Bull. Environm. Contam. Toxicol. 23,044-050 (1979)

Reversible Bioconcentration of Monochlorobiphenyls by *Rhodotorula rubra:* Correlations with Aqueous Solubility of Substrate

M. A. Cole[1], P. B. Reichart[1,2], and D. K. Button[2]

[1]*Department of Chemistry and* [2]*Institute of Marine Science, University of Alaska, Fairbanks, Alaska 99701*

Polychlorinated biphenyls (PCBs) are recognized as ubiquitous contaminants of aquatic ecosystems. Their toxicity to a wide range of biological systems, environmental persistence, and propensity for bioconcentration have caused concern.

A key step in environmental contamination by PCBs is their uptake by biota. While the importance of biomagnification in the food chain has been questioned (HARVEY *et al.* 1974, HAMELINK *et al.* 1971) the bioconcentration of such substances by organisms low in the food chain remains one important point of uptake. Bioconcentration not only introduces PCBs into the food chain, it has been reported to alter species composition as well (FISHER *et al.* 1974). Although it is generally accepted that PCB components with higher chlorine content are bioconcentrated more extensively than their more lightly chlorinated counterparts (HAMMOND *et al.* 1972, CLAYTON *et al.* 1977), more information is needed on the bioconcentration susceptibility of structurally similar PCB components (e.g. isomers) before a complete predictive methodology for uptake is available. This study was directed toward an understanding of the bioconcentration of the isomeric monochlorobiphenyls by a marine strain of the yeast *Rhodotorula rubra*.

MATERIALS AND METHODS

Organism. *R. rubra*, a euryhalic yeast of marine origin (BUTTON *et al.* 1973) was grown in batch culture on a glucose (20 mM), mineral salts [$(NH_4)_2SO_4$, 2.5 mM; NaH_2PO_4, 1 mM] medium (BUTTON 1969). After growth was complete the organisms were allowed to settle and the supernatant decanted. The cells were washed and resuspended in 0.1% saline to obtain a population of about 10^9 organisms per mL. Cell concentrations and volumes were determined electronically.

Substrates. Biphenyl-U-^{14}C (4.9 mCi/mmol) was diluted with radioinactive biphenyl and recrystallized from 95% ethanol.

2-Chlorobiphenyl-1',2',3',4',5',6'-$^{14}C_6$ (2-Cl-BP) was synthesized and purified according to the published procedure (REICHARDT & SCHUTTNER 1976). The corresponding ^{14}C-labeled 3- and 4-chlorobiphenyls (3-Cl-BP and 4-Cl-BP) were synthesized

0007-4861/79/0023-0044 $01.40

from benzene-U-^{14}C and 3- and 4- chloroaniline, respectively, by modifications of this method. Specific activities of purified chlorobiphenyls (ca. 0.2 mCi/mmol) were obtained from liquid scintillation counting. Purity of all samples was checked by thin layer chromatography (Silica Gel 60 F-254; hexane).

Equilibration Experiments. For each substrate, 50 mL portions of equilibration medium were added to three 125-mL Erlenmeyer flasks fitted with ground glass stoppers. Cells of *R. rubra* were added by pipet to two of the flasks to give final concentrations of 10^7 to 10^{10} cells/L (0.5 to 500 mg/L). A similar set of three flasks containing ^{14}C-BP was run in parallel with each ^{14}C-Cl-BP experiment. All incubations were agitated by gentle magnetic stirring at 25°C.

Equilibration Medium. Samples of solid ^{14}C-BP and ^{14}C-4-Cl-BP were added directly to ampules containing 1.0 L of 0.1% saline. Oily samples of ^{14}C-2- and ^{14}C-3-Cl-BP were vacuum transferred to the inner wall of a short length of 8 mm glass tubing and the tubing added to similar ampules. The sealed ampules were autoclaved (121°C). Final concentrations were about 0.3 ppm for Cl-BPs and 0.3-2.0 ppm for BP.

At periodic intervals 10 mL samples of each cell-containing flask were transferred to 30-mL centrifuge tubes and centrifuged at 3000 g for 15 min. Portions (1.0 mL) of supernatant were removed for counting and the remainder discarded. The pellet from each was suspended in one mL of 0.1% saline and removed for counting. Pellet blanks were obtained by similar treatment of the control flask to which no cells were added.

In some cases the pellet from one flask was not counted but was suspended in 10 mL of 0.1% saline, agitated, and allowed to stand for 10 min. Repetition of the above procedure provided data for bioconcentration coefficients in "reverse experiments".

Localized Uptake of Pyrene by *R. rubra*. The location of pyrene in *R. rubra* organisms was determined by equilibration of the cells with a saturated aqueous solution of pyrene and observation of the fluorescence with an A.O. Spencer microscope fitted with a mercury lamp, U.V. light source, and 2074 filter. The observations were recorded on 10,000 ASA Polaroid film.

Calculations. All bioconcentration coefficients were calculated as averages of duplicate runs on a volume/volume basis (dpm mL^{-1} cells/dpm mL^{-1} solution).

RESULTS AND DISCUSSION

A study of the bioconcentration of biphenyl from saline solution (without the complicating contribution of organic cosolvent)

by *R. rubra* allowed calculation of a bioconcentration coefficient of 307 ± 63 (90% confidence interval; N=14). Approximately 75% of the added radioactivity was recovered in each experiment (including controls) and thin layer chromatography showed all radioactivity to be associated with biphenyl. Since the losses were relatively constant in all experiments, they are attributed to problems associated with sorption on glassware and volatility. Equilibrium was established in less than 15 min (minimum analysis time of this method) and the bioconcentration coefficient remained constant over three days. The same bioconcentration coefficient (within experimental error) was found for systems with biphenyl concentration varying by an order of magnitude and cell density varying by three orders of magnitude. The rather large uncertainty limit is derived, at least in part, from experimental protocol with the largest source of error (ca. ± 10%) coming from nonquantitative removal of the pellet after centrifugation.

Because of the uncertainty of the bioconcentration coefficient for biphenyl and its possible dependence upon differing properties of cells grown in different batches, the bioconcentration coefficients for the monochlorobiphenyls in each run were calculated relative to that for biphenyl. The absolute bioconcentration coefficients were obtained by multiplying the relative values by the average absolute value for biphenyl (307). The results are summarized in Table 1. As with biphenyl all equilibria were rapidly established and remained constant for over 24 h.

The absolute bioconcentration coefficients of these substances are plotted against reported water solubilities (VESALA 1974, WALLNÖFER *et al.* 1973) in Figure 1. It is readily seen that within this set of closely related compounds the bioconcentration coefficients strongly correlate with water solubilities of substrates.

Attempts have been made to quantify this type of correlation with empirical equations. CHIOU *et al.* (1977) measured bioconcentration (substrate concentration in tissue/substrate concentration in medium) of various organics by rainbow trout and plotted the log of observed bioconcentrations against the log of molar aqueous solubility of substrate (S). A fit of the data to the best linear plot allowed calculation of expected bioconcentrations (termed "biological factors", BF) from solubility according to the equation log (BF) = 3.41 - 0.508 log S. METCALF *et al.* (1975) did a similar study with mosquito fish and calculated expected bioconcentrations (termed "ecological magnifications", EM) from the equation log (EM) = 4.4806 - 0.4732 log X where X is the aqueous solubility of substrate in ppb. Comparison of the measured bioconcentration coefficients reported here as well as a sampling of those available from the literature on related systems with calculated values for BF and EM show good agreement (Table 2). The BF and EM calculated for the chlorobiphenyls are probably erroneously low because the determinations of WALLNÖFER *et al.* (1973) overestimated the water solubilities of these substances (HAQUE

TABLE 1

Bioconcentration of Monochlorobiphenyls by *R. rubra*

Compound	Observed Bioconcentration*	Bioconcentration relative to biphenyl	Absolute Bioconcentration coefficient†	
2-chlorobiphenyl	547(258)	2.12	651	737
	948(354)	2.68	823	
3-chlorobiphenyl	1313(521)	2.52	774	1178
	2555(496)	5.15	1581	
4-chlorobiphenyl	1089(230)	4.74	1455	1547
	1204(225)	5.34	1639	

* Number in parenthesis is the observed bioconcentration of biphenyl in a parallel run.

† Values are calculated for a bioconcentration coefficient of 307 for biphenyl.

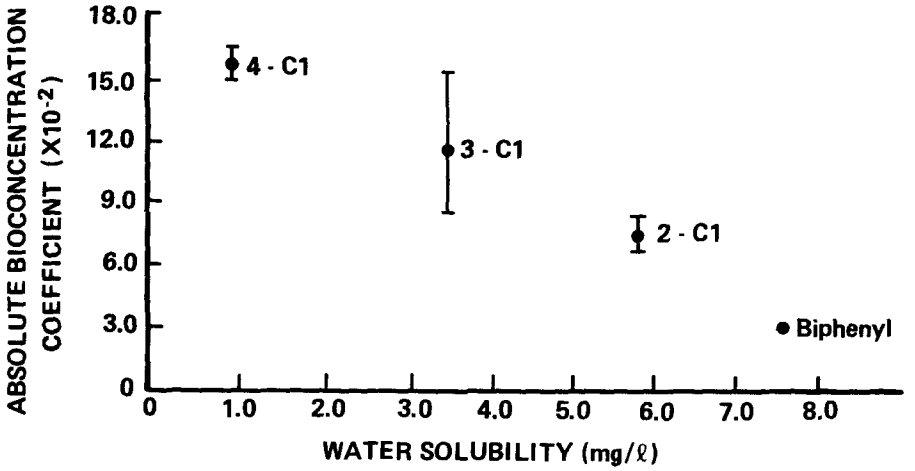

Figure 1. Relationship between *R. rubra* bioconcentrations and water solubilities of chlorinated biphenyls.

and SCHMEDDING 1976). The generally good agreement for a variety of biological systems and chlorinated biphenyls may indicate a wider applicability of the empirical equations for approximation of bioconcentration potential than has been previously recognized.

Certainly deviations from any such correlation which ignores lipid content of biota are known (METCALF *et al.* 1975, GRIMES &

TABLE 2

Comparison of Observed Bioconcentration Coefficients (BC) of Chlorinated Aromatic Compounds with Calculated Bioconcentration Factors (BF) and Ecological Magnifications (EM).

Substrate[a]	Organism	BF ($x10^{-3}$)	EM ($x10^{-3}$)	BC ($x10^{-3}$)	Reference for observed BC
BP	*R. rubra* (yeast)	0.357	0.443	0.307[b]	this work
2-Cl-BP	"	0.447	0.503	0.737[b]	"
3-Cl-BP	"	0.583	0.644	1.18[b]	"
4-Cl-BP	"	1.00	1.07	1.55[b]	"
TCBP	*Chlorella pyrenoidosa* (chlorophyte)	6.6	5.0	4.8[c]	UREY *et al.* 1976
HCBP	"	17	11	10[c]	"
OCBP	"	51	30	11[c]	"
DDT	*Thalassiosira fluviatilis* (diatom)	51	30	25[c]	COX 1970
	Amphidinium carteri (dinoflagellate)	51	30	80[c]	"

a. BP = biphenyl; 2-ClBP = 2-chlorobiphenyl; 3-ClBP = 3-chlorobiphenyl; 4-ClBP = 4-chlorobiphenyl; TCBP = 2,2',5,5'-tetrachlorobiphenyl; HCBP = 2,2',4,4',5,5'-hexachlorobiphenyl; OCBP = 2,2', 3,3', 4,4',5,5'-octachlorobiphenyl

b. $\frac{\text{g substrate/ml total cell volume}}{\text{g substrate/ml supernatant}}$

c. $\frac{\text{g substrate/g cells}}{\text{g substrate/ml supernatant}}$

MORRISON 1975). Based on solubility concepts one expects lipophilic substances to concentrate in the lipid-rich portion of biota. Indeed, pyrene (another neutral arene) was fluorometrically observed to locate predominantly in the mitochondria of *R. rubra*. These organelles appeared as four to six brightly glowing cytoplasmic inclusions while the cytoplasmic membrane appeared as a faint surrounding line. Electron microscopy confirmed the general location of the pyrene-absorbing structures.

The mitochondrial content of organisms varies with growth conditions and will thus affect the bioconcentration coefficients reported here. Since yeasts are approximately 2% lipid (wet weight; LONG 1961) a biphenyl bioconcentration coefficient of 3×10^2 in whole cells corresponds to 1.5×10^4 in their lipid portion. This agrees well with the biphenyl partition coefficient of 2.5×10^4 observed for the model system of cetyltrimethylammonium bromide micelles in water (GRIESER, personal communication).

Confirmation that these studies deal with equilibrium states was obtained by studying the approach to equilibrium by systems consisting initially of sterile saline and substrate-laden cells. Such systems rapidly (<25 min) produced essentially the same partitioning observed in the forward direction (Table 3).

The rapid, reversible nature of the partitioning reported here is consistent with the proposal that equilibrium partitioning is the controlling factor in the bioaccumulation of hydrophobic compounds in aquatic systems (HARVEY *et al.* 1974, HAMELINK *et al.* 1971. The data do not allow a choice between an absorption mechanism or one involving active transport, although the latter would require unusually large rate constants for transport across the cell envelope. The partitioning observed here seems to be quite different from the essentially irreversible process seen in related experiments (SÖDERGREN 1968, GRIMES & MORRISON 1975). It should be noted, however, that the partitioning equilibria in all these studies lie far to the side of the organisms and that the equilibrium concentration of aqueous substrate in desorption experiments poses severe analytical difficulties.

In summary, biphenyl and the three monochlorobiphenyls are rapidly and reversibly concentrated by *R. rubra* from saline solutions containing subsaturating levels of substrate. The measured bioconcentration coefficients correlate (inversely) with reported water solubilities and can be approximately calculated from water solubilities of the compounds with the aid of empirical equations.

ACKNOWLEDGMENT

We thank K. Thomas and M. Pollard for assistance at the University of Notre Dame and the National Science Foundation for financial support (OCE 76-84266).

TABLE 3

Bioconcentration Coefficients Determined from Forward and Reverse Experiments

Substrate	Bioconcentration coefficient: Forward experiment	Bioconcentration coefficient: Reverse experiment
biphenyl	307 ± 63 (90%; N=14)	300 ± 123 (90%; N=6)
2-Cl-biphenyl	848 461	565 369
3-Cl-biphenyl	775 1560	684 1580
4-Cl-biphenyl	1470	1260

REFERENCES

BUTTON, D. K.: Limnol. Oceanogr. 14, 95 (1969).

BUTTON, D. K., S. S. DUNKER, and M. L. MORSE: J. Bacteriol, 113, 599 (1973).

CHIOU, C. T., V. H. FREED, D. W. SCHMEDDING, and R. L. KOHNERT: Environ. Sci. Technol. 11, 475 (1977).

CLAYTON JR., J. R., S. P. PAVLOU, and N. F. BREITNER: Environ. Sci. Technol. 11, 676 (1977).

COX, J. L.: Bull. Environ. Contam. Toxicol. 5, 218 (1970).

FISHER, N. S., E. J. CARPENTER, C. C. REMSEN, and C. F. WURSTER: Microb. Ecol. 1, 39 (1974).

GRIMES, D. J. and S. M. MORRISON: Microb. Ecol. 2, 43 (1975).

HAMELINK, J. L., R. C. WAYBRANT, and R. C. BALL: Trans. Amer. Fish. Soc. 100, 207 (1971).

HAMMOND, P. B., I. C. T. NISBET, A. F. SAROFIM, W. H. DRURY, N. NELSON, and D. P. RALL: Environ. Res. 5, 249 (1972).

HAQUE, R. and D. SCHMEDDING: Bull. Environ. Contam. Toxicol. 14, 13 (1975).

HARVEY, G. R., H. P. MIKLAS, V. T. BOWEN, and W. G. STEINHAUER: J. Mar. Res. 32, 103 (1974).

LONG, C.: Biochemists' Handbook. New York - D. van Nostrand Co. 1961. p. 1052.

METCALF, R. L., J. R. SANBORN, P-Y. LU, and D. NYE: Arch. Environ. Contam. Toxicol. 3, 151 (1975).

REICHARDT, P. B. and S. E. SCHUTTNER: J. Labelled Compd. Radiopharm. 12, 243 (1976).

SÖDERGREN, A.: Oikos 19, 126 (1968).

UREY, J. C., J. C. KRICHER, and J. M. BOYLAN: Bull. Environ. Contam. Toxicol. 16, 81 (1976).

VESALA, A.: Acta Chem. Scand. A28, 839 (1974).

WALLNÖFER, P. R., M. KÖNIGER, and O. HUTZINGER: Analabs Res. Notes 13, 14 (1973).