MEASURING BIOCONCENTRATION FACTORS AND RATE CONSTANTS OF CHEMICALS IN AQUATIC ORGANISMS UNDER CONDITIONS OF VARIABLE WATER CONCENTRATIONS AND SHORT EXPOSURE TIME

Frank A.P.C. Gobas* and Xin Zhang

School of Resource & Environmental Management
Simon Fraser University
Burnaby, British Columbia. V5A 1S6
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ABSTRACT

A novel method, based on iterative numerical integration, is presented for deriving bioconcentration factors and rate constants of chemicals in aquatic organisms from experimental data of bioconcentration tests in which the chemical concentration in the water is variable over time and the test duration is too short to reach steady-state. The method is applied to reported data from fish and plant bioconcentration tests. The results demonstrate that this method can derive bioconcentration factors and rate constants with considerably less experimental error than other methods currently used, thus reducing uncertainty and variability in bioconcentration measurements.

INTRODUCTION

Bioconcentration factors (BCF), rate constants and half-lives are widely used in hazard and risk assessment, environmental modelling, and in the development of environmental standards and water quality criteria [1-3]. A common criticism of the practical use of bioconcentration data for regulatory purposes is the often considerable variability or uncertainty of the experimental measurements [4]. This variability or uncertainty is due to a combination of factors including the preparation of the aqueous solution [5], the test duration [6], variations of the chemical concentrations in the water during the test [7], chemical bioavailability [7,8], differences between fish species including size [9], lipid content [10] and capacity for metabolic transformation [11], differences between individuals of a particular species

[12], fish growth and differences in analytical methodologies. Although some of these factors are difficult to control in experiments, the variability introduced by some other factors can easily be reduced. For example, in our experience a significant error in the measurement of bioconcentration factors (i.e. up to an order of magnitude) can be introduced after the bioconcentration test when deriving the bioconcentration factor and rate constants from observed water and fish concentrations if during the bioconcentration test the water concentration varied and/or the duration of the bioconcentration experiment was too short to reach a fish-water steady-state or equilibrium. In particular for chemicals with high bioconcentration factors, this is often the case. For example, current OECD guidelines [13] recommend a water flow-through rate of 1.13 L/d or more for a bioconcentration test with 25 goldfish of 4.5 g in a 100 L tank. However, the gill ventilation rate of a single gold fish is approximately 2.4 L/day [9], resulting in a total fish gill ventilation rate in the tank of approximately 60 L/d. If the BCF is large enough (e.g. BCF greater than 1,000) for the fish to absorb a significant fraction of the chemical in the tank, the low renewal rate of the chemical solution in the tank will cause a significant drop of the chemical concentration in the water of the tank at the beginning of the bioconcentration test. In static or semi-static bioconcentration experiments, variations in water concentration are often greater than in flow-through experiments. OECD guidelines [13] also recommend bioconcentration tests to be 28 days in duration. However, for many high K_{ow} chemicals this period is too short to reach a steady-state. For example, 2,5-dichlorobiphenyl requires approximately 3/0.066 or 46 days to reach 95% of its ultimate steady-state in a 4.5 g gold fish [12].

Since variable water concentrations and short test durations are often inevitable, we have developed a technique to derive BCFs, uptake and elimination rate constants from typical bioconcentration experiments under conditions of variable water concentrations and short exposure times. The advantage of this method is that it reduces the error (and resulting variability) introduced in the derivation of the bioconcentration factor and rate constants from experimental data. We have already applied this technique successfully to bioconcentration studies with fish [7] and aquatic plants [14]. In this paper, we will present the method, outline its theoretical basis, demonstrate its practical application and illustrate its ability to reduce the experimental error in the measurement of the BCF.

THEORY

The rate of uptake and bioconcentration of organic chemicals from water by fish and other organisms can often be described by the following differential equation [15]:

$$dC_B/dt = k_1.C_W - k_2.C_B \quad (1)$$

where C_B is the chemical concentration in the organism (ng/kg organism), C_W is the chemical

[12], fish growth and differences in analytical methodologies. Although some of these factors are difficult to control in experiments, the variability introduced by some other factors can easily be reduced. For example, in our experience a significant error in the measurement of bioconcentration factors (i.e. up to an order of magnitude) can be introduced after the bioconcentration test when deriving the bioconcentration factor and rate constants from observed water and fish concentrations if during the bioconcentration test the water concentration varied and/or the duration of the bioconcentration experiment was too short to reach a fish-water steady-state or equilibrium. In particular for chemicals with high bioconcentration factors, this is often the case. For example, current OECD guidelines [13] recommend a water flow-through rate of 1.13 L/d or more for a bioconcentration test with 25 goldfish of 4.5 g in a 100 L tank. However, the gill ventilation rate of a single gold fish is approximately 2.4 L/day [9], resulting in a total fish gill ventilation rate in the tank of approximately 60 L/d. If the BCF is large enough (e.g. BCF greater than 1,000) for the fish to absorb a significant fraction of the chemical in the tank, the low renewal rate of the chemical solution in the tank will cause a significant drop of the chemical concentration in the water of the tank at the beginning of the bioconcentration test. In static or semi-static bioconcentration experiments, variations in water concentration are often greater than in flow-through experiments. OECD guidelines [13] also recommend bioconcentration tests to be 28 days in duration. However, for many high K_{ow} chemicals this period is too short to reach a steady-state. For example, 2,5-dichlorobiphenyl requires approximately 3/0.066 or 46 days to reach 95% of its ultimate steady-state in a 4.5 g gold fish [12].

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concentration in the water (ng/L), k_1 is the first order uptake rate constant (L/kg.d), k_2 is the first order elimination rate constant (1/d) and t is time (d). If C_W is constant during the exposure period, equation 1 can be solved to give

$$C_B = (k_1/k_2).C_W.(1 - \exp(-k_2.t)) \quad (2)$$

It is possible to determine the rate constants k_1 and k_2 and the steady-state bioconcentration factor (BCF), i.e. k_1/k_2, in equation 2 from a series of experimental data of C_W and C_B at different times t, through non-linear regression [16,17]. If, in addition, the exposure is sufficiently long to reach steady-state, the bioconcentration factor BCF can be determined as the ratio of the organism and water concentrations, i.e. BCF equals C_B/C_W. If both conditions do not apply, we propose a new method to derive k_1 and k_2 and the steady-state BCF. This method is based on a first degree approximation of equation 1, where an incremental change in the chemical concentration in the organism $\Delta C_{B,i}$ or $C_{B,i}$-$C_{B,i-1}$ is calculated over a specified short time interval Δt_i (e.g. 0.01 day) as

$$\Delta C_{B,i} = (k_1.C_{W,i-1} - k_2.C_{B,i-1}).\Delta t_i \quad (3)$$

where i is the number of time intervals in time t_i, i.e. $t_i/\Delta t_i$, $C_{W,i-1}$ is the chemical concentration in the water at t_{i-1} and $C_{B,i-1}$ is the chemical concentration in the organisms at t_{i-1}. The chemical concentration in the organism at time t_i, i.e. $C_{B,i}$, thus follows as $(C_{B,i-1}+\Delta C_{B,i})$ or

$$C_{B,i} = C_{B,i-1} + (k_1.C_{W,i-1} - k_2.C_{B,i-1}).\Delta t_i \quad (4)$$

For $C_{B,1}$, i.e. the concentration in the organisms after one time interval, to $C_{B,i-1}$, we can derive similar equations, i.e.

$$C_{B,1} = C_{B,0} + (k_1.C_{W,0} - k_2.C_{B,0}).\Delta t_i \quad (5)$$

:

$$C_{B,i-1} = C_{B,i-2} + (k_1.C_{W,i-2} - k_2.C_{B,i-2}).\Delta t_i \quad (6)$$

Substitution of equations 5 to 6 in equation 4 gives the following general expression for $C_{B,i}$:

$$C_{B,i} = C_{B,0} + k_1.\sum_{j=0}^{i-1} C_{W,j}.\Delta t_i - k_2.\sum_{j=0}^{i-1} C_{B,j}.\Delta t_i \quad (7)$$

If the time intervals Δt_i are chosen to be sufficiently small (e.g. less than approximately $1/(25.k_2)$), equation 7 is a very accurate numerical approximation of the real solution of equation 1 at any time during the bioconcentration test and at any value of the concentration in the water. From the water

concentration measurements during the bioconcentration experiment time it is possible to approximate $C_{W,i}$ for every time increment (e.g. by linear interpolation) to reconstruct the actual water concentration time relationship during the bioconcentration experiment. If this information is introduced in equation 7 and values for k_1 and k_2 are chosen, then chemical concentrations in the organism can be calculated for every time t_i during the bioconcentration experiment. The best agreement between calculated and observed chemical concentrations in the organism indicates the best set of k_1 and k_2 values. The agreement between calculated and observed concentrations F can be expressed by the sum of squares of the relative deviations between calculated $C_{B,i}(calc)$ and experimental $C_{B,i}(exp)$ chemical concentrations in the organism:

$$F = \sum_{m=1}^{n} \left[\frac{C_{B,i}(\exp) - C_{B,i}(calc)}{C_{B,i}(\exp)}\right]^2 \tag{8}$$

where n is the number of experimental data points. Substitution of equation 7 in equation 8 then gives

$$F = \sum_{m=1}^{n} \left[\frac{C_{B,i}(\exp) - C_{B,0} - k_1 \cdot \sum_{j=0}^{i-1} C_{W,j} \cdot \Delta t_i + k_2 \cdot \sum_{j=0}^{i-1} C_{B,j} \cdot \Delta t_i}{C_{B,i}(\exp)}\right]^2 \tag{9}$$

The best agreement between calculated and experimental concentrations is reached when F is minimum, i.e.:

$$\frac{\partial F}{\partial k_1} = 0 \qquad and \qquad \frac{\partial F}{\partial k_2} = 0 \tag{10}$$

resulting in two simultaneous linear equations with respect to k_1 and k_2, i.e.

$$k_1 \cdot \sum_{m=1}^{n} \left[\frac{1}{C_{B,i}(\exp)} \cdot \sum_{j=0}^{i-1} C_{W,j} \cdot \Delta t_i\right]^2 - k_2 \cdot \sum_{i=1}^{n} \left[\frac{1}{C_{B,i}(\exp)^2} \cdot \sum_{j=0}^{i-1} C_{B,j} \cdot \Delta t_i \cdot \sum_{j=0}^{i-1} C_{W,j} \cdot \Delta t_i\right]$$

$$= \sum_{m=1}^{n} \left[\frac{C_{B,i}(\exp) - C_{B,0}}{C_{B,i}(\exp)^2} \cdot \sum_{j=0}^{i-1} C_{W,j} \cdot \Delta t_i\right] \tag{11}$$

$$k_1 \cdot \sum_{m=1}^{n} \left[\frac{1}{C_{B,i}(\exp)^2} \cdot \sum_{j=0}^{i-1} C_{W,j} \cdot \Delta t_i \cdot \sum_{j=0}^{i-1} C_{B,j} \cdot \Delta t_i\right] - k_2 \cdot \sum_{m=1}^{n} \left[\frac{1}{C_{B,i}(\exp)} \cdot \sum_{j=0}^{i-1} C_{B,j} \cdot \Delta t_i\right]$$

$$= \sum_{m=1}^{n} \left[\frac{C_{B,i}(\exp) - C_{B,0}}{C_{B,i}(\exp)^2} \cdot \sum_{j=0}^{i-1} C_{B,j} \cdot \Delta t_i\right] \tag{12}$$

By providing initial estimates for k_1 and k_2, a set of $C_{B,i}$(calc) values can be derived from equation 7. Then, $C_{B,i}$(calc), $C_{B,i}$(exp) and $C_{W,i}$ for each sampling time t_i during the bioconcentration experiment are introduced in equations 11 and 12, which can then be solved to give new k_1 and k_2 values. These new k_1 and k_2 values are then used in equation 7 to derive a new set of $C_{B,i}$(calc) values, which with $C_{B,i}$(exp) and $C_{W,i}$ are introduced in equations 11 and 12 to derive new k_1 and k_2 values. This iterative procedure is continued until the calculated k_1 and k_2 values do not differ significantly (e.g. $P < 0.05$) between iterations. This set of k_1 and k_2 values provides the best fit of the organism-water two-compartment model (i.e. equation 1) to the experimental bioconcentration data. The quality of the fit can be expressed by the mean deviation E between experimental and fitted fish concentration data, i.e.

$$E = \frac{\sum_{m=1}^{n} \frac{\sqrt{(C_{B,m}(\text{exp}) - C_{B,m}(calc))^2}}{C_{B,m}(\text{exp})}}{n} \qquad (13)$$

The steady-state or equilibrium bioconcentration factor BCF follows from the ratio of the uptake and elimination rate constants, i.e. k_1/k_2, and is independent of the duration of the bioconcentration experiment.

Theoretically, it is possible that this regression method diverges if the initial estimates of k_1 and k_2 are inadequate. However, in our experience the method outlined above converges rapidly as long as the initial estimates are within a factor of approximately 1000 and equation 1 is an adequate representation of the bioconcentration process.

METHOD

Table 1 presents the numerical integration procedure in QuickBasic format.

RESULTS AND DISCUSSION

We applied the method outlined above to experimental bioconcentration data reported by Bruggeman et al. [12] in fish and Gobas et al. [14] in aquatic plants. These studies were selected because (i) observed concentrations in water and organism were reported or available, (ii) the experimental concentrations of the test chemicals in the water varied several fold during the uptake period, (iii) the bioconcentration tests of respectively 23 and 25 days were too short to reach steady-state for the test chemicals and (iv) results from independent elimination experiments are presented that can be used to test if the estimates of k_2 that are derived from the uptake experiment are accurate. Typical results of the data fitting procedure are given for 2,5-dichlorobiphenyl in fish in Table 2. Table 3 summarizes

Table 1

Outline of the iterative numerical integration method for determining bioconcentration factors and rate constants of chemicals in aquatic organisms under conditions of variable water concentrations and short exposure time in Quick-BASIC format.

```
CLS
DIM CW(1000): DIM CF(1000)
DIM CFE(1000): DIM TIME(100)
INPUT "TIME STEP (days)"; DT
INPUT "ENTER NUMBER OF DATA POINTS"; DATAPOINTS%
INPUT "CONFIDENCE LEVEL (%)"; CONFIDENCE
DEV = 1 - (CONFIDENCE / 100)
CLS
PRINT "ENTER TIMES IN DAYS"
FOR N% = 1 TO DATAPOINTS%
INPUT TIME(N%)
NEXT N%
PRINT "ENTER EXPERIMENTAL WATER CONCENTRATIONS"
FOR N% = 1 TO DATAPOINTS%
TIMECOUNTER% = TIME(N%) / DT
INPUT CW(TIMECOUNTER%)
NEXT N%
PRINT "ENTER EXPERIMENTAL FISH CONCENTRATIONS"
FOR N% = 1 TO DATAPOINTS%
TIMECOUNTER% = TIME(N%) / DT
INPUT CFE(TIMECOUNTER%)
NEXT N%
CLS
ENDTIME% = TIME(DATAPOINTS%)
INPUT "ENTER INITIAL ESTIMATE FOR k1 "; H1
INPUT "ENTER INITIAL ESTIMATE FOR k2 "; H2
PRINT "FITTING RATE CONSTANTS!"
PRINT ""
PRINT "K1", "K2"
PRINT "-----------------------------------------------------"
'CALCULATE WATER CONCENTRATIONS
FOR N% = 1 TO DATAPOINTS% - 1
TIMECOUNTER1% = TIME(N%) / DT
TIMECOUNTER2% = TIME(N% + 1) / DT
A = CW(TIMECOUNTER1%)
B = CW(TIMECOUNTER2%)
DCW = (B - A) / (TIMECOUNTER2% - TIMECOUNTER1%)

T% = 1
DO UNTIL TIMECOUNTER1% + T% = TIMECOUNTER2%
CW(TIMECOUNTER1% + T%) = CW(TIMECOUNTER1%) + (T% * DCW)
T% = T% + 1
LOOP
NEXT N%

FIT:
'INITIALIZE
CF(0) = CFE(0): SA = 0: SB = 0: CW2 = 0: F2 = 0: FW = 0
FOR I% = 1 TO ENDTIME% / DT
  SW = 0: SF = 0
FOR J% = 0 TO I% - 1
   SW = SW + CW(J%) * DT
   SF = SF + CF(J%) * DT
NEXT J%
CF(I%) = CF(0) + H1 * SW - H2 * SF
IF CFE(I%) <> 0 THEN
SA = SA + (CFE(I%) - CFE(0)) * SW / CFE(I%) / CFE(I%)
SB = SB + (CFE(I%) - CFE(0)) * SF / CFE(I%) / CFE(I%)
CW2 = CW2 + SW * SW / CFE(I%) / CFE(I%)
F2 = F2 + SF * SF / CFE(I%) / CFE(I%)
FW = FW + SW * SF / CFE(I%) / CFE(I%)
ELSE
END IF
NEXT I%
K1 = (SB * FW - F2 * SA) / (FW * FW - CW2 * F2)
K2 = (-SA + K1 * CW2) / FW
IF ABS(1 - H1 / K1) < DEV AND ABS(1 - H2 / K2) < DEV THEN
CLS
PRINT "BEST FIT FOR K1 : "; K1
PRINT "BEST FIT FOR K2 : "; K2
IF K2 <= 0 THEN
PRINT "BIOCONCENTRATION TEST WAS TOO SHORT TO MEASURE K2"
PRINT "OR KINETIC MODEL DOES NOT APPLY!"
ELSE
PRINT "BIOCONCENTRATION FACTOR: "; K1 / K2
END IF
IF DT > (1 / (25 * K2)) THEN PRINT "USE SMALLER TIME STEP!"
PRINT ""
PRINT "TIME", "Cf(FIT)", "Cf(EXP)", "DEVIATION", "% DEV"
FOR I% = 1 TO ENDTIME% / DT
IF CFE(I%) <> 0 THEN
DEVCOUNTER% = DEVCOUNTER% + 1
DIFF = CFE(I%) - CF(I%)
SUMDEV = SUMDEV + (ABS(DIFF) / CFE(I%))
PRINT I% * DT, CF(I%), CFE(I%), DIFF, DIFF * 100 / CFE(I%)
END IF
NEXT I%
PRINT ""
PRINT "MEAN DEVIATION (%)"; SUMDEV * 100 / DEVCOUNTER%
ELSE
H1 = (K1 + H1) / 2
H2 = (K2 + H2) / 2
GOTO FIT
END IF
END
```

Table 2 : Experimental concentrations of 2,5-dichlorobiphenyl in water (C_W, ng/L) and gold fish (C_F(exp), ng/kg) from Bruggeman et al. [12] and fitted concentrations in the gold fish (C_F(calc), ng/kg) derived by the numerical integration procedure.

TIME (days)	$C_{W,I}$	$C_{F,I}$(exp)	$C_{F,I}$(calc)
0	7	ND	ND
1	1.24	2230	2333
2	0.48	2360	2548
3	0.30	3290	2732
6	0.24	2850	2693
8	0.29	2740	2479
23	0.76	3790	3779

the results of the data fitting procedure for all chemicals in fish, including the uptake and elimination rate constants and BCFs reported by Bruggeman et al. [12]. Table 3 shows that given the experimental error, the agreement between elimination rate constants calculated by our method from uptake data is in good agreement with elimination rate constants derived from an independent elimination experiment. For 2,3',4',5-tetrachlorobiphenyl an elimination rate constant could not be determined from the available data because the duration of the bioconcentration test was too short to detect the non-linearity of the time response of the fish concentration expressed by k_2. These results suggest that as long as the duration of the bioconcentration test is sufficiently long, it may not be necessary to conduct an elimination experiment in combination with an uptake experiment to derive k_2. In particular for slowly eliminating chemicals (e.g. very hydrophobic chemicals), it is suggested that instead of conducting a short uptake and then a short elimination experiment, a single uptake study of longer duration is performed to determine the BCF and bioconcentration rate constants.

Table 3 also shows that substantial differences in the uptake rate constant k_1 and the BCF can arise as a result of the method used to derive k_1 and BCF from the experimental water and fish concentrations. For example, we derived an uptake rate constant and a BCF for 2,5-dichlorobiphenyl that are approximately 60% of those reported by Bruggeman et al. [12]. The reason for these differences is that during the uptake period the fish did not achieve steady-state because of fluctuating water concentration (i.e. the water concentration first fell from 7 to 0.2 μg/L in the first 7 days and then increased from 0.2 to 0.76 μg/L) and the long time (i.e. approximately 46 d) that 2,5-dichlorobiphenyl requires to reach steady-state in the gold fish. Due to the drop in water concentration, the concentration in the fish "overshoots" its steady-state value (by approximately 30%) from day 3 to 8.

Table 3 : Uptake rate constants k_1 (L/kg.d), elimination rate constants k_2 (1/d), the bioconcentration factor BCF (L/kg) and the mean deviation E (%) of experimental and fitted concentrations in fish for various PCBs in gold fish derived by the iterative numerical integration method and those (i.e. k_1^a, k_2^b and BCF^c) reported by Bruggeman et al. [12].

CHEMICAL	k_1	k_2	BCF	E	k_1^a	k_2^b	BCF^c
2,5-dichlorobiphenyl	550	0.068	8,200	8.40	920	0.066(± 0.008)	13,900
2,2',5-trichlorobiphenyl	830	0.040	20,800	6.98	950	0.048(± 0.006)	19,800
2,4',5-trichlorobiphenyl	890	0.041	21,600	9.48	890	0.021(± 0.003)	42,400
2,2',5,5'-tetrachlorobiphenyl	720	0.022	32,200	8.14	740	0.015(± 0.002)	49,300
2,3',4',5-tetrachlorobiphenyl	400	ND	ND	12.0	420	0.010(± 0.001)	42,000

a. derived as BCF^c.k_2^b; b. k_2 and their standard errors (n=7) measured in a 150 day elimination experiment [12]; c. BCF determined as the ratio of chemical concentrations in fish and water at the end of the bioconcentration experiment [12].

The following 3.8 fold increase in the water concentration causes the fish concentration to be approximately 40% below its steady-state value at the end of the 23 day bioconcentration experiment. The eye-ball method used by Bruggeman et al. [12] tends to "average" the fish concentrations, giving an estimate of the BCF that is higher than that derived by our method.

Additional evidence that the method of deriving bioconcentration factors and rate constants from experimental organism and water concentrations can cause a considerable variability in the determination of the BCF is presented in Figure 1, which shows BCFs for several PCBs, chlorobenzenes and octachlorostyrene in aquatic plants [14] derived from observed plant and water concentration data by 3 regularly used methods and the method outlined above. The 3 methods that are presented derive the BCF as the ratio of (i) the fish and water concentrations at the end of the bioconcentration test, (ii) the fish concentration at the end of the bioconcentration test and the mean of the observed water concentrations during the test, and (iii) the fish concentration at the end of the bioconcentration test and the time-averaged water concentration during the bioconcentration test. Figure 1 illustrates that reported BCFs can range up to an order of magnitude depending on the method used to derive the BCF from the experimental data. This variability tends to become larger with increasing BCF and time to reach steady-state, which are often related to the K_{ow} of the chemical. This variability can be reduced by adopting the method that we have presented. The most important advantages of our method are that (i) it can be applied to the results from different types of

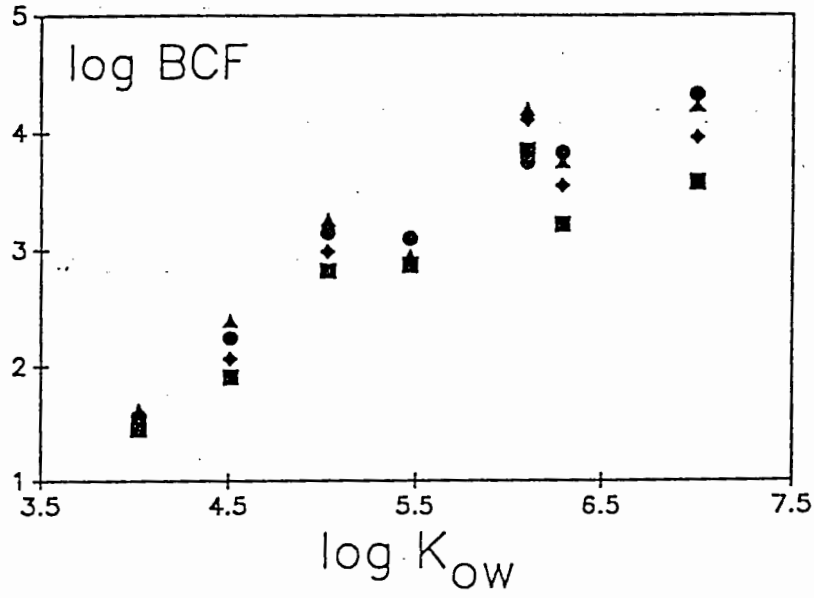

Figure 1 : The logarithm of the bioconcentration factors (BCF) of some PCBs, chlorobenzenes and octachlorostyrene in Myriophyllum spicatum as a function of log K_{ow}. BCFs were derived from the experimental data by the iterative numerical integration procedure (•); as the ratio of the fish and water concentrations at the end of the bioconcentration test (▲); as the ratio of the fish concentration at the end of the bioconcentration test and the mean of the observed water concentrations (■); and as the ratio of the fish concentration at the end of the bioconcentration test and the time-averaged water concentration during the bioconcentration test (♦).

bioconcentration tests (i.e. static, semi-static or flow-through), (ii) it provides a theoretically sound method for the derivation of bioconcentration factors and rate constants from data of bioconcentration tests in which the chemical concentration was not constant during the test and the duration of the bioconcentration test was too short to reach steady-state, and (iii) it has high statistical power by using all observed data points in determining the bioconcentration factor and rate constants.

CONCLUSIONS

To reduce experimental error and variability in experimentally determined bioconcentration factors and rate constants, it is preferable to use a single method for deriving BCFs and rate constants from experimental data. We believe that the iterative numerical integration procedure that we presented above is a theoretically sound and reliable method for deriving bioconcentration factors and rate constants under a wide range of typical experimental conditions. This method can be applied to data from bioconcentration experiments, in which the concentration of the test chemical in the water was constant throughout the duration of the experiment and steady-state was achieved during the test. The

merit and advantage of our method is that it also provides a theoretically sound method for deriving BCFs and rate constants from data of bioconcentration experiments, in which fluctuations in water concentrations occurred during the bioconcentration test and/or the duration of the bioconcentration test was too short to reach steady-state. Our method is believed to derive bioconcentration factors and rate constants from experimental bioconcentration data more accurately and with less error than other methods currently used, thus reducing uncertainty and variability in BCFs and rate constants.

ADDENDUM

A user-friendly IBM-PC version of the iterative numerical integration procedure with graphical presentation of the results can be obtained by writing to the corresponding author. Please, provide an IBM compatible formatted diskette.

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