Bull. Environ. Contam. Toxicol. (1994) 53:627–632

Environmental Contamination and Toxicology

Acute Toxicity of Technical Trichlorphon to Cyprinid Fish

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Received: 1 October 1992/Accepted: 1 May 1994

The pollution problems in relation to fish and their environment have received increasing attention during past decades. Many research studies have revealed the effects of environmental pollutants, especially pesticides, on nontarget freshwater organisms such as fish (Spehar et al 1982). Nowadays, the use of fish toxicity testing is well established in many countries by law. Moreover, the extrapolations from laboratory to aquatic freshwater ecosystems requires data on a number of organisms representative of the ecosystem to be protected. Field studies are difficult and the laboratory bioassays are therefore required to gain insight into the potential risk of chemicals for ecosystems. The use of laboratory data requires that test systems resemble natural situations as much as possible (Committee to Review Methods for Ecotoxicology 1981). In ecotoxicology, laboratory toxicity data are used to predict the potential impact of chemicals (as pesticides) on fish populations in the aquatic ecosystems, to assess the potential hazard of chemicals and establish "safe" values of pollutants in the aquatic ecosystems. Furthermore to protect them, the knowledge of these laboratory toxicity data is the first needed aspect to the registration of new chemicals. These laboratory studies are needed also to predict the levels of no concern for the aquatic ecosystems.

Trichlorphon (dimethyl-2,2,2-trichloro-1-hydroxyethyl phosphonate), is a contact and ingested insecticide recommended for forest and agricultural use against fruit flies, lepidopterous and coleopterus larvae, household pests, ectoparasites on domestic animals. Also it has been used as antiparasitic on fish cultures (Kozlovskaya et al 1984).

Several scientific papers have described the general characteristics, metabolism in soil and water and the environmental features of trichlorphon (IRTPC/UNEP 1985),

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as its influence on water quality (Shiu et al 1990), toxicological aspects and their effects on other aquatic freshwater organisms (IRPTC/UNEP 1985). The effects of this insecticide were also studied on other fish species (Spehar et al 1982), but only can be found few papers related with the ecotoxicological effects on cyprinids (Dzuvic et al 1981; Kozlovskaya et al 1984; Studnicka and Sopinska 1983; Cossarini et al 1990; Siwicki-Andrzej et al 1990; Antón et al 1991).

The aim of the present study is to assess in vivo the acute toxic effects of technical insecticide Trichlorphon (97% a.i., Explosivos RioTinto S.A., Madrid, Spain) on two freshwater fish: Carassius auratus L. goldfish and Cyprinus carpio L. carp (Fam. Cyprinidae). Frequently both species of cyprinid fish live in the aquatic freshwater ecosystems, and also can be used as nontarget organisms to assess the toxicity of chemicals.

MATERIALS AND METHODS

The experimental procedures used were according to the OECD and EEC guidelines for testing chemicals on freshwater fish (USEPA 1975; EEC 1984; OECD 1984).

The assayed doses of technical insecticide (97%, a.i.) on Carassius auratus were from 0.001 mg/L to 400 mg/L (97 % a.i., equivalent to 0.00097 mg/L to 388 mg/L of pure insecticide). Several bioassays were conducted. On Cyprinus carpio the assayed concentrations in the aquatic medium of fish were from 50 to 100 mg/L of the same technical trichlorphon (97% a.i.) in a bioassay only, after known the LC50 values on the other specie Carassius auratus.. Then, it was searched a suitable range of concentrations of technical insecticide that, into the aquatic medium of fish, could be hazardous to Cyprinus carpio: these selected concentrations were ranged between 48.5 to 97 mg/L of pure insecticide. In Table 1 can be seen the concentrations that were tested in all experiments with fish and technical trichlorphon.

Carassius auratus L. (7 ± 1 cm length and 8 ± 2 g weight), were purchased in an aquaria-shop (Mundo Azul S.A., Madrid, Spain), and Cyprinus carpio L. (12.5 ± 2.5 cm length, 60 ± 20 g weight) were purchased in an official fishery ("Las Vegas del Guadiana" Badajoz, Spain). Fish were brought away to the laboratory. Therein, they were kept 7 d in acclimation before the bioassays, in 60 L or 270 L aquaria, respectively, in conditions of suitable aeration and illumination with running tapwater (pH near neutrality, 64 mg/L as conductivity, 80-100% of dissolved oxygen and 18°C temperature) that was previously dechlorinated. They were fed a commercial and standard food for cold freshwater fish. Temperature of the aquatic medium was 18 ± 1°C on both species of fish.

Acute toxicity bioassays were made in static exposure to insecticide for 96 hr. Both fish species were exposed to technical trichlorphon (97%, a.i.), in dechlorinated tapwater. In the exposed aquaria, the chemical was added to water without solvent because its high level of solubility in water at ambient temperature (Shiu et al 1990). A control aquarium had only received dechlorinated running tapwater, but without technical trichlorphon.

The experimental conditions for all bioassays were the following: six fish in 12 L illuminated aquaria (16 hr light and 8 hr darkness as photoperiod), with aeration and 18 ± 1°C of temperature, for C. auratus, and four fish in each 12 L illuminated aquaria with the same photoperiod, aeration and temperature for C. carpio. Four to eight concentrations were tested with C. auratus with two replicates of each concentration of trichlorphon. However only one test was carried out to study the effect of trichlorphon on C. carpio. Bioassays were carried out to assess all the exposures that are described in Table 1. Fish were not fed during the exposure to pesticide. Carassius auratus were exposed to 27 different concentrations, and Cyprinus carpio to 7 different exposure concentrations (from 50 to 100 mg/L). All fish were weighed when bioassays began and finished. Every 24 hr interesting notes were also taken about their state and the visible anomalies appearent. Every day pH, temperature, conductivity and percentage of dissolved oxygen in the aquatic medium were recorded in all aquaria. Before the last bioassays, on C. auratus, preliminar tests that gave us an useful information about the range of the effective doses of insecticide were made on both fish species.

In the all bioassays with the two species of cyprinid fish, temperature did not vary too much (16.0°C to 20°C) in 96 hr. The dissolved oxygen in the aquatic medium of fish was near to 80-100%, pH was always between 6.8 to 8.0, and only the conductivity was slightly increased up to 120 mg/L in the higher assayed concentrations of technical trichlorphon.

When bioassays were finished we calculated the mortality percentages for each concentration in the aquatic media. Then, comparing the "concentration-effect" data in a regression analysis "logarithmic-probit" using a computer program (Abou-Setta et al 1986) the estimated values of LC50 (lethal mean concentration) (96 hr) were calculated. Finally, it was also verified that the regression coefficients were in the interval of the 95% confidence limits.

RESULTS AND DISCUSSION

In Table 1 the 96 hr-LC50 values of assayed trichlorphon on the two fish species can be seen.

In the first three bioassays on _Carassius auratus_ L., 96 hr-LC50 value of technical trichlorphon (97%, a.i.) was higher than 16.00 mg/L. In the fourth test, 96 hr-LC50 was 48.36 ± 1.21 mg/L, equivalent to 46.90 ± 1.17 mg/l of pure insecticide. In the last bioassay, 96 hr-LC50 was 54.00 ± 3.45 mg/L (equivalent to 52.38 ± 3.34 mg/L, a.i.). Technical trichlorphon (97%, a.i.) in _Cyprinus carpio_ L., showed an acute toxicity value (96 hr-LC50) of 92.72 ± 6.74 mg/L (LC50), equivalent to 89.92 ± 6.53 mg/L of pure insecticide. The NOEC values (concentration without observable effects) were not calculated, but the lowest level of mortality on _C. auratus_ was found when fish were exposed to 27.5 mg/L (26.67 mg/L, a.i.). On _C. carpio_ the lower mortality percentage was found at 50 mg/L (48.5 mg/L, a.i.), which also was the lowest assayed concentration.

For _Carassius auratus_, from concentrations of 0.1 to 0.001 mg/L, in a time exposure 192 hr longer than in the standardized bioassay, less than a 10% mortality was observed, and no lethal effects were observed at 0, 4, 24, 48, 96, 144 and 172 hr of time with these assayed doses of technical trichlorphon in the aquatic medium of fish.

C. auratus mortality was 100% at 190 mg/L of technical insecticide in water. At the higher assayed concentrations in the medium of _C. auratus_ (150 to 190 mg/L of technical insecticide), fish showed symptoms of a clear intoxication (lethargic movements, gills and mouth opened by the need to accelerate their respiration, probably due to suffering a lack of oxygen, although the levels of dissolved oxygen in the aquatic medium would be sufficient, convulsions and a loss of its equilibrium to swim, especially at the 96 hr of bioassay). Similar symptoms were observed in _Cyprinus carpio_ fish exposed to concentrations from 60 to 75 mg/L and higher concentrations at the 72 hr. The fish also showed erratic movements and convulsions.

Several authors have studied the toxic effects of this organophosphate insecticide on cyprinid fish (Studnicka and Sopinska 1983; Kozlovskaya et al 1984; Cossarini et al 1990; Siwicki-Andrzej et al 1990; Antón et al 1991). Neubert (1986) has pointed out that there was a recovery time after the exposure of _Cyprinus carpio_ and other fish and aquatic invertebrates to sublethal concentrations of trichlorphon. Dzuvic et al (1981) have found hepatotoxic symptoms on exposed carps to 0.002 mg/L, and mortalities after 1 mon. Siwicki-Andrzej et al (1990) and Cossarini et al (1990) studying "in vivo" effects of this insecticide on _Cyprinus carpio_ have found an unspecific immunological response on the fish organs, as consequence of trichlorphon exposure at higher residual levels. Moreover different metabolic and biochemical effects caused by this insecticide on fish are also described (Dzuvic et al 1981; Kozlovskaya et al 1984; Cossarini et al 1990; Antón et

Table 1. Acute toxicity values of technical insecticide trichlorphon (97%, a.i.) on Carassius auratus L. and on Cyprinus carpio L. fish.

Species	Test	Nominal Concentration Range (mg/L)	Dilution Factor	LC50 (s.d.) (mg/L)	LC50 (s.d.) based on a.i. (mg/L)
C. auratus	I-III	0.001-16	1.5, 2	>16.00	>16.00
	IV	40-90	1.1	48.36 ± 1.21	46.90 ± 1.17
	V	27.5-400	1, 1.5	54.00 ± 3.45	52.38 ± 3.34
C. carpio	I	50-100	1.5	92.72 ± 6.74	89.92 ± 6.53

al,1991).

It is important to know the acute toxicity of trichlorphon on these fish species, but additional studies are needed to know more about the environmental fate and degradation of this insecticide in freshwater aquatic medium. It is also important to know something about the chronic toxicity of trichlorphon on fish, and other aspects as the brain acetylcholinesterase inhibition caused by sublethal exposures to low insecticide concentrations on these fish, as Antón et al (1991) studied.

In conclusion, trichlorphon (97 %, a.i.) has demonstrated to be very toxic to cyprinids fish, but to a lesser degree than on other fish such as salmonids (Neubert 1986), although their sublethal effects could be also very hazardous to Cyprinids. Moreover its risks in the freshwater ecosystems should be better known because this insecticide can be degraded to the more toxic compound Dichlorvos (IRPTC/UNEP 1985). All these questions should be studied with more detail in subsequent investigations.

Acknowledgments. We thank to other members of Environmental Sciences Center their collaboration.

REFERENCES

Abou-Setta MMA, Sorrell RW, Childers CC (1986) A computer program in basic for determining probit and log-probit or logit correlation for toxicology and biology. Bull Environ Contam Toxicol 36:242-249

Antón FA, Alia M, Gil A, Laborda E (1991) Toxicidad aguda del Triclorfón en Cyprinus carpio L., y Rata Wistar. Rev Toxicol 8(3):199 (Abstract)

Committee to Review Methods for Ecotoxicology (1981) Testing for effects of chemicals on ecosystems. National Academy of Science, Academic Press, Washington, DC

Cossarini DM, Demael A, Siwicki AK (1990) In vivo effect of the organophosphorus insecticide trichlorphon on the inmune response of carp Cyprinus carpio. I. Effect of contamination on antibody production in relation to residue level in organs. Ecotoxicol Environ Safety 19:93-98

Dzuvic A, Kiskaroly M, Ibrovic M, Sabic S (1981) Experimental study of the effects of trichlorphon in carp ponds on liver and kidney tissue. Veterinaria (Yugosl.) 30:257-262

European Economic Community (1984) Mètodes de determination de l'ecotoxicité: toxicité aigué pour les poissons. J Off Commun Europ 1 251/146 (19 September)

U.S. Environmental Protection Agency (1975) Methods for the acute toxicity tests with fish, macroinvertebrates and amphibians. The Committee on Methods for Toxicity Tests with Aquatic Organisms. Ecol Res Ser EPA-600-75-009

International Register of Potentially Toxic Chemicals (1985) IRTPC data profile: Trichlorphon. IRTPC/UNEP. United Nations Environment Programme, Gèneve (Suisse). 17 pp

Kozlovskaya VI, Maier FL, Petti DD (1984) Acetyl-cholinesterase activity in the brain and collagen content in the vertebral column of Carassius in Chlorophos intoxication. Gidrobiol Zh 20:53-58

Neubert J (1986) Acute toxicity of trichlorphon to selected aquatic organisms. Acta Hydrochim Hydrobiol 14: 643-651

Organization for Economic Cooperation and Development (1984) Tests guidelines. Directrice 203. Decision of the Council C, 30 final, Paris

Shiu WY, Ma KC, Mackay D, Seiber JN, Wauchope RD (1990) Solubilities of pesticide chemicals in water. Part I. Environmental physical chemistry. Res Rev 116:1-13

Siwicki-Andrzej K, Cossarini-Dunier M, Studnicka M, Demael A (1990) In vivo effect of the organophosphorus insecticide trichlorphon on inmune response of carp (Cyprinus carpio). II. Effect of high doses of trichlorphon on nonspecific inmune response. Ecotox Environ Safety 19:99-105

Spehar RL, Christensen GM, Curtis C, Lemke AE, Norberg TJ, Pickering QH (1982) Effects of pollution on freshwater fish. J Water Pollut Control Fed (EPA 600/J81-084) 52: 877-922

Studnicka M, Sopinska A (1983) Toxicity of technical trichlorphon for fish. Rocz Nauk Polnic 100:111-116