Bull. Environm. Contam. Toxicol. 20,786-792 (1978)

Application of a New Bioassay to Screen the Toxicity of Polychlorinated Biphenyls on Blue-green Algae

Ninette Zullei and Gisela Benecke

Institut für Wasserforschung GmbH, Dortmund, Dortmunder Stadtwerke AG, 5840 Schwerte 6 (Geisecke), Federal Republic of Germany

INTRODUCTION

Many recent publications are concerned with the effects of polychlorinated biphenyls (PCB) on algae. The different organisms, chemicals, methods, and last but not least the problems in question gave a large spectrum of results.
Of great importance is the very fast accumulation of these ubiquitious chemicals in biological materials (UREY et al, 1976; SÖDERGREN, 1971). The growth of several diatoms is strongly inhibited (MOSSER, 1972; FISHER, 1975), while the green algae Chlorella pyrenoidosa appears to be more resistant against PCB (UREY et al, 1976; SÖDERGREN, 1971). Furthermore the PCB's have an influence on the photosynthetic activity (GLOOSCHENKO and GLOOSCHENKO, 1975), the RNA-synthesis, and the chlorophyll index (KEIL, 1971) of several species of algae as well as on the motility of the blue-green algae Phormidium autumnale (NOLL and BAUER, 1973).

In this work the effect of defined PCB's with a different degree of chlorination was tested by a standardized bioassay using the motility of blue-green algae as a criterion for the toxicity of a substance.

MATERIALS AND METHODS

1. Testorganism

Filamentous cyanophyceae of the genus Phormidium (Oscillatoria) served as testorganism. Phormidium has been collected from slow-sand-filter basins of the artificial ground water recharge plant of the Dortmunder Stadtwerke AG in the Ruhr-Valley.

2. Cultivation

The cultivation was carried out in batch-cultures with BG-11 nutrient media (BENECKE, 1975).

0007-4861/78/0020-0786 $01.40

3. Test preparation

After 7 days of cultivation the algae were separated from the media by membrane filtration (Sartorius,pore size 5 µ). Algae discs with a diameter of 5 mm and with a dry weight of 2,1 µg were cut from the algae-covered membranes. 1, 5o, and 1oo µg of the test substances (Clophen A 3o and A 6o from Bayer AG, single PCB's from Analabs) were applicated for each test at three marked spots on a paper filter (Schwarzband, 7 cm diameter). The algae discs were placed on these spots. So prepared for the test run, the paper-filter was set into a Petri dish which contained 2 ml of fresh nutrient media (pH 8).

4. Procedure of measurement

The motility of the algae filaments can be recorded by a photoelectric cell (Valvo LDR 03), which to a greater or smaller extent is covered by the increasing algae discs (BENECKE in preparation). Thus the light-dependent current is inversely correlated to the movement of the algae . By comparing the signals of three test discs with those of three control discs, the inhibition can be measured as the difference between the undisturbed and the PCB-inhibited movement.
A Wheatstone bridge with a constant tension of 5 V DC is coupled to a recorder (Siemens Kompensograph III). In order to determine the relative inhibition of the algae-filaments displacement, the total inhibition caused by 1o µg per test spot of the herbicide Deiquat after 3 hours was taken as 1oo % and compared with the three-hour values of the respective PCB-test.

RESULTS AND DISCUSSION

Table 1 shows the reaction of test-algae on the applied PCB's after a three-hour test run using 1oo µg per test spot.

Those substances marked with (-) are within the security-range of the blanks. The different intensity is not marked in this table. For detailed inhibition diagrams for some of the tested substances look at figures 1 - 6.
Most of the mono-, di-, and trichlorobiphenyls as well as tetrachlorodibenzo-p-dioxin showed a significant reaction. TCDD is considered as a toxic contaminant of commercial PCB (VOS, 1972).
2,4-dichlorobiphenyl, 2,2',5-trichlorobiphenyl and the higher chlorinated chemicals gave no toxic effect on the algae motility.

TABLE 1

Tested substance	Inhibition at 1oo µg PCB per test spot
o-monochlorobiphenyl	+
m-monochlorobiphenyl	+
p-monochlorobiphenyl	+
2,2'-dichlorobiphenyl	+
2,3 -dichlorobiphenyl	+
2,3'-dichlorobiphenyl	+
2,4 -dichlorobiphenyl	-
2,4'-dichlorobiphenyl	+
2,5 -dichlorobiphenyl	+
4,4'-dichlorobiphenyl	+
2,2',5-trichlorobiphenyl	-
2,4,4'-trichlorobiphenyl	+
3,4,4'-trichlorobiphenyl	+
2,2,',5,5'-tetrachlorobiphenyl	-
2,2',3,4,5,5'-hexachlorobiphenyl	-
decachlorobiphenyl	-
2,3,7,8-tetrachlorodibenzo-p-dioxin	+ (1 µg)
	key: + inhibition - no inhibition

Figure 1 shows the effect caused by 5o µg of 2,2'- chlorobiphenyl. The photoelectric response is presented in figure 2 together with the 1 µg and 1oo µg test of the same substance.

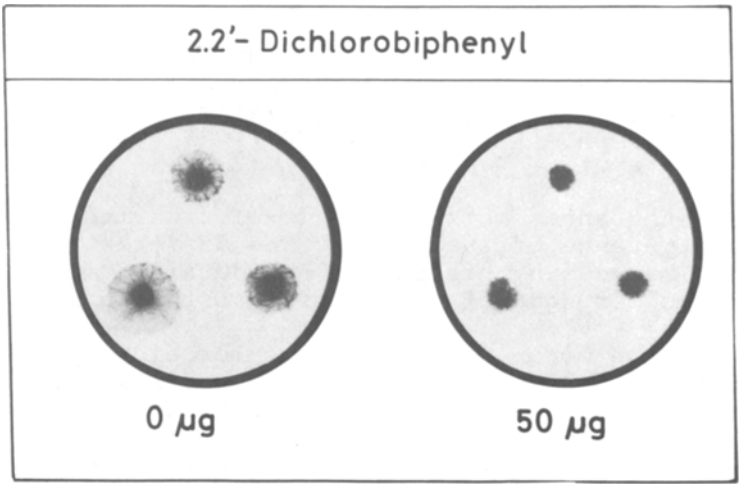

Fig. 1: Algae discs after 3-hour test run

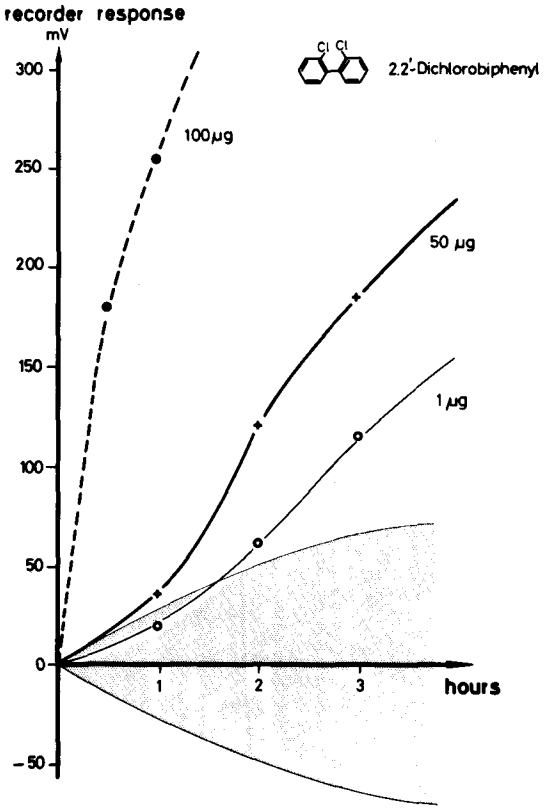

Fig. 2 The hatched range marks the possible variation of the "blank"

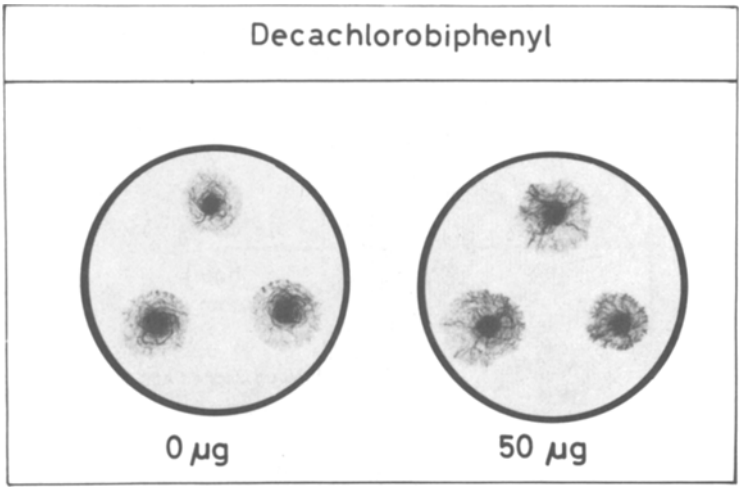

Fig. 3 Algae discs after 3-hour test run

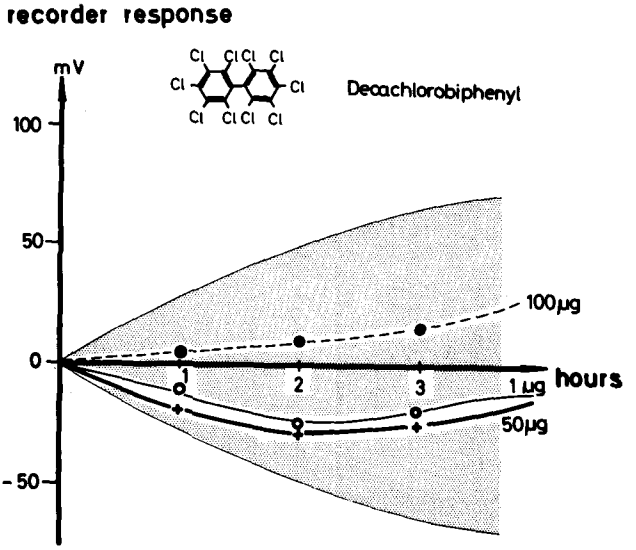

Fig. 4 The hatched range shows the possible variation of the blank assays

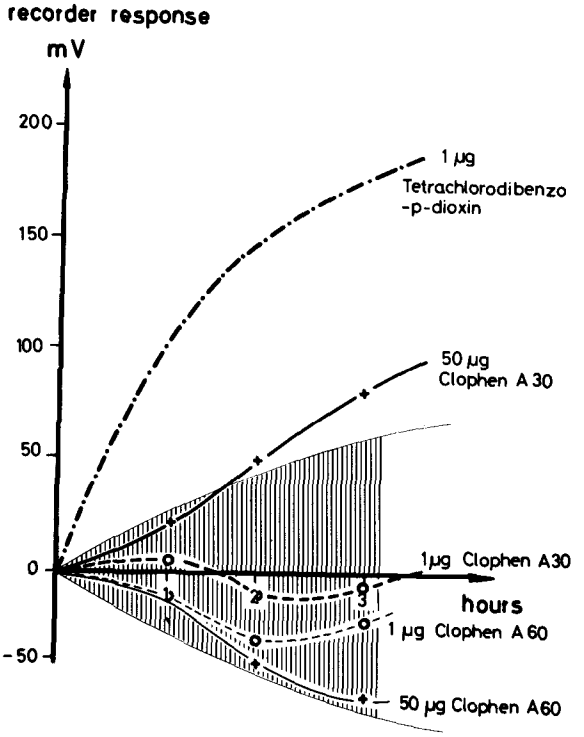

Fig. 5 Recorder response of the commercial products with the security range of the blanks

With decachlorobiphenyl no inhibition is achieved (fig. 3). No signal received exceed the blank range (fig. 4).

The tests with the different PCB's indicate that biphenyls with a low degree of chlorination show a stronger toxic effect on the algae than those with a higher degree. Hence we expect that a comparison between the commercial product Clophen A 3o (average mean 3 Cl-atoms per molecule) and Clophen A 6o (6 Cl-atoms per molecule) leads to similar results. As shown in fig. 5 this could be confirmed. But the effect is weakened by the fact, that commercial Clophen A 3o mixture is unexpectedly richer in higher chlorinated compounds. This can be seen in the GC-separation (fig. 6). Therefore, 5o µg of A 3o have a little effect compared with applied e.g. 5o µg of 2,4,4'- trichlorobiphenyl. Because of its very great content of highly chlorinated biphenyls the inhibiting effect of A 6o is undetectable.

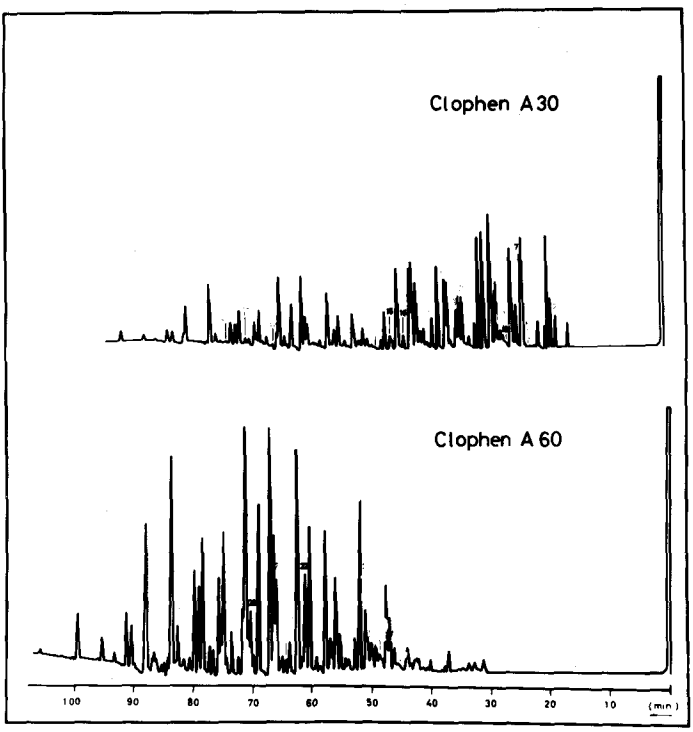

Fig. 6: PCB-chromatograms
(GC-Carlo Erba 23o1 AC with Ni-63 ECD, 5o m glass-column, OV-1o1, Ø o,25 mm, oven 12o-22o° C, 1° per min, 1,6 ml N_2 per min)

This standardized bioassay was proved to be useful in water quality control. In comparison to other bioassays the results are available in a relative short time (3 hours). Preparation of the test takes only few minutes.

SUMMARY

A new bioassay was applied testing the toxicity of several PCB's and tetrachlorodibenzo-p-dioxin against blue-green algae.

TCDD, mono-, di-, and trichlorobiphenyls inhibited the motility of Phormidium spec., while higher chlorinated biphenyls had no effect.

LITERATURE

G. BENECKE: Veröffentlichungen des Instituts für Wasserforschung GmbH Dortmund und der Hydrologischen Abteilung der Dortmunder Stadtwerke AG, Nr. 21 (1975)

N.S.FISHER: Science 189 (1975)

V.GLOOSCHENKO, W.GLOOSCHENKO: Can. J. Bot. 53 (1975) 653-659

J.E. KEIL: Bull. Envir. Cont. Tox. 6 (2),(1971) 156-159

J.L. MOSSER, N.S. FISHER, C.F. WURSTER: Science 176 (1972) 533-535

M. NOLL, U. BAUER: Zbl. Bakt. Hyg. 157 (1973) 178-183

A. SÖDERGREN: Oikos 22 (1971) 215-220

J.C. UREY, J.C. KRICHER, J.M. BOYLAN: Bull. Envir. Cont. Tox. 16 (1), (1976) 81-85

J.G. VOS: Environm. Health Perspect. April 1972, 105-117

1700 further PCB-references are stored in the EDV-literature documentation of the Dortmunder Stadtwerke AG and the Institut für Wasserforschung GmbH