Technical Report

N° 9

ASSESSMENT OF REVERSE - PHASE CHROMATOGRAPHIC METHODS FOR DETERMINING PARTITION COEFFICIENTS

Brussels, December 1983

CONTENTS

SUMMARY

The possibility of using reverse-phase High Performance Liquid Chromatography (HPLC) and Thin Layer Chromatography (TLC) for determining partition coefficients is assessed. Whereas HPLC has been adequately validated and is recommended, TLC requires further validation. A method of calculating partition coefficients from molecular fragmental constants is also noted as being useful in certain cases.

A. INTRODUCTION

One important interest in determining a partition coefficient (P) is that empirical correlations exist between the n-octanol/water partition coeffient of a chemical and its partitioning between other organic systems and water (Collander, 1951; Leo et al., 1971). In particular, some ecotoxicologically important descriptors such as the bioconcentration factor in fish (Neely et al., 1974; Lyman et al. 1981) or soil adsorption (Brown and Flagg, 1981) have been found to correlate well with log P, which is therefore used as one of the indicators in the hazard assessment of a chemical.

The OECD in their Test Guideline 107 describe the "Flask-shaking method"(we prefer the term "Shake-Flask" method) for determining the n-octanol/water partition coefficient. The fact that the retention of a chemical in a reverse-phase chromatographic system is related to its partition coefficient suggests that a valuable alternative technique might be available. Two such techniques, High Performance Liquid Chromatography (HPLC) and Thin-Layer Chromatography (TLC), are assessed in this paper, taking into account their ultimate purpose as screening tests for assessing the bioconcentration potential of organic chemicals.

B. DETERMINATION OF PARTITION COEFFICIENTS BY REVERSE-PHASE CHROMATOGRAPHY

1. General

Relevant reverse-phase chromatographic systems where the mobile phase is partly aqueous and the stationary phase is lipoidal have been described by Unger et al.,1978; Veith et al.,1979; Renberg et al.,1980; Ellgehausen et

al.,1981; McDuffie, 1981; Bruggeman et al., 1982; and Hafkenscheid and Tomlinson, 1983. These methods offer a rapid and inexpensive way of determining P provided that their limits of applicability, as described below, are taken into account.

The partition coefficient (P) is defined according to the Nernst Partition Law as the ratio of the equilibrium concentrations of a dissolved substance in a two-phase system consisting of two largely-immiscible solvents, e.g. n-octanol and water:

$$P = \frac{C_{n\text{-octanol}}}{C_{water}}$$

The partition coefficient is therefore dimensionless, being the quotient of two concentrations, and is usually given as its logarithm to base ten (log P).

Two alternatives to the Shake-Flask method for the estimation of n-octanol/water partition coefficients, i.e. Reverse-Phase High Performance Liquid Chromatography (HPLC) and Reverse-Phase Thin-Layer Chromatography (TLC) may be considered. The methods are primarily applicable to neutral compounds but not to substances which associate in solution, are fully or partly ionised, or are surface active. Measurements should be made on ionisable substances only in their non-ionised form (free acid or free base) produced by the use of an appropriate buffer with a pH below (free acid) or above (free base) the pK. These conditions are not always environmentally relevant and the results so obtained should be interpreted with caution. The pH should be within the operating range of the column, i.e. usually between pH 2 and 8. HPLC measurements at above pH 8 are not advisable since the use of an alkaline mobile phase may cause rapid deterioration in the performance of the column. Care must be taken to avoid salt precipitation and column or plate deterioration which occur with some organic phase/buffer mixtures.

In order to correlate the measured HPLC or TLC data of a compound with its P, a calibration graph of log P vs chromatographic data is needed for a number (typically between 5 and 10) of reference compounds adequate to define the straight-line graph over the desired range. To minimise errors in this correlation it is preferable when possible to choose reference

compounds which are structurally related to the test chemical. Extensive lists of values of log P (n-octanol/water) for many groups of chemicals are available (Hansch and Leo, 1979; Pomona Project, 1982).

It is a simple and rapid matter to introduce reference compounds of known P into the chromatographic system, determine their chromatographic data and construct the calibration graph. If data on the partition coefficients of structurally-related compounds are not available, then a more general calibration graph has to be established on other reference compounds, in which case the correlation for the test compound is less accurate.

Specific solvation effects or particular interactions with the stationary phase can lead to systematic errors (Bruggeman et al.,1982).

Precision. In order to increase confidence in the measurement, duplicate determinations must be made. The values of the partition coefficient of individual compounds expressed as their common logarithm should fall within a range of ± 0.1 log units for HPLC and ± 0.3 log units for TLC.

Sensitivity. The reverse-phase methods usually enable partition coefficients to be determined over a log P range of about 0-6 for HPLC and 0-12 for TLC (Renberg et al., 1980). Adjustment of the mobile phase may extend this range for HPLC.

Specificity. Reverse-phase chromatographic methods are less sensitive to the presence of impurities in the test compound than is the Shake-Flask method. Nevertheless, in some cases impurities may make the interpretation of the results difficult because peak or spot assignment becomes uncertain. For mixtures which give unresolved bands, upper and lower limits of log P should be stated (Renberg et al.,1980).

Accuracy. Normally, the partition coefficient of a compound can be estimated to within ± 1 log unit of the Shake-Flask value. Typical correlations can be found in the literature (McDuffie, 1981; Renberg et al.,1980; Ellgehausen et al.,1981; Bruggeman et al., 1982). Improved accuracy can usually be achieved when correlation plots are based on structurally-related reference compounds (Fujisawa and Masuhara, 1981; Bruggeman et al.,1982).

2. Reverse-Phase High Performance Liquid Chromatography (HPLC).

Principle of method. HPLC is performed on commercially-available analytical columns packed with a solid phase containing a long-chain hydrocarbon (e.g. C_{18}, C_8). Chemicals injected onto such a column move along it by partitioning between the mobile phase and the hydrocarbon stationary phase. Provided that the residual polar groups on the packing can be kept at a minimum, mixtures of chemicals are eluted in order of their hydrophobicity, with water-soluble chemicals eluted first and oil-soluble chemicals last, in proportion to their hydrocarbon/water partition coefficient. This enables the relationship between the retention time on a reverse-phase column and the n-octanol/water partition coefficient to be established.

Although the retention time (t_R) is measured, the capacity factor (k) derived from it is actually used as the parameter expressing the lipophilicity of a compound

$$k = \frac{t_R - t_o}{t_o}$$

t_R = retention time of test compound
t_o = retention time of an unretained polar chemical (e.g. formamide; McCall, 1975).

Apparatus. The following are required :

- A liquid chromatograph fitted with a pulse-free pump and a refractive index detector, or other detection device (e.g. a UV spectrophotometer) for determining the test and reference compounds. The injection system should be capable of operating against a pressure of about 6 MPa (1 Pa = 1 Newton.m^{-2}; 1 bar = 10^5 Pa; 1 torr = 133 Pa) and the use of an injection valve with injection loop is recommended.
- As stationary phase, commercial microparticulate reverse-phase packings or ready-packed columns are used, eg. Partisil (Whatman), Zorbax (Dupont), Lichrosorb (E. Merck), µBondapak (Waters Associates) and Hypersil ODS (Ahrin). Typically, an HPLC column is 25 cm in length x 0.5 cm in diameter. A guard column (e.g. packed with Whatman CO:PELL-ODS, available as a column survival kit) may be positioned between the injection system and the analytical column.

A UV-visible spectrophotometer is recommended for determining the λ_{max} of test and reference compounds. Currently available equipment permits the automation of solvent mixing, injection and data processing which could improve the precision of the log k, and hence log P, determination.

Procedure

a) Estimation of the partition coefficient. Normally, before carrying out an HPLC determination, an estimate of log P and a consideration of the structure of the test compound will permit a judicious choice of reference compounds. The partition coefficient can be estimated by calculation (Rekker,1977; Hansch and Leo, 1979) or as the quotient of the solubilities of the test substance in n-octanol and water (Jübermann, 1958; Ellgehausen et al.,1981). The calculation is based on additivity rules in which it is assumed that the partition coefficient of a molecule can be computed as the sum of coefficients specific for each portion of the molecule (cf.section E). The reliability of prediction decreases with increasing structural complexity of the compound under study.

b) Preparation of materials.

- Mobile phases. HPLC-grade methanol and distilled water are used to prepare the eluting solvent, which is degassed before use. Isocratic elution with a methanol/water mixture is normally carried out. Typically, a 3:1 (v/v) methanol/water mixture is satisfactory for eluting compounds of log P = 6 within an hour, at a flow rate of 1 ml/min. N,N-dimethyl-n-octylamine can be added to the methanol/water mixture to minimize the effect of residual polar groups on the packing. Other solvent mixtures can also be used, e.g. ethanol/water, and acetonitrile/water. The pH of the eluent is critical for ionizable compounds. It should be within the operating pH range of the column, which is usually between 2 and 8. Buffering is recommended. It may be necessary to shorten the elution times of compounds of high log P (and those of the reference compounds) either by decreasing the polarity of the mobile phase or the column length, or by increasing the eluent flow rate within the operating range of the column pressure.

- Solutes. The test and reference compounds should be the purest available. Compounds to be used for test or calibration purposes are

dissolved in a methanol/water mixture, methanol, or occasionally a less polar solvent (e.g. hexane, for the least water-soluble compounds) at a concentration of about 1 mg ml^{-1}.

c) Detection. Monitoring of the refractive index of the eluate is the most universal method of detection. UV absorption at or close to the λ_{max} of the test and the reference compounds can also be used. This can, however, give misleading results when impurities have a greater absorbance than the pure test compound.

d) Performance of test. The first step is to construct a correlation plot of log k versus log P for appropriate reference compounds. In practice, a set of between 5 and 10 reference compounds of log P around the expected range are injected simultaneously and the retention times are determined, preferably on a reporting integrator linked to the injection system. The corresponding capacity factors, k, are calculated and plotted as a function of the log P determined by the Shake-Flask method. The calibration is performed at regular intervals, at least once daily, so that possible changes in column performance can be allowed for.

The test compounds are injected separately and their retention times are also determined, in duplicate, permitting the calculation of the capacity factor k. From the correlation graph of the reference compounds, the partition coefficients of the test compounds can be interpolated.

The temperature during the measurements should not vary by more than $\pm$ 2°C.

e) Reporting. The following information should be included in reporting the results :

- test and reference substances, and their purity;
- temperature range of the determinations;
- pH at which the determination was made, if appropriate;
- estimate of the partition coefficient and its manner of determination;
- details of the analytical and guard column, mobile phase and means of detection;
- retention data and literature log P values for reference compounds used in calibration;

- details of fitted regression line (log k versus log P);
- average retention data and interpolated log P value for the test compound.

Variant. A more direct simulation of partitioning between n-octanol and water in the Shake-Flask method can be obtained by the use of an n-octanol-coated column, and n-octanol-saturated water as the mobile phase, (Mirrlees et al., 1976; Miyake and Terada, 1978; Unger et al., 1978). Although such columns appear to give an accurate estimate of partition coefficient they suffer from column instability and hence require periodical recoating. At present they have a limited range of applicability.

3. Reverse-Phase Thin-Layer Chromatography (TLC).

Principle of method. In TLC, a small aliquot of a solution of the compound is applied as a spot onto a plate supporting an adsorbent as stationary phase. Under the influence of a solvent the compound moves over the surface in the direction of the solvent front. Partitioning on the plate occurs in order of hydrophobicity when a suitable mobile phase is used. The spot or spots of the compound(s) are detected under ultraviolet light or by spraying with a chemical which reacts to give a colour. This enables the retention factor (R_F) and thence the R_M value (see below) to be determined. From the relationship between the derived R_M values and the n-octanol/water partition coefficient for reference compounds, the partition coefficient of an unknown chemical is calculated.

For each test and reference compound an R_M value is calculated :

$$R_M = \log(1/R_F - 1)$$

R_F is the ratio of the distance moved by the substance to the distance of the solvent front on the plate, after elution by the mobile phase.

Apparatus. Commercially available reverse-phase thin-layer plates, coated with a long-chain (eg. C_{18}) hydrocarbon to give a C loading of up to 10%, are used as stationary phase. Precoated plates (e.g. Whatman KC 18F RP, Merck RP-18 F 254 Art. No. 13724, or similar types) are also effective.

Methanol/water mixtures are preferably used as the mobile phase, although other organic solvents mixed with water have also been used, e.g. ethanol,

acetone, acetonitrile. R_M values depend strongly on the solvent composition. Different log P ranges may require different solvent ratios.

To minimise errors resulting from temperature fluctuations it is advisable to thermostat the TLC tank.

Procedure

a) Estimation of the partition coefficient. This is the same as for HPLC - see section 2, above.

b) Preparation of materials. Solutions of reference and test compounds are prepared in a suitable solvent. All compounds should be of the highest purity available, i.e. puriss. or equivalent grade.

c) Test conditions. These should be as follows :
   - Ambient temperature, with no temperature gradient in the TLC tank.
   - Typical solvent mixture : methanol/water (85/15 v/v). Other ratios may be required depending on the lipophilicity and solubility of the test and reference compounds.
   - The concentration of the compounds in the solution applied to the plate is not critical but must be such as to give easily measurable spots.
   - The R_F value for each test or reference compound should be between 0.05 and 0.95 otherwise the solvent composition ratio should be changed.

d) Performance of test. A solution containing from 5 to 10 reference compounds for which the log P is known (Shake-Flask method) is chromatographed. The R_F values are determined, and R_M values are calculated and plotted against their log P to give the calibration graph. The chemical(s) being tested are chromatographed on the same plate as are the reference compounds. They are detected as dark spots on a plate impregnated with a fluorescent indicator under ultraviolet light at 254 nm, or by a suitable spraying technique, and the R_M values are determined. Log P for the unknown compound is estimated by interpolation from the calibration plot of R_M against log P.

   Duplicate measurements are performed on two different plates.

TABLE 1
Methods for log P determination

Method	Measurable log P range	Applicability	Advantages	Disadvantages
Estimation from the solubilities in water and n-octanol	0 to 6	- compounds with low solubility in both solvents, but not those highly soluble in one phase	- inexpensive - fast	- affected by impurities - low accuracy - solubilities in both solvents are often not available
Calculation by summation of molecular fragments	not limited	- only method for compounds with extreme log P values which cannot be determined experimentally - all compounds for which constants are available - selection of optimum conditions in experimental determinations - confirmation of experimental values	- inexpensive - fast	- requires expertise for proper use on complex molecules - limited by applicability of additivity and interaction rules, and availability of fragment constants - less accurate than Shake-Flask method
Shake-Flask	-2.5 to 4.5	- all classes of compounds except surface active compounds and organometallics	- thermodynamic method	- affected by impurities - difficult for compounds of low solubility - time consuming - may require development of analytical method
Reverse-Phase HPLC	0 to 6	- neutral compounds; but not charged, partly charged or very polar compounds	- relatively fast - reproducible - less sensitive to impurities	- requires calibration - occasional outliers occur
Reverse-Phase TLC	0 to 12	- neutral compounds; but not charged, partly charged, very polar, or volatile compounds	- cheap - relatively fast - inexpensive - less sensitive to impurities	- requires calibration - less reproducible than HPLC method - resolution inferior to HPLC - less accurate than Shake-Flask method (occasional outliers occur)

e) Reporting. The following information should be included in reporting the results.

- test and reference substances, including their purity;
- temperature of the determination;
- pH at which the determination was made, if appropriate;
- preliminary estimate of the partition coefficient and its manner of determination;
- details of type of plate, mobile phase and means of detection;
- R_M and literature log P values for reference compounds used in calibration;
- details of the linear relation, R_M versus log P.
- R_M and interpolated log P value for the test compound.

C. COMPARISON OF METHODS

1. General

For determining partition coefficients it is desirable to have alternative methods available, and to be aware of the range of applicability and pitfalls of each so that serious errors can be avoided. In addition to their determination by the experimental methods discussed above, it has been proposed to calculate them from empirically-derived rules; see Section E. A brief comparison of the methods is given in this section. In Table 1 are listed the main advantages and problems associated with the different methods.

Calculation methods are based on empirical rules and therefore the values of log P so obtained should be regarded as approximations. Confidence in the calculated value, in general, decreases as the complexity of the molecule under investigation increases. Such values may, however, be more trustworthy than experimental values for compounds whose log P is at the extremes of, or outside of, the normal measurable range.

It is emphasised that relatively simple substances were used in the development of the chromatographic procedures, whereas partition coefficients may sometimes have to be determined for substances with rather extreme properties such as strong acids and bases; metal complexes or surface active agents; extremely lipo- and hydro-philic compounds;

extremely insoluble compounds and impure chemicals or mixtures. Reverse-phase chromatographic methods are inappropriate for the first two groups of compounds listed.

2. Validation

HPLC Method. Some intra-laboratory comparative measurements (Eadsforth, unpublished) have shown that the reproducibility of the results obtained with reverse-phase HPLC (without automated solvent mixing and solute injection) is slightly superior to that of the Shake-Flask method (see Table 2). An inter-laboratory comparison of log P values determined by either the Shake-Flask or the HPLC methods has been made (see Table 3). In both cases a minimum of three determinations by different laboratories was generally sufficient to justify inclusion in the Table. The data confirm that the inter-laboratory precision of reverse-phase HPLC is equivalent to that of the Shake-Flask method.

The results of the above comparative testing combined with the abundant literature permits the conclusion that the HPLC method gives an adequate estimate of log P, comparable to that obtained by the Shake-Flask method. The accuracy can be improved if structurally-related reference compounds are used. The ASTM is at present (mid-1983) carrying out a ring test with seven unknown compounds and Veith's standard reference compounds (Veith et al.,1979, 1980). Data from this study will be published.

It is concluded that no further validation by ring-testing of the HPLC method is necessary.

TLC-Method

The corresponding information on the TLC method is more limited. It is expected that its precision will not be as good as that of the HPLC method since it cannot be as extensively automated, and it may sometimes be difficult to detect the spots. It is, however, very cheap in equipment and man-hours and would be an excellent screening method. Further ring-testing would be necessary to complete the validation of this method.

TABLE 2

Single-laboratory comparison of log P values determined by Shake-Flask and HPLC methods

Compound	Shake-Flask method			HPLC method		
	n(1)	mean	range	n(1)	mean	range
2,4-Difluoroaniline	4	1.54	1.49-1.59	3 3	1.26 1.56	1.21-1.27 1.51-1.58(2)
3-Chloro-4-fluoroaniline	12	2.06	1.85-2.23	3 3	1.57 1.94	1.53-1.60 1.90-1.96(2)
1,3,5-Cycloheptatriene	4	2.63	2.54-2.81	3	3.03	3.00-3.05
2,6-Dichlorobenzonitrile	4	2.65	2.62-2.67	3	2.57	2.52-2.60
Cycloocta-1,5-diene	4	3.16	3.06-3.21	3	3.94	3.88-3.99
Tetrachlorvinphos	4	3.53	3.49-3.60	3	3.53	3.47-3.57
Chlorfenvinphos	4	3.82	3.75-3.89	3	3.79	3.72-3.82
Cyclododecatriene	4	4.12(3)	3.85-4.28	3	5.77	5.69-5.80
	Coefficient of variation for P values; range 5-34%, mean 16% (4)			Coefficient of variation for P values; range 6-13%, mean 10% (4)		

(1) n = number of individual determinations.

(2) Values obtained when structurally-related reference compounds are used.

(3) Outside Shake-Flask test range.

(4) Coefficient of variation (%) = $\frac{\text{standard deviation} \times 100}{\text{mean}}$; all calculations are based on P (not log P) values.
Data for cyclododecatriene were excluded from the calculation as the log P value was outside the Shake-Flask test range.

TABLE 3

Inter-laboratory comparison of log P values determined by Shake-Flask and HPLC methods

Compound	Shake-Flask Method			HPLC method		
	n(1)	mean	range	n(2)	mean	range
Butylamine	3	0.80	0.68-0.88	-	-	-
Aniline	7	0.90	0.85-0.98	4	1.09	0.89-1.36
Phenol	11	1.62	0.62-2.20	3	1.16	1.00-1.37
4-Chloroaniline	-	-	-	3	1.64	1.40-1.83
Acetophenone	5	1.63	1.58-1.73	5	1.65	1.56-1.72
2-Nitroaniline	3	1.72	1.44-1.83	-	-	
Benzene	3	2.02	1.86-2.15	7	2.38	2.01-2.69
Indole	3	2.14	2.00-2.25	2	1.92	1.81-2.01
3,4-Dichloroaniline	3	2.62	2.12-2.78	4	2.30	2.08-2.62
Toluene	4	2.65	2.11-2.80	7	2.88	2.51-3.06
Bromobenzene	1	2.99	-	4	3.02	2.80-3.17
Naphthalene	3	3.31	3.01-3.45	3	3.35	3.20-3.43
Diphenylamine	3	3.37	3.22-3.50	-	-	-
1,3-Dichlorobenzene	4	3.52	3.38-3.62	4	3.73	3.62-3.95
Biphenyl	3	3.91	3.16-4.09	4	4.05	3.91-4.15
1,2,4-Trichlorobenzene	3	4.09	3.93-4.18	4	4.21	4.12-4.32
Phenanthrene	2	4.52(3)	4.46-4.57	3	4.31	4.10-4.45
Chlorpromazine	3	5.28(3)	5.16-5.35	-	-	-
Hexachlorobenzene	6	5.66(3)	4.13-6.27	4	6.38	6.27-6.48
DDT	6	5.90(3)	3.98-6.36	6	6.12	5.56-6.33
	Coefficient of variation for P values; range 9-95%, mean 41% (4)			Coefficient of variation for P values; range 16-72%, mean 41% (4)		

(1) n = number of individual determinations; data taken from Pomona College Medicinal Chemistry Project (1982).

(2) n = number of individual determinations; data taken from Hilgeheuser et al. (1981); Könemann et al. (1979), McDuffie (1981); McCall (1979); Nahum and Horvath (1980); Renberg et al.(1980); Veith et al.(1980) and Eadsforth (unpublished).

(3) Outside Shake-Flask test range.

(4) Coefficient of variation (%)= $\frac{\text{standard deviation} \times 100}{\text{mean}}$;all calculations based on P (not log P) values.

Data for phenanthrene, chlorpromazine, hexachlorobenzene and DDT were excluded from the calculation as the log P values were outside the Shake-Flask test range.

D. SUMMARY AND CONCLUSIONS

Reverse-phase HPLC and TLC methods have been widely used to derive the n-octanol/water partition coefficients of organic compounds over a log P range of 0 to 6 for HPLC, and 0 to 12 for TLC. Their usefulness has been demonstrated by the existence of a linear correlation between log P values (n-octanol/water) and both the logarithms of the capacity factor (k) obtained by HPLC and the R_M values derived from TLC. These methods, and the traditional Shake-Flask method, are not applicable to certain types of compound, i.e. surface-active and organometallic compounds, and partly- or fully-ionized acids and bases.

For sufficiently-soluble non-polar substances the chromatographic methods normally give results for log P which are within $\pm$ 1 log unit of the value determined by the Shake-Flask method. The accuracy can be further increased if structurally-related reference compounds are used for calibration.

The reverse-phase chromatographic methods have a number of advantages over the Shake-Flask method. Quantitative analytical methods are not required and only the determination of elution times or R_F values is necessary. If standard reference compounds are available and experimental conditions can be standardised, the chromatographic methods are generally quicker and cheaper than the Shake-Flask method. The HPLC method has the advantage over the TLC method that the compounds can be detected more easily. Also, if solvent mixing, solute injection and data processing can be automated, the precision of the retention data measured by HPLC can be much improved.

The calculation method based on fragmental constants may be of considerable value for compounds whose log P is at the extremes of, or outside of, the normal measurable P range. In these cases the calculated values may be more trustworthy than those obtained by experiment.

E. APPENDIX

1. Calculation of Partition Coefficients from Fragmental Constants.

It was discovered by Hansch that partition coefficients have an additive-constitutive character. By simple summation of the lipophilicity of molecular fragments it is possible to calculate the log P of a complete molecule.

The original hydrophobic substituent constant, π, is defined as

$$\pi = \log P_{PhX} - \log P_{PhH}$$

where P_{PhX} is the partition coefficient of an aromatic derivative and P_{PhH} that of the parent compound (e.g. $\pi_{Cl} = \log P_{C\ H\ Cl} - \log P_{C\ H} = 2.84 - 2.13 = 0.71$). According to the definition, the π-method is applicable only for aromatic substitution. π-values for a large number of substituents have been tabulated (Hansch and Leo, 1979; Hansch et al.,1973) and are used for the calculation of log P for aromatic molecules or substructures.

Rekker (1977), and later Hansch and Leo (1979), developed a fragmental method aimed at being more universal than the π-method. The two methods differ in the manner in which the hydrophobic increments of each fragment are derived and in their ease of application. As shown in a critical review by Mayer et al.(1982), both methods yield roughly the same result. In our opinion, Rekker's approach is more straightforward to use "by hand" and needs fewer rules. Leo's method is already commercially available on computer programmes (e.g. MACCS from Molecular Design Ltd; see reference).

The calculation of log P is based on the general equation

$$\log P = \Sigma f_i + \Sigma (\text{interactions})$$

where f_i are the hydrophobic constants of the various fragments making up a given molecule. Table 4 shows fragment values, most of them taken from Rekker (1977). For most molecules a few simple rules suffice for the corrections necessary to account for intramolecular interactions. These correction terms, called "proximity effects" by Rekker, are listed in the lower right hand corner of Table 4. One kind of correction, leading to higher log P values, is

necessary for structures in which two polar (N,O,S,P,halogen) atoms are separated by no (pe^0), one (pe^I) or two (pe^{II}) saturated C-atoms. Another important correction, leading to a higher lipophilicity, is for the presence of intramolecular hydrogen bonds. To estimate the possibility of their occurrence, an inspection of the 2-dimensional structural formula is usually sufficient, although in some cases the construction of a 3-dimensional molecular model is advisable.

There are no explicit rules for corrections necessary for the parallel stacking of aliphatic/aromatic structural elements. A rule of thumb is to reduce log P by 1 unit for those chains of more than 5 carbons atoms which can give rise to molecular hydrophobic interactions.

Finally, the effects of charge have to be accounted for. For permanently charged moieties, such as N^+_{quat} or SO_3^{--}, 5 log P units must be subtracted. For partial dissociation of weak acids and bases, log P is reduced by roughly $[pH-pK]$, but by not more than 5 log P units.

Rekker's scheme as described above suffices to calculate the log P values of most relatively simple molecules with an accuracy of $\pm$ 0.5 log P units. Increased accuracy can be achieved by applying further correction terms as detailed by Hansch and Leo (1979), Rekker (1977),and Rekker and de Kort, (1979). Greatly increased reliability for more complex molecules is obtained if the calculation can be started from the log P of large substructures of the molecule under investigation. These substructure log P values can be found in, or derived from, the Pomona tabulation (1982), the book of Hansch and Leo (1979) or from experimental values.

TABLE 4
LIPOPHILIC FRAGMENTAL CONSTANTS (Rekker)

Fragment	Aliphat.	Aromat.
H		
C		0.182
CH		0.150
CH2		0.337
CH3		0.519
C6H5		0.701
C6H4		1.840
C6H3		1.658
C6H2		1.476
CH2=CH		1.294
CH=C		0.856
COOH		0.730
COH	-0.938	
OCH3	-1.172	-0.071
OCH2COOH	-0.894	-0.305
CONH2	-1.160	-0.262
CONH	-1.975	-0.582
CONHNH	-2.446	-1.108
OOCNH2	-3.290	-1.579
OOCNH	-1.453	-2.134
NHOC	-1.924	-0.875
NHCOO	-2.446	-1.346
CF3	-1.924	-1.290
CF2	0.750	-0.768
CCl3	0.360	1.328
CCl2	2.060	
CBr3	1.136	
CN	2.636	
	-1.041	-0.174
COO^-	-1.251	
COO^-	-4.998	-0.384
CO	-1.643	-0.131
CON	-2.917	-0.776
NCS	0.524	-2.050
SCN	-0.348	1.391

Fragment	Hetero	Aliphat.	Aromat.
OH		-1.470	-0.314
NH2		-1.420	-0.842
NH	-0.60	-1.814	-0.947
SO2NH2			-1.468
SO2NH			-1.939
SH		0.000	0.62
F		-0.476	0.391
Cl		0.057	0.924
Br		0.249	1.116
I		0.570	1.437
N	-0.98	-2.085	-0.929
NO2		-0.920	-0.053
O	0.10	-1.595	-0.439
NOOH			-2.410
S	0.44	-0.51	0.11
S-S		0.37	
SO		-2.75	-2.05
SO2			-1.87
Imidazolyl			
Pyrrolyl		-0.081	
Pyridinyl		0.610	
Naphthalenyl		0.520	
Indolyl		3.113	
Acridinyl		1.884	
Quinolinyl		3.066	
Benzimidazolyl		1.794	
Uracilyl		1.193	
Barbituryl		-1.314	
		-1.516	
Proximity effects:			
pe	(-N-O-, -N-N-)	1.65	
pe'	(-N-C-N-, -N-C-O-, -O-C=O)	0.867	
pe''	(-N-C-C-O-, N-C-C=O)	0.578	
H-Bond		0.6	

F. BIBLIOGRAPHY

- Brown, D.S. and Flagg, E.W. (1981). Empirical prediction of organic pollutant sorption in natural sediments. J. Environ. Qual., 10(3), 382.

- Bruggeman, W.A., Van der Steen, J. and Hutzinger, O.(1982). Reversed-phase thin-layer chromatography of polynuclear aromatic hydrocarbons and chlorinated biphenyls. Relationship with hydrophobicity as measured by aqueous solubility and n-octanol-water partition coefficient. J. Chromatogr., 238(2), 335.

- Collander, R.(1951). Partition of organic compounds between higher alcohols and water. Acta Chem. Scand.,5, 774.

- Ellgehausen, H., D'Hondt, C. and Fuerer, R.(1981). Reversed-phase chromatography as a general method for determining octane-1-ol/water partition coefficients. Pestic. Sci.,12(2), 219.

- Fujisawa, S. and Masuhara, E.(1981). Determination of partition coefficients of acrylates, methacrylates and vinyl monomers using high performance liquid chromatography (HPLC). J. Biomed. Mat. Res.,15, 787.

- Hafkenscheid, T.L. and Tomlinson, E. (1983). Correlations between alkane-water and octane-1-ol-water distribution coefficients and isocratic reversed-phase liquid chromatographic capacity factors of acids, bases and neutrals. Int. J. Pharmaceutics, in press.

- Hansch, C. and Leo, A.(1979). Substituent constants for correlation analysis in chemistry and biology. J. Wiley and Sons, New York.

- Hansch, C., Leo, A., Unger, S.H., Kim, K.H., Nikaitani, D. and Lien, E.J.(1973). "Aromatic" substituent constants for structure-activity correlations. J. Med. Chem., 16, 1207.

- Jübermann, O.(1958). Verteilen und Extrahieren. In: Methoden der Organischen Chemie (Houben Weyl), Band I/1, Allgemeine Laboratoriumspraxis, Ed., E. Müller, publ. Georg Thieme Verlag, Stuttgart, pp. 223-339.

- Könemann, H., Zelle, R., Busser, F. and Hammers, W.E.(1979). Determination

of log P_{oct} values of chloro-substituted benzenes, toluenes and anilines by high-performance liquid chromatography on ODS-silica. J. Chromatogr., 178, 559.

- Leo, A., Hansch, C. and Elkins, D. (1971). Partition coefficients and their uses. Chemical Reviews, 71(6), 525.

- Lyman, W.J., Reehl, W.F. and Rosenblatt, D.M. (1981). Handbook of Chemical Property Estimation Methods : Environmental Behaviour of Organic Compounds, section 5, McGraw Hill Book Co, New York.

- MACCS, Molecular Design Ltd, 1121 B-street, Hayward, California 94541.

- Mayer, J.H., van de Waterbeemd, H. and Testa, B.(1982). A comparison between the hydrophobic fragmental methods of Rekker and Leo. Eur. J. Med. Chem., 17, 17.

- McCall, J.M.(1975). Liquid-liquid partition coefficients by high-pressure liquid chromatography. J. Med. Chem., 18(6), 549.

- McDuffie, B.(1981). Estimation of octanol/water partition coefficients for organic pollutants using reverse-phase HPLC. Chemosphere, 10(1),73.

- Mirrlees, M.S., Moulton, S.J., Murphy, C.T. and Taylor, P.J.(1976). Direct measurement of n-octanol-water partition coefficients by high-pressure liquid chromatography. J. Med. Chem., 19, (5), 615.

- Miyake, K. and Terada, H.(1978). Preparation of a column with n-octanol-like properties for high-performance liquid chromatography. Direct measurements of partition coefficients in an n-octanol-water system. J. Chromatogr., 157, 386.

- Nahum, A. and Horvath, C.(1980). Evaluation of octanol-water partition coefficients by using high-performance liquid chromatography. J. Chromatogr., 192, 315.

- Neely, W.B., Branson, D.R. and Blau, G.E.(1974). Partition coefficient to measure bioconcentration potential of organic chemicals in fish. Environ. Sci. Technol., 8(13), 1113.

- Pomona College Medicinal Chemistry Project, (1982). Log P and Parameter Database : A tool for the quantitative prediction of bioactivity. Hansch C. and Leo A., Comtex Scientific Corporation, New York.

- Rekker, R.F.(1977). The hydrophobic fragment constant, its derivation and application. A means of characterizing membrane systems. In: Pharmacochemistry Library, Volume 1., Ed., Nauta, W.Th. and Rekker, R.F., Elsevier Scientific Publishing Co, Amsterdam.

- Rekker, R.F. and de Kort, H.(1979). The hydrophobic fragmental constant; an extension to a 1000 data point set. Eur. J. Med. Chem., 14, 479.

- Renberg, L., Sundström, G. and Sundh-Nygard, K.(1980). Partition coefficients of organic chemicals derived from reversed phase thin-layer chromatography. Evaluation of methods and application on phosphate esters, polychlorinated paraffins and some PCB-substitutes. Chemosphere, 80(9), 683.

- Unger, S.H., Cook, J.R. and Hollenberg, J.S.(1978). Simple procedure for determining octanol-aqueous partition, distribution, and ionization coefficients by reversed phase high pressure liquid chromatography. J. Pharm. Sci.,67(10), 1364.

- Veith, G.D., Austin, W.M. and Morris, R.T.(1979). A rapid method for estimating log P for organic chemicals. Water Res.,13, 43.

- Veith, G.D., Macek, K.J., Petrocelli, S.R. and Carroll, J.(1980). An evaluation of using partition coefficients and water solubility to estimate bioconcentration factors for organic chemicals in fish. Aquatic Toxicology ASTM, STP 707. Eds., J.G. Eaton, P.R. Parrish and A.C. Hendricks. American Society for Testing and Materials (1980), pp. 116-129.

G. MEMBERS OF TASK FORCE

Dr. C.V. Eadsforth	SHELL (Sittingbourne)
Dr. P. Moser	CIBA-GEIGY (Basel)

H. MEMBERS OF ECETOC SCIENTIFIC COMMITTEE

I. PURCHASE (Chairman), Director, Central Toxicity Laboratory	ICI (Alderley Park)
M. SHARRATT, (Vice-chairman), Senior Toxicologist	BP (Sunbury
J. BÄCKSTROM, Consultant Toxicologist	ASSOCIATION OF SWEDISH CHEMICAL INDUSTRIES (Stockholm)
B. BROECKER, Coordinator, Product-related Environmental Problems	HOECHST (Frankfurt)
L. CAILLARD, Industrial Toxicological and Ecotoxicological Service	RHONE POULENC (Paris)
H.O. ESSER, Head of Biochemistry, Agrochemie Division	CIBA-GEIGY (Basel)
P. GELBKE, Head, Department of Toxicology	BASF (Ludwigshafen)
U. KORALLUS, Medical Director	BAYER (Leverkusen)
R. MATTIUSSI, Responsible for Medicine and Industrial Hygiene	MONTEDISON (Milan)
H.G. NOSLER, Head, Coord. Centre for Consumer Safety and Environmental Protection	HENKEL (Dusseldorf)
J.F. NEWMAN, Consultant to ICI	(Reading)
C.L.M. POELS, Head, Envir. Toxicol. and Risk Assessment Section	SHELL (den Haag)
J.P. TASSIGNON, Counsellor, Direction Centrale Pharmacie	SOLVAY (Brussels)
W.F. TORDOIR, Head, Environmental, Product and Process Toxicology	SHELL (den Haag)
H. VERSCHUUREN, Head, Department of Toxicology	DOW CHEMICAL (Horgen)