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OECD GUIDELINE FOR THE TESTING OF CHEMICALS

Estimation of the Adsorption Coefficient (K_{oc}) on Soil and on Sewage Sludge using High Performance Liquid Chromatography (HPLC)

INTRODUCTION

1. The sorption behaviour of substances in soils or sewage sludges can be described through parameters experimentally determined by means of the OECD Test Guideline 106. An important parameter is the adsorption coefficient which is defined as the ratio between the concentration of the substance in the soil/sludge and the concentration of the substance in the aqueous phase at adsorption equilibrium. The adsorption coefficient normalised to the organic carbon content of the soil K_{oc} is a useful indicator of the binding capacity of a chemical on organic matter of soil and sewage sludge and allows comparisons to be made between different chemicals. This parameter can be estimated through correlations with the water solubility and the n-octanol/water partition coefficient (1)(2)(3)(4)(5)(6)(7).

2. The experimental method described in this Test Guideline uses HPLC for the estimation of the adsorption coefficient K_{oc} in soil and in sewage sludge (8). The estimates are of higher reliability than those from QSAR calculations (9). As an estimation method it cannot fully replace batch equilibrium experiments used in the OECD Test Guideline 106. However, the estimated K_{oc} may be useful for choosing appropriate test parameters for adsorption/desorption studies according to the Guideline 106 by calculating K_d (distribution coefficient) or K_f (Freundlich adsorption coefficient) according to the equation 3 (see paragraph 12).

PRINCIPLE OF THE TEST

3. HPLC is performed on analytical columns packed with a commercially available cyanopropyl solid phase containing lipophilic and polar moieties. A moderately polar stationary phase based on a silica matrix is used:

$-O-Si$	$-CH_2-CH_2-CH_2$	$-CN$
silica	non-polar spacer	polar moiety

The principle of the test method is similar to that of the OECD Test Guideline 117 (10). While passing through the column along with the mobile phase the test substance interacts with the stationary phase. As a result of partitioning between mobile and stationary phases the test substance is retarded. The dual-composition of the stationary phase having polar and non-polar sites allows for interaction of polar and non-polar groups of a molecule in a similar way as is the case for organic matter in soil or sewage sludge matrices. This enables the relationship between the retention time on the column and the adsorption coefficient on organic matter to be established.

4. pH has a significant influence on sorption behaviour in particular for polar substances. For agricultural soils or tanks of sewage treatment plants pH normally varies between pH 5.5 and 7.5. For ionisable substances, two tests should be performed with both ionised and non-ionised forms in appropriate buffer solutions but only in cases where at least 10 % of the test compound will be dissociated within pH 5.5 to 7.5.

5. Since only the relationship between the retention on the HPLC column and the adsorption coefficient is employed for the evaluation, no quantitative analytical method is required and only the determination of the retention time is necessary. If a suitable set of reference substances is available and standard experimental conditions can be used, the method provides a fast and efficient way to estimate the adsorption coefficient K_{oc}.

APPLICABILITY OF THE TEST

6. The HPLC method is applicable to chemical substances (unlabelled or labelled) for which an appropriate detection system (e.g. spectrophotometer, radioactivity detector) is available and which are sufficiently stable during the duration of the experiment. It may be particularly useful for chemicals which are difficult to study in other experimental systems (i.e. volatile substances; substances which are not soluble in water at a concentration which can be measured analytically; substances with a high affinity to the surface of incubation systems). The method can be used for mixtures, which give unresolved elution bands. In such a case, upper and lower limits of the log K_{oc} values of the compounds of the test mixture should be stated.

7. Impurities may sometimes cause problems for interpretation of HPLC results, but they are of minor importance as long as the test substance can analytically be clearly identified and separated from the impurities.

8. The method is validated for the substances listed in Table 1 in the Annex and was also applied to a variety of other chemicals belonging to the following chemical classes:

- aromatic amines (e.g. trifluralin, 4-chloroaniline, 3,5-dinitroaniline, 4-methylaniline, N-methylaniline, 1-naphthylamine);
- aromatic carbonic acid esters (e.g. benzoic acid methylester, 3,5-dinitrobenzoic acid ethylester);
- aromatic hydrocarbons (e.g. toluene, xylene, ethylbenzene, nitrobenzene, 1,2,3-trichlorobenzene);
- aryloxyphenoxypropionic acid esters (e.g. diclofop-methyl, fenoxaprop-ethyl, fenoxaprop-P-ethyl);
- benzimidazole and imidazole fungicides (e.g. carbendazim, fuberidazole, triazoxide);
- carbonic acid amides (e.g. 2-chlorobenzamide, N,N-dimethylbenzamide, 3,5-dinitrobenzamide N-methylbenzamide, 2-nitrobenzamide, 3-nitrobenzamide);
- chlorinated hydrocarbons (e.g. endosulfan, DDT, hexachlorobenzene, quintozene);
- organophosphorus insecticides (e.g. azinphos-methyl, disulfoton, fenamiphos, isofenphos, pyrazophos, sulprofos, triazophos);
- phenols (e.g. phenol, 2-nitrophenol, 4-nitrophenol, pentachlorophenol, 2,4,6-trichlorophenol, 1-naphtol);
- phenylurea derivatives (e.g. isoproturon, monolinuron, pencycuron);
- pigment dyestuffs (e.g. Acid Yellow 219, Basic Blue 41, Direct Red 81);
- polyaromatic hydrocarbons (e.g. acenaphtene, naphthalene);
- 1,3,5-triazine herbicides (e.g. prometryn, propazine, simazine, terbutryn);

- triazole derivatives (e.g. tebuconazole, triadimefon, tradimenol, triapenthenol).

9. The method is not applicable for substances which react either with the eluent or the stationary phase. It is also not applicable for substances that interact in a specific way with inorganic components (e.g. formation of cluster complexes with clay minerals). The method may not work for surface active substances, inorganic compounds and moderate or strong organic acids and bases. Log K_{oc} values ranging from 1.5 to 5.0 can be determined. Ionisable substances must be measured using a buffered mobile phase, but care has to be taken to avoid precipitation of buffer components or test substance.

DEFINITIONS AND UNITS

10. K_d : Distribution coefficient is defined as the ratio of equilibrium concentrations C of a dissolved test substance in a two phase system consisting of a sorbent (soil or sewage sludge) and an aqueous phase; it is a dimensionless value when concentrations in both phases are expressed on a weight/weight base. In case the concentration in the water phase is given on a weight/volume base the dimension then is $ml \cdot g^{-1}$. K_d can vary with sorbent properties and can be concentration dependent.

$$K_{\mathbf{d}} = \frac{C_{soil}}{C_{water}} \text{ or } \frac{C_{sludge}}{C_{water}} \qquad [1]$$

where:

C_{soil} = concentration of substance in soil at equilibrium ($\mu g \cdot g^{-1}$)
C_{sludge} = concentration of substance in sludge at equilibrium ($\mu g \cdot g^{-1}$)
C_{water} = concentration of substance in water at equilibrium ($\mu g \cdot g^{-1}$, $\mu g \cdot ml^{-1}$)

11. K_f : Freundlich adsorption coefficient is defined as the concentration of the test substance in soil or sewage sludge (x/m) when the equilibrium concentration C_{water} in the aqueous phase is equal to one; dimension is $\mu g.g^{-1}$ sorbent. The value can vary with sorbent properties**.**

$$\log \frac{x}{m} = \log K_f + \frac{1}{n} \cdot \log C_{water} \qquad [2]$$

where:

x/m = amount of test substance (µg) adsorbed on amount of sorbent m (g) at equilibrium
1/n = slope of sorption isotherm
C_{water} = concentration of test substance in aqueous phase at equilibrium ($\mu g \cdot ml^{-1}$)

At $C_{water} = 1$; $\log K_f = \log \frac{x}{m}$

12. K_{oc}: Distribution coefficient (K_d) or Freundlich adsorption coefficient (K_f) normalized to the organic carbon content (f_{OC}) of a sorbent; particularly for non-ionised chemicals, it is an approximate indicator for the extent of adsorption between a substance and the sorbent and allows comparisons to be made between different

chemicals. Depending on the dimensions of K_d and K_f, K_{oc} can be dimensionless or have the dimensions $ml \cdot g^{-1}$ or $\mu g \cdot g^{-1}$ organic matter.

$$K_{oc} = \frac{K_d}{f_{oc}} \text{ (dimensionless or } ml \cdot g^{-1}\text{) or } \frac{K_f}{f_{oc}} \ (\mu g \cdot g^{-1}) \qquad [3]$$

The relationship between K_{oc} and K_d is not always linear and thus K_{oc} values can vary from soil to soil but their variability is greatly reduced compared to K_d or K_f values.

13. The adsorption coefficient (K_{oc}) is deduced from the capacity factor (k') using a calibration plot of log k' versus log K_{oc} of the selected reference compounds.

$$k' = \frac{t_R - t_O}{t_O} \qquad [4]$$

where:

t_R : HPLC retention time of test and reference substance (minutes)
t_O : HPLC dead time (minutes) (see paragraph 27)

14. P_{OW} : The octanol-water partition coefficient is defined as the ratio of the concentrations of dissolved substance in octanol and water; it is a dimensionless value.

$$P_{ow} = \frac{C_{octanol}}{C_{water}} (= K_{ow}) \qquad [5]$$

QUALITY CRITERIA

Accuracy

15. Normally, the adsorption coefficient of a test substance can be estimated to within +/- 0.5 log unit of the value determined by the batch equilibrium method (see Table 1 in the Annex). Higher accuracy may be achieved if the reference substances used are structurally related to the test substance.

Repeatability

16. Determinations should be run at least in duplicate. The values of log K_{oc} derived from individual measurements should be within a range of 0.25 log unit.

Reproducibility

17. Experience gained so far in the application of the method is supportive of its validity. An investigation of the HPLC method, using 48 substances (mostly pesticides) for which reliable data on K_{oc} on soils were available gave a correlation coefficient of R = 0.95 (11) (12).

18. An inter-laboratory comparison test with 11 participating laboratories was performed to improve and validate the method (13). Results are given in Table 2 of the Annex.

INFORMATION ON THE TEST SUBSTANCE

19. The structural formula, the purity and the dissociation constant (if appropriate) should be known before using the method. Information on solubility in water and organic solvents, on octanol-water partition coefficient and on hydrolysis characteristics is useful.

REFERENCE SUBSTANCES

20. To correlate the measured HPLC-retention data of a test substance with its adsorption coefficient K_{oc}, a calibration graph of log K_{oc} versus log k' has to be established. A minimum of six reference points, at least one above and one below the expected value of the test substance should be used. The accuracy of the method will be significantly improved if reference substances that are structurally related to the test substance are used. If such data are not available, it is up to the user to select the appropriate calibration substances. A more general set of structurally heterogeneous substances should be chosen in this case. Substances and K_{oc} values which may be used are listed in the Annex in Table 1 for sewage sludge and in Table 3 for soil. The selection of other calibration substances should be justified.

DESCRIPTION OF THE METHOD

Preliminary Estimation of the Adsorption Coefficient

21. The octanol-water partition coefficient P_{ow} (=K_{ow}) and, to some extent, the water solubility can be used as indicators for the extent of adsorption, particularly for non-ionised substances, and thus may be used for preliminary range finding. A variety of useful correlations have been published for several groups of chemicals (1)(2)(3)(4)(5)(6)(7).

Apparatus

22. A liquid chromatograph fitted with a pulse-free pump and a suitable detection device is required. The use of an injection valve with an injection loop is recommended. Commercial cyanopropyl chemically bound resins on a silica base shall be used (e.g. Hypersil and Zorbax CN). A guard column of the same material may be positioned between the injection system and the analytical column. Columns from different suppliers may vary considerably in their separation efficiency. As a guidance, the following capacity factors k' should be reached: log $k' > 0.0$ for log $K_{oc} = 3.0$ and log $k' > -0.4$ for log $K_{oc} = 2.0$ when using methanol/water 55/45 % as mobile phase.

Mobile phases

23. Several mobile phases have been tested and the following two are recommended:

- methanol/water (55/45% v/v)
- methanol/0.01M citrate-buffer pH 6.0 (55/45% v/v)

HPLC grade methanol and distilled water or citrate-buffer are used to prepare the eluting solvent. The mixture is degassed before use. Isocratic elution should be employed. If methanol/water mixtures are not appropriate, other organic solvent/water mixtures may be tried, e.g. ethanol/water or acetonitrile/water mixtures. For ionisable compounds the use of buffer solution is recommended to stabilize pH. Care must be taken to avoid salt precipitation and column deterioration, which may occur with some organic phase/buffer mixtures.

24. No additives such as ion pair reagents may be used because they can affect the sorption properties of the stationary phase. Such changes of the stationary phase may be irreversible. For this reason, it is mandatory that experiments using additives are carried out on separate columns.

Solutes

25. Test and reference substances should be dissolved in the mobile phase.

PERFORMANCE OF THE TEST

Test conditions

26. The temperature during the measurements should be recorded. The use of a temperature controlled column compartment is highly recommended to guarantee constant conditions during calibration and estimation runs and measurement of the test substance.

Determination of dead time t_o

27. For the determination of the dead time t_o two different methods may be used (see also paragraph 13).

Determination of the dead time t_o by means of a homologous series

28. This procedure has proven to yield reliable and standardised t_o values. For details see OECD Test Guideline 117: Partition Coefficient (n-octanol/water), HPLC Method (10).

Determination of the dead time t_o by inert substances which are not retained by the column

29. This technique is based on the injection of solutions of formamide, urea or sodium nitrate. Measurements should be performed at least in duplicate.

Determination of the retention times t_R

30. Reference substances should be selected as described in paragraph 20. They may be injected as a mixed standard to determine their retention times, provided it has been confirmed that the retention time of each reference standard is unaffected by the presence of the other reference standards. The calibration should be

performed at regular intervals at least twice daily in order to account for unexpected changes in column performance. For best practice the calibration injections should be carried out before and after injections of the test substance to confirm retention times have not drifted. The test substances are injected separately in quantities as small as possible (to avoid column overload) and their retention times are determined.

Evaluation

31. The capacity factors k' are calculated from the dead time t_o and retention times t_R of the selected reference substances according to equation 4 (see paragraph 13). The log k' data of the reference substances are then plotted against their log K_{oc} values from batch equilibrium experiments given in Tables 1 and 3 of the Annex. Using this plot, the log k' value of a test substance is then used to determine its log K_{oc} value. If the actual results show that the log K_{oc} of the test substance is outside the calibration range the test should be repeated using different, more appropriate reference substances.

DATA AND REPORTING

32. The report must include the following information:

- identity of test and reference substances and their purity, and pK_a values if relevant;
- description of equipment and operating conditions, e.g. type and dimension of analytical (and guard) column, means of detection, mobile phase (ratio of components and pH), temperature range during measurements;
- dead time and the method used for its determination;
- quantities of test and reference substances introduced in the column;
- retention times of reference compounds used for calibration;
- details of fitted regression line (log k' *vs* log K_{oc}) and a graph of the regression line;
- average retention data and estimated d log K_{oc} value for the test compound;
- chromatograms.

LITERATURE

(1) W.J. Lyman, W.F. Reehl, D.H. Rosenblatt (ed). (1990). Handbook of *c*hemical property estimation methods, chapt. 4, McGraw-Hill, New York.

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(12) B. von Oepen, W. Kördel, W. Klein (1991). Sorption of nonpolar and polar compounds to soils: Processes, measurements and experience with the applicability of the modified OECD Guideline 106, Chemosphere, 22, 285-304.

(13) W. Kördel, G. Kotthoff, J. Müller (1995). HPLC-screening method for the determination of the adsorption coefficient on soil-results of a ring test. Chemosphere, 30(7), 1373-1384.

ANNEX

Table 1

Comparison of K_{oc} values for soils and sewage sludges, and calculated values by the HPLC method[1,2]

Substance	CAS Number	log K_{oc} sewage sludges	log K_{oc} HPLC	Δ	log K_{oc} soils	log K_{oc} HPLC	Δ
Atrazine	1912-24-9	1.66	2.14	0.48	1.81	2.20	0.39
Linuron	330-55-2	2.43	2.96	0.53	2.59	2.89	0.30
Fenthion	55-38-9	3.75	3.58	0.17	3.31	3.40	0.09
Monuron	150-68-5	1.46	2.21	0.75	1.99	2.26	0.27
Phenanthrene	85-01-8	4.35	3.72	0.63	4.09	3.52	0.57
Benzoic acid phenylester	93-99-2	3.26	3.03	0.23	2.87	2.94	0.07
Benzamide	55-21-0	1.60	1.00	0.60	1.26	1.25	0.01
4-Nitrobenzamide	619-80-7	1.52	1.49	0.03	1.93	1.66	0.27
Acetanilide	103-84-4	1.52	1.53	0.01	1.26	1.69	0.08
Aniline	62-53-3	1.74	1.47	0.27	2.07	1.64	0.43
2,5-Dichloroaniline	95-82-9	2.45	2.59	0.14	2.55	2.58	0.03

[1] W. Kördel, D. Hennecke, M. Herrmann (1997). Application of the HPLC-screening method for the determination of the adsorption coefficient on sewage sludges. Chemosphere, 35 (1/2), 121-128.

[2] W. Kördel, D. Hennecke, C. Franke (1997). Determination of the adsorption-coefficients of organic substances on sewage sludges. Chemosphere, 35 (1/2), 107–119.

Table 2

Results of a laboratory inter-comparison test (11 participating laboratories) performed to improve and validate the HPLC-method[3]

Substance	CAS Number	log K_{oc} [OECD 106]	K_{oc} [HLPC-method]	log K_{oc} [HLPC-method]
Atrazine	1912-24-9	1.81	78 ± 16	1.89
Monuron	150-68-5	1.99	100 ± 8	2.00
Triapenthenol	77608-88-3	2.37	292 ± 58	2.47
Linuron	330-55-2	2.59	465 ± 62	2.67
Fenthion	55-38-9	3.31	2062 ± 648	3.31

[3] W. Kördel, G. Kotthoff, J. Müller (1995). HPLC-screening method for the determination of the adsorption coefficient on soil - Results of a ring test. Chemosphere, 30 (7), 1373-1384.

Table 3

Recommended reference substances for the HPLC screening method based on soil adsorption data.

Reference substance	CAS Number	log K_{oc} mean values from batch equilibrium	Number of K_{oc} data	log S.D.	Source
Acetanilide	103-84-4	1.25	4	0.48	a
Phenol	108-95-2	1.32	4	0.70	a
2-Nitrobenzamide	610-15-1	1.45	3	0.90	b
N.N-dimethylbenzamide	611-74-5	1.52	2	0.45	a
4-Methylbenzamide	619-55-6	1.78	3	1 76	a
Methylbenzoate	93-58-3	1.80	4	1.08	a
Atrazine	1912-24-9	1.81	3	1.08	c
Isoproturon	34123-59-6	1.86	5	1.53	c
3-Nitrobenzamide	645-09-0	1.95	3	1.31	b
Aniline	62-53-3	2.07	4	1.73	a
3,5-Dinitrobenzamide	121-81-3	2.31	3	1.27	b
Carbendazim	10605-21-7	2.35	3	1.37	c
Triadimenol	55219-65-3	2.40	3	1.85	c
Triazoxide	72459-58-6	2.44	3	1.66	c
Triazophos	24017-47-8	2.55	3	1.78	c
Linuron	330-55-2	2.59	3	1.97	c
Naphthalene	91-20-3	2.75	4	2.20	a
Endosulfan-diol	2157-19-9	3.02	5	2.29	c
Methiocarb	2032-65-7	3.10	4	2.39	c
Acid Yellow 219	63405-85-6	3.16	4	2.83	a
1,2,3-Trichlorobenzene	87-61-6	3.16	4	1.40	a
γ-HCH	58-89-9	3.23	5	2.94	a
Fenthion	55-38-9	3.31	3	2.49	c
Direct Red 81	2610-11-9	3.43	4	2.68	a
Pyrazophos	13457-18-6	3.65	3	2.70	c
α-Endosulfan	959-98-8	4.09	5	3.74	c
Diclofop-methyl	51338-27-3	4.20	3	3.77	c
Phenanthrene	85-01-8	4.09	4	3.83	a
Basic Blue 41 (mix)	26850-47-5 12270-13-2	4.89	4	4.46	a
DDT	50-29-3	5.63	1	-	b

a: W. Kördel, J. Müller (1994). Bestimmung des Adsorptionskoeffizienten organischer Chemikalien mit der HPLC. UBA R & D Report No. 106 01 044 (1994).

b: B.V. Oepen, W. Kördel, W. Klein. (1991). Chemosphere, 22, 285-304.

c: Data provided by industry.