Section 1
Physical-Chemical properties

# Test Guideline No. 123

## Test No. 123: Partition Coefficient (1-Octanol/Water): Slow-Stirring Method

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# *OECD GUIDELINE FOR THE TESTING OF CHEMICALS*

## Partition Coefficient (1-Octanol/Water): Slow-Stirring Method

## INTRODUCTION

1. 1-octanol/water partition coefficient ($P_{OW}$) values up to a log $P_{OW}$ of 8.2 have been accurately determined by the slow-stirring method (1). Therefore it is a suitable experimental approach for the direct determination of $P_{OW}$ of highly hydrophobic substances.
2. Other guidelines describing methods for the determination of the 1-octanol/water partition coefficient ($P_{OW}$) are OECD guidelines 107 and 117 (2) (3). The former refers to the 'shake-flask' method, the latter describes the determination of the $P_{OW}$ from reversed phase HPLC-retention behavior. The 'shake-flask' method is prone to artifacts due to transfer of octanol microdroplets into the aqueous phase. With increasing values of $P_{OW}$ the presence of these droplets in the aqueous phase leads to an increasing overestimation of the concentration of the test chemical in the water. Therefore, its use is limited to substances with log $P_{OW} < 4$. The second method relies on solid data of directly determined $P_{OW}$ values to calibrate the relationship between HPLC-retention behavior and measured values of $P_{OW}$.
3. This guideline has been developed in the Netherlands. The precision of the methods in this guideline has been validated and optimized in a ring-test validation study in which 15 laboratories participated (4).

## INITIAL CONSIDERATIONS

### Significance and use

4. For inert organic substances highly significant relationships have been found between 1-octanol/water partition coefficients (POW) and their bioaccumulation in fish. Moreover, POW has been demonstrated to be correlated to fish toxicity as well as to sorption of chemicals to solids such as soils and sediments. An extensive overview of the relationships has been given in reference (5).
5. A wide variety of relationships between the 1-octanol/water partition coefficient and other substance properties of relevance to environmental toxicology and chemistry have been established. As a consequence, the 1-octanol/water partition coefficient has evolved as a key parameter in the assessment of the environmental risk of chemicals as well as in the prediction of fate of chemicals in the environment.

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**Scope**

6. The slow-stirring experiment is thought to reduce the formation of microdroplets from 1-octanol droplets in the water phase. As a consequence, overestimation of the aqueous concentration due to test chemical molecules associated to such droplets does not occur. Therefore, the slow-stirring method is particularly suitable for the determination of $P_{OW}$ for substances with expected log $P_{OW}$ values of 5 and higher, for which the shake-flask method (2) is prone to yield erroneous results.

## DEFINITION AND UNITS

7. The partition coefficient of a substance between water and a lipophilic solvent (1-octanol) characterizes the equilibrium distribution of the chemical between the two phases. The partition coefficient between water and 1-octanol ($P_{OW}$) is defined as the ratio of the equilibrium concentrations of the test chemical in 1-octanol saturated with water ($C_O$) and water saturated with 1-octanol ($C_W$).

$$P_{OW} = C_O / C_W$$

As a ratio of concentrations it is dimensionless. Most frequently it is given as the logarithm to the base 10 (log $P_{OW}$). $P_{OW}$ is temperature dependent and reported data should include the temperature of the measurement.

## PRINCIPLE OF THE METHOD

8. In order to determine the partitioning coefficient, water, 1-octanol, and the test chemical are equilibrated with each other at constant temperature. Then the concentrations of the test chemical in the two phases are determined.

9. The experimental difficulties associated with the formation of microdroplets during the shake-flask experiment can be reduced in the slow-stirring experiment proposed here. In the slow-stirring experiment, water, 1-octanol and the test chemical are equilibrated in a thermostated stirred reactor. Exchange between the phases is accelerated by stirring. The stirring introduces limited turbulence which enhances the exchange between 1-octanol and water without microdroplets being formed (1).

## APPLICABILITY OF THE TEST

10. Since the presence of substances other than the test chemical might influence the activity coefficient of the test chemical, the test chemical should be tested as a pure substance. The highest purity commercially available should be employed for the 1-octanol/water partition experiment.

11. The present method applies to pure substances that do not dissociate or associate and that do not display significant interfacial activity. It can be applied to determine the 1-octanol/water partition ratio of such substances and of mixtures. When the method is used for mixtures, the 1-octanol/water partition ratios determined are conditional and depend on the chemical composition of the mixture tested and on the electrolyte composition employed as aqueous phase. Provided additional steps are taken, the method is also applicable to dissociating or associating compounds (paragraph 12).

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12. Due to the multiple equilibria in water and 1-octanol involved in the 1-octanol/water partitioning of dissociating substances such as organic acids and phenols, organic bases, and organometallic substances, the 1-octanol/water partition ratio is a conditional constant strongly dependent on electrolyte composition (6)(7). Determination of the 1-octanol/water partition ratio therefore requires that pH and electrolyte composition be controlled in the experiment and reported. Expert judgement has to be employed in the evaluation of these partition ratios. Using the value of dissociation constant(s), suitable pH-values need to be selected, such that a partitioning ratio is determined for each ionization state. Non-complexing buffers must be used when testing organometallic compounds (7). Taking the existing knowledge on the aqueous chemistry (complexation constants, dissociation constants) into account, the experimental conditions should be chosen in such a manner that the speciation of the test chemical in the aqueous phase can be estimated. The ionic strength should be identical in all experiments by employing a background electrolyte.

13. Difficulties in the test may arise in conducting the test for substances with low water solubility or high $P_{OW}$, due to the fact that the concentrations in the water become very low such that their accurate determination is difficult. This Guideline provides guidance on how to deal with this problem.

## INFORMATION ON THE TEST CHEMICAL

14. Chemical reagents should be of analytical grade or of higher purity. The use of non-labelled test chemicals with known chemical composition and preferably at least 99% purity, or of radiolabelled test chemicals with known chemical composition and radiochemical purity, is recommended. In the case of short half-life tracers, decay corrections should be applied. In the case of radiolabelled test chemicals, a chemical specific analytical method should be employed to ensure that the measured radioactivity is directly related to the test chemical.

15. An estimate of log $P_{OW}$ may be obtained by using commercially available software for estimation of log $P_{OW}$, or by using the ratio of the solubilities in both solvents.

16. Before carrying out a slow-stirring experiment for determination of $P_{OW}$, the following information on the test chemical should be available:

    a. structural formula;

    b. suitable analytical methods for determination of the concentration of the substance in water and 1-octanol;

    c. dissociation constant(s) of ionisable substances [OECD Guideline 112] (8);

    d. aqueous solubility [OECD Guideline 105] (9);

    e. abiotic hydrolysis [OECD Guideline 111] (10)

    f. ready biodegradability [OECD Guideline 301] (11);

    g. vapour pressure [OECD Guideline 104] (12).

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## DESCRIPTION OF THE METHOD

### Equipment and apparatus

17. Standard laboratory equipment is required, in particular, the following:

- magnetic stirrers and Teflon coated magnetic stir bars are employed to stir the water phase;
- analytical instrumentation, suitable for determination of the concentration of the test chemical at the expected concentrations;
- stirring-vessel with a tap at the bottom. Dependent on the estimate of log $P_{OW}$ and the Limit of Detection (LOD) of the test compound, the use of a reaction vessel of the same geometry larger than one litre has to be considered, so that a sufficient volume of water can be obtained for chemical extraction and analysis. This will result in higher concentrations in the water extract and thus a more reliable analytical determination. A table giving estimates of the minimum volume needed, the LOD of the compound, its estimated log $P_{OW}$ and its water solubility is given in Annex 1. The table is based on the relationship between log $P_{OW}$ and the ratio between the solubilities in octanol and water, as presented by Pinsuwan et al. (13):

$$\log P_{OW} = 0.88 \log SR + 0.41$$

where SR = $S_{oct}/S_w$ (in molarity);

- and the relationship given by Lyman (14) for predicting water solubility. Water solubilities calculated with the equation given in Annex 1 must be seen as a first estimate. It should be noted that the user is free to generate an estimate of water solubility by means of any relationship that is considered to better represent the relationship between hydrophobicity and solubility. For solid compounds, inclusion of melting point in the prediction of solubility is for instance recommended. In case a modified equation is used, it should be ascertained that the equation for calculation of solubility in octanol is still valid. A schematic drawing of a glass-jacketed stirring-vessel with a volume of ca. one litre is given in Annex 2. The proportions of the vessel shown in Annex 2 have proven favorable and should be maintained when apparatus of a different size is used;
- a means for keeping the temperature constant during the slow-stirring experiment is essential.

18. Vessels should be made from inert material such that adsorption to vessel surfaces is negligible.

### Preparation of the test solutions

19. The $P_{OW}$ determination should be carried out with the highest purity 1-octanol that is commercially available (at least +99 %). Purification of 1-octanol by extraction with acid, base and water and subsequent drying is recommended. In addition, distillation can be used to purify 1-octanol. Purified 1-octanol is to be used to prepare standard solutions of the test chemicals. Water to be used in the $P_{OW}$ determination should be glass or quartz distilled, or obtained from a purification system, or HPLC-grade water may be used. Filtration through a 0.22 μm filter is required for distilled water, and blanks should be included to check that no impurities are in the concentrated extracts that may interfere with

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the test chemical. If a glass fiber filter is used, it should be cleaned by baking for at least three hours at 400 ºC.

20. Both solvents are mutually saturated prior to the experiment by equilibrating them in a sufficiently large vessel. This is accomplished by slow-stirring the two-phase system for two days.

21. An appropriate concentration of test chemical is selected and dissolved in 1-octanol (saturated with water). The 1-octanol/water partition coefficient needs to be determined in dilute solutions in 1-octanol and water. Therefore the concentration of the test chemical should not exceed 70 % of its solubility with a maximum concentration of 0.1 M in either phase (1). The 1-octanol solutions used for the experiment must be devoid of suspended solid test chemical.

22. The appropriate amount of test chemical is dissolved in 1-octanol (saturated with water). If the estimate of log $P_{OW}$ exceeds five, care has to be taken that the 1-octanol solutions used for the experiment are devoid of suspended solid test chemical. To that end, the following procedure for chemicals with an estimated value of log $P_{OW} > 5$ is followed:

    - the test chemical is dissolved in 1-octanol (saturated with water);
    - the solution is given sufficient time for the suspended solid substance to settle out. During the settling period, the concentration of the test chemical is monitored;
    - after the measured concentrations in the 1-octanol-solution have attained stable values, the stock solution is diluted with an appropriate volume of 1-octanol;
    - the concentration of the diluted stock solution is measured. If the measured concentration is consistent with the dilution, the diluted stock solution can be employed in the slow-stirring experiment.

## Extraction and analysis of samples

23. A validated analytical method should be used for the assay of test chemical. The investigators have to provide evidence that the concentrations in the water saturated 1-octanol as well as in the 1-octanol saturated water phase during the experiment are above the method limit of quantification of the analytical procedures employed. Analytical recoveries of the test chemical from the water phase and from the 1-octanol phase need to be established prior to the experiment in those cases for which extraction methods are necessary. The analytical signal needs to be corrected for blanks and care should be taken that no carry-over of analyte from one sample to another can occur.

24. Extraction of the water phase with an organic solvent and preconcentration of extract are likely to be required prior to analysis, due to rather low concentrations of hydrophobic test chemicals in the water phase. For the same reason it is necessary to reduce eventual blank concentrations. To that end, it is necessary to employ high purity solvents, preferably solvents for residue analysis. Moreover, working with carefully pre-cleaned (e.g. solvent washing or baking at elevated temperature) glassware can help to avoid cross-contamination.

25. An estimate of log $P_{OW}$ may be obtained from an estimation program or by expert judgment. If the value is higher than six then blank corrections and analyte carry-over need to be monitored closely. Similarly, if the estimate of log $P_{OW}$ exceeds six, the use of a surrogate standard for recovery correction is mandatory, so that high preconcentration factors can be reached. A number of software programs for the estimation of log $P_{OW}$ are commercially

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available[1], e.g., Clog P (15), KOWWIN (16), ProLogP (17) and ACD log P (18). Descriptions of the estimation approaches can be found in references (19-21).

26. The limits of quantification (LOQ) for determination of the test chemical in 1-octanol and water are established using accepted methods. As a rule of thumb, the method limit of quantification can be determined as the concentration in water or 1-octanol that produces a signal to noise ratio of ten. A suitable extraction and pre-concentration method should be selected and analytical recoveries should also be specified. A suitable pre-concentration factor is selected in order to obtain a signal of the required size upon analytical determination.

27. On the basis of the parameters of the analytical method and the expected concentrations, an approximate sample size required for an accurate determination of the compound concentration is determined. The use of water samples that are too small to obtain a sufficient analytical signal should be avoided. Also, the use of excessively large water samples should be avoided, since otherwise there might be too little water left for the minimum number of analyses required (n = 5). In Annex 1, the minimum sample volume is indicated as a function of the vessel volume, the LOD of the test chemical and the solubility of the test chemical.

28. Quantification of the test chemicals occurs by comparison with calibration curves of the respective compound. The concentrations in the samples analyzed must be bracketed by concentrations of standards.

29. For test compounds with a log $P_{OW}$ estimate higher than six a surrogate standard has to be spiked to the water sample prior to extraction in order to register losses occurring during extraction and pre-concentration of the water samples. For accurate recovery correction, the surrogates must have properties that are very close to, or identical with, those of the test chemical. Preferably, (stable) isotopically-labelled analogs of the substances of interest (for example, perdeuterated or $^{13}C$-labelled) are used for this purpose. If the use of labelled stable isotopes i.e., $^{13}C$ or $^{2}H$, is not possible it should be demonstrated from reliable data in the literature that the physical-chemical properties of the surrogate are very close to those of the test chemical. During liquid-liquid extraction of the water phase emulsions can form. They can be reduced by addition of salt and allowing the emulsion to settle overnight. Methods used for extracting and pre-concentrating the samples need to be reported.

30. Samples withdrawn from the 1-octanol phase may, if necessary, be diluted with a suitable solvent prior to analysis. Moreover, the use of surrogate standards for recovery correction is recommended for substances for which the recovery experiments demonstrated a high degree of variation in the recovery experiments (relative standard deviation > 10%).

31. The details of the analytical method need to be reported. This includes the method of extraction, pre-concentration and dilution factors, instrument parameters, calibration routine, calibration range, analytical recovery of the test chemical from water, addition of surrogate standards for recovery correction, blank values, detection limits and limits of quantification.

[1] This information is only given for the convenience of users. Other equivalent computer programmes may be used if they can be shown to produce the same results.

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## Performance of the Test

*Optimal 1-octanol/water volume ratios*

32. When choosing the water and 1-octanol volumes, the LOQ in 1-octanol and water, the pre-concentration factors applied to the water samples, the volumes sampled in 1-octanol and water, and the expected concentrations should be considered. For experimental reasons, the volume of 1-octanol in the slow-stirring system should be chosen such that the 1-octanol layer is sufficiently thick (>0.5 cm) in order to allow for sampling of the 1-octanol phase without disturbing it.

33. Typical phase ratios used for the determinations of compounds with log $P_{OW}$ of 4.5 and higher are 20 to 50 ml of 1-octanol and 950 to 980 ml of water in a one litre vessel.

*Test conditions*

34. During the test the reaction vessel is thermostated to reduce temperature variation to below 1 °C. The assay should be performed at 25 °C.

35. The experimental system should be protected from daylight by either performing the experiment in a dark room or by covering the reaction vessel with aluminum foil.

36. The experiment should be performed in a dust-free (as far as possible) environment.

37. The 1-octanol-water system is stirred until equilibrium is attained. In a pilot experiment the length of the equilibration period is assessed by performing a slow-stirring experiment and sampling water and 1-octanol periodically. The sampling time points should be interspersed by a minimum period of five hours.

38. Each $P_{OW}$ determination has to be performed employing at least three independent slow-stirring experiments.

*Determination of the equilibration time*

39. It is assumed that the equilibrium is achieved when a regression of the 1-octanol/water concentration ratio against time over a time span of four time points yields a slope that is not significantly different from zero at a p-level of 0.05. The minimum equilibration time is one day before sampling can be started. As a rule of thumb, sampling of substances with a log $P_{OW}$ estimate of less than five can take place during days two and three. The equilibration might have to be extended for more hydrophobic compounds. For a compound with log $P_{OW}$ of 8.23 (decachlorobiphenyl) 144 hours were sufficient for equilibration. Equilibrium is assessed by means of repeated sampling of a single vessel.

*Starting the experiment*

40. At the start of the experiment the reaction vessel is filled with 1-octanol-saturated water. Sufficient time should be allowed to reach the theremostated temperature.

41. The desired amount of test chemical (dissolved in the required volume of 1-octanol saturated with water) is carefully added to the reaction vessel. This is a crucial step in the experiment, since turbulent mixing of the two phases has to be avoided. To that end, the 1-octanol phase can be pipetted slowly against the wall of the experimental vessel, close to the water surface. It will subsequently flow along the glass wall and form a film above the water phase. The decantation of 1-octanol directly into the flask should always be avoided; drops of 1-octanol should not be allowed to fall directly into the water.

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42. After starting the stirring, the stirring rate should be increased slowly. If the stirring motors cannot be appropriately adjusted the use of a transformer should be considered. The stirring rate should be adjusted so that a vortex at the interface between water and 1-octanol of 0.5 to maximally 2.5 cm depth is created. The stirring rate should be reduced if the vortex depth of 2.5 cm is exceeded; otherwise microdroplets may be formed from 1-octanol droplets in the water phase, leading to an overestimation of the concentration of the test chemical in the water. The maximum stirring rate of 2.5 cm is recommended on the basis of the findings in the ring-test validation study (4). It is a compromise between achieving a rapid rate of equilibration, while limiting the formation of 1-octanol microdroplets.

*Sampling and Sample Treatment*

43. The stirrer should be turned off prior to sampling and the liquids should be allowed to stop moving. After sampling is completed, the stirrer is started again slowly, as described above, and then the stirring rate is increased gradually.

44. The water phase is sampled from a stopcock at the bottom of the reaction vessel. Always discard the dead volume of water contained in the taps (approximately 5 ml in the vessel shown in the Annex 2). The water in the taps is not stirred and therefore not in equilibrium with the bulk. Note the volume of the water samples, and make sure that the amount of test chemical present in the discarded water is taken into account when setting up a mass balance. Evaporative losses should be minimized by allowing the water to flow quiescently into the separatory funnel, such that there is no disturbance of the water/1-octanol layer.

45. 1-Octanol samples are obtained by withdrawing a small aliquot (ca. 100 µl) from the 1-octanol layer with a 100 microliter all glass-metal syringes. Care should be taken not to disturb the boundary. The volume of the sampled liquid is recorded. A small aliquot is sufficient, since the 1-octanol sample will be diluted.

*46.* Unnecessary sample transfer steps should be avoided. To that end the sample volume should be determined gravimetrically. In case of water samples this can be achieved by collecting the water sample in a separatory funnel that contains already the required volume of solvent.

## DATA AND REPORTING

47. According to the present guideline, $P_{OW}$ is determined by performing three slow-stirring experiments (three experimental units) with the compound under investigation employing identical conditions. The regression used to demonstrate attainment of equilibrium should be based on the results of at least four determinations of $C_O$ / $C_W$ at consecutive time points. This allows for calculating variance as a measure of the uncertainty of the average value obtained by each experimental unit.

48. The $P_{OW}$ can be characterized by the variance in the data obtained for each experimental unit. This information is employed to calculate the $P_{OW}$ as the weighted average of the results of the individual experimental units. To do so, the inverse of the variance of the results of the experimental units is employed as weight. As a result, data with a large variation (expressed as the variance) and thus with lower reliability have less influence on the result than data with a low variance.

49. Analogously, the weighted standard deviation is calculated. It characterizes the repeatability of the $P_{OW}$ measurement. A low value of the weighted standard deviation indicates that the $P_{OW}$ determination was very repeatable within one laboratory. The formal statistical treatment of the data is outlined below.

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### *Treatment of the results*

*Demonstration of attainment of equilibrium*

*50.* The logarithm of the ratio of the concentration of the test compound in 1-octanol and water (log ($C_O/C_w$)) is calculated for each sampling time. Achievement of chemical equilibrium is demonstrated by plotting this ratio against time. A plateau in this plot that is based on at least four consecutive time points indicates that equilibrium has been attained, and that the compound is truly dissolved in 1-octanol. If not, the test needs to be continued until four successive time points yield a slope that is not significantly different from 0 at a p-level of 0.05, indicating that log $C_o/C_w$ is independent of time.

*Log POW-calculation*

51. The value of log $P_{OW}$ of the experimental unit is calculated as the weighted average value of log $C_o/C_w$ for the part of the curve of log $C_o/C_w$ vs. time, for which equilibrium has been demonstrated. The weighted average is calculated by weighting the data with the inverse of the variance so that the influence of the data on the final result is inversely proportional to the uncertainty in the data.

*Average log POW*

52. The average value of log $P_{OW}$ of different experimental units is calculated as the average of the results of the individual experimental units weighted with their respective variances.

The calculation is performed as follows:

$$\log P_{OW,Av} = (\Sigma w_i \times \log P_{OW,i}) \times (\Sigma w_i)^{-1}$$

where

| | | |
|---|---|---|
| $\log P_{OW,i}$ | = | the log $P_{OW}$ value of the individual experimental unit i; |
| $\log P_{OW,Av}$ | = | the weighted average value of the individual log $P_{OW}$ determinations; |
| $w_i$ | = | the statistical weight assigned to the log $P_{OW}$ value of the experimental unit i. |

The reciprocal of the variance of log $P_{OW,i}$ is employed as $w_i$ ($w_i = \text{var}(\log P_{OW,i})^{-1}$).

53. The error of the average of log $P_{OW}$ is estimated as the repeatability of log $C_o/C_w$ determined during the equilibrium phase in the individual experimental units. It is expressed as the weighted standard deviation of log $P_{OW,Av}$ ($\sigma_{\log Pow,Av}$) which in turn is a measure of the error associated with log $P_{OW,Av}$. The weighted standard deviation can be computed from the weighted variance ($\text{var}_{\log Pow,Av}$) as follows:

$$\text{var}_{\log Pow,Av} = (\Sigma w_i \times (\log P_{OW,i} - \log P_{OW,Av})^2) \times (\Sigma w_i \text{ x } (n-1))^{-1}$$
$$\sigma_{\log Pow,Av} = (\text{var}_{\log Pow,Av})^{0.5}$$

The symbol n stands for the number of experimental units.

### *Test Report*

54. The test report should include the following information:

*Test chemical:*

- common name, chemical name, CAS number, structural formula (indicating position of label when radiolabelled substance is used) and relevant physical-chemical properties (see paragraph 17);

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- purity (impurities) of test chemical;
- label purity of labelled chemicals and molar activity (where appropriate);
- the preliminary estimate of log $P_{ow}$, as well as the method used to derive the value.

*Test conditions:*

- dates of the performance of the studies;
- temperature during the experiment;
- volumes of 1-octanol and water at the beginning of the test;
- volumes of withdrawn 1-octanol and water samples;
- volumes of 1-octanol and water remaining in the test vessels;
- description of the test vessels and stirring conditions (geometry of the stirring bar and of the test vessel, vortex height in mm, and when available: stirring rate) used;
- analytical methods used to determine the test chemical and the method limit of quantification;
- sampling times;
- the aqueous phase pH and the buffers used, when pH is adjusted for ionizable molecules;
- number of replicates.

*Results:*

- repeatability and sensitivity of the analytical methods used;
- determined concentrations of the test chemical in 1-octanol and water as a function of time;
- demonstration of mass balance;
- temperature and standard deviation or the range of temperature during the experiment;
- the regression of concentration ratio against time;
- the average value log $P_{ow,Av}$ and its standard error;
- discussion and interpretation of the results;
- examples of raw data figures of representative analysis (all raw data have to be stored in accordance with GLP standards), including recoveries of surrogates, and the number of levels used in the calibration (along with the criteria for the correlation coefficient of the calibration curve), and results of QA/QC;
- when available: validation report of the assay procedure (to be indicated among references).

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# Annex 1- Spreadsheet for computation of minimum volumes of water required for detection of test compounds of different log $p_{ow}$ values in aqueous phase

Assumptions:

- Maximum volume of individual aliquots = 10 % of total volume; 5 aliquots = 50% of total volume.
- Concentration of test chemicals = 0.7 × solubility in either phase. In case of lower concentrations, larger volumes would be required.
- Volume used for LOD determination = 100 ml.
- log $P_{ow}$ vs. log $S_w$ and log $P_{ow}$ vs. SR ($S_{oct}/S_w$) are reasonable representations of relationships for test compounds.

## Table A.1. Estimation of $S_w$

| log $P_{ow}$ | Equation | log $S_w$ | $S_w$ (mg/L) |
|---|---|---|---|
| 4 | (-)0.922*log $P_{ow}$ + 4.184 | 0.496 | 3.133E+00 |
| 4.5 | (-)0.922*log $P_{ow}$ + 4.184 | 0.035 | 1.084E+00 |
| 5 | (-)0.922*log $P_{ow}$ + 4.184 | -0.426 | 3.750E-01 |
| 5.5 | (-)0.922*log $P_{ow}$ + 4.184 | -0.887 | 1.297E-01 |
| 6 | (-)0.922*log $P_{ow}$ + 4.184 | -1.348 | 4.487E-02 |
| 6.5 | (-)0.922*log $P_{ow}$ + 4.184 | -1.809 | 1.552E-02 |
| 7 | (-)0.922*log $P_{ow}$ + 4.184 | -2.270 | 5.370E-03 |
| 7.5 | (-)0.922*log $P_{ow}$ + 4.184 | -2.731 | 1.858E-03 |
| 8 | (-)0.922*log $P_{ow}$ + 4.184 | -3.192 | 6.427E-04 |

## Table A.2. Estimation of $S_{oct}$

| log $P_{ow}$ | Equation | $S_{oct}$ (mg/L) |
|---|---|---|
| 4 | log $P_{ow}$ = 0.88log SR + 0.41 | 3.763E+04 |
| 4.5 | log $P_{ow}$ = 0.88log SR + 0.42 | 4.816E+04 |
| 5 | log $P_{ow}$ = 0.88log SR + 0.43 | 6.165E+04 |
| 5.5 | log $P_{ow}$ = 0.88log SR + 0.44 | 7.890E+04 |
| 6 | log $P_{ow}$ = 0.88log SR + 0.45 | 1.010E+05 |
| 6.5 | log $P_{ow}$ = 0.88log SR + 0.46 | 1.293E+05 |
| 7 | log $P_{ow}$ = 0.88log SR + 0.47 | 1.654E+05 |
| 7.5 | log $P_{ow}$ = 0.88log SR + 0.48 | 2.117E+05 |
| 8 | log $P_{ow}$ = 0.88log SR + 0.49 | 2.710E+05 |

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| Total Mass test compound (mg) | $Mass_{oct}/Mass_{water}$ | $Mass_{H2O}$ | $Conc_{H2O}$ | $Mass_{oct}$ | $Conc_{oct}$ |
|---|---|---|---|---|---|
| | | (mg) | (mg/L) | (mg) | (mg/L) |
| 1319 | 526 | 2.5017 | 2.6333 | 1 317 | 26 333 |
| 1686 | 1664 | 1.0127 | 1.0660 | 1 685 | 33 709 |
| 2158 | 5263 | 0.4099 | 0.4315 | 2 157 | 43 149 |
| 2762 | 16644 | 0.1659 | 0.1747 | 2 762 | 55 230 |
| 3535 | 52632 | 0.0672 | 0.0707 | 3 535 | 70 691 |
| 4524 | 166436 | 0.0272 | 0.0286 | 4 524 | 90 480 |
| 5790 | 526316 | 0.0110 | 0.0116 | 5 790 | 115 807 |
| 7411 | 1664357 | 0.0045 | 0.0047 | 7 411 | 148 223 |
| 9486 | 5263158 | 0.0018 | 0.0019 | 9 486 | 189 713 |

## Table A.3. Computation of volumes

| | Minimum volume required for $H_2O$ phase at each LOD concentration | | | | | |
|---|---|---|---|---|---|---|
| log $K_{ow}$ | LOD (micrograms/L)→ | 0.001 | 0.01 | 0.10 | 1.00 | 10 |
| 4 | | 0.04 | 0.38 | 3.80 | 38 | 380 |
| 4.5 | | 0.09 | 0.94 | 9.38 | 94 | 938 |
| 5 | | 0.23 | 2.32 | 23.18 | 232 | 2 318 |
| 5.5 | | 0.57 | 5.73 | 57.26 | 573 | 5 726 |
| 6 | | 1.41 | 14.15 | 141 | 1 415 | 14 146 |
| 6.5 | | 3.50 | 34.95 | 350 | 3 495 | 34 950 |
| 7 | | 8.64 | 86.35 | 864 | 8 635 | 86 351 |
| 7.5 | | 21.33 | 213 | 2 133 | 21 335 | 213 346 |
| 8 | | 52.71 | 527 | 5 271 | 52 711 | 527 111 |
| Volume used for LOD (L) | 0.1 | | | | | |

### Key to Computations

| |
|---|
| Represents <10% of total volume of aqueous phase, 1 liter equilibration vessel. |
| Represents <10% of total volume of aqueous phase, 2 liter equilibration vessel. |
| Represents <10% of total volume of aqueous phase, 5 liter equilibration vessel. |
| Represents <10% of total volume of aqueous phase, 10 liter equilibration vessel. |
| Exceeds 10% of even the 10 liter equilibration vessel. |

## Table A.4. Overview of volumes required, as a function of water solubility and Log Pow

| | | Minimum volume required for $H_2O$ phase at each LOD concentration (ml) | | | | | |
|---|---|---|---|---|---|---|---|
| log $P_{ow}$ | $S_w$ (mg/L) | LOD (micrograms/L)→ | 0.001 | 0.01 | 0.10 | 1.00 | 10 |
| 4 | 10 | | 0.01 | 0.12 | 1.19 | 11.90 | 118.99 |
| | 5 | | 0.02 | 0.24 | 2.38 | 23.80 | 237.97 |
| | 3 | | 0.04 | 0.40 | 3.97 | 39.66 | 396.62 |
| | 1 | | 0.12 | 1.19 | 11.90 | 118.99 | 1189.86 |
| 4.5 | 5 | | 0.02 | 0.20 | 2.03 | 20.34 | 203.37 |
| | 2 | | 0.05 | 0.51 | 5.08 | 50.84 | 508.42 |
| | 1 | | 0.10 | 1.02 | 10.17 | 101.68 | 1016.83 |
| | 0.5 | | 0.20 | 2.03 | 20.34 | 203.37 | 2033.67 |

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| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 5 | 1 | | 0.09 | 0.87 | 8.69 | 86.90 | 869.01 |
| | 0.5 | | 0.17 | 1.74 | 17.38 | 173.80 | 1738.02 |
| | 0.375 | | 0.23 | 2.32 | 23.18 | 231.75 | 2317.53 |
| | 0.2 | | 0.43 | 4.35 | 43.45 | 434.51 | 4345.05 |
| 5.5 | 0.4 | | 0.19 | 1.86 | 18.57 | 185.68 | 1856.79 |
| | 0.2 | | 0.37 | 3.71 | 37.14 | 371.36 | 3713.59 |
| | 0.1 | | 0.74 | 7.43 | 74.27 | 742.72 | 7427.17 |
| | 0.05 | | 1.49 | 14.85 | 148.54 | 1485.43 | 14854.35 |
| 6 | 0.1 | | 0.63 | 6.35 | 63.48 | 634.80 | 6347.95 |
| | 0.05 | | 1.27 | 12.70 | 126.96 | 1269.59 | 12695.91 |
| | 0.025 | | 2.54 | 25.39 | 253.92 | 2539.18 | 25391.82 |
| | 0.0125 | | 5.08 | 50.78 | 507.84 | 5078.36 | 50783.64 |
| 6.5 | 0.025 | | 2.17 | 21.70 | 217.02 | 2170.25 | 21702.46 |
| | 0.0125 | | 4.34 | 43.40 | 434.05 | 4340.49 | 43404.93 |
| | 0.006 | | 9.04 | 90.43 | 904.27 | 9042.69 | 90426.93 |
| | 0.003 | | 18.09 | 180.85 | 1808.54 | 18085.39 | 180853.86 |
| 7 | 0.006 | | 7.73 | 77.29 | 772.89 | 7728.85 | 77288.50 |
| | 0.003 | | 15.46 | 154.58 | 1545.77 | 15457.70 | 154577.01 |
| | 0.0015 | | 23.19 | 231.87 | 2318.66 | 23186.55 | 231865.51 |
| | 0.001 | | 46.37 | 463.73 | 4637.31 | 46373.10 | 463731.03 |
| 7.5 | 0.002 | | 19.82 | 198.18 | 1981.77 | 19817.73 | 198177.33 |
| | 0.001 | | 39.64 | 396.35 | 3963.55 | 39635.47 | 396354.66 |
| | 0.0005 | | 79.27 | 792.71 | 7927.09 | 79270.93 | 792709.32 |
| | 0.00025 | | 158.54 | 1585.42 | 15854.19 | 158541.86 | 1585418.63 |
| 8 | 0.001 | | 33.88 | 338.77 | 3387.68 | 33876.77 | 338767.72 |
| | 0.0005 | | 67.75 | 677.54 | 6775.35 | 67753.54 | 677535.44 |
| | 0.00025 | | 135.51 | 1355.07 | 13550.71 | 135507.09 | 1355070.89 |
| | 0.000125 | | 271.01 | 2710.14 | 27101.42 | 271014.18 | 2710141.77 |
| **Volume used for LOD (L)** | | 0.1 | | | | | |

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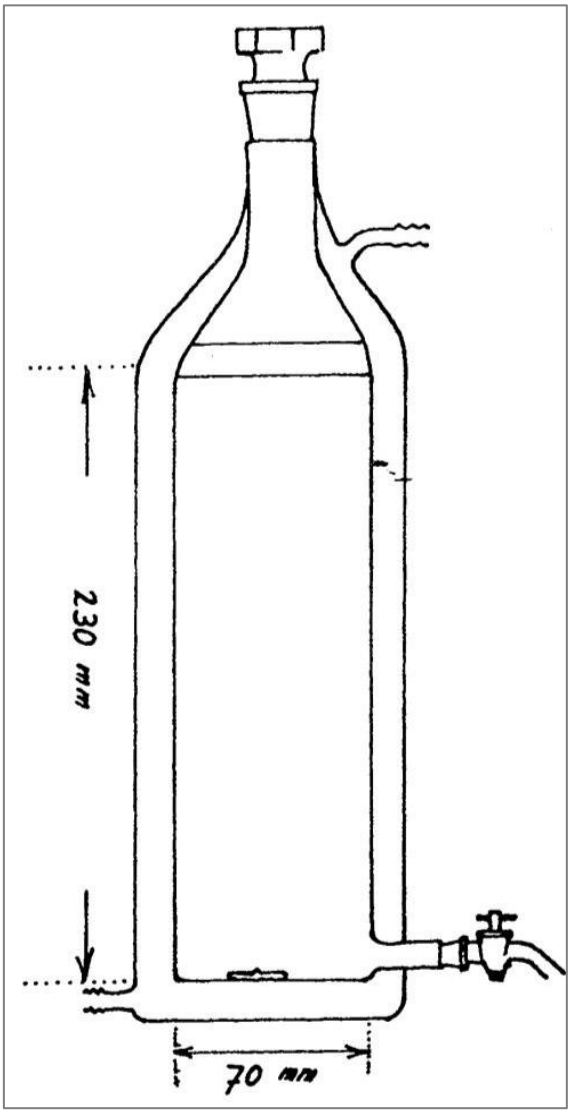
## Annex 2

**Figure 1: An example of glass-jacketed test vessel for the slow-stirring experiment for determination of $P_{OW}$**

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