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Acute Toxicity of Two Compounds with Different Modes of Action to the Zebrafish, *Danio rerio*

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According to Verhaar et al. (1992), four classes of aquatic organic toxicants can be distinguished: nonpolar narcotic chemicals, polar narcotic chemicals, reactive chemicals and specifically acting chemicals. The first class consists of a broad group of mainly industrial pollutants with a non-specific mode of action. Their acute toxicity can mainly be explained by their octanol/water partition coefficient, expressed as log K_{ow}. Quantitative Structure Activity Relationships (QSAR) with log K_{ow} as only variable give good estimations of the toxicity for most of the representatives of this class (Könemann 1981; Veith et al. 1983; McCarty et al. 1985; Klopman et al. 1999). This class represents baseline or minimum toxicity, because any chemical is at least as toxic as its baseline toxicity.

The class of specifically acting chemicals consists mainly of pesticides. The group of organophosphorus (OP) pesticides, whose mode of action is the inhibition of the enzyme acetylcholinesterase (AChE) is one of the major groups. However, upon acute exposure, sometimes a nonspecific narcosis-like mode of action seems to be active, depending on the metabolic characteristics of the test species and the physio-chemical properties of the OP (Keizer et al. 1993; Deneer et al. 1999). Attempts to model the acute toxicity of this group by means of QSARs is more complex than for the class of non-polar narcotic chemicals and has not been very successful until now (DeBruijn and Hermens, 1992). The enhanced acute toxicity of these groups of compounds, compared to baseline toxicity, is expressed in the Toxic Ratio (TR). This TR is defined as the baseline LC_{50} divided by the experimental LC_{50} (Verhaar et al. 1992).

The aims of this study were to determine the acute toxicity of representatives of the two classes mentioned above to the zebrafish (*Danio rerio*) and to compare these with LC_{50} values obtained from the literature, using the same test organism. The two model compounds are the nonpolar narcotic 1,2,3-trichlorobenzene (123TCB) and the AChE inhibitor parathion. In order to explain the acute toxicity of both compounds, the LC_{50} was related to the hydrophobicity and the Toxic Ratio (TR) was calculated.

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MATERIALS AND METHODS

Adult zebra fish (*Danio rerio*) were purchased from a commercial supplier and were approximately three months old at arrival in our laboratory. The fish were maintained in a climate room with a temperature of 28°C and a photoperiod of 12:12 h light: dark, and were fed frozen adult brine shrimp (*Artemia salina*). The fish were acclimated in glass aquaria containing copper free Amsterdam tap water for two weeks before use in the experiment.

1,2,3-Trichlorobenzene (123TCB, purity 99%) was obtained from Aldrich Chemical Company (Milwaukee, WI). Pentachlorobenzene (>99%) was obtained from Fluka AG (Buchs, Switserland). Parathion-ethyl (98.9%), chlorpyrifos (99%) and isopropanol (>99.8%) were purchased from Riedel-de Haën (Seelze, Germany) and hexane (>99.5%) from JT Baker (Phillipsburg, NJ).

Fish were exposed to four concentrations of parathion or 123TCB. Bellavere and Gorbi (1984) established a 48h LC_{50} value of 5.64 mg/L for parathion. Calamari et al. (1983) found a 48h LC_{50} of 3.1 mg/L for 123TCB. Based on these values, the following concentration ranges were composed: 1, 3, 9, and 12 mg/L for 123TCB and 0.3, 1, 3, and 9 mg/L for parathion. Different stock solutions of parathion and 123TCB were obtained by dissolving them in 2-propanol. Of these stock solutions, 150 µL was added to 10-L vessels, which contained 10 L of copper free Amsterdam tap water. Because of the volatility of 123TCB, a continuous flow-through system was employed. A peristaltic pump (Watson Marlow 205S/BA12) was used to deliver a constant flow of freshly contaminated water to the exposure aquaria. The flow was set at 6.2 mL/min and was checked at the start and the end of the exposure period. Solutions were prepared freshly every day and were stirred at least four hours before use. For parathion a static system was used. The aquaria had a volume of 14 L. Each aquarium contained five fish, which were randomly distributed among the treatments and were not fed during the experiment. Two solvent-control treatments were incorporated, a flow-through and a static one. During the experiment, several water parameters were measured: oxygen, nitrite and nitrate content, pH and hardness. At first, mortality was checked every hour, later in longer intervals. The LC_{50} was determined using the Spearmann-Karber method (Hamilton et al. 1977, 1978). The Toxic Ratio of both compounds were calculated, according to Verhaar et al. (1992). As no QSARs were available for zebrafish, the baseline LC_{50} was calculated according to the QSAR for guppies (Könemann 1981) Values for log K_{ow} were extracted from the literature for 123TCB (De Bruijn et al. 1989) or the ClogP program for parathion (Biobyte 1994).

Actual concentrations of parathion and 123TCB were checked daily. Aliquots of 20 mL of water were extracted with 10 mL of hexane. Before extraction 50 µL of an internal standard in isopropanol was added to the water samples to determine the recovery of the extraction procedure. For 123TCB, pentachlorobenzene was added as internal standard, chlorpyrifos was used in the case of parathion. After

extraction, the hexane fractions were analyzed on a gas chromatograph. For the analysis of 123TCB, a Carlo Erba 8000 series gas chromatograph, equipped with a 30 m DB5 column (J&W Scientific, Folson, CA) and an electron capture detector (ECD) was used in the splitless mode. The injection volume was 1 µL. Injection took place cold on column. The temperature of the detector was 300°C, the temperature of the oven was programmed at 70°C for 1 minute, raised at 40°C min^{-1} to 160°C, kept at 160°C for 1 minute, raised at 30°C min^{-1} to 250°C, and kept there for 2 minutes. For the analysis of parathion a Carlo Erba 8000 series gas chromatograph, equipped with a 30m DB1701 column (J&W Scientific, Folson, CA) and a Nitrogen Phosphorous Detector (NPD), was used in the splitless mode. Aliquots of 2 µL were injected cold on column. Temperature of the detector was 280°C. The oven temperature was programmed at 80°C for 1 minute, raised at 30°C min^{-1} to 150°C, then at 10°C min^{-1} to 260°C and kept at 260°C for 4 minutes. Results were corrected for recovery.

RESULTS AND DISCUSSION

The average aqueous concentrations of parathion and 123TCB are shown in Table 1. The concentrations of the lowest treatments were in good agreement with the nominal ones for both compounds. For the two highest treatments, actual concentrations never reached the nominal ones. In the two highest parathion treatments, a yellow coloration of the water phase took place, caused by the primary breakdown product of the parent compound, *p*-nitrophenol, which was also found in other studies (Jarvinen and Tanner 1982). The calculated LC_{50} values are based on time-weighed actual concentrations.

In Table 2, the LC_{50} values for both compounds at different time intervals are presented. The LC_{50} of both compounds decreased with increasing exposure time, but the LC_{50} values for different time intervals were not significantly different. Bellavere and Gorbi (1984) also found the LC_{50} for parathion to remain stable after 24 hours. Longer exposure duration may give rise to an enhanced formation of toxic metabolites, causing a higher toxicity and consequently a lower LC_{50}. However, the duration of our acute test is probably still too short to cause this decrease in LC_{50}.

Table 1. Actual aqueous concentrations of parathion and 123TCB (mg/L) plus standard deviations during the LC_{50} experiment with *Danio rerio*.

parathion		123TCB	
nominal	actual	nominal	actual
0	0.03 ± 0.015	0	0.01 ± 0.003
1	1.45 ± 0.203	0.3	0.29 ± 0.18
3	2.59 ± 0.438	1	0.79 ± 0.34
9	4.72 ± 0.529	3	0.63 ± 0.08
12	4.50	9	4.23

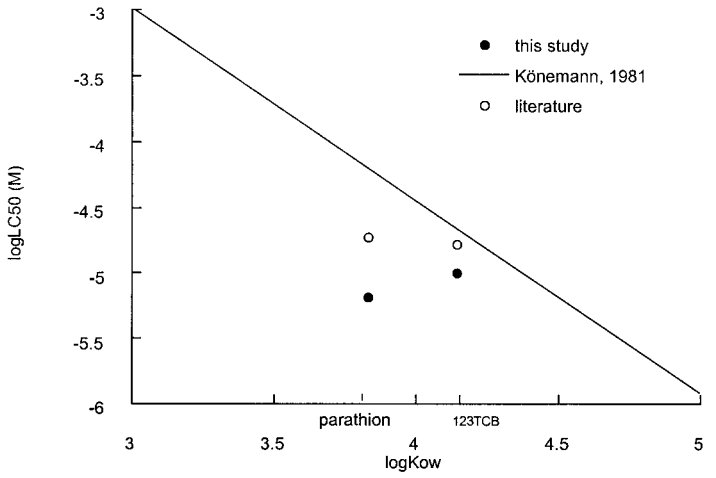

Figure 1. LC_{50} versus log K_{ow} for 123TCB and parathion, compared with a QSAR from the literature.

Table 2. Calculated LC_{50} values (mg/L) including 95% confidence intervals for parathion and 123TCB on the survival of *Danio rerio*.

Exposure time	parathion	123TCB
24 h	2.43 (1.90 - 3.13)	2.28 (-)*
48 h	2.17 (1.77 – 2.66)	1.85 (-)*
96 h	1.94 (1.45 – 2.59)	1.85 (-)*

*no reliable confidence interval could be calculated.

For both compounds the LC_{50} was lower than the values reported in other studies. In the case of parathion this discrepancy is the biggest, probably caused by the fact that in other studies (Bellavere and Gorbi, 1984) nominal concentrations are reported, while in this study the LC_{50} was calculated on the basis of actual concentrations. This stresses the importance of measuring actual exposure concentrations of the toxicant in the water phase.

In Figure 1, the acute toxicity of both compounds is plotted against their log K_{ow}. The solid line represents the QSAR equation for the 96h LC_{50} of chlorobenzenes to fish, as calculated by Könemann (1981). As can be seen, the acute toxicity of 123TCB to zebrafish in this study can merely be explained by its log K_{ow}, the TR is 1.47.

Parathion shows a small-enhanced toxicity, compared to the baseline toxicity QSAR, with a TR of 5.6. This is relatively low compared to other published TRs for organophosphorus compounds that were calculated to be between 0.7 and

4,130 (Russom et al. 1997). Based on the QSAR for organophosphates, with log K_{ow} as only parameter, as proposed by De Bruijn and Hermens (1992) a TR of 0.78 could be calculated. This indicates that upon acute exposure the toxicity of organophosphates is merely caused by the mechanism of nonpolar narcosis, and that the exposure duration is too short to elicit more specific modes of action (De Bruijn et al. 1991). It is therefore recommended to prolong the acute exposure duration in toxicity test with fish, especially when compounds are involved, which are supposed to act via a specific mode of action.

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