

The Toxicity of Manganese Ethylenebisdithiocarbamate to the Adult Newt, *Triturus cristatus*

Nicoletta Paces Zaffaroni, Elio Arias, Giorgio Capodanno,¹ and Teresa Zavanella
Istituto di Zoologia, Università di Milano, 20133, Italy ¹*Present address: Istituto dei Tumori, Fondazione Senatore Pascale, 80100 Napoli*

SUMMARY

The toxicity of ethylenebisdithiocarbamate (maneb), a widely used agricultural fungicide, to the adult newt (*Triturus cristatus carnifex*) was evaluated after percutaneous exposure. Maneb was lethal to all the animals at the 50 ppm level within fifteen days. Male newts seem to be less resistant to the toxicant than females. At concentrations as low as 25 ppm all males died within 25 days, whereas some of the females were still alive after 5 months. Microscopic examination showed that the skin and the kidney were the most severely affected organs. It has been hypothesized that an osmoregulatory breakdown and an impairment of cutaneous respiratory exchanges play a role in the death of the animals. Renal failure must also be taken into account. However, additional mechanisms can not be ruled out at present.

INTRODUCTION

The metal derivatives of the ethylenebisdithiocarbamates are used extensively as fungicides in agriculture. In Italy about 41 million kilos of dithiocarbamates were sold for agricultural use in 1974 (Bollettino Mensile di Statistica, 1976). Therefore the presence of these chemicals in water courses and flooded soils may be a hazard to the health of aquatic animals.

The data on the chemical and toxicological properties, use patterns and residues of dithiocarbamate fungicides have been reviewed by FISHBEIN (1976). However, little information is available concerning the toxicity of these compounds in aquatic vertebrates.

Data on the acute toxicity of manganese ethylenebisdithiocarbamate (maneb) in the adult newt (*Triturus cristatus*) were obtained as a preliminary to a research program on the assessment of the carcinogenic and teratogenic risk of this chemical in the same species and are presented in this report.

MATERIALS AND METHODS

Adult newts (Triturus cristatus carnifex) were collected from irrigation streams near Perugia and maintained in running water (14-16°C) for two months prior to experimentation.

Manganese ethylenebisdithiocarbamate of technical grade, with an active component of 80% (maneb 80) was used (°). The animals were exposed to the toxicant percutaneously.

The results of preliminary trials indicated that maneb 80 was lethal at a concentration of 100 parts per million (ppm) to all animals within 26 hours. Therefore the following levels of maneb were tested: 0, 25, 50, 75, 100 and 125 ppm.

For each concentration tested, twelve newts (six males and six females) were used. The animals were distributed as equally as possible with regard to their body size: in each group the median length was 7.4 cm for males (7.2 - 7.5 cm) and 7.8 cm for females (7.6 - 8.0 cm); the median weights were about 8 g and 12 g, respectively.

Experimental and control groups, each of six animals, were kept in glass bowls containing approximately 500 ml of tap water (18°C \pm 2). Water hardness (300 mg/liter as CaCO₃ at 18°C) and pH were determined only at the initiation of the test. The pH was 7.4. Water and test solutions were renewed every 24 hours (at 10 a.m.). During the course of the experiment the newts were not fed.

A replicate experiment was set up three months after the first, testing only the concentrations of 100 and 125 ppm. In this second experiment median length was 7.1 cm for the males (median weight 6.5 g) and 7.5 cm for the females (median weight 8.5 g).

Mortalities were recorded every hour the first 15 hours, then at intervals of 9 and 15 hrs through the second day and at daily intervals thereafter for a total period of five months. For each concentration tested the LT₅₀ values (lethal time for 50% of the individuals) with 95% confidence limits were calculated from the cumulative mortality data by the method of LITCHFIELD (1949).

Gross and microscopic pathological examination were made on all animals dying during the course of the experiment and on the control animals, which were killed at the end of the test (males after 25 days and females after 5 months). The tissues examined included skin, skeletal muscle, liver, spleen, heart, stomach,

(°) Maneb 80 was kindly supplied by SIPCAM, Milano.

intestine, pancreas, gonads, kidneys, eye, brain and spinal cord. The thyroid was examined only in the controls and in the females exposed to 25 ppm. Tissues were fixed in Bouin's fluid and paraffin sections were stained with Mayer's haemalum and eosin.

RESULTS AND DISCUSSION

Toxicity Studies

The LT₅₀ values after exposure of the newts to different concentrations of maneb are presented in Table 1 and in Figure 1.

Death after the first exposure to the three highest concentrations tested in most instances occurred within 7 to 33 hours.

In the first experiment there was no clear relationship between the LT₅₀ values and the concentration tested above the level of 50 ppm. This finding may be due to the different individual susceptibility. In the second experiment, carried out after three months, the results were less divergent from a dose-response effect.

TABLE 1

LT₅₀ values for newts (Triturus cristatus) after percutaneous exposure to maneb 80

maneb 80 (ppm)	LT ₅₀ (hrs) (95% confidence limits)		
	Male		Female
125	8.4	(7.6 - 9.2)	28.5 (16.4 - 49.6)
125 (°)	8.8	(8.3 - 9.3)	16.0 (7.6 - 33.8)
100	28.0	(16.0 - 49.0)	19.5 (9.5 - 40.2)
100 (°°)	11.0	(8.9 - 13.5)	16.0 (9.2 - 27.7)
75	19.0	(7.0 - 51.3)	25.5 (11.6 - 55.8)
50	76.0	(57.6 - 100.3)	168.0 (85.7 - 329.3)
25	255.0	(147.4 - 441.1)	(°°) -

(°) Second experiment.

(°°) Some female newts were still alive after 5 months.

Female newts seem to be less susceptible than the males to the toxicant. Comparison of the mortality data showed a significant sex difference at concentration levels of 125 and 50 ppm. Maneb was lethal to all the males at the 50 ppm level within five days

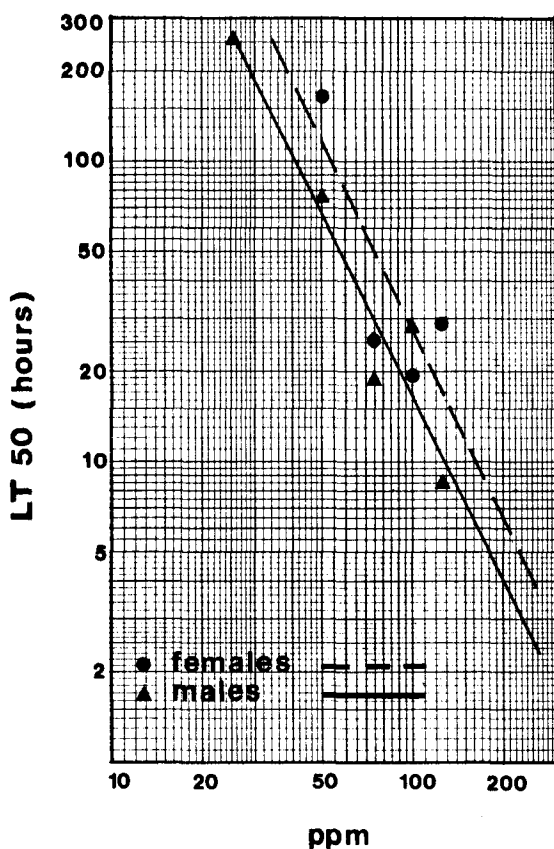


Figure 1. The toxicity of manganese ethylenedisithiocarbamate (maneb) to the adult newt.

Males. $Y = 5.202X^{-1.99}$, $r = -0.961$

Females. $Y = 5.493X^{-2.03}$, $r = -0.819$

Regression does not include 25 ppm, since two females were still alive after three months.

and to all the females within fifteen days. At the concentration of 25 ppm, all the male newts died within 25 days, whereas in the female group the first death occurred only after 58 days. Two females were still alive after five months and were killed.

The observed sex differences in susceptibility may partly be due to the greater body size of the females. In fact, in the first experiment a linear relationship was found between mortality and length of the animals, as calculated by regression analysis of the whole sample. Moreover, the susceptibility is sex related because a striking sex difference was observed in the animals exposed to 25 ppm.

No mortality occurred in control animals tested under identical conditions.

Pathological Effects

Upon gross examination the animals exposed to maneb appeared to be slightly edematous. The sections of skin often revealed a dramatic increase in the serous secretory material which accumulated between the dermis and epidermis and, even more frequently, under the superficial horny layer (Fig. 2).

Histopathological examination of the kidneys revealed variable degrees of alterations in most of the animals exposed to maneb. The early signs of the maneb lesions were seen at the glomerular level after only ten hours. Proteinaceous material was present within Bowman's space and the tubular lumen: the presence of red cells, leukocytes and shed cells is also indicative of severe glomerular lesions. In part of the animals which died after two or more days tubular degeneration and focal areas of necrosis, with pycnotic nuclei and karyorrhexis were observed. Most of the pathologic changes were found in the proximal convoluted tract (Fig. 3). However, there was no apparent relationship between the severity of the tubular changes and the concentration of the toxicant. This discrepancy may be due to the earlier deaths of newts exposed to the highest levels of maneb, when the lesions could not be seen by light microscopy.

Impairment of kidney function has also been reported in dogs treated with maneb (CLAYTON, quoted in "Evaluation of the Toxicity of Pesticide Residues in Food", 1965) and in rats treated with other dithiocarbamates (BLACKWELL SMITH et al., 1953).

In the livers of some newts exposed to maneb vacuolar degeneration of the hepatocytes was observed. More frequently, there was a slight to marked sinusoid congestion, sometimes with associated

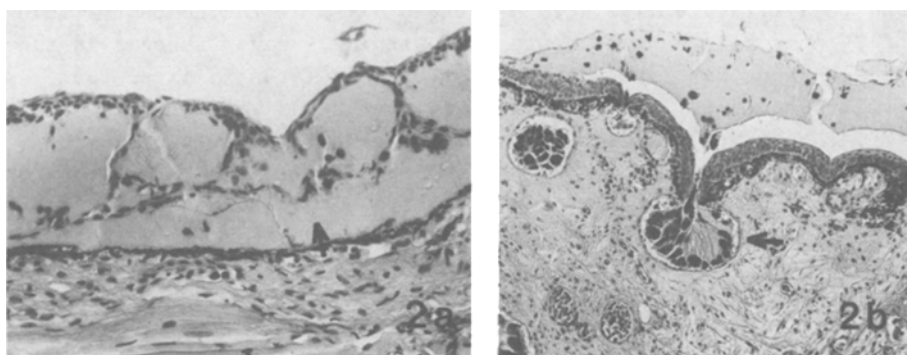


Fig. 2. Skin from newts treated with maneb (100 ppm), H&E.
 (a) Serous secretion (arrow) between epidermis and dermis after 24 hrs, x 50.
 (b) Serous secretion with scattered glandular cell nuclei under the superficial horny layer after 31 hrs. Note emptying of a serous gland (arrow), x 40.

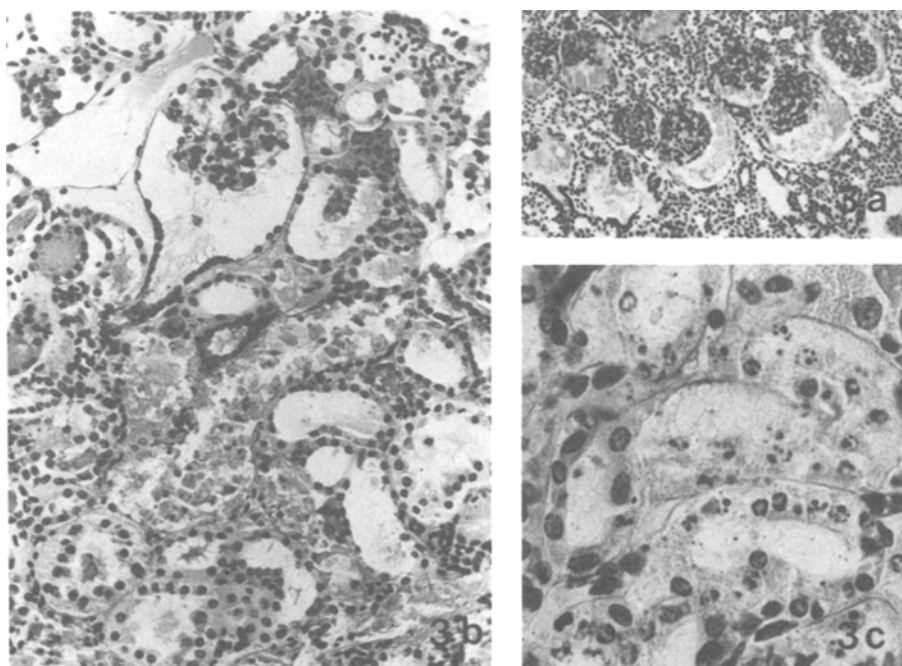


Fig. 3. Kidneys from newts treated with maneb, H&E.
 (a) 100 ppm: glomerular lesions after 24 hrs, x 50.
 (b) 50 ppm: focal tubular necrosis after 46 hrs, x 120.
 (c) 25 ppm: tubular cells showing karyorrhexis after 19 days, x 220.

hemorrhage.

Varying degrees of vascular congestion were also present in other organs, such as the spleen, lung, adipose tissue and brain.

Inflammatory cells were present in the cavity and in the wall of the lung in sixteen per cent of the animals exposed to 50-125 ppm.

Occasionally, other histopathological abnormalities were seen. Thrombi with inflammatory invasion were present in some large vessels and in the heart cavity. Marked hydrocephalous, with enlargement of all the ventricles and thinned brain walls, was observed in two newts, one exposed to 100 ppm and dead after 49 hrs and the other exposed to 25 ppm and dead after 19 days. No nervous tissue degenerative changes as reported in sodium diethyl-dithiocarbamate-treated rabbits (EDINGTON et al., 1966) were observed. Thyroid hyperplasia was found in three female newts dead or killed after about five months.

It is noteworthy that although a difference in LT₅₀ was observed between female and male newts, the pathological pictures were the same in both sexes.

From these findings, one can speculate that the mechanism of the lethal effects of maneb is related to an impairment of the osmoregulatory or the respiratory function of the skin or both. In turn, the damage to the skin could increase the absorption rate of the toxicant. Renal failure must also be taken into account. However, additional mechanisms can not be ruled out at present.

REFERENCES

- BLACKWELL SMITH, R., Jr., FINNEGAN, J.K., LARSON, P.S., SAHYOUN, P.F., DREYFUSS, M.L., and H.B. HAAG : J.Pharmacol.Exp.Ther. 109, 159 (1953).
- BOLLETTINO MENSILE DI STATISTICA No. 1, 212 (1976).
- CLAYTON, J.L., HOOD, D.B., BARNES, J.R., and A.R. BORGMANN: quoted in Evaluation of the Toxicity of Pesticide Residues in Food, FAO mtg. Rep. No. PL/1965/10/1 WHO/Food ADD/27.65, p.142, Rome: FAO 1965.
- EDINGTON, N., and J. McC. HOWELL: Nature 210, 1060 (1966).
- FISHBEIN, L.: J.Toxicol.Environ.Health 1, 713 (1976).
- LITCHFIELD, J.T.: Jr.: J.Pharmacol.Exp.Ther. 97, 399 (1949).