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Paramecium Bioassay Test in Studies on Cartap

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In previous papers, KOMALA (1975, 1976, 1978) reported on the toxic effect of several pesticides commonly used in Poland by using paramecia in a bioassay test.

The compound studied in the present work was the insecticide cartap (S,S'-[2-(dimethylamino)-1,3-propanediyl]dicarbamothioate), an insecticide which according to LAKOTA et al. (1981) is finding an increasingly widespread application in Poland both in plant protection and in sanitary hygiene. It is likely that this pesticide can enter the aquatic environment; hence studies on its toxicity to aquatic organisms are necessary.

According to the present state of knowledge, cartap is not highly toxic to mammals and belongs to toxicity class III (SZCZUCKI et al. 1978) with its LD-50 established for rats ranging from 325 to 345 mg per kg of body weight (TAKEDA CHEMICAL INDUSTRY LTD 1976). The effect of cartap on insects differs from that of organochlorine and organophosphorus insecticides, since cartap paralyzes the central nervous system and causes the rapid death of an insect without symptoms of indirect intoxication (BYRDY et al. 1976).

In the study on the side-effects of cartap, carbaryl, and propoxur on some aquatic organisms, LAKOTA et al. (1981) found that cartap was the most hazardous insecticide in the aquatic environment, whereas propoxur was the least hazardous. The authors showed that after 96 h of exposure to cartap, the compound caused 100% mortality at the following concentrations: 0.2 mg/dm^3 of water for the fry of Salmo trutta morpha fario, 4.0 mg/dm^3 for the fry of Cyprinus carpio, 2.0 mg/dm^3 for Lebistes reticulatus, 0.1 mg/dm^3 for Daphnia magna, and 0.8 mg/dm^3 for larvae of Aedes aegypti. In the last case, the effect was observed after 48 h of exposure. LC-50 of cartap was: 0.8 mg/dm^3 for the fry of Salmo trutta morpha fario, 1.4 mg/dm^3 for the fry of Cyprinus carpio, 0.71 mg/dm^3 for Lebistes reticulatus, 0.01 mg/dm^3 for Daphnia magna, and 0.29 mg/dm^3 for larvae of Aedes aegypti.

The aim of the present study was to investigate the toxic effect of cartap on Paramecium primaurelia, one of the most widespread infusorians in the world, and to determine the LC-50 of cartap.

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MATERIALS AND METHODS

Cartap monohydrochloride (Padan, Takeda Chemical Industries Ltd.) is a white crystalline substance with a slight odour, easily soluble in water, poorly soluble in methanol and ethanol and insoluble in organic solvents. Its melting point is 179-181°C (BYRDY et al. 1976).

Cartap used in the present investigations was obtained through the courtesy of Dr. S. Lakota, Institute of Organic Industry, Pszczyna.

For experiments, a Polish standard strain L of Paramecium primaurelia was used. Methods of culturing paramecia and preparing medium as well as obtaining complementary mating types were according to SONNEBORN (1950, 1970). The culture medium was prepared from dried lettuce in the ratio of 1 g/L of distilled water, sterilized in an autoclave, and inoculated with E. aerogenes.

Paramecium bioassay test. The test (KOMALA 1975) was performed on paramecia coming from a genetically homogeneous vegetative culture and being at the synchronized stage of life. Homogeneity of the test cultures was obtained by the isolation of progenitor paramecia from the stock postautogamic culture derived from a single postautogamic paramecium. Several progenitor paramecia were isolated separately, each individual into a separate depression slide, with 0.5 cm^3 of medium. The thus obtained slide cultures were transferred into test tubes and cultivated until the paramecia reached sexual activity. Complementary mating types were then established. (Each tube culture represents one of the two mating types.) Thus, the synchronization of the life cycle of paramecia was also achieved by bringing individual test tube cultures to the state of readiness for conjugation (i.e., conjugative maturity and activity between the two mating types). For bioassay purposes paramecia belonging to one of the two mating types are used, no matter which, since they react similarly to the investigated compound as shown in the case of P. primaurelia (KOMALA, in press). Each experiment included 3 series, 100 animals in each treated with 0.5 cm^3 of cartap solution. Observations were conducted at regular time intervals starting immediately after treatment. For each experiment a control series of 100 animals kept only in tap water was established and observed at identical time intervals as was with the experimental series. The surviving individuals were used for establishing daily isolation lines in which the abnormalities in the development of progeny could be analysed and compared with the behaviour of the control animals and lines.

Stock cartap solution in tap water 100 mg/100 cm^3 was used to prepare the required test solutions containing 10, 1, 0.5, 0.25 and 0.1 mg of the compound per 100 cm^3 of tap water.

RESULTS AND DISCUSSION

The results of the present study, confirm the high toxicity of cartap to Paramecium primaurelia. It was found that cartap solutions containing 100, 10, and 1 mg of the compound per 100 cm^3 of

water caused 100% mortality of all investigated paramecia after about 27 min, 2 h, and approximately 18 h, respectively. It was also shown that the LC-50 of cartap for P. primaurelia is 0.25 mg/100 cm^3 of water during 24 h (At cartap concentration of 0.1 mg/100 cm^3, all the treated animals survived).

At cartap concentration of 0.5 mg/100 cm^3, mean survival rate was about 5%. Using this concentration of cartap, two groups of experiments (A and B) were performed, each group composed of 3 experimental series (I, II, III), 100 animals in each. The total number of isolated and observed paramecia was 600, with 18 surviving individuals found in group A and 15 in group B. (In the present work the group A is only considered, because only in this group the abnormal paramecia were found.) In two different isolation lines derived from the surviving paramecia in AI experimental series, 3 cases of abnormal individuals with a disturbance of the mechanism of vegetative division and locomotion were found. The abnormality was manifested by a defective separation of animals during the division, resulting in the formation of doublets connected at different angles (acute or obtuse) and showing a rotatory movement around their axis. Such forms were not stable and died after a short time. No abnormal individuals were observed in the control lines. (The latter observation is important since the experiments with paramecia have shown that control animals cultured under improper conditions can occasionally show various abnormalities of the vegetative development and locomotion.)

The disturbance in the mechanism of vegetative fission was previously observed in paramecia treated with another pesticide, prometryne (KOMALA 1975), which induced formation of chain forms and in one case a monstrous form (kind of vegetative triplet). Other pesticides studied previously (KOMALA 1978) as Sadoplon, Karbatox, and Terrafun showed similarly like cartap very high toxicity to paramecia. They caused 100% mortality of the animals at all concentrations tested (0.1, 0.01, and 0.001%). Miedzian brought about 100% mortality of paramecia at a concentration of 0.01% (KOMALA 1976). Prometryne, in turn, was less toxic bringing about the survival of a small percentage of paramecia (6%) at this concentration. The least toxic for paramecia was lindane which even at a concentration of 0.1% revealed about 5% of survivors. Lindane, however, is known as a compound potentially dangerous to other organisms, since it exerts a toxic effect on the central nervous system of mammals (LITTERST et al. 1975, HANIG et al. 1976, HULTH et al. 1976), causes paramyeloblastic leukaemia in man (JEDLIČKA et al. 1958) and also has a mutagenic effect on plants (EPSTEIN & LEGATOR 1971). As far as cartap is concerned JORDAN & NADACHOWSKA (1981) found that at a cartap concentration of 1 and 0.5 mg % tadpoles of Xenopus laevis died during 4.5 - 8 h. At concentrations of 0.1 - 0.001 mg % they survived showing no cytological changes on the preparations. It seems that cartap may be recommended as a component of different pesticides since, despite its considerable toxicity to aquatic organisms, it has no mutagenic properties detected so far and thus can be regarded as less dangerous on a general biological scale.

REFERENCES

BYRDY, S., K. GORECKI, and E. LASZCZ: In Pestycydy, p. 81, Warszawa: PWRL (1976).

EPSTEIN, S., M. S. LEGATOR: In Mutagenicity of Pesticides. Concepts and Evaluation, pp. 181-182, Cambridge: MIT Press (1971).

HANIG, J. P., P. D. YODER, and S. KROP: Appl. Pharmacol. 38, 463 (1976).

HULTH, L., M. LARSSON, R. CARLLSON, and I. E. KIHLSTRÖM: Bull. Environ. Contam. Toxicol. 16, 133 (1976).

JEDLIČKA, V. L., Z. HEŘMANSKA, I. ŠMIDA, and A. KOUBA: Acta Medica Scand. 161, 447 (1958).

JORDAN, M., A. NADACHOWSKA: personal communications (1981).

KOMALA, Z.: Fol. Biol. (Krakow) 23, 231 (1975).

KOMALA, Z.: Fol. Biol. (Krakow) 24, 65 (1976).

KOMALA, Z.: Fol. Biol. (Krakow) 26, 355 (1978).

KOMALA, Z.: Fol. Biol. (Krakow) 30, in press.

LITTERST, CH. L., and F. MILLER: Bull. Environ. Contam. Toxicol. 13, 619 (1975).

LAKOTA, S., A. RASZKA, and I. KUPCZAK: Acta Hydrobiol. 23, in press.

SZCZUCKI, B., W. HAJCZAKOWA, M. PODOLAK, and J. KRZYŻANOWSKI: In Chemiczne Środki Ochrony Roslin, pp. 54-55, Warszawa: PWRL (1978).

Takeda Chemical Industries Ltd. Japan: Padan pp. 1-52 (1976).

SONNEBORN, T. M.: J. Exp. Zool. 113, 87 (1950).

SONNEBORN, T. M.: In Methods in Cell Physiology 4, pp. 242-339, D. M. Prescott (ed.). New York: Academic Press (1970).