Bull. Environm. Contam. Toxicol. 28, 728–732 (1982)

Uptake of 4-Chlorobiphenyl and 4,4′-Dichlorobiphenyl in Six Species of Plant Tissue Cultures

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The fate of chlorobiphenyls in different ecosystems has attracted much interest. There are many reports on the metabolism of chlorobiphenyls in plants (MOZA et al. 1973, 1974, 1976a, b, 1979,MAASS et al. 1975). However, only limited data are available on the uptake of chlorobiphenyls in plants. The uptake of 2,2'-dichlorobiphenyl (DCB), 2,4',5-trichlorobiphenyl, and 2,2',4,4',6-pentachlorobiphenyl by carrots was shown to be much higher than that by sugar beets (MOZA et al. 1976b, 1979).

We have studied the relationship between the uptake of 4-chlorobiphenyl (CB) and 4,4'-DCB and lipid content in plants using arcangelisia (AfPe), tobacco (BYSt), safflower (CtF), carrot (DcR), datura (DiE), and gromwell (LeSe) tissue suspension cultures.

MATERIALS AND METHOD

Table 1 gives the codes and origin of the callus cultures used in this study, and the best composition of plant growth regulators in a stock culture medium for each callus culture. Stock cultures were subcultured on RM-1964 basal medium (LINSMAIER & SKOOG 1965) containing 0.9% agar and 3% sucrose every 45 days at 25°C in the dark. The stock culture medium for each callus culture was used as the basal medium for test.

About 5 g of each callus tissue was aseptically transferred to a 100-ml flask containing 50 ml of liquid medium and grown with gentle shaking for 3 days. After the pre-incubation, 4 mg of 4-CB or 4,4'-DCB (Aldrich Chemical Co., U.S.A) in 0.5 ml of acetone was added into the liquid medium, and the treated callus tissue cultutes were further incubated for 7 days. The callus tissues were collected on a Buchner funnel and washed with water. The washed tissues were stored at -20°C prior to extraction.

0007-4861/82/0028-0728 $1.00

TABLE 1

Origin of the callus cultures and the composition of growth regulators in stock culture medium

Callus code	Origin of callus culture		Growth regulators
	Species	Organ	
AfPe	Arcangelisia flava	Petiole	10^{-6}M IAA, 10^{-6}M kinetin
BYSt	Nicotiana tabacum var. Bright Yellow	Stem	10^{-5}M IAA, 10^{-6}M kinetin
CtF	Carthamus tinctorius	Tubulous flower	10^{-6}M IAA, 10^{-5}M kinetin
DcR	Daucus carota	Root	5×10^{-6}M 2,4-D, 5×10^{-7}M kinetin
DiE	Datura innoxia	Embryo	10^{-6}M 2,4-D
LeSe	Lithospermum erythrorhizon	Seedling	10^{-6}M IAA, 10^{-5}M kinetin

Abbreviations: IAA, indol-3-acetic acid; 2,4-D, 2,4-dichlorophenoxy-acetic acid

The frozen tissues were ground in a blender with 95% ethanol. The homogenates were filtered and the filtrate was concentrated and extracted with diethyl ether. 4-CB and 4,4'-DCB in the extracts were determined by gas chromatography on an OV-1 column with electron-capture detection. The residual percentages of chlorobiphenyls shown in Table 2 are averages of 3 replicates.

Lipid content in each species of the callus stock cultures was determined by the method of BLIGH and DYER (1959).

RESULTS AND DISCUSSION

Table 2 shows the percentage recovery of the applied chlorobiphenyls from plant tissue cultures after 7 days incubation. The uptake of 4,4'-DCB was higher than that of 4-CB in all the tissue cultures except AfPe. The highest uptake of 4-CB and 4,4'-DCB was observed in CtF, while the lowest uptake was in DcR.

The toxicity of chlorobiphenyls to the tissue cultures was observed. Both 4-CB and 4,4'-DCB markedly inhibited the growth of DcR and slightly inhibited AfPe, CtF, and LeSe, but had no effect on BYSt and DiE. Although the lowest uptake of 4-CB and 4,4'-DCB was found in DcR that showed the lowest growth, no linear relationship between the uptake of chlorobiphenyls and growth of tissue cultures was observed.

Carrots had been used as a model for accumulators of lipophilic xenobiotics in the studies regarding the fate of chlorobiphenyls in plants (MOZA et al. 1976b,

TABLE 2

Percentage recovery of the applied dose of 4-CB and 4,4'-DCB from plant tissue cultures after 7 days incubation

Chloro-biphenyl	Plant tissue culture					
	AfPe	BYSt	CtF	DcR	DiE	LeSe
4-CB	39	23	46	14	20	43
4,4'-DCB	35	56	68	29	29	43

1979). Therefore, the relationship between the uptake of 4-CB and 4,4'-DCB and lipid content in the tissue cultures was examined. As shown in Fig. 1, the uptake of chlorobiphenyls per weight of wet tissue decreased as follows: DcR > CtF > LeSe ≒ AfPe > BYSt ≒ DiE. On the other hand, the lipid content in the callus stock

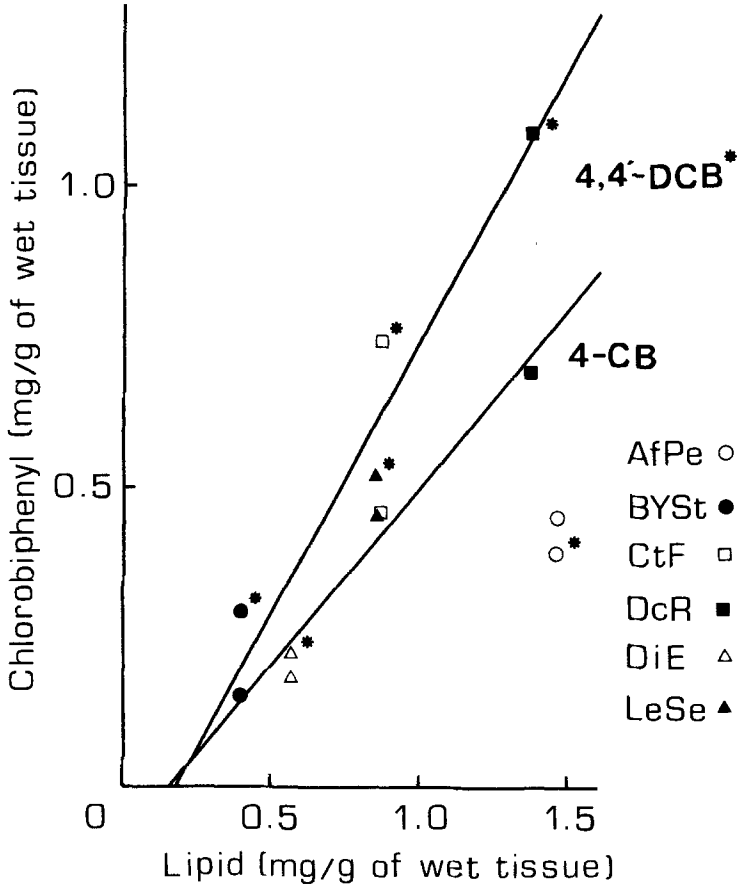

Fig. 1 Relationship between the uptake of 4-CB and 4,4'-DCB and lipid content in the five species of plant tissue cultures except AfPe.
$Y = 0.59X - 0.09$ ($r = 0.98$) for 4-CB and $Y = 0.90X - 0.16$ ($r = 0.95$) for 4,4'-DCB.

cultures decreased as follows: AfPe > DcR > CtF ≒ LeSe > DiE > BYSt. In the five tissue cultures except AfPe, there was a significant linear relationship between the uptake of 4-CB and 4,4'-DCB and lipid content. AfPe might give a higher lipid value than the true value because of its pigments. The highest uptake of chlorobiphenyls per weight of wet tissue in DcR seems to be attributable to its higher lipid content.

These results suggest that the uptake of other chlorobiphenyls into plant tissue cultures may depend on the lipid content of tissues. The uptake of aldrin and dieldrin into plant tissue suspension cultures was reported to be affected by the medium composition and growth parameters (BRAIN & LINES 1977). In addition, the uptake of pentachloronitrobenzene into peanut tissue suspension cultures was studied as a function of time by using noninhibitory concentrations of this compound (LAMOUREUX et al. 1981). These findings might present a model for the uptake of lipophilic xenobiotics into plants, especially into aquatic plants, but, to extrapolate them to events in ecosystems, further work must be done with great care.

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