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Bioconcentration of Organic Chemical Vapors in Plant Leaves: Experimental Measurements and Correlation

Eros Bacci,*,† Davide Calamari,‡ Carlo Gaggi,† and Marco Vighi‡

Dipartimento di Biologia Ambientale, Università di Siena, Via delle Cerchia 3, 53100 Siena, Italy, and Istituto di Entomologia Agraria, Università di Milano, Via Celoria 2, 20133 Milano, Italy

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Introduction

Higher plant leaves, as well as lichens, mosses, and grasses, constitute an important environmental compartment, in which the bioconcentration of trace organic chemicals can occur. Among the three main pathways for the uptake of organics in plant leaves (translocation from contaminated soils via roots, atmospheric deposition of contaminated particulates, and vapor intake from the air), the air to leaf transfer of gaseous organics can be considered a key process, particularly for less water soluble compounds (*1–4*).

In previous translocation experiments carried out in small glass greenhouses (200-L volume), with plants and contaminated soils, it was observed that the concentrations of the vapors of some organochlorine compounds were,

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Table I. Compoun Studied[a]

compound	MW	vp, Pa	H, Pa m^3/mol	log K_{ow}
trifluralin	335.29	0.011	4.02	3.0
hexachlorobenzene	284.8	0.001	131.5	6.0
mirex	545.59	0.0001	839.4	6.9
thionazin	248.2	0.4 (30 °C)	0.087	1.24
sulfotep	322.3	0.0226	0.29	3.02

[a] Molecular weight (MW), vapor pressure (vp) (20 °C), H (20 °C), and log K_{ow} values from Suntio et al. (*11*). For sulfotep and thionazin only: vapor pressure (and water solubility to calculate H) from Worthing and Walker (*12*); log K_{ow} estimated by means of the fragment constant method (*13*).

within a factor of 2, constants for 4 weeks (*5*). This property of the system, mainly due to the relatively constant volatilization rate of contaminants from fortified soils and to a constant air turnover in the greenhouses, was then applied in the measurement of the equilibrium leaf/air partition coefficient (BCF) of *p,p'*-DDT and DDE, of α- and γ-HCH, and of a PCB mixture in azalea leaves (*6*). These preliminary results were used to try to predict the BCF from the Henry's law constant, H (*7*), from the 1-octanol/water partition coefficient, K_{ow} (*8*) and from H and K_{ow} (*8*, *9*).

In this study the azalea BCF of five more compounds obtained by the same apparatus are reported. Chemicals with a wide range of H (Pa m^3/mol) and K_{ow} values were selected.

The aim of the study was to develop a method of predicting BCF from the 1-octanol/water partition coefficient, K_{ow}, and the air/water dimensionless partition coefficient, K_{aw}, obtained from the relationship $K_{aw} = H/RT$, where H is the Henry's law constant (Pa m^3/mol), R is the gas constant, 8.314 Pa m^3/(mol K), and T is the working temperature (K).

Materials and Methods

The selected chemicals, with their relevant physicochemical properties, are reported in Table I. All, including the two organophosphates thionazin and sulfotep (*10*), were assumed to not degrade during the experiments.

The azalea (*Azalea indica*, var. Knut Erwên) was chosen as test plant, using for the analysis only the old leaves, whose growth during the experiment was negligible.

During the uptake phase, four plants in pots, with their "clean" soil, were placed in two glass 200-L greenhouses (two in each greenhouse), maintained at constant temperature by means of a warm-water system in the bottom, and continuously illuminated by 3 × 20 W True-Lite Fluorescent tubes (*3*). The working temperatures were 24 °C at the bottom, ~25 °C at the level of the azalea leaves, and 27 °C at the top of the greenhouses, because of the lamps. The pots were arranged on a glass tray, to reduce the impact of air flowing in through a 3-cm-diameter hole near the bottom of the greenhouses. Two similar holes near the top and slight gaps between the cover and the walls guaranteed sufficient air turnover. The turnover time of each system was ~80 min and the mixing of the air inside the "glass boxes" was enough to obtain similar mean levels of contamination both near the corners and in the middle (*6*). As vapor sources, 200-g aliquots of dried (80 °C) Pliocene sand, pH 7.6, 0.2% organic carbon, with the texture of a "fine sandy medium sand" (*14*) were fortified with 50 mg each of the selected compounds, after the addition of 200 mL of *n*-hexane. The solvent was then evaporated by vacuum rotary evaporator. Two vessels containing 100 g of the treated sand were prepared for each chemical and placed in the greenhouses. Because of its high vapor pressure, the thionazin-fortified sand vessels were each covered with 100 g of "clean sand", to reduce the evaporation rate. In the first box trifluralin, hexachlorobenzene, and mirex were tested; in the second thionazin and sulfotep.

In the release phase, the vessels containing the contaminated sand and the cover of the greenhouses were removed, and the walls were lifted (~ 4 cm) to increase air turnover.

The plants were watered daily, as were the vessels containing the contaminated sand, to maintain a constant volatilization potential.

Periodically, 15 old leaves were taken at random from each box, by means of a little noose introduced through one of the lateral aeration holes. Uptake was studied for 420 h in the first box and 340 h in the second; elimination was followed for 280 h in both boxes. Air samples were taken by means of Florisil traps, prepared by filling disposable Pasteur pipets with 700 mg of Florisil as suggested by Giam et al. (*15*), with minor modifications. Apparent sample volume was measured by soap-bubble flow meter, connected in series with the air trap and a tap-water vacuum pump. These volumes were corrected only for internal pressure (~50 kPa), measured by a vacuometer on the suction line.

The vapors adsorbed onto the Florisil were then eluted with 100 mL of *n*-hexane and 50 mL of 4% acetone in *n*-hexane, in glass chromatography columns. After appropriate concentration (needed only for mirex), the samples were ready for GLC analysis.

Plant foliage samples (10 leaves, 1–2-g fresh weight, water content ~70%) were extracted for 2 min in an Ultra-Turrax homogenizer, after the addition of 5 mL of distilled water, 10 mL of acetone, and 15 mL of *n*-hexane (all solvents for pesticide residue analysis). After centrifugation, the "green" upper layer was recovered and the aqueous phase reextracted with a further 15 mL of *n*-hexane, as before. The second "green" phase was recovered and added to the previous one. The volume of the extract was then reduced to ~3 mL, with the elimination of the acetone. The cleanup procedure was carried out on Florisil columns (1.5 g, Florisil height 3 cm), eluting first with 100 mL of *n*-hexane, recovering ~75% trifluralin and hexachlorobenzene (HCB) and 30% mirex, and then with 50 mL of 4% acetone in *n*-hexane, recovering the remaining trifluralin and HCB (discarded), 30% more mirex and ~100% thionazin and sulfotep. The samples were then ready for GLC analysis, without concentration, except for mirex.

Trifluralin, HCB, and mirex were detected by a Perkin-Elmer F-22 gas chromatograph equipped with on-column injectors, ^{63}Ni electron capture detectors (ECDs), 2 m × 2 mm i.d. borosilicate glass column packed with GP 4% SE-30, 6% SP-2401 on 100–120-mesh Supelcoport. The carrier gas was argon–methane, 95/5%; flow, 60 and 40 (to the scavenger) mL/min. Injector, oven, and detector temperatures were 210, 200, and 280 °C, respectively. Retention times were 1.90, 2.30, and 28.5 min for trifluralin, HCB, and mirex, respectively.

Thionazin and sulfotep were detected by a Perkin-Elmer Sigma 3 B gas chromatograph, equipped with nitrogen–phosphorus detector (NPD), J&W 15 m × 0.544 mm fused-silica wide-bore column, liquid-phase DB-1, 5.0-μm film thickness, was used. The carrier gas, nitrogen, was regulated at 10 mL/min and air and hydrogen pressure at 80 and 180 kPa, respectively. The rubidium bead temperature was regulated at 450 °C. The injector and de-

Table II. Vapor Concentrations (ng/g) of the Various Chemicals in Greenhouse Air during Uptake (Air Density 1.19 g/L)

	trifluralin	HCB	mirex	thionazin	sulfotep
mean	24.9	4.1	0.023	16.1	17.2
n	5	5	5	6	6
%CV[a]	30.8	20.7	46.0	16.9	29.5

[a] Percent coefficient of variation, (standard deviation/mean) × 100.

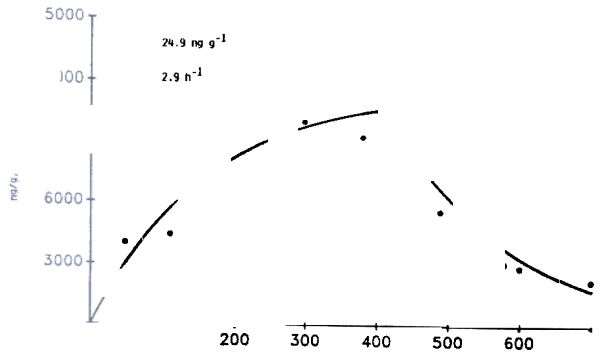

Figure 1. Uptake and release kinetics of trifluralin vapors in azalea leaves. BCFm is the leaf/air bioconcentration factor (ng/g of dry leaf)/(ng/g of air). Air density 1.19 g/L.

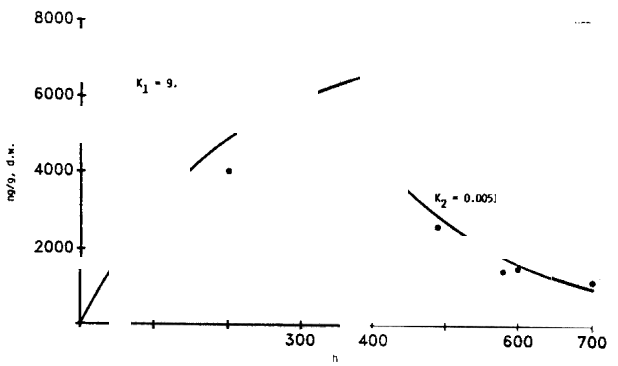

Figure 2. Uptake and release kinetics of hexachlorobenzene vapors in azalea leaves. BCFm, as in Figure 1.

tector were set at 200 and 250 °C. Oven temperature was 180 °C for 2 min, then raised to 210 °C in 1 min, and held for 3 min. Retention times were 4.5 and 5.5 min for thionazin and sulfotep, respectively.

Results

The vapor levels of the compounds tested, measured during uptake, generally ranged within a factor of 2–3. The results are reported in Table II as nanograms per gram of air (air density 1.19 g/L).

As in previous experiments (*5*), the mean vapor concentrations in the greenhouse air are in acceptable agreement (except for thionazin, because of the covering of the treated soil, to avoid levels in the air as high as 100 ng/g or more) with predictions based on the vapor pressure and molecular weight values, as suggested by Hartley (*16*).

Figures 1–5 give the levels of the different compounds in old azalea leaves, during both the uptake and release phases. The mass/mass dry leaf/air bioconcentration factors, BCFm, calculated from a two-compartment first-order model, are also given. During uptake, the concentration of the chemicals in the leaves, $C_{l(t)}$, (ng/g, dry weight) varied as a function of time t (h), according to the following equation:

$$C_{l(t)} = C_a K_1/K_2(1 - e^{-K_2 t}) \quad (1)$$

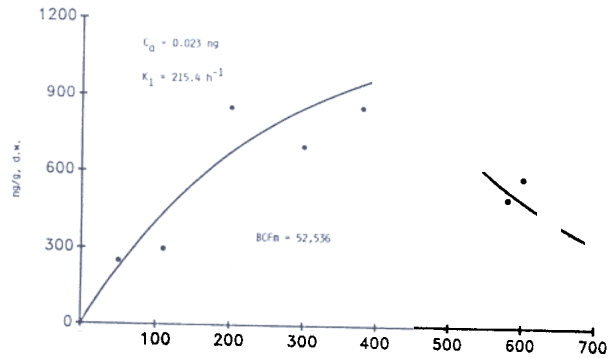

Figure 3. Uptake and release kinetics of mirex vapors in azalea leaves. BCFm, as in Figure 1.

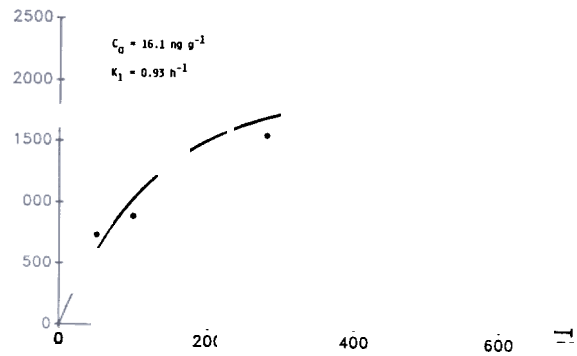

Figure 4. Uptake and release kinetics of thionazin vapors in azalea leaves. BCFm, as in Figure 1.

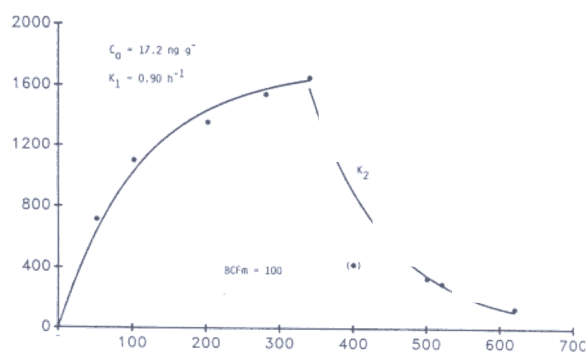

Figure 5. Uptake and release kinetics of sulfotep vapors in azalea leaves. BCFm, as in Figure 1.

where C_a is the concentration of the chemical, as vapor, in the air (ng/g, air density 1.19 g/L) and K_1 and K_2 are input (air to leaf) and output rate constants (h^{-1}).

When $t \rightarrow \infty$, eq 1 becomes

$$C_{l(t=\infty)} = C_a K_1/K_2 \quad (2)$$

where $C_{l(t=\infty)}$ is the equilibrium concentration in the leaf (ng/g of dry weight) for a given C_a value. The leaf/air equilibrium bioconcentration factor, BCFm, can be obtained from eq 2 as follows:

$$\text{BCFm} = C_{l(t=\infty)}/C_a = K_1/K_2 \quad (3)$$

During elimination, C_a is assumed to be zero so that the equation is

$$C_{l(t)} = C_{l0} e^{-K_2 t} \quad (4$$

where C_{l0} is the concentration of the chemical in the leaf (ng/g, dry weight) at the beginning of elimination phase. From eq 4, the half-life ($t_{1/2}$, h) is ln $2/K_2$.

Table III. Parameters Evaluated[a]

compound (or mixture)	BCFm	BCF	log BCF	H^b	K_{aw}	log K_{ow}[c]	log (BCFK_{aw})
p,p'-DDT	1.92×10^5	4.32×10^7	7.64	6.02	2.47×10^{-3}	6.0	5.03
p,p'-DDE	1.35×10^5	3.03×10^7	7.48	7.95	3.26×10^{-3}	5.7	4.99
α-HCH	4.60×10^3	1.03×10^6	6.01	0.87	3.57×10^{-4}	3.8	2.57
γ-HCH	3.40×10^3	7.63×10^5	5.88	0.13	5.34×10^{-5}	3.8	1.61
PCBs (60% Cl)	8.54×10^4	1.91×10^7	7.28	7.11	2.92×10^{-3}	6.1	4.75
trifluralin	4.65×10^2	1.04×10^5	5.02	4.02	1.65×10^{-3}	3.0	2.24
HCB	1.86×10^3	4.18×10^5	5.62	131.5	5.40×10^{-2}	6.0	4.35
mirex	5.25×10^4	1.18×10^7	7.07	839	3.44×10^{-1}	6.9	6.61
thionazin	1.21×10^2	2.71×10^4	4.43	0.087	3.57×10^{-5}	1.2	−0.01
sulfotep	1.00×10^2	2.24×10^4	4.35	0.29	1.19×10^{-4}	3.0	0.43

[a] Mass/mass leaf/air bioconcentration factor, BCFm, as (ng/g of dry leaf)/(ng/g of air), air density 1.19 g/L; volume/volume leaf/air bioconcentration factor, BCF, as (ng/L of wet leaf)/(ng/L of air), leaf density 0.89 g/cm³; log BCF; H (Pa m³/mol); K_{aw}, obtained from H/RT, where R is the gas constant, 8.314 Pa m³/(mol K), and T the temperature (K); log K_{ow} and log (BCFK_{aw}) values for 10 organic chemical vapors. [b] From Suntio et al. (*11*), with the following exception: for thionazin and sulfotep H was obtained from vapor pressure and water solubility data reported in the literature (*12*); PCBs from Mackay and Leinonen (*19*). [c] From Suntio et al. (*11*), except thionazin and sulfotep, where the values were calculated by the fragment constant method (*13*).

In order to obtain the value of the elimination rate constants, K_2, the least-squares method was applied to the eq 4, after logarithmic transformation:

$$\ln C_{l(t)} = \ln C_{l0} - K_2 t \quad (5)$$

This is a straight line with intercept ln C_{l0} and slope $-K_2$.

The input rate constants, K_1, were calculated from the initial accumulation rate, $C_{l(t)}/t$, (t = 50–100 h), assuming negligible, in that phase, the elimination of the chemicals from the azalea leaves: for $t \leq 100$ h

$$C_{l(t)}/t = K_1 C_a \quad (6)$$

and

$$K_1 = (C_{l(t)} / t) / C_a \quad (7)$$

The BCFm values obtained by this approach are dimensionless mass/mass partition coefficients: i.e., (ng/g of dry leaf)/(ng/g of air), with air density of 1.19 g/L.

Discussion and Conclusions

Once the BCFm had been obtained, the possibility of predicting it from empirical relationships with some basic partitioning properties of the chemicals was investigated.

Following the indications of Travis and Hattemer-Frey (*8*), the Henry's law constant H and the 1-octanol/water partition coefficient K_{ow} were considered. Table III lists the mass/mass leaf/air bioconcentration factor, BCFm, the volume/volume leaf/air bioconcentration factor, BCF (see below), log BCF, H, the dimensionless volume/volume air/water partition coefficient, K_{aw}, log K_{ow}, and log (BCFK_{aw}) for these five chemicals and for the five chemicals previously tested in the same way (*6*). The few prior studies in this field have usually had different aims, such as the investigation of the accumulation of organic chemicals in growing plants (*17*). Recent research on the partition coefficient of five chlorinated benzenes between aerial parts of growing sprouts of barley and cress and air has shown that there is a positive linear correlation (log/log) with K_{ow} (*18*). Similar results have been reported by Travis and Hattemer-Frey (*8*) for the first five chemicals reported in Table III.

If the same correlation is applied to the 10 chemicals in Table III, a poor linear correlation coefficient is found (r = 0.86). Better results are obtained if the BCF is assumed to be related to K_{ow} and H, as first suggested by Travis and Hattemer-Frey (*8*): the BCF is by definition the ratio of the equilibrium concentration of a chemical in the leaf to its concentration in the air. Dividing the two terms by the equilibrium concentration in water, the BCF could also be defined as K_{lw}/K_{aw}, where K_{lw} is the leaf/water partition coefficient, and K_{aw} the air/water partition coefficient. K_{lw} can be quantified by K_{ow}, and K_{aw} is essentially H''. Thus (BCFH) should be related to K_{ow}.

The data reported in Table III were processed by following this suggestion, obtaining a first equation (*21*)

$$\log (\text{BCFm}H) = -0.92 + 1.14 \log K_{ow} \quad r = 0.96 \quad (8)$$

where BCFm is the mass/mass dimensionless leaf/air partition coefficient, as (ng/g of dry leaf)/(ng/g of air), air density 1.19 g/L; H is the Henry's law constant, Pa m³/mol; and K_{ow} is the volume/volume dimensionless 1-octanol/water partition coefficient.

A more clear expression of eq 8 can be obtained if H is expressed in the dimensionless form $K_{aw} = H/RT$, where K_{aw} is the air/water partition coefficient, R the gas constant, 8.314 Pa m³/(mol K), and T the temperature (K).

Considering now that both K_{aw} and K_{ow} are volume/volume partition coefficients, the expression of BCF as mass/mass ratio could generate some confusion. So, the BCF should also be expressed as a volume/volume partition coefficient: e.g., (ng/L of wet leaf)/(ng/L of air). Considering that the water content of the azalea leaves is 70% of the wet weight, the leaf density is 890 g/L, and the air density 1.19 g/L, the volume/volume bioconcentration factor, BCF, can be calculated from the previous one as follows:

$$\text{BCF} \quad 224.37\text{BCFm} \quad (9$$

where BCF is the leaf/air bioconcentration factor as mass/volume ratio (e.g., mol/m³, ng/L, ng/cm³, etc. in both leaf and air), and BCFm is the leaf/air bioconcentration factor as mass/mass ratio, e.g., (ng/g of dry leaf)/(ng/g of air).

From all these considerations, eq 8 can be rearranged as follows:

$$\log (\text{BCF}K_{aw}) = -1.95 + 1.14 \log K_{ow} \quad r = 0.96 \quad (10)$$

where BCF, K_{aw}, and K_{ow} are volume/volume dimensionless partition coefficients (Figure 6).

The slope of the correlation in eq 10 is very near to 1, indicating, to a first approximation, that the leaf/air BCF is directly proportional to K_{ow} and inversely proportional to K_{aw}, i.e.: BCF $\simeq$ constant K_{ow}/K_{aw}. However, the meaning of the ratio K_{ow}/K_{aw} seems at present more difficult to grasp than the meaning of K_{ow} and log K_{ow}, widely used to indicate the lipoaffinity of different chemicals.

From eq 10, chemicals with high lipoaffinity need low K_{aw} values to be accumulated to a considerable extent, and

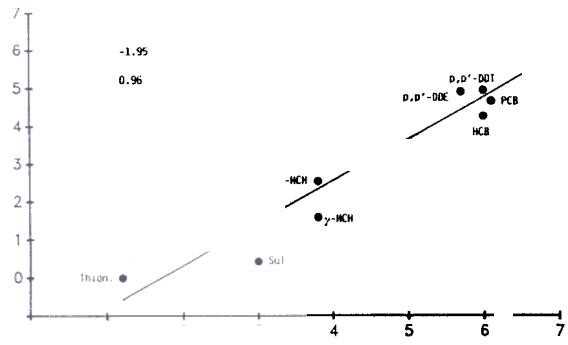

Figure 6. Relationship between the volume/volume bioconcentration factor of organic chemicals in azalea leaves, BCF as (ng/L of wet leaf)/(ng/L of air), the air/water partition coefficient K_{aw}, and the 1-octanol/water partition coefficient, K_{ow}.

polar organic compounds may have relatively high BCF levels if K_{aw} is low.

The apparent contradiction inherent in the possibility of good correlations between BCF and K_{ow} (without H or K_{aw}) is overcome by the observation that the range of H values of the chlorobenzenes before mentioned (*18*) lies within 1 order of magnitude (from 24 to 591 Pa m^3/mol; ref 20), as did the range of H values of four out of five of the organochlorine compounds investigated in this respect by Travis and Hattemer-Frey (*8*).

The number of chemicals available to date with a view to predicting the BCF is still very small. So before generalizing the results shown in Figure 6, further research is needed; however, at least with the present chemicals, the equilibrium leaf/air bioconcentration factor seems to be controlled by two opposite driving forces: K_{aw} and K_{ow}. The former, when high, tends to reduce the bioconcentration potential of highly lipophilic chemicals such as mirex; when small, it may increase the bioaffinity of poorly liposoluble compounds such as thionazin.

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