Acute Toxicity of Carbofuran to Selected Species of Aquatic and Terrestrial Organisms

RADKA DOBŠÍKOVÁ

Department of Veterinary Ecology and Environmental Protection, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic

Abstract

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Carbofuran is an anticholinesterase carbamate commonly used as an insecticide, nematicide and acaricide in agricultural practice throughout the world. The aim of the study was to investigate the acute toxicity of carbofuran to selected species of aquatic organisms (the guppy *Poecilia reticulata* Peters, the water flea *Daphnia magna* Straus and the green alga *Raphidocelis subcapitata* Korsikov), and to a terrestrial organism (a white mustard *Sinapis alba* Linné). *Daphnia magna* Straus was found to be the most sensitive organism.

Keywords: carbofuran; *Poecilia reticulata*; *Daphnia magna*; *Raphidocelis subcapitata*; *Sinapis alba*; acute toxicity

Pesticides are widely used substances in current agricultural practices. Owing to their toxic effects on nontarget organisms, most of them may produce serious detrimental effects on ecosystems. Agricultural applications done in an incorrect way may result in the pollution of terrestrial habitats. In aquatic environments, an accidental spraying with pesticides presents a minor risk but a more serious one is leaching and runoff water from the treated fields.

Carbofuran (2,3-dihydro-2,2-dimethylbenzofuran-7-yl methylcarbamate) is a broad-spectrum systemic insecticide, nematicide, and acaricide used throughout the world. As a result of its widespread use, carbofuran has been detected in ground, surface and rain waters, in soils, air, foods and wildlife (RICHARDS *et al.* 1987; WAITE *et al.* 1992; FISHER *et al.* 1999).

In the Czech Republic, it is used to protect field crop-plants, decorative plants and vegetables from various insect pests – for example *Leptinotarsa decenlineata*, *Chortophila brassicae*, *Phyllotreta nemorum*, *Atomaria linearis*, *Phorodon humuli*, etc. (Collective SPA 2002).

The low persistence of carbofuran is an important factor in its substitution for pesticides persisting in the environment for a long period of time (MORA *et al.* 1996). Although MORA *et al.* (1996) found a short half-life of carbofuran in soil suspensions in water ($t_{1/2}$ 1–2 days), its half-time in most soil types is indicated typically as < 60 days (ANTON *et al.* 1993). In soils, the biodegradation of carbofuran is activated by bacterial populations. AMBROSOLI *et al.* (1996) found that the genera *Arthrobacter*, *Pseudomonas*, *Bacillus* and *Actinomyces* metabolised carbofuran in soil.

In aquatic environments, the toxicity of carbofuran is reduced by microbial populations, sediments and flooded soils (TROTTER *et al.* 1991). The compound degrades within 1 to 8 weeks in neutral and moderate alkaline water depending upon water temperature (ANTON *et al.* 1993). Car-

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bofuran is stable in acid waters. In alkaline waters, the compound is subjected to chemical hydrolysis with major metabolites 3-hydroxycarbofuran and 3-ketocarbofuran (Gupta 1994). Its relatively high water solubility (0.32 g/l at 20°C) and minimal adsorption on soil and sediments poses a threat to natural surface waters after its accidental escape from the treated areas (Trotter *et al.* 1991).

Carbofuran is classified as very toxic to bees, fish, and other water animals, and dangerous to wild game and birds (Collective SPA 2002). It is frequently involved in malicious poisoning of animals as well as in human suicides (Gupta 1994; Ameno *et al.* 2001).

The specific toxicodynamic effect of carbofuran is the inhibition of cholinesterase in nerve synapses in the central nervous system. The intoxication itself is considered to be an induced internal intoxication by acetylcholine accumulated in nerve synapses (Zapletal *et al.* 2001). Carbofuran has carcinogenic, teratogenic, and genotoxic effects, it crosses the placental barrier and produces serious effects on the maternal-placental-fetal unit (Gupta 1994). Yolk sac resorption was incomplete and the transformation into larvae did not take place in pike embryos exposed to 250 μg/l of carbofuran, and sublethal exposures to carbofuran affected ontogenetic changes (Amblard *et al.* 1998).

The objective of the study was to evaluate the sensitivity of selected aquatic and terrestrial organisms to various concentrations of carbofuran by determining the concentration at which a 50% effect [LC_{50} – lethal concentration for fish, EC_{50} – effect, immobilisation concentration for daphnids, IC_{50} – inhibition concentration for a green alga (growth inhibition) and white mustard seedlings (root growth inhibition)] could be observed.

MATERIAL AND METHODS

Toxicity tests on *Poecilia reticulata* Peters, *Daphnia magna* Straus, *Raphidocelis subcapitata* Korsikov and *Sinapis alba* Linné were performed according to ČSN EN ISO 7346 – 2 (Water quality. Determination of the acute lethal toxicity of substances to a freshwater fish [*Brachydanio rerio* Hamilton-Buchanan (Teleostei, Cyprinidae)]. Part 2: Semi-static method 1999), ČSN EN ISO 6341 (Water quality. Determination of the inhibition of the mobility of *Daphnia magna* Straus (Cladocera, Crustacea) – Acute toxicity test 1997) and ČSN EN ISO 28692 (Water quality. Fresh water algal growth inhibition test with *Scenedesmus subspicatus* and *Selenastrum capricornutum* (ISO 8692: 1989) 1995), and the methodology by Máchová *et al.* (1994), respectively.

The acute toxicity of carbofuran was evaluated in the study. The concentrations of carbofuran were prepared by diluting the stock solution of carbamate pesticide Furadan 350 F containing 1 g/l of carbofuran for *P. reticulata*, *D. magna* and *R. subcapitata* toxicity tests and 36 g/l of carbofuran for *S. alba* toxicity test with dilution water prepared according to relevant ČSN EN ISO standards.

Acute (96h) tests on *Poecilia reticulata* were performed as semistatic tests (renewal of the medium after 48 h, 10 individuals in 1 l of the test medium), acute (48h) tests on *Daphnia magna* were performed as static tests (10 individuals in 75 ml of the test medium). Acute tests on *Raphidocelis subcapitata* (250ml Erlenmayer flasks, 100 ml of the test medium, continuous lighting, the cell density 30 000 cells/ml) and *Sinapis alba* (30 seedlings in a Petri dish, 10 ml of the test medium, incubation in the dark) were performed as 72h static tests.

Sublethal effects and mortality in *Poecilia reticulata*, immobilisation in *Daphnia magna* and the inhibition of growth in *Raphidocelis subcapitata* and *Sinapis alba*, and the parameters of the test media (temperature, pH, dissolved oxygen content) were recorded in the study.

Table 1 presents the mean values of the parameters of the test media (temperature, dissolved oxygen concentration, pH) and lighting.

The acute toxicity of carbofuran for fish (LC_{50}) and for daphnids (EC_{50}) was determined using a logarithmic probability regression, i.e., probit procedure. This analysis technique is commonly used for the prediction of the dose-response relation and 95% confidence intervals in the test data (Fisher *et al.* 1999). The acute toxicity of carbofuran for the green alga tested (IC_{50}) and for seedlings of the white mustard was evaluated using the Toxicity software (TG Masaryk's Water Research Institute Prague, Ostrava Branch Off).

RESULTS AND DISCUSSION

Values of the acute toxicity of carbofuran for the test organisms are given in Table 2.

Poecilia reticulata ***toxicity tests***. Fish in life stages are known to be extremely vulnerable and were used as a sensitive species for toxicity studies (De Silva & Samayawardhena 2002). In our toxicity tests, the guppy *Poecilia reticulata* was used to

Table 1. Mean values of parameters of test media and lighting

Organism	*Poecilia reticulata*	*Daphnia magna*	*Raphidocelis subcapitata*	*Sinapis alba*
Test medium according to	ČSN EN ISO 7346	ČSN EN ISO 6341	ČSN EN ISO 28692	ČSN EN ISO 7346#
Temperature (°C)	22.6	21.8	25.6	21.9
DOC*	26.7	3.1	–	–
pH	7.39	7.47	8.08	–
Lighting	daylight	daylight	6500 lx	kept in the dark

*DOC – dissolved oxygen concentration: *P. reticulata* (%), *D. magna* (mg/l)

\# Test media for tests on *P. reticulata*, *D. magna* and *S. alba* were identical

Table 2. Values of acute toxicity of carbofuran*

Organism	*Poecilia reticulata*		*Daphnia magna*		*Raphidocelis subcapitata*	*Sinapis alba*
Acute toxicity	48h LC_{50} (mg/l)	96h LC_{50} (mg/l)	24h LC_{50} (mg/l)	48h LC_{50} (mg/l)	72h IC_{50} (mg/l)	72h IC_{50} (mg/l)
Mean	0.3468	0.2245	0.0447	0.0187	0.1582	13.0825
SD	0.0471	0.0294	0.0101	0.0009	0.0420	3.8918
95% CI	0.3006–0.3930	0.1957–0.2533	0.0398–0.0496	0.0182–0.0191	0.1171–0.1993	11.1755–14.9895

*StatPlus statistics software

evaluate the toxicity of carbofuran and its effect on the swimming activity, social interactions and behavioural changes of exposed fish. Clinical and pathoanatomical symptoms of the carbofuran exposure were non-specific. In carbofuran concentrations of 5 and 10 mg/l, an immediate increase in the locomotor activity, burst swimming and nipping was observed in fish. The exposed guppies then became uncoordinated, side- and back-positioned, and within a few minutes of exposure died in agony. In comparison, in the acute toxicity test on 1-year-old common carps (*Cyprinus carpio* L.), carbofuran concentration of 2.44 mg/l killed all the test animals after 4 h of exposure (Dembele *et al.* 2000).

Carbofuran is very toxic to fish. Heavy carbofuran usage was highly toxic to fish such as rainbow trout, fathead minnow, and bluegill sunfish (Kidd & James 1991). Generally, fish LC_{50} values of the compound are below 1 mg/l (Trotter *et al.* 1991). The acute toxicity values for *Poecilia reticulata* were in our tests 0.3468 mg/l (48h LC_{50}) and 0.2245 mg/l (96h LE_{50}). In the concentration range of 0.10 to 0.50 mg/l, the main symptoms were hyperaemia of gills, uncoordinated surface swimming, nipping and behavioral signs of muscle tetany. Identical symptoms were found in carbofuran acute toxicity tests on juvenile *Carrasius auratus* (weighing 5.2 ± 1.3 g) exposed to concentrations of 0.005, 0.05, and 0.50 mg/l (Bretaud *et al.* 2001). Nevertheless, no mortality was observed in goldfish after 48h exposure to the highest concentration of carbofuran (0.50 mg/l) although in common carp (*Cyprinus carpio*) 0.50 mg/l corresponds to the 96h EC_{50}. LC_{50} values appear to differ in species within the same family (goldfish and common carp). *Poecilia reticulata* was found to be more sensitive to carbofuran exposure in comparison with *Carrasius auratus* and *Cyprinus carpio*.

Fisher *et al.* (1999) evaluated the sensitivity of juvenile and adult flathead chubs (*Platygobio gracilis*) to various concentrations of carbofurans. A greater increase in the carbofuran concentration was required to raise the mortality probability of adult chubs (96h LC_{50} of 2.64 ppm) than that needed to raise the death likelihood by the same amount for juveniles (96h LC_{50} of 1.96 ppm). Also, in flathead chubs, they found several symptoms such as spinal dislocations, vertebral hemorrhage near the dorsal tin, anterior discolouration, loss of caudal fin mobility, and quick colonisation by the fungus *Saprolegnia* sp. in the discoloured area.

Daphnia magna ***toxicity tests***. The results of the acute toxicity tests on *Daphnia magna* confirmed the generally high sensibility of daphnids to pesticides. The values of 24h EC_{50} and 48h EC_{50} of carbofuran were 0.0447 mg/l and 0.0187 mg/l, respectively. The results of the toxicity tests with *Daphnia magna* differed in the order of magnitude as compared to the test results with the other selected aquatic organisms.

A comparison of the sensitivity of the test organisms to carbofuran using *Daphnia magna*, the luminescent bacterium *Vibrio fischeri* (Biotox™), and MitoScan™ assays was made by Fernandez-Alba *et al.* (2001). Carbofuran EC_{50} values for *Daphnia magna* were 0.092 mg/l (24 h incubation) and 0.030 mg/l (48 h incubation), for Biotox™ 9.60 mg/l (15 min incubation) and MitoScan™ 7.60 mg/l. *D. magna* bioassay was found the most sensitive. Biotox™ and MitoScan™ assay results may be generally correlated with the EC_{50} values of the *Daphnia magna* test; nevertheless, *Daphnia magna* bioassay has no substitute because of its sensitivity to a great variety of toxicants and because of its substantially more complex biochemistry in comparison with the other two test systems. In another carbofuran toxicity assessment, BioTox™, MitoScan™, and Daphtoxkit™ bioassays were used (Fernandez-Alba *et al.* 2002). The latter was found to be the most sensitive test. The comparison of the carbofuran toxicity values in *Daphnia magna* "standard" bioassay and Daphtoxkit™ revealed that the sensitivity of both bioassays was similar – 48h EC_{50} of 0.030 mg/l for *D. magna* "standard" bioassay, and 0.018 mg/l for Daphtoxkit™, respectively (Fernandez-Alba *et al.* 2001, 2002).

Physical stress may affect the susceptibility of organisms to chemical impact by changing the toxicodynamics of the chemical/organism interaction. Herbrandson *et al.* (2003), explored the effect of stress from suspended solids in combination with carbofuran exposure to *Daphnia magna*. They found that in the absence of suspended solids, the carbofuran effect was dose dependent and resulted in an EC_{50} of 0.092 mg/l but at a suspended solid concentration of 1000 mg/l, the EC_{50} was reduced by half to 0.045 mg/l.

Raphidocelis subcapitata ***toxicity tests***. In algal populations, pesticides negatively influence cell metabolism by decreasing nutrient intake, protein syntesis, etc. The sensitivity of various algal species to chemical compounds is diverse and depends on algal genera phylogenesis, morphology, cytology, physiology, and genetics (Blanck *et al.* 1984; Lewis 1990). The value of 72h IC_{50} of carbofuran for *Raphidocelis subcapitata* was in our study 0.1582 mg/l. In comparison, the value of 96h IC_{50} for *Chlorella pyrenoidosa* was 204.48 mg/l (Anton *et al.* 1993). The toxicity values of carbofuran confirm *Chlorella* sp. to be a less sensitive organism compared to *Selenastrum* sp. (Nyholm & Källquist 1989). Megharaj *et al.* (1989) found as the lethal dose for *Scenedesmus bijugatus* 5 mg/l. In toxicity tests, *Selenastrum capricornutum* is more practical in comparison with *Scenedesmus* sp. since it is one-cellular, immobile, morphologically stable and non-clustering algal species (Blaise 1993).

Sinapis alba ***toxicity tests***. The acute toxicity test on *Sinapis alba* seedlings with the carbofuran concentration of 100 mg/l (limiting test) was negative. In definitive toxicity tests, 72h IC_{50} value for *Sinapis alba* was found to be 13.08 g/l. The low toxicity of carbofuran may be caused by generally high metabolic rate of the compound in plants. *Sinapis alba* was not found to be a suitable (sensitive enough) plant species for the evaluation of carbofuran toxicity.

Conclusion

The tests of acute toxicity with carbofuran confirmed the high toxicity of the chemical to selected species of aquatic organisms. The results proved *Daphnia magna* to be the most sensitive test organism. It is necessary to protect natural surface waters from accidental escape of carbofuran from treated areas. Assuming that the application of carbofuran is done in an appropriate way, the pesticide should degrade quickly and never be present in surface waters at lethal concentrations.

Toxicity is a biological response and this needs to be taken into account in formulating realistic guidelines on the acceptable upper limits on pesticide contamination of the environment. Toxicity testing has a clear role in safeguarding environmental quality but a considered selection of the testing methods is essential for obtaining relevant results. Toxicity of pesticides is highly dependent on the duration, frequency, intensity of exposure, and the susceptibility of the target organism which is influenced by age, sex, fitness and genetic variation. Though a new generation of bioassays (toxkits, biomarkers, bioprobes) has been developed and is used in ecotoxicological practice, "standard" toxicity tests on living organisms (daphnids, fish, etc.) are still essential and more relevant in the evaluation of the toxicity of compounds for higher animals, including humans.

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Souhrn

Dobšíková R. (2003): **Akutní toxicita carbofuranu pro vybrané druhy vodních a suchozemských organismů**. Plant Protect. Sci., **39**: 103–108.

Carbofuran je anticholinesterázový karbamát v zemědělství běžně používaný jako insekticid, nematicid a akaricid. Vyhodnocovali jsme akutní toxicitu carbofuranu pro vybrané druhy vodních organismů (živorodka duhová *Poecilia reticulata* Peters, perloočka *Daphnia magna* Straus a zelená řasa *Raphidocelis subcapitata* Korsikov) a pro suchozemskou rostlinu (hořčice bílá *Sinapis alba* L.). Nejcitlivějším organismem byla perloočka *Daphnia magna*.

Klíčová slova: carbofuran; *Poecilia reticulata*; *Daphnia magna*; *Raphidocelis subcapitata*; *Sinapis alba*; akutní toxicita

Corresponding author:

MVDr. Radka Dobšíková, Veterinární a farmaceutická univerzita Brno, Fakulta veterinární hygieny a ekologie, Ústav veterinární ekologie a ochrany životního prostředí, Palackého 1-3, 612 42 Brno, Česká Republika
tel.: + 420 541 562 573, fax: + 420 549 243 020, e-mail: dobsikovar@vfu.cz