide in the water which causes 50% growth inhibition with respect to the controls. For the other organisms a LC_{50} was determined after 2 days.

Results and discussion

Short-term toxicity study

An EC_{50} respectively LC_{50} (2 days) of the 3 fungicides could be determined to all tested organisms, except to Lebistes in the case of BCM. The results are shown in

Table 2. Where possible the 95% confidence limits were calculated according to the method of LITCHFIELD and WILCOXON (1949).

TABLE 2

EC_{50} (Chlorella; E = growth inhibition) - respectively LC_{50}-values (2 days) in mg/l of 3 fungicides to 4 freshwater organisms

Species	Benomyl	Thiophanate-methyl	BCM
Chlorella	1.4	8.5	0.34
Daphnia	0.64 (0.63-0.65)*	16 (14-18)	0.46 (0.38-0.56)
Lebistes	3.4 (3.0-3.8)	130 (106-159)	-
Salmo	0.48 (0.29-0.81)	7.8 (6.2-9.8)	1.8 (1.4-3.1)

- : no effect within the area of solubility (max. ca. 8 mg/l)

* : numbers between brackets are the 95% confidence limits

The LC_{50}-value of thiophanate-methyl to Salmo (7.8 mg/l) is in agreement with the value of 8.8 mg/l found by HASHIMOTO et al. (1972).

Long-term toxicity study

The influence of BCM on the reproductive capacity of Daphnia was studied with concentrations of ca. 1/10, 1/100 and 1/1000 of the LC_{50} 2 days (i.e. 50, 5 respectively 0.5 µg BCM/l water). At 50 µg BCM/l water a complete inhibition of reproductive capacity was seen, while at 5 and 0.5 µg BCM/l no differences could be noticed in respect to the control.

Since this first experiment did not reveal a dose-response relation in a second experiment the influence on the reproductive capacity was examined with test-solutions containing 10, 20, 30 and 40 µg BCM/l. The results are summarized in figure 1.

Because of the linear connection between number of young per parent and exposure time, a time-independent EC_{50} was determined (E = the reproductive capacity percentages of the controls). This EC_{50}-value proved to be ca. 20 µg BCM/l. By extrapolation from this value an EC_{10} (which means 10% reproduction inhibition) of 16 µg BCM/l was calculated. Assuming that a 10% inhibition of reproduction will not influence the population-density of the daphniae in a natural eco-system (see also CANTON et al., 1975), a provisional E.L. can be stated, based on this EC_{10}-value. Furthermore a safety factor is necessary for the extrapolation from the laboratory to the field and therefore the E.L. should be < 16 µg BCM/l. Because of the limitations of this study, like the lack of water analyses, long-

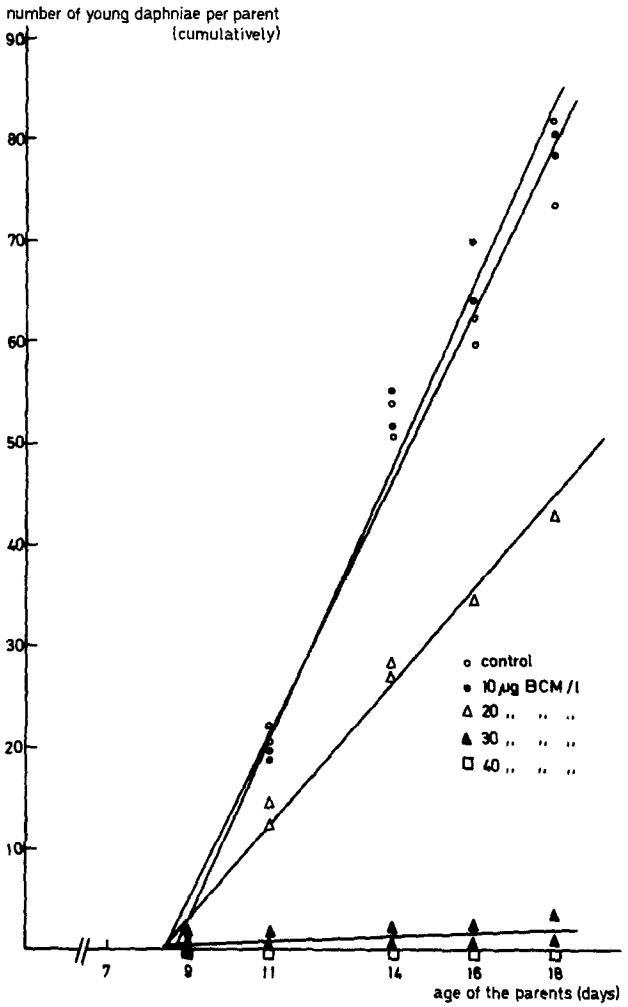

Fig. 1 Daphnia magna: Reproductive capacity as a function of the BCM concentration in the water

term experiments with other organisms, accumulation-elimination and persistence studies, this must be considered as a preliminary approach in establishing the E.L. for BCM.

Conclusions

In the 2-day studies with 3 fungicides, thiophanate-methyl proved to be the least toxic to all tested freshwater organisms. BCM was most toxic for Chlorella and Daphnia, while benomyl was the most toxic for the 2 test fishes.

Based on the influence of BCM on the reproductive capacity of Daphnia, a provisional E.L. < 16 µg BCM/l could be stated, but more research has to be done to confirm this provisional figure.

Summary

The EC_{50} or LC_{50} values after a 2-day exposure of the three fungicides benomyl, thiophanate-methyl and BCM, have been determined using four freshwater organisms: Chlorella pyrenoidosa, Daphnia magna, Lebistes reticulatus and Salmo gairdneri. The influence of BCM on the reproductive capacity of Daphnia was also investigated and from these data a provisional ecological limit was established.

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