Bull. Environ. Contam. Toxicol. (1997) 58:263-267

Bulletin of Environmental Contamination and Toxicology

Determination of Acute Toxicity of Polychlorinated Biphenyls to *Photobacterium phosphoreum*

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Received: 13 November 1995/Accepted: 25 October 1996

Polychlorinated biphenyls(PCBs) are a highly lipophilic group of global pollutants, consisting of 209 congeners(WHO ,1993). PCBs were discovered before the turn of the century and their usefulness for industry, because of their physical properties, was recognized early. The distribution of PCBs in the environment was not noticed until Jensen and his colleagues found PCBs in wildlife samples(Jensen 1969). Since then, investigations in many parts of the world have revealed the widespread distribution of PCBs in environmental samples and PCBs are persistent and accumulate in food webs. Thus, determination of toxicities of commercial PCB mixtures and PCB congeners are required.

Toxicity tests using luminous bacteria developed since the 1970's have shown high correlation to traditional bioassays. The bioluminescent bacterial test utilizes a standardized culture of a selected strain of a marine bacterium, *Photobacterium phosphoreum,* the light output of which can be measured. The addition of a toxic substance to the bacterial suspension results in most cases in a rapid decrease of its light emission, and the toxicity is recorded as the percent decrease in luminescence after a certain time (Brenner 1993). As a simple, fast and comparatively inexpensive alternative to *in-vivo* bioassay with higher organisms, the bioluminescent bacteria-test has been widely applied. There are numerous luminescent bacteria test data for 5-min, 15-min and 30-min exposure to pesticides, herbicides and other chemicals such as DDT, 2,4-D, chlorophenols etc. (Kaiser 1991, 1994), Hence it is very important to assess the toxicity values of PCBs with the bioluminescent bacteria test and try to find out whether there are correlations between the luminescent bacteria toxicity test data and those from other acute toxicity tests. China manufactured PCBs for about a decade before 1974 with the trade names of the PCB

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mixtures as PCB3 and PCB 5 . One of the goals of our study was to compare the EC_{50} values of the commercial mixtures, PCB3 and PCB 5 , with those of Aroclor 1242 and Aroclor 1254. Simultaneously, eight PCB congeners were also to be tested.

MATERIALS & METHODS

The PCB3 and PCB 5 mixtures were provided by the Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, and Aroclor 1242 and Aroclor 1254 were purchased from the Supelco Company, U.S.A. The eight PCB congeners were purchased from the Analabs, Inc., Japan (2,3-diCB, 2,4′,5-triCB, 2,2′,5,5′-tetraCB, 3,3′, 4,4′-tetraCB, 2,2′, 3,4,5′-pentaCB & 2,2′,3,4,4′,5-hexaCB)and AccuStandard Company, U.S.A. (3,3′,4,4′,5-pentaCB & 3,3′,4,4′,5,5′-hexaCB). Mercuric chloride and other chemicals used in the test were analytical grade. The luminometer model DXY-2 and freeze-dried *Photobacterium phosphoreum* T3 spp. cells were obtained from the Institute of Soil Science, Chinese Academy of Sciences, Nanjing.

Each of the four commercial PCB mixtures was dissolved in acetone and then added to water. The eight PCB congeners were directly added to water separately the aqueous solutions were used as test samples after one week.

The test procedures followed the national standard method of China (Yuan 1994), and are briefly outlined here . A working solution of luminescent bacteria was prepared by reconstituting a vial of freeze-dried *Photobacterium phosphoreum,* using 1 mL of 2 % NaCl aqueous solution under the temperature of 2-5°C and fully homogenized. The hydrated cell suspension, when kept on ice, was usable for about 4-5 hours. 0.01 mL of the bacterial suspension was added to 2 mL sample solution containing 3 % NaCl in a test tube. Luminescence was measured at 21-23°C after 15 minutes, and compared to the control sample. For the sample of which the relative luminescence of original solution was lower than 50 %, a dilution series of the sample could be measured directly in this way, The EC_{50} was calculated as the effective sample concentration causing a 50 % decrease in activity compared to the control and shown with the unit of mg/L. For the sample whose relative luminescence of original solution was higher than 50 %, the relative luminescence of a series of 11 $HgCl_2$ solultions which ranged from 0.02 mg/L to 0.22mg/L with same concentration lags had to be determined. The toxicity of tested sample ‘was measured and expressed as the concentration of the $HgCl_2$ solution which caused the same relative luminescence decrease as that caused

by the sample solution in the unit of mg/L $HgCl_2$.

RESULTS & DISCUSSION

To determine acute toxicity of commercial PCB mixtures, it is important to keep every congener in the mixture unsaturated. The rather low water solubility of PCB congeners make it very difficult to prepare such aqueous solution. In order to resolve this problem, the commercial PCB mixtures are recommended to be dissolved in acetone and then diluted with distilled water while the acetone concentration cannot be higher than 0.08 % (0.063 % in this work). It was reported that methanol could be used at up to 5 % concentration to get complete solution without interfering with the toxicity test (Ribo 1983), but in our experiment the relative luminescence of the sample solution containing 5% methanol is only 8 %, therefore the application of methanol is proved not suitable for this work.

The *Photobacterium phosphoreum* toxicity values of the four commercial PCB mixtures are shown in Table 1. It is clear that Aroclor 1242 and PCB_3 have similar acute toxicity along with Aroclor 1254 and PCB_5 .

Table 2 shows the toxicity values of the saturated aqueous solutions of the eight PCB congeners, ranging from 0.040 mg/L $HgCl_2$ to 0.158 mg/L $HgCl_2$. In Table 2 the congeners with asterisk mark belong to coplanar congeners , which are generally regarded as the most toxic because of their capabilities for inducing similar toxic effects as 2,3,7,8-TCDD (Safe 1992). However, this characteristic does not appear in this work, the toxicity values of these three coplanar congeners are not elevated compared with those of the other tested congeners especially in consideration of the fact that the solubilities of each couple of tetraCB, pentaCB, and hexaCB are similar (Nirmalakhandam 1989).

The toxic and carcinogenic effects elicited by commercial PCB mixture and individual congeners have been extensively investigated in laboratory animals . from fish to mammals (Safe 1987). The toxicity of many chemicals is dependent on their biotransformation to reactive metabolites by cytochrome P-450-dependent monooxygenases or other drugmetabolizing enzymes. Induction of hepatic microsomal enzymes is one of the most sensitive parameters and the results show that PCB-induced effects are dependent on many factors, e.g. the species of animals, age, sex and so on. Gooch et al. have studied the effects of some ortho- and non-ortho- substituted polychlorinated biphenyl congeners on the hepatic monooxygenase system in scup *(Stenotomus chysops)* . Although 3,3’,4,4’-TCB is a potent P450E

Table 1 Acute toxicity values of commercial PCB mixtures

Name	EC_{50} (mg/L)	n	r^2
Aroclor 1242	0.14	6	0.81
Aroclor 1254	0.018	6	0.99
PCB_3	0.12	6	0.83
PCB_5	0.017	6	0.99

Table 2 Acute toxicity values of saturated aqueous solutions of PCB congeners

IUPAC no.	Structure	Toxicity value' (mg/L $HgCl_2$)
5	2,3-	0.085±0.010
31	2,4',5-	0.040±0.00l
52	2,2' ,5,5'-	0.158±0.002
77*	3,3' ,4,4'-	0.044±0.002
87	2,2' ,3,4,5'-	0.045±0.006
126*	3,3',4,4' ,5-	0.143±0.003
138	2,2',3,4,4' ,5-	0.048±0.007
169*	3*3',4,4',5;5'--	0.0573.0,009

' Three determinations

inducer in scup, it can also inhibit P450E activity by acting as a alternate substrate *in vitro,* and probably *in vivo* (Gooch, 1989). Miranda et al. have studied the multiple effects of 3,3', 4,4',5–HCB administration on hepatic cytochrome P450 isozymes and associated mixed-function oxidase activities in rainbow trout *(Oncorhynchus mykiss)* and the results are analogous to Gooch's (Miranda, 1990). *Photobacterium phosphoreum* belongs to bacteria and its enzyme system is not the same as that of higher organisms. This might be the best explanation for the inconsistency of our data shown in Table 2 with some early results. This result should be attended in other toxicity test.

Acknowledgements . This work was supported by National Natural Science Foundation of China.

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