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ORIGINAL PAPER

Joint acute toxicity of tributyl phosphate and triphenyl phosphate to *Daphnia magna*

Kunde Lin

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Abstract In this study, the joint acute toxicity of tributyl phosphate (TBP) and triphenyl phosphate (TPP) was investigated using *Daphnia magna* as the test organism. The median lethal concentrations for TBP and TPP at 24 and 48 h were 5.48 and 0.51 mg/L, 1.17 and 0.089 mg/L, respectively. When mixed at either equal concentration or various toxic unit ratios, TBP and TPP mixtures displayed an additive toxicity after both 24- and 48-h exposure. This work suggests that the joint acute toxicity of TBP and TPP towards *D. magna* can be predicted by calculating the toxic units of mixtures.

Keywords Tributyl phosphate · Triphenyl phosphate · *Daphnia magna* · Joint toxicity

Introduction

Tributyl phosphate (TBP) and triphenyl phosphate (TPP) are important organophosphorus compounds used in flame retardants and plasticizers industry. TBP is mainly utilized as solvent for cellouse esters, lacquers, and natural gums, as primary plasticizers in the manufacture of plastics and vinyl resins and as an antifoam agent for concrete and hydraulic fluids. TBP is also used as an additive in the textile and clothing dyeing industry, as well as a solvent for the extraction of uranium and plutonium from other radionuclides in nuclear fuel processing (Thomas and Macaskie 1996). TPP is predominately used as hydraulic fluids and flame retardant (Huckins et al. 1991). TBP and TPP are listed as European Union high production volume chemicals.

The broad application of TBP and TPP results in their diffusive spreading into the environment by volatilization, leaching, and abrasion. TBP and TPP were detected in samples of indoor air from 17 domestic and occupational environments, with TBP concentrations ranging from 3.8×10^{-3} to 0.12 ng/L while TPP ranging from 0 to 2.3×10^{-2} ng/L (Marklund et al. 2005a). TBP and TPP were also frequently identified in the aquatic environment. For example, the occurrence of TBP in German river water was found to be 17–1,510 ng/L in the Rhine, Elbe, Main, Oder, Nidda, and Schwarzbach Rivers (Fries and Püttmann 2001). It was supposed that wastewater treatment plants (WWTPs) discharge TBP and TPP into the aquatic environment as they were detected in both the influent and effluent (Meyer and Beser 2004; Marklund et al. 2005b). The highest concentrations for TBP and TPP in the influent from German WWTPs were 5.5 μg/L and 290 ng/L (Meyer and Beser 2004), respectively, while 52 μg/L for TBP and 290 ng/L for TPP in the influent from Swedish WWTPs (Marklund et al. 2005b). The elimination rates in WWTPs ranged 55–67% and 57–75% for TBP and TPP, respectively (Meyer and Beser 2004).

The presence of TBP and TPP in the environment may have a direct adverse impact on the aquatic organisms and pose a threat to human beings through the contamination of drinking water supplies. However, toxicity and environmental safety of TBP and TPP are poorly understood and have so far received limited research. The median lethal concentration (LC50) at 96 h for TPP to rainbow trout is 0.36 mg/L (WHO 1991a). TBP is about one order of magnitude less toxic to the same species with a LC50 of 4.2–12 mg/L (WHO 1991b). Since TBP and TPP are always concurrently present in the aquatic environment, the

K. Lin (✉)
College of Biological and Environmental Engineering, Zhejiang University of Technology, 310032 Hangzhou, Zhejiang, China
e-mail: link@zjut.edu.cn; linkd1974@hotmail.com

toxicity of their mixtures should be considered when evaluating their ecological risks.

Traditionally, the toxicity of chemical pollutants is mainly evaluated on the basis of a single test substance. However, it is probably that coexisting toxicants may interact with each other and produce unexpected combination effects, such as antagonism and synergism. For example, diazinon and copper exhibited less than additive effect on the mayfly (*Ephoron virgo*) (van der et al. 2000). On the other hand, additive effect to *Ceriodaphnia dubia* was found for diazinon and chlorpyrifos when present together (Bailey et al. 1997). However, the lack of information on the joint toxicity of TBP and TPP on aquatic species warranties further study.

The objective of the present work was to investigate the interactive toxicity effect of TBP and TPP towards *Daphnia magna*.

Experiments

Chemicals

Analytical standard of TBP (>99%) and TPP (>99%) was purchased from Acros Organics (NJ, USA). Other chemicals and solvents were of analytical or high-performance liquid chromatography grade.

Bioassays

The acute toxicity was measured for individual TBP or TPP solution, and TBP and TPP mixtures using *D. magna* as the test organism. The test organisms were obtained from a continuous culture maintained at 22 ± 1°C in M4 culture medium (OECD 1997) with a photoperiod of 12 h/day and a density of <50 animals per liter. Stock organisms were originally obtained from the Chinese Academy of Protection and Medical Science (Beijing, China). The medium was renewed three times a week and daphnids were fed daily with the alga *Scenedesmas obliquus*, which were cultured in the laboratory using a nutrient medium. The test animals used in this experiment were juveniles aged between 8 and 24 h. Prior to the test, a sensitive test for daphnids to potassium dichromate was performed as a positive control and the LC50 (24 h) value was in the range of 0.6–1.7 mg/L. The overall acute toxicity test was conducted according to the standard protocol (OECD 1997). Briefly, five neonates were transferred into glass beakers filled with 20 mL of blank or test solutions of known TBP or TPP concentrations. The test solutions with the highest concentration were prepared by adding a known amount of TPP or TBP into the dilution water. Subsequent dilutions were made from the highest concentration to derive the lower concentration solutions. The nominal concentrations were 0.5, 1.0, 2.5, 6.25, 12.5, and 25 mg/L for TBP, 0.025, 0.05, 0.1, 0.25, 0.5, 1.0, and 2.0 mg/L for TPP. For TBP and TPP mixtures, the mixture concentrations were chosen according to the LC50 (24 h) values of TBP and TPP. For example, the nominal concentration for the mixture with a toxic unit ratio of 4:1 were 0.063:0.025, 0.125:0.05, 0.25:0.1, 0.63:0.25, 1.25:0.5, and 2.5:1.0 (mg/L TBP: mg/L TPP). Four replicates were prepared for each treatment. The test animals were not further fed and were incubated at 22 ± 1°C for 48 h. Mortality of daphnids was observed after incubation for 24 and 48 h.

Joint toxicity assay

Concentration addition is employed to predict the toxicity of organic compounds that usually pose a nonspecific mode of action, e.g., narcosis (Evans and Nipper 2007, 1997; Bailey et al. 2001). According to the definition of concentration addition, toxicity of mixtures can be expressed as toxic units of the mixtures (TU_{mix}), which is a ratio of the measured concentration of a chemical in a mixture to the corresponding effect concentration of the single compound in the same medium (DiToro and McGrath 2000). Assuming concentration addition, the TUs for individual components in a mixture can be added to estimate the total toxicity. The TU_{mix} could be determined by the following equation:

$$TU_{mix} = \frac{LC50_{TPP\ in\ the\ mixture}}{LC50_{TPP\ alone}} + \frac{LC50_{TBP\ in\ the\ mixture}}{LC50_{TBP\ alone}}.$$

Accordingly, 1.0 TU is considered equivalent to the LC50 of an individual chemical when tested alone. Assuming a strictly additive effect, the LC50 concentration of a mixture should equal unity when expressed as TUs. For example, if TBP concentration associated with the LC50 of a mixture is 0.5 mg/L, 0.5 TU of TBP would be present at this concentration, assuming the LC50 for TBP alone is 1.0 mg/L. The TUs for TPP in the mixtures were similarly calculated and added to the TBP TUs to determine the total TU_{mix} for mixtures. The sum of these values was then used to evaluate the joint effect of TBP and TPP mixtures. A TU_{mix} value equal to 1.0 ± 0.2 indicates concentration addition. A TU_{mix} less than 0.8 indicates a synergistic effect (more than additive effect), while a TU_{mix} greater than 1.2 indicates an antagonistic effect (less than additive effect) (Broderius et al. 1995).

Results and discussion

Acute toxicity of TBP and TPP

The LC50 values obtained from the tests are summarized in Table 1. The toxicity of TPP at both 24 and 48 h was more

Table 1 The median lethal concentration (LC50, mg/L) of TBP and TPP to *Daphnia magna* (data are given as mean and 95% confidence intervals)

Compound	LC50 (24 h)	LC50 (48 h)
TBP	5.48 (4.86–6.09)	1.17 (1.02–1.32)
TPP	0.51 (0.47–0.55)	0.09 (0.08–0.10)

potent than TBP. The acute toxicity of TPP to *D. magna* was about 10.7–13.1 times as that of TBP after 24- and 48-h exposure (Table 1). This observation is consistent with previous reports (WHO 1991a, b), showing that TPP was about 11.7–33.1 times potent to rainbow trout as that of TBP. Previous tests showed that the 24-h LC50 for TBP and 48-h LC50 for TPP to *D. magna* were 33 and 1.0 mg/L, respectively (WHO 1991a, b), both displaying a less toxic than those observed in this study. However, the result is close and the discrepancy can be reasonably expressed by the fact that the biological response of the organisms to toxicants can vary slightly among different labs.

It is evident that TPP was generally more toxic than TBP. Although the toxicity difference is uncertain for different species, it might be as high as ten times or more. The identified concentration of TPP in the environmental samples was usually lower than that of TBP; for example, the concentration of TBP in German sewage was about 19 times as that of TPP (Meyer and Beser 2004). These results together indicate that although TPP present in lower concentration in the environment, it might have a comparable toxicity with the coexisting TBP with higher concentrations.

Joint acute toxicity

To understand the interaction of TBP and TPP in the joint acute toxicity to *D. magna*, the values of LC50 at 24 and 48 h for different binary mixtures were determined. Based on the LC50 values from the tests on the individual chemicals and binary mixtures, the results for the mixtures were converted to TUs of the individual chemical and summed (Tables 2, 3). When TBP and TPP were mixed at equal-concentration, the calculated TU_{mix} at 24 and 48 h were 0.85 and 0.88 (Table 2), respectively, suggesting an additive effect. For further verification, the joint acute toxicity was evaluated by a suite of mixtures with different toxic unit ratios ranging from 4:1 to 1:4 (TBP:TPP). In 24-h test, the values of calculated TU_{mix} ranged between 0.93 and 1.05, with an average of 0.97 ($n = 5$). Similarly, the values of calculated TU_{mix} in 48-h test ranged between 0.93 and 1.15, with an average of 1.06 ($n = 5$). Collectively, these values were very close to the hypothetical value of 1.0, suggesting that mixtures of TBP and TPP exhibited additive toxicity to *D. magna*. This observation implies that the toxicity to *D. magna* for TBP and TPP mixtures with different mixing ratios can be predicted by the calculation of TU_{mix}.

Table 2 The median lethal concentration (LC50, mg/L) of TBP and TPP to *Daphnia magna* for the equal-concentration mixtures and their corresponding calculated toxic units

Exposure time (h)	LC50		TU_{mix}
	TBP	TPP	
24	0.39	0.39	0.85
48	0.07	0.07	0.88

Table 3 The median lethal concentration (LC50, mg/L) of TBP and TPP to *Daphnia magna* for mixtures with different toxic unit ratios and their corresponding calculated toxic units

Exposure time (h)	Mixing ratios[a]	LC 50		TU_{mix}
		TBP	TPP	
24	1:4	0.95	0.38	0.93
	2:3	1.71	0.31	0.93
	1:1	2.50	0.25	0.95
	3:2	3.33	0.22	1.05
	4:1	4.39	0.11	1.01
48	1:4	0.22	0.09	1.15
	2:3	0.41	0.06	1.11
	1:1	0.47	0.05	0.93
	3:2	0.68	0.05	1.08
	4:1	0.89	0.02	1.01

[a] The mixing ratios are given as $\frac{\text{Concentration of TBP in the mixture}}{\text{LC50 of TBP at 24 h}} : \frac{\text{Concentration of TPP in the mixture}}{\text{LC50 of TPP at 24 h}}$

The additive effect between TBP and TPP may be reasonably explained by the fact that both chemicals have a similar mode of toxic action by binding with acetylcholinesterase in *D. magna*. Likely for the same reason, the joint toxicity to *C. dubia* for two organophosphorus insecticides, diazinon and chlorpyrifos, was also additive (Bailey et al. 1997).

Conclusions

This study showed that TPP was more potent towards *D. magna* than that of TBP. This work also demonstrated that the mixtures of TBP and TPP produced additive toxicity towards *D. magna*. The additive toxicity suggests that the ecotoxicological risks of concurrent presence of TBP and TPP in the environment may be considered additively. Since TBP and TPP were found to concurrently present with other organophosphorus compounds such as tris(2-butoxyethyl) phosphate, tris(chloropropyl) phosphate, and 2-ethylhexyl diphenyl phosphate in the wastewater, more comprehensive study on their joint toxicity effect should be addressed in the future.

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