Estimating the octanol-water partition coefficient for chemical substances:

Feasibility of extending the log KOW range of OECD test guideline 117

Chief Scientist's Group report

January 2025

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Dr Robert Bradburne
Chief Scientist

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Executive summary

Validated guideline studies for determining the n-octanol:water partition coefficient (K_{ow}) include shake-flask, slow-stirring and high-performance liquid chromatography (HPLC) methods. The method used depends on the type of substance, the expected K_{ow} value, and its chemical properties. The HPLC method is suitable for substances with log K_{ow} values in the range of 0 to 6, though there is potential for the method to be extended up to a log K_{ow} of 10. HPLC methods do not involve direct analysis of the test substance. The log K_{ow} is determined from the capacity factor (k) of the test substance and the linear regression line constructed from capacity factors of reference substances with known log K_{ow} values.

To identify potential reference substances that could help extend the HPLC method up to log K_{ow} values of 10, a review of publicly available study reports and databases was conducted. This review assessed the reliability and relevance of available data with reported log K_{ow} > 6 and identified limitations of the current HPLC method(s). Additionally, as extended range methods are currently being used in practice, a questionnaire was sent to three Contract Research Organisations (CROs) to gather further information about their use.

During the initial review of the experimental study and database records, and from the responses to the CRO questionnaire, several important limitations were identified. Specifically, there is currently a lack of validated reference substances with log K_{ow} values > 6. This hinders the ability to perform structural similarity assessments (as recommended by the guidelines). Additional limitations include the requirements to change the mobile phase, the potential for calibration to lose linearity, and difficulties in the identification of the log K_{ow} values for all constituents in a substance of Unknown or Variable Composition, complex reaction products or Biological materials (UVCB) or a multi-constituent substance.

As part of this assessment 437 publicly available experimental study records and greater than 4 000 database records were reviewed. Based on a pre-defined and agreed list of criteria, the available data were categorised for the purpose of this project as either ’reliable and acceptable’, ’potentially reliable with restrictions’, or ‘unreliable or unsuitable’. In total 69 substances were determined to be ‘reliable and acceptable’ and a further 22 substances were identified as already being used by CROs as reference substances for log K_{ow} > 6.

The studies for substances that were determined to be ’reliable and acceptable’, as well as the additional standards currently in use, were assessed to confirm the reported experimental log K_{OW} values from other data sources. The assessed substances were assigned a category of ‘confirmed’, ‘not fully confirmed’ or ‘could not be confirmed’ based on a defined set of criteria. Thirty-two substances with log K_{ow} values > 6 were assigned the ‘confirmed’ category and they were subsequently assigned a log K_{ow} value from a hierarchy of sources.

The guidance for the HPLC method states that reference substances should preferably be structurally related to the test substance being assessed. It has been noted that this a limitation of current studies with reported log K_{OW} > 6. Therefore, a proof-of-concept approach has been conducted using an established structural similarity measure and free-to-use software. In this proof-of-concept analysis, four test substances with expected log K_{OW} > 6 were assessed against potential additional reference substances and the current reference substances with log K_{OW} values between 4 and 6.5. The four substances (two mono-constituent and two multi-constituent substances) were selected based on their similarity (or lack of similarity) with the reference substances and to determine the effect isomers and different chain-lengths may have on the similarity score. This approach showed that theoretically structural similarity can be accounted for when determining appropriate reference substances for substances with an expected log K_{OW} > 6.

1 Introduction

The n-octanol:water partition coefficient (K_{ow}) is a mandatory information requirement under the United Kingdom (UK) Registration, Evaluation, Authorisation and restriction of CHemicals (REACH) Regulations at Annex 7 and above (i.e. substances registered at ≥ 1 tonnes per annum). It is an important property for understanding the fate and behaviour of substances in the environment and in environmental test systems. For example, it can be used in the prediction of bioaccumulation potential and other environmental hazards, and it is a parameter in long range transport modelling.

There are some circumstances where differences in relatively high log K_{OW} values may impact the conclusions of a hazard assessment or the outcome of a risk assessment. For example, the REACH guidance (ECHA, 2017a) indicates that substances with a log K_{OW} value above 4.5 are potentially bioaccumulative but a value of 10 and above is one of the indicators that the bioaccumulation criteria are unlikely to be met. Long range transport modelling results can be sensitive to the value of the log K_{OW} inputted, even when the log K_{OW} value is high.

Although K_{OW} can itself be predicted using software tools, it is generally considered more reliable to perform an experimental determination using appropriate methods. K_{ow} values are typically reported in the logarithmic form to the base ten (i.e. log K_{ow}) which is the dimensionless unit of measure and provides greater ease for the interpretation of the result.

1.1 Currently available methodologies

There are several guideline methods that have been validated for the measurement of K_{ow} values, and they can be summarised as shake-flask, for example Organisation for Economic Co-operation and Development (OECD) test guideline (TG) 107 (OECD, 1995), slow-stirring, for example OECD TG 123 (OECD, 2022a), and high-performance liquid chromatography (HPLC) methods, for example OECD TG 117 (OECD, 2022b). There are known limitations with each method and the appropriate method to be used depends on the type of substance, the expected K_{ow} value, and chemical properties such as hydrophobicity.

Shake-flask methods can be used for the assessment of log K_{ow} values in the expected range -2 to 4 (European Commission, 2008; OECD, 1995). These methods do not require the use of reference substances as the concentration of the substance of interest is measured directly in the n-octanol and water phases, ideally using substance-specific methods. The K_{ow} is calculated by division of the concentration in the n-octanol phase by the concentration determined in the water phase.

Slow-stir methods have been used to accurately determine log K_{ow} values up to 8.2, and they are particularly suitable for substances with expected log K_{OW} values of 5 and above, and therefore are suitable for highly hydrophobic substances (EU, 2014). However, the

method is not appropriate for substances with very high log K_{OW} values due to concentrations in the water phase becoming very low (for example where concentrations in the water phase are below the limit of quantification of the analytical method). Additional modifications are required for substances with log K_{OW} > 6, as detailed in the guidance (European Commission, 2014; OECD, 2022a). As with the shake-flask method no reference substances are explicitly required, except if the estimate of log K_{OW} exceeds 6 in which case a surrogate standard for recovery correction is mandatory, via substance-specific analysis of each phase, with the method employed having a sufficiently sensitive limit of detection.

The third type of experimental method for the evaluation of K_{OW} uses HPLC and example methods are detailed in Table 1.1. The US EPA guideline (OPPTS 830.7570) and OECD TG 117 are quite similar and it is noted that the EU A.24 method is a copy of OECD TG 117:

Table 1.1 Guideline HPLC methods

Guideline producer	Guideline name	Reference
Organisation for Economic Co-operation and Development (OECD)	Test Guideline No. 117 Partition Coefficient (n-octanol/water), HPLC Method	OECD (2022b)
European Commission (EC)	A.24. Partition coefficient (n-octanol/water), high performance liquid chromatography (HPLC) method	European Commission (2016)
United States Environmental Protection Agency (US EPA)	Product Properties Test Guidelines OPPTS 830.7570 Partition coefficient (n-octanol/water), estimation by liquid chromatography	US EPA (1996)

OECD TG 117 states that the method is suitable for substances with log K_{OW} values in the range of 0 to 6, though it may be extended up to 10 in exceptional circumstances (European Commission, 2016; OECD, 2022b). The extension of the method up to log K_{OW} of 10 may require changes to the mobile phase or extrapolation (OECD, 2022b) or additional reference substances. This type of method does not involve direct analysis of the test substance, with log K_{OW} determination based on the capacity factor of the test substance (determined from the retention time of the test substance on the column and the HPLC dead time) and the regression line created from the capacity factors of reference substances with known log K_{OW} values. OECD TG 117 includes a list of recommended reference substances, with log K_{OW} values ranging from 0.3 (2-butanone) up to 6.5 (DDT). The guideline recommends that structural similarity should be accounted for where possible in the selection of reference substances.

1.2 Project aims

The aims of this desk-based project were to:

- Evaluate the reliability and limitations of experimentally derived log K_{ow} values > 6, including substances that have multiple constituents or are UVCBs.
- Compile a list of substances compatible with the HPLC method with reliable log K_{ow} values > 6 that could potentially be used to extend the range of the OECD TG 117 method.
- Assess whether it is possible to account for structural similarity when selecting reference substances for the evaluation of test substances with an expected log K_{ow} > 6.

1.3 Report structure

After this brief introduction, Section 2 of this report describes the reliability of reported log K_{ow} values in the range of 6 to 10 produced from experimental studies following OECD TG 117 and OECD TG 123, and it highlights important limitations in the method for results above 6.0. Section 3 details substances that have been identified as suitable for use in OECD TG 117 studies and their potential for use as new reference substances to extend the applicability range of the method. In Section 4 a proof-of-concept approach is applied to propose a method for accounting for the requirement to assess structural similarity as part of the extended range of the test. A summary of the main findings of the project, and recommendations, are presented in Section 5.

2 Experimental log K_{OW} values in the range 6 to 10

Experimentally derived log K_{OW} values > 6 have been reported in published studies conducted according to different guidelines (e.g. OECD, 2022a, 2022b). An assessment is therefore required to determine the suitability and reliability of the reported log K_{OW} values, in particular in relation to the method used. Each available method has some limitations, and these are discussed in more detail in Section 2.5. Extended OECD TG 117 studies are already being applied by Contract Research Organisations (CROs) and so can provide invaluable information regarding the application of extended range studies in practice. Therefore, CROs are important sources of information for this project, and we have attempted to access their expertise through the use of a questionnaire.

This section of the report summarises the data sources reviewed (Section 2.1), the procedure applied for assessing the reliability and suitability of the reported results (Section 2.2). A summary of the results of this exercise are presented in Section 2.3, with feedback from the CROs and conclusions regarding the identified limitations are presented in Section 2.4 and Section 2.5 respectively.

2.1 Sources of data

Two main sources were used to obtain data on substances with experimental log K_{OW} values > 6: the OECD eChemPortal (OECD, 2023a) and the National Library of Medicine, National Institutes of Health (NIH) PubChem database (NIH, 2023).

2.1.1 OECD eChemPortal

The eChemPortal contains experimental K_{OW} data disseminated from sources such as the European Chemicals Agency's (ECHA) REACH registration portal (ECHA, 2023), Japan CHEmicals Collaborative Knowledge database (J-CHECK) (NITE, 2023), and OECD Existing Chemicals Screening Information Dataset (SIDS) Database (OECD, 2023c). It contains approximately 10 000 experimental partition coefficient studies. All experimental studies conducted following the OECD TG 117 or OECD TG 123 guidelines with reported log K_{OW} in the range 6 up to 10 were downloaded from the portal. This resulted in 372 individual OECD TG 117 and 65 individual OECD TG 123 studies for review. Some studies were included more than once in the eChemPortal extract due to being submitted for the purposes of EU REACH in a joint-submission opt-out registration; in these cases, the study was flagged as being included in the dataset on more than one occasion.

Prior to further assessment, substances without a publicly available name (this information can be claimed as confidential as part of EU REACH), EC number or CAS number were excluded from further assessment as were studies with only lower and upper bounded results reported, as they would not be suitable for further assessment. This resulted in 377 OECD TG 117 and 65 OECD TG 123 studies progressing to the reliability and applicability

assessment. The complete raw (unprocessed or formatted) datasheets accompany this report in the spreadsheet named ‘eChemPortal_OECD 117 and OECD 123 studies raw data’. The results from OECD TG 107 (shake-flask) studies were not downloaded as the upper range of this method is a log K_{OW} of 4, potentially up to 5, and therefore not in range of log K_{OW} values of interest. Any studies reporting log K_{OW} values > 6 would have deviated significantly from the OECD TG 107 guideline.

2.1.2 PubChem database

PubChem contained 22 222 records with a log K_{OW} associated at the date of download on 19th September 2023; an Microsoft Excel® export of these records was downloaded. Due to the large amount of data available, and the reported log K_{OW} not being included within the export, the records for screening were reduced to 4 141 by assessing only substances with a predicted log K_{OW} (based on the XLOGP prediction) of 4 to 20. XLOGP is quantitative structure-activity relationship (QSAR) model included in PubChem that estimates log K_{OW} using the known log K_{OW} value of a reference substance as a starting point. The difference in the log K_{OW} values of the substance and the reference substance is then estimated by an additive model. During this initial extraction of data, the applicability domain of the QSAR compared to the substance of interest was not considered.

2.1.3 Other sources

Due to the relatively large quantity of data available from the OECD eChemPortal and PubChem, it was decided that targeted literature searches were not required. Additional sources of literature were used for confirmation of reported experimental values deemed reliable (as discussed in Section 3).

2.2 Reliability and applicability assessment

2.2.1 OECD eChemPortal

All data to be assessed further from the OECD eChemPortal were disseminated by ECHA from REACH registration dossiers, and links were provided to the individual study records. The reliability and applicability of the studies following either OECD TG 117 or OECD TG 123 were assessed using the following information:

- The type of substance (mono-constituent; multi-constituent or UVCB).
- Whether an additional guideline was followed.
- The temperature used.
- The pH used.
- Other:
    - This category was used to collate any additional information of note, for example changes in the mobile phase from the guideline or individual peak results if not noted in other sections.

Where possible OECD TG 117 or OECD TG 123 repeatability and reproducibility criteria as detailed in the guideline documents were also assessed. The quality criteria from OECD TG 117 were also part of the confirmation assessment (see Section 3.1).

Due to the different methodologies of OECD TG 117 and OECD TG 123 studies, guideline specific criteria were also required. For OECD TG 117 studies the additional information requirements were:

- Whether the reference substances used were reported (and if they were listed).
- Whether the result reported was extrapolated beyond the highest reference substance capacity factor. If a limit value was also reported this was noted.
- Whether the reported result was weighted.

The additional information requirements for the OECD TG 123 studies were:

- The stirring time.
- Whether the substance was measured in the octanol phase.
- Whether the substance was measured in the aqueous phase.
- Whether a limit value was reported.

After completion of this assessment, the reliability and suitability of the study for the purpose of this project was determined. This was based on Klimisch (Klimisch *et al.*, 1997) scoring but included specific criteria (see next paragraph) and used one of the following conclusions, based on the Klimisch scoring conclusions:

- ‘Reliable and acceptable’.
- ‘Potentially reliable with restrictions’.
- ‘Unreliable or unsuitable’.

A substance was determined to be ‘reliable and acceptable’ if all the required information was reported and if extrapolation beyond the maximum log K_{OW} value of the reference standards used for the calibration was not required to determine the reported log K_{OW} value (for OECD TG 117 studies). Examples of studies deemed ‘potentially reliable with restrictions’ are where there was limited extrapolation of no greater than 0.5 log K_{OW} units (or extrapolation of a single constituent) and all other information was available or if some information was missing (e.g., pH) but generally the study was well reported. Studies were determined to be ‘unreliable’ for the purpose of this project if there was extrapolation of over 0.5 log K_{OW} units or if important information was missing.

Additionally, during this review of OECD TG 117 studies the use of reference substances with log K_{OW} values above the highest guideline value (DDT; log K_{OW} of 6.5 (OECD, 2022b)) were noted and they progressed to the potential additional reference substances assessment (Section 3). Furthermore, substances that may be potentially useful for the proof-of-concept structural similarity assessment were also noted (Section 4). The criteria for identification of these substances were either unbounded log K_{OW} values or there was insufficient information available to determine the study result to be ‘reliable and acceptable’.

2.2.2 PubChem database

The PubChem database extract does not include information as detailed as the data from the OECD eChemPortal. Therefore, each record was screened to determine whether the reported result was based on two different criteria. The two criteria were:

- Whether the reported log K_{ow} value < 4, between 4 and 6 or > 6.
- Whether the reported result was experimentally derived, estimated (either via calculation or QSAR) or was extrapolated.

Experimental results were used in preference to predicted or extrapolated results for records with more than one log K_{ow} reported, and they fall into different log K_{ow} bands.

2.3 Findings

2.3.1 OECD eChemPortal

The conclusions for each log K_{ow} record are included in the spreadsheet "Reliability and relevance assessment" that accompanies this report, with one workbook for screened OECD TG 117 study records and one for OECD TG 123 study records. A summary of the conclusions assigned to each study is presented in Table 2.1.

Table 2.1 Summary of the conclusions for each OECD TG 117 and OECD TG 123 study assessed

Guideline	Klimisch Score Assigned by Data Submitter	'Reliable and acceptable'	'Potentially reliable with restrictions'	'Unreliable or unsuitable'
OECD TG 117	1	31	83[a]	92[a]
	2	9	54[b]	39[a]
	3	0	0	2
	4	0	0	2
OECD TG 123	1	27	4	20
	2	7	0	6
	Not reported	0	0	1

[a] excludes 1 duplicate result (see spreadsheet 'reliability and relevance assessment')

[b] excludes 6 duplicate results (see spreadsheet 'reliability and relevance assessment')

From the assessment performed it was determined that 40 OECD TG 117 studies and 34 OECD TG 123 studies were 'reliable and acceptable' for the purpose of this project. The test substances from these studies were further assessed to determine whether the reported log K_{ow} value could be confirmed (Section 3). The substances that were assigned the criteria 'potentially reliable with restrictions' or 'unreliable or unsuitable' for the purpose

of this project were not further assessed unless a study deemed ‘reliable and acceptable’ was also available.

The major reasons for studies being removed at this stage were extrapolation of the results beyond the highest log K_{ow} value of the reference substances, only weighted log K_{ow} values being available (a weighted log K_{ow} value for a substance is determined based on the log K_{ow} values for the constituents of the substance and the composition of the substance), or missing information. Further details on the reasoning for scoring for each study are included in the ‘Reliability and relevance assessment’ spreadsheet. A further 22 substances were identified as already being used by CROs as reference substances with log K_{ow} values > 6 (Section 2.4); these substances were collated (including the reported log K_{ow} value) and progressed to the compatibility assessment (Section 3).

2.3.2 PubChem database

4 141 records from the PubChem database were screened as part of this project. During this screening it was noted that:

- 222 substances have measured log K_{ow} values ≥ 6.
- 363 substances have log K_{ow} values > 6 based on QSAR modelling but were reported as measured by PubChem (a further discussion on QSAR results is included in Section 3).
- 2 493 substances have measured log K_{ow} values > 6 based on extrapolation.
- 870 substances have measured or estimated log K_{ow} values in the range of ≥ 4 < 6.
- 191 substances have measured or estimated log K_{ow} values < 4.

The full screened dataset with the reported result(s) and the study record link is included in the spreadsheet “Reliability and relevance assessment” that accompanies this report. Records with a measured log K_{ow} value ≥ 6 are highlighted in green text.

PubChem collates data from various sources and is submitted directly to it by other bodies. There is often limited information regarding the source of the data (and they are often non-peer reviewed sources) and no information regarding the methods used. An in-depth assessment of the reported results as performed for the eChemPortal data is therefore not possible. Consequently, the substances identified were not taken forward directly as potential reference substances, although the results were used to aid the confirmation assessment (Section 3).

2.4 Contract research organisation questionnaires

A number of CROs already perform OECD TG 117 studies with extended ranges, as indicated by information on the ECHA dissemination portal via the OECD eChemPortal export. Therefore, a brief questionnaire was prepared comprising eight questions about different aspects of the study; the specific questions are presented in Table 2.2.

The questionnaire was sent to three CROs, one each in Germany, the Netherlands, and the United Kingdom; to maximise the potential for responses the contacted CROs were informed that their responses would be anonymised in the final report. Responses were received from two CROs and these are presented in Table 2.2.

Table 2.2 Responses to the questionnaire regarding the extension of the OECD TG 117 guideline to log Kow values > 6

Question	CRO A	CRO B
Do you extend the upper range of the OECD TG 117 study to log K_{OW} values above 6?	We can up to 7.2	Yes
Are any other guidelines also followed (e.g. EU Method A.8; EPA OPPTS 830.7570)	Yes, EPA	OECD TG 117; OECD TG 123
To what log K_{ow} can the method be extended to?	7.2 due to linearity	Log P: 8.2
What additional standards are used? (e.g. Benzo[ghi]perylene)	Benzo[ghi]perylene	Decachlorobiphenyl (PCB 209)
Is structural similarity assessed when selecting reference materials?	Depends	No, there are not enough validated reference compounds at log K_{OW} > 6
Are reference standards removed from calibration where required to improve correlation coefficient (r^2)?	Usually not	No, correlation has been sufficient; method extension was validated in GLP study with a known reference substance at log K_{OW} 6
Is a different column used when the range is extended?	No, only for different pH	No, but the eluent composition is adapted to the higher log K_{OW} range
Is the extended range method only recommended for certain types of substances?	When needed	No, the method is universally used. There are not enough reference substances yet that cover all substances

Both of the CROs that responded perform extended OECD TG 117 guideline studies, with CRO B noting that the method was validated within their laboratory according to GLP. The two laboratories do not perform the extension to the same extent, with CRO A extending the range of the method to log K_{OW} values of 7.2 and CRO B to 8.2 (Table 2.2). CRO A stated that linearity in the calibration can become an issue at log K_{OW} values above 7.2.

Neither CRO uses different columns when extending the assessment range of the OECD TG 117 method, although both use modifications of the mobile phase. CRO A adjusts the pH and CRO B changes the eluent (i.e., mobile phase) composition. Neither CRO routinely alters the calibration requirements nor excludes certain substances from being

suitable for the determination of the range extension, based on the responses they have provided (Table 2.2). One of the main differences between CRO A and CRO B was in the application of a structural similarity assessment of reference substances. CRO A accounts for structural similarity where possible, whereas CRO B does not due to the lack of validated reference substances with log K_{OW} values > 6. These conclusions highlight the issues faced with extending the range of the OECD TG 117 study and are discussed in Sections 3 and 4 of this report.

The responses to the questionnaire correlate with some of the findings from the OECD TG 117 study reliability assessment. In the study records assessed, it was reported (publicly) in some reports that structural similarity could not be assessed, some records included additional substances (for example benzo[ghi]perylene), but extrapolation was still required, and in others changes to the mobile phase were required (see the accompanying OECD TG 117 reliability scoring spreadsheet for more details and specific cases).

2.5 Identified limitations

OECD TG 117 states that the method is not suitable for the determination of K_{OW} values of strong acids and bases, metal complexes, substances that react with the eluent, or surface-active substances (OECD, 2022b). The guideline states that the method can be used for ionisable substances only in their non-ionised form (free acid or free base) by using an appropriate buffer (OECD, 2022b), though an ECETOC report concluded that because charged molecules have more complex retention behaviour than the neutral species, the OECD TG 117 guideline is not recommended as being suitable for determining the K_{OW} of ionisable compounds (ECETOC, 2014). Other studies have highlighted that the method is unsuitable for nanomaterials Rasmussen *et al.* (2019) or multifunctional ionisable substances (Schönsee and Bucheli, 2020). Additionally, Saranjampour and Armbrust (2018) highlighted, using a select group of substances, that although there is good reproducibility within methods for the experimental determination of log K_{OW}, there is less reproducibility between methods.

From the review of the study records conducted as part of this project and the responses to the questionnaires from the two CROs, further limitations have been identified. Due to the lack of recommended reference substances with log K_{OW} values > 6.5, extrapolation is required to calculate the test substance log K_{OW}. Of the OECD TG 117 studies assessed, 91 studies (out of 327) had confirmed extrapolation for at least one constituent of the substances tested, with other studies having unconfirmed extrapolation based on the publicly available information. This, coupled with the responses from the CRO questionnaire (Table 2.2), highlights the need for additional reference substances to use as calibration standards for this method to be extended to higher log K_{OW} values.

The lack of higher log K_{OW} reference substances has also been noted as an issue by both a CRO (CRO B; Table 2.2) and in some of the study records for accounting for structural similarity during the selection of the reference substances (see Section 4 for further discussion). For example, in one study record for reaction products of 2,2'-(1,3-phenylenebis(oxy))diethanol with 2-(phenoxymethyl)oxirane and 2-isocyanatoethyl

methacrylate, it is stated that the study is scored Klimisch 2 (reliable with restrictions) due to the guideline reference substances used bearing very little structural similarity to the test substance. Based on the evidence from the CROs (Table 2.2), this same conclusion could potentially be drawn for other studies assigned Klimisch 1. If substances with higher log K_{ow} values compatible with the HPLC method can be confirmed (Section 3) it would aid in the ability for structural similarity to be accounted for (Section 4).

The guideline states that at least 6 reference substances are required for calibration when using the HPLC method (OECD, 2022b). However, in at least 4 studies where the reference substances are stated, fewer than 6 reference substances were used. Additionally, in one study it is explicitly stated that 2 reference substances were removed to improve the correlation coefficient of the calibration graph, indicating a potential lack of linearity when the calibration is extended using certain reference substances. If a greater number of higher log K_{ow} reference substances become available, there is greater potential for linear calibration graphs to be produced within the minimum guideline requirement of using 6 substances.

Another identified limitation is that changes need to be made to the mobile phase when testing substances with higher log K_{ow} values, as identified from both the CRO responses and the records assessed. This is also noted within the guideline (OECD, 2022b). This change is unlikely to cause a significant issue with mono-constituent substances due to only one structure being present in the test substance. However, for multi-constituent and UVCB substances where the substance log K_{ow} may cover a large range, the changes in the mobile phase could have negative effects at the extremes of the range affecting the calibration curve and thus potentially negatively impacting the quantification of the test substance. This is compounded by the fact that isocratic operations should be performed according to the OECD TG 117 guideline, though gradient profiles of mobile phases have been used in published studies, for example Saranjampour and Armbrust (2018).

A further limitation for UVCB substances (though it is also relevant for some multi-constituent substances) is that the log K_{ow} of individual constituents could not be identified. If it is not possible to identify all constituents (for example as not all constituents have eluted to be detected), or the peaks cannot be attributed to a constituent (for example if the retention time cannot be associated with a constituent), it may lead to underestimation of K_{ow}, due to the whole substance not being assessed. Additionally, it is important that the detection technique used matches the structural characteristics of the test substance. In some cases, simplified test substances (e.g., fewer hydrocarbon chain lengths) have been used and the data read across to a more complex UVCB substance.

2.6 Summary

Experimental log K_{ow} values > 6 were obtained from two main sources for the assessment of reliability of existing data and limitations, the OECD eChemPortal and the PubChem database. A total of 437 OECD TG 123 or OECD TG 117 guideline studies were assessed, from which 40 OECD TG 117 studies and 34 OECD TG 123 studies were

determined to be ‘reliable and acceptable’ for the purpose of this project and so were assessed further (Section 3).

Of the 4 141 records screened from the PubChem database, only 222 records were identified as having measured K_{OW} values ≥ 6. Due to the limited information contained in this database, these substances were not taken forward directly as potential reference substances though they are used as part of the confirmation assessment performed in Section 3.

A number of limitations in the extension of the OECD TG 117 guideline to log K_{OW} values > 6 were identified in the targeted literature search, the CRO questionnaire responses, and the publicly available study records. The lack of reference substances with higher log K_{OW} values (> 6) has been reported by the CROs and in the reviewed study records. This also hinders structural similarity assessments (as indicated by a CRO and in the study records). Other limitations include requirements to change the mobile phase, the potential for calibration to lose linearity and difficulties (particularly for UVCB substances) in the identification of the log K_{OW} of all constituents in a substance.

3 Potential substances for reference range extension

The review of available OECD TG 117 and OECD TG 123 studies identified 74 substances that had measured log K_{OW} values > 6 that were deemed 'reliable and acceptable' for the purpose of this project, as well as 22 substances identified as already being used in OECD TG 117 studies (Section 2.3). This included 8 multi-constituent substances and 15 UVCB substances. As the OECD TG 117 method is based on linear regression from calibration based on the capacity factors of reference substances, a log K_{OW} value needs to be assigned (Section 3.2). Prior to ascribing these substances as suitable, the reported experimental result therefore needed to be confirmed (Section 3.1), a log K_{OW} value selected where the result was confirmed, and a list of potential reference substances prepared (Section 3.2).

3.1 Confirmation of experimental log K_{OW} values

Several information sources were interrogated for confirmatory purposes. The public EU REACH registration information was reviewed for all substances to determine whether any other experimental studies were available. If data were available, these were extracted and a reference to the study obtained. Additional sources checked were the PubChem database (NIH, 2023), the US EPA Comptox dashboard (US EPA, 2022), targeted grey literature searches (using Google and Google Scholar), and the SRC PHYSPROP database that is included in the KOWWIN model dataset of EPI Suite™ (US EPA, 2012). If the KOWWIN prediction from EPI Suite™ had not previously been obtained from other sources, this was also obtained using publicly available SMILES codes or substance CAS number (if it was included in the model database); note that the applicability domain was not checked during the initial data extraction. Alternative approaches to QSAR estimation are possible, such as the approach used by COSMOTHERM (Glüge and Scheringer, 2023), but are still limited by the fact that they are not based on experimentally measured data. All data, including QSAR results, were compiled and reviewed, and the suitability of the substance was assessed and assigned to one of the following categories:

- 'Confirmed'.
- 'Not fully confirmed'.
- 'Could not be confirmed'.

A substance was assigned to each category based on the factors detailed in Table 3.1:

Table 3.1 Information requirements to meet different categorisations

Category	Requirement
Confirmed	Chemically suitable for the HPLC method
	Experimental or peer-review published log K_{OW} values were within ± 0.5 log K_{OW} units of the experimental result[a]
Not fully confirmed	Only QSAR data with good agreement between the experimental value and QSAR (± 0.5 log K_{OW} units[a])
	Experimental data were available but the difference was ± 1 log K_{ow} units
Could not be confirmed	No other data available for comparison
	Greater than ± 0.5 log K_{ow} units difference in QSAR results
	Greater than ± 1 log K_{ow} units in experimental results

[a] The threshold of ± 0.5 log K_{OW} units was used as this is the reproducibility requirement of the OECD TG 117 guideline (OECD, 2022b).

The number of substances assigned to each category are presented in Table 3.2, and the complete assessment is included in the spreadsheet "K_{OW} confirmation assessment" that accompanies this report.

Table 3.2 Summary of the number of substances in each category following the confirmation assessment

Source of substance Data	'Confirmed'	'Not fully confirmed'	'Could not be confirmed'
OECD TG 117 study	5	22	13
OECD TG 123 study	10	7	15[a]
In use as additional reference substance in OECD TG 117 studies	20	0	2

[a] Excluding duplicate substances

Based on this assessment 35 potential reference standards have been identified. One substance (phenol, 4-methyl-, reaction products with dicyclopentadiene and isobutylene; CAS no.: 68610-51-5), had a 'confirmed' log K_{OW} value, but it was removed from use as a potential reference standard as it is reported to be a UVCB as the composition between batches may vary making it unsuitable. Another substance ([3R-(3α,3aβ,6α,7β,8aα)]-octahydro-3,6,8,8-tetramethyl-1H-3a,7-methanoazulen-5-yl acetate; CAS no.: 77-54-3) also fell into the 'confirmed' category though the overall weight of evidence suggests that this substance is likely to have a log K_{ow} value < 6 based on published data (log K_{OW} =

5.67; Kang *et al.* (2007)) and an Environment agency report (log K_{OW} = 5.33; Environment Agency (2010)). Bis(2-ethylhexyl)phthalate (CAS no.: 117-81-7) was present in both the OECD TG 123 study and the additional reference substances lists and therefore there were 32 potential reference standards identified.

Substances that fall into the 'not fully confirmed' (Table A 1 in the Appendix) or 'could not be confirmed' categories (Table A 2 in the Appendix) could potentially be used as reference substances in future, but currently there is insufficient information to confirm the log K_{ow} result (as discussed in Section 5).

3.2 Selection of log K_{OW} values for the additional reference standards

To assign a definitive log K_{OW} value to the substances falling into the 'confirmed' category of the compatibility assessment, a hierarchy of sources was agreed with the Environment Agency project team:

1. Data from the peer-reviewed SRC PHYSPROP database that is included in the peer-reviewed KOWWIN model dataset included in EPI Suite™ (US EPA, 2012).
2. Confirmation of the experimental value from other peer-reviewed sources, including regulatory reviews (for example risk assessment reports (RAR) published under the EU Existing Substances Regulation).
3. Average of measured values (or value) used by CROs.
4. 'Reliable and acceptable' experimental result (as assigned during the initial assessment).

These criteria were applied to give confidence to the assigned log K_{ow} value. However, it is recognised that further assessment of assigned log K_{OW} values might be required in some cases. The final substances and the assigned value (including the reasoning for the selection) are presented in Table 3.3.

Table 3.3 List of proposed reference standards, assigned log K_{ow} values and justification for value selected

Substance	CAS number	log K_{ow}	Justification
Dodecane	112-40-3	6.10	SRC PHYSPROP database
Tetradecanoic acid	544-63-8	6.11	SRC PHYSPROP database
Benzo[k]fluoranthene	207-08-9	6.11	SRC PHYSPROP database
Benzo[a]pyrene (benzo[def]chrysene)	50-32-8	6.13	SRC PHYSPROP database
(4-Methyl-4-phenylpent-1-en-2-yl)benzene	6362-80-7	6.20	'Reliable and acceptable' experimental result
3,6,9-Trioxaundecamethylene bis(2-ethylhexanoate)	18268-70-7	6.27	Arithmetic mean of OECD TG 117 values (n = 2)

Substance	CAS number	log K_{ow}	Justification
3,3,5-Trimethylcyclohexyl salicylate	118-56-9	6.31	Arithmetic mean of experimental values (n = 2)
2,2,4,4,6,6-Hexamethyl-3,5-dioxa-2,4,6-trisilaheptane	107-51-7	6.60	Value from all sources
2,2,4,4,6,6,8,8-Octamethyl-1,3,5,7,2,4,6,8-tetroxatetrasilocane	556-67-2	6.74	SRC PHYSPROP database
1-Phenylnonane	1081-77-2	7.11	SRC PHYSPROP database
2-Ethylhexyl 3,5,5-trimethylhexanoate	70969-70-9	7.16	'Reliable and acceptable' experimental result
Hexadecanoic acid	57-10-3	7.17	SRC PHYSPROP database
Benzo[ghi]perylene	191-24-2	7.20	Value currently in use by CROs
Tetradecane	629-59-4	7.20	SRC PHYSPROP database
Methyl hexadecanoate	112-39-0	7.38	SRC PHYSPROP database
1-(1-Acetyl-2,2,6,6-tetramethylpiperidin-4-yl)-3-dodecylpyrrolidine-2,5-dione	106917-31-1	7.44	'Reliable and acceptable' experimental result
Bis(2-ethylhexyl)phthalate	117-81-7	7.50	Experimental OECD TG 117 and EU RAR
1,4-Bis(2-ethylhexyl) butanedioate	2915-57-3	7.54	'Reliable and acceptable' experimental result
2,2,4,4,6,6,8,8,10,10-Decamethyl-1,3,5,7,9,2,4,6,8,10-pentoxapentasilecane	541-02-6	8.03	SRC PHYSPROP database
1-Phenylundecane	6742-54-7	8.14	SRC PHYSPROP database
2,2,4,4,6,6,8,8-Octamethyl-3,5,7-trioxa-2,4,6,8-tetrasilanonane	141-62-8	8.21	SRC PHYSPROP database
Octadecanoic acid	57-11-4	8.23	SRC PHYSPROP database
Decachlorobiphenyl	2051-24-3	8.27	SRC PHYSPROP database
Methyl octadecenoate (methyl stearate)	112-61-8	8.35	SRC PHYSPROP database
1-Phenyldodecane	123-01-3	8.65	SRC PHYSPROP database
1,2-Benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich	68515-48-0	8.80	'Reliable and acceptable' experimental result
Icosanoic acid (eicosanoic acid)	506-30-9	9.29	SRC PHYSPROP database
Methyl icosanoate (eicosanoic acid methyl ester)	1120-28-1	9.30	SRC PHYSPROP database
1-Phenyltridecane	123-02-4	9.36	SRC PHYSPROP database
2,2,4,4,6,6,8,8,10,10-Decamethyl-3,5,7,9-tetraoxa-2,4,6,8,10-pentasilaundecane	141-63-9	9.41	OECD TG 123 value, used by ECHA and Environment

Substance	CAS number	log K_{ow}	Justification
			and (ECCC and Health Canada, 2019)
1-Phenyltetradecane	1459-10-5	9.95	SRC PHYSPROP database
Methyl docosanoate (docosanoic acid methyl ester)	929-77-1	10.20	SRC PHYSPROP database

A total of 32 substances with log K_{ow} values > 6 have been identified as potential additional reference substances suitable for use in the HPLC method. The substances identified can be grouped as:

- 2 alkanes
- 4 alkylbenzenes
- 1 aromatic hydrocarbon
- 4 esters
- 4 methyl esters
- 3 polycyclic aromatic hydrocarbons (PAHs)
- 2 phthalates
- 1 polychlorobiphenyl
- 1 polyphenylalkene
- 1 salicylate
- 4 saturated fatty acids
- 5 siloxanes

There is a variety of different substance types with 8 of the 12 groups having more than one substance. However, some groups are not well represented, for example halogenated groups, in particular brominated substances.

No consideration has been given to the availability of analytical standards of sufficient purity for these substances. The expected retention times of the potential substances have not been considered within this exercise either.

3.3 Summary

The confirmation and compatibility assessment concluded that 32 substances are recommended as potential reference substances, with log K_{ow} values ranging from 6.1 to 10.2. The definitive log K_{ow} value assigned to each substance was based on a hierarchy of sources. As this project was solely desk-based, the expected retention times of the potential substances and how these substances interact in practice in the HPLC column are areas of uncertainty and highlight potential further work.

A further 30 substances were determined to be ‘not fully confirmed’ and 28 substances were assigned the ‘could not be confirmed’ category. With further experimental work, some of these substances could potentially become additional reference substances in the future if a definitive log K_{ow} value could be assigned.

4 Structural relationship between test and reference substances

OECD TG 117 states that it is preferable that reference substances should be structurally related to the substance being tested (OECD, 2022b), although it does not provide any further information regarding how this should be assessed in practice. Additionally, it has been noted by one of the CROs that responded to the questionnaire that when log K_{OW} values > 6 are expected, structural similarity cannot be performed due to the lack of reference substances. In Section 4.1, we present an example of how an assessment could be performed using readily available tools containing structural similarity profilers (OECD QSAR toolbox; OECD (2023b)) and provide the results of the structural similarity assessment of the substances that have been assigned confirmed log K_{OW} values (Table 3.3). A proof-of-concept approach has been undertaken for 4 substances that have previously been tested using OECD TG 117, but where the results were determined to be 'unreliable' or 'reliable with restrictions' (Section 4.2). A brief summary of the findings is presented in Section 4.3.

4.1 Potential approaches to assess structural similarity

Several measures have been developed to assess structural similarity, including Dice (Dice, 1945), Tanimoto (Jaccard, 1912; Tanimoto, 1958), Tversky (Tversky, 1977) and Euclidean scores (described in Willett (2014)). These measures can be applied to different molecular features of a substance and have been compiled into free-to-use software.

One of the principal development aims of the OECD QSAR toolbox was the identification of chemicals that have similar structural characteristics as the target substance of interest. The latest version (Version 4.6) was released in 2023 (OECD, 2023c) and contains the Tanimoto (Jaccard), Dice, Kulczynski-2, Ochiai (Cosine) and Yule measures to determine structural similarity. The molecular features that can be used in the assessment are atom pairs, topologic torsions, atom centred fragments, path, cycles and PubChem features; further information on each of these molecular features are included within the program. The substances of interest (i.e., in this case the substances detailed in Table 3.3) can be uploaded to the OECD QSAR Toolbox and used as source substances for the assessment the user wishes to perform for a novel substance that needs to be tested.

To test the use of the OECD QSAR Toolbox, an initial assessment for structural similarity was applied to the substances identified as potential reference standards (Table 3.3). For the structural similarity profiler to operate, both a measure and a molecular feature must be selected. This assessment was performed using the Dice coefficient similarity measure and the PubChem molecular features system:

- The Dice coefficient assesses the number of features in common to 2 different molecules relative to the average size of the total number of features present with a weighting factor applied.

- The PubChem feature system molecular description generates fragments of the substances to be assessed and compares the presence and absence of these fragments between each substance.

The profilers selected for this proof-of-concept approach are not intended to be definitive recommendations that these profilers should always be used.

The initial assessment showed that all of the substances had a structural similarity score of ≥ 50% when compared to at least 4 other potential reference standards; 22 of the substances had at least 5 other potential reference substances with similarity scores of ≥ 80% (based on the Dice method utilising the PubChem system for molecular features) (Table 4.1). The full assessment is included in the accompanying spreadsheet called "Structural similarity assessment".

Table 4.1 Summary of the structural similarity assessment (%) of the potential reference substances

Substance	CAS number	Number of substances in each similarity assessment band									
		10 - 20%	10 - 20%	20 - 30%	30 - 40%	40 - 50%	50 - 60%	60 - 70%	70 - 80%	80 - 90%	90 - 100 %
Dodecane	112-40-3	0	0	7	2	11	10	0	0	0	1
Tetradecanoic acid	544-63-8	0	1	3	11	3	3	0	3	0	7
Benzo[k]fluoranthene	207-08-9	0	5	14	0	0	1	3	0	6	2
Benzo[a]pyrene (benzo[def]chrysene)	50-32-8	0	5	14	0	0	1	3	0	6	2
(4-Methyl-4-phenylpent-1-en-2-yl)benzene	6362-80-7	0	3	2	14	0	0	3	1	3	5
3,6,9-Trioxaundecamethylene bis(2-ethylhexanoate)	18268-70-7	0	0	4	11	3	3	0	4	4	2
3,3,5-Trimethylcyclohexyl salicylate	118-56-9	0	4	2	1	11	4	6	0	2	1
2,2,4,4,6,6-Hexamethyl-3,5-dioxa-2,4,6-trisilaheptane	107-51-7	1	6	7	11	0	2	0	0	0	4
2,2,4,4,6,6,8,8-Octamethyl-1,3,5,7,2,4,6,8-tetroxatetrasilocane	556-67-2	1	7	6	11	2	0	0	0	1	3
1-Phenylnonane	1081-77-2	0	0	5	12	2	0	3	1	3	5
2-Ethylhexyl 3,5,5-trimethylhexanoate	70969-70-9	0	0	4	11	2	3	0	4	5	2
Hexadecanoic acid	57-10-3	0	1	3	11	2	4	0	3	0	7
Benzo[ghi]perylene	191-24-2	0	5	14	0	0	1	3	0	6	2
Tetradecane	629-59-4	0	0	7	2	11	10	0	0	0	1
Methyl hexadecanoate	112-39-0	0	1	3	10	2	5	0	0	3	7

Substance	CAS number	Number of substances in each similarity assessment band									
		10 - 20%	10 - 20%	20 - 30%	30 - 40%	40 - 50%	50 - 60%	60 - 70%	70 - 80%	80 - 90%	90 - 100 %
1-(1-Acetyl-2,2,6,6-tetramethylpiperidin-4-yl)-3-dodecylpyrrolidine-2,5-dione	106917-31-1	0	0	9	9	2	11	0	0	0	0
Bis(2-ethylhexyl)phthalate	117-81-7	0	4	3	0	2	11	5	4	1	1
1,4-Bis(2-ethylhexyl) butanedioate	2915-57-3	0	0	4	11	3	3	0	4	5	1
2,2,4,4,6,6,8,8,10,10-Decamethyl-1,3,5,7,9,2,4,6,8,10-pentoxapentasilecane	541-02-6	1	7	6	10	2	0	0	0	1	4
1-Phenylundecane	6742-54-7	0	0	5	12	2	0	2	2	3	5
2,2,4,4,6,6,8,8-Octamethyl-3,5,7-trioxa-2,4,6,8-tetrasilanonane	141-62-8	1	6	7	11	0	2	0	0	0	4
Octadecanoic acid	57-11-4	0	1	3	11	2	4	0	3	0	7
Decachlorobiphenyl	2051-24-3	5	8	6	0	1	2	8	1	0	0
Methyl octadecenoate (methyl stearate)	112-61-8	0	1	3	10	2	5	0	0	3	7
1-Phenyldodecane	123-01-3	0	0	5	12	2	0	2	2	3	5
1,2-Benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich	68515-48-0	0	5	2	0	5	8	4	5	1	1
Icosanoic acid (eicosanoic acid)	506-30-9	0	1	3	11	2	4	0	3	0	7
Methyl icosanoate (eicosanoic acid methyl ester)	1120-28-1	0	1	3	10	2	5	0	0	3	7
1-Phenyltridecane	123-02-4	0	0	5	12	2	0	2	2	3	5
2,2,4,4,6,6,8,8,10,10-Decamethyl-3,5,7,9-tetraoxa-2,4,6,8,10-pentasilaundecane	141-63-9	1	6	7	7	6	0	0	0	0	4
1-Phenyltetradecane	1459-10-5	0	0	5	12	2	0	2	2	3	5
Methyl docosanoate (docosanoic acid methyl ester)	929-77-1	0	1	3	10	2	5	0	0	3	7

4.2 Proof of concept assessment

It has been shown in Section 4.1 that the potential reference substances share a level of structural similarity. Assigning a definitive minimum threshold for structural similarity was not a necessary aim of this assessment (or project). Nevertheless, the same approach has been applied to 4 further potential reference substances that currently do not have 'reliable and acceptable' OECD TG 117 log K_{ow} results (Section 2):

- 1,1,5,5,5-Hexamethyl-3-phenyl-3-[(trimethylsilyl)oxy]trisiloxane (CAS number: 2116-84-9) was selected as 5 siloxanes were included in the list of potential reference substances with log K_{ow} values > 6. It is a mono-constituent substance with a reported log K_{ow} value of 8.27 from a previously conducted OECD TG 117 study (and a reported water solubility of 0.0066 mg/L (ECHA, 2018a). This substance was also assessed using the Tanimoto method to investigate differences between the Dice and Tanimoto methods.
- Oxacycloheptadecan-2-one (CAS number: 109-29-5) is a mono-constituent macrocyclic substance with a log K_{ow} value of 7.3 from an OECD TG 117 study (and a reported water solubility of 0.103 mg/L (ECHA, 2018b). It was selected because there is a limited number of (macro)cyclic substances represented.
- 5-Cyclohexadecen-1-one (CAS number: 37609-25-9) is a multi-constituent synthetic musk consisting of two isomers, with a log K_{ow} of 6.4 for each isomer in an OECD TG 117 study (and a reported water solubility of 0.65 mg/L (ECHA, 2017b). It was selected to assess the effect different isomers may have on the results of the structural similarity assessment.
- "Reaction mass of 2-(palmitoylamino)ethyl acrylate and 2-(stearoylamino)ethyl acrylate" (CAS number not applicable) is a multi-constituent substance with the two constituents differing in carbon chain lengths (C16 and C18) and has a reported weighted average log K_{ow} of 8.1, with a range of 5.7 to 8.6 (and a reported water solubility of 0.7 mg/L (ECHA, 2021). It was selected to assess the effects differing chain lengths may have on the outcomes of the structural similarity assessment.

The initial aim was to also include a UVCB substance, but this was not possible due to the lack of publicly available structural information (e.g. SMILES codes) for all constituents.

The full results of the structural similarity assessment are available in the "Structural similarity assessment" spreadsheet that accompanies this report, and a summary of the findings are presented in Table 4.2.

Table 4.2 Comparison of 4 selected substances with 32 potential reference substances

Substance assessed		Number of reference substances with structural similarity percentage of:				
		≥ 50%	≥ 60%	≥ 70%	≥ 80%	≥ 90%
1,1,5,5,5-Hexamethyl-3-phenyl-3-[(trimethylsilyl)oxy]trisiloxane		13	11	6	5	0
Oxacycloheptadecan-2-one		16	11	11	10	4
5-Cyclohexadecen-1-one	(Z)-cyclohexadec-5-enone	21	8	0	0	0
	(E)-cyclohexadec-5-enone	21	8	0	0	0
Reaction mass of 2-(palmitoyl-amino)ethyl acrylate and 2-(stearoylamino)-ethyl acrylate	2-Propenoic acid, 2-[(1-oxohexadecyl)-amino]ethyl ester	14	5	0	0	0
	2-Propenoic acid, 2-[(1-oxooctadecyl)-amino]ethyl ester	14	5	0	0	0

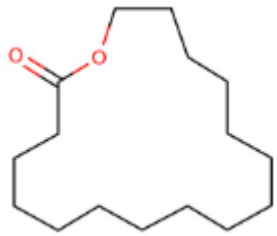
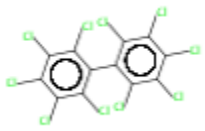
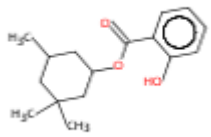
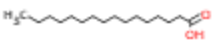
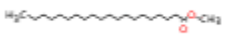
Similar to the reference substance comparison, all 4 substances have potential references with ≥ 60% structural similarity score, with oxacycloheptadecan-2-one having four reference substances with ≥ 90% structural similarity.

For both 5-cyclohexadecen-1-one and the reaction mass of 2- (palmitoylamino)ethyl acrylate and 2-(stearoylamino)ethyl acrylate, no differences were observed between the two constituents when the Dice measure with the PubChem molecular features profiler are applied.

When the Tanimoto (rather than Dice) similarity score was applied to 1,1,5,5,5-hexamethyl-3-phenyl-3-[(trimethylsilyl)oxy]trisiloxane, the similarity scores were in the range of 11 to 17% lower, with an average difference of 14.23%. However, even when the Tanimoto method is used, 5 potential reference substances had similarity scores of greater than 70%.

OECD TG 117 states that a minimum of 6 references standards should be used, with a minimum of one substance having a log K_{ow} value below that expected for the test substance, and a minimum of one above (OECD, 2022b). Therefore, each of the 4 substances that underwent the structural similarity assessment were also assessed against the reference substances recommended in the guideline with log K_{ow} values ≥ 4. All substances had at least 2 existing reference standards with a structural similarity score of > 50%, with 2 having at least 1 substance with a similarity score of > 70%. An example of the differences for different similarity scores are presented in Table 4.3 and the full results from this assessment are included in the spreadsheet titled “Structural similarity assessment” that accompanies this report.

Table 4.3 Examples of differences in structural similarity score for the substance oxacycloheptadecan-2-one

Substance (CAS number)	Structure	Structural Similarity Range[a]	Specific Structural Similarity Score
Substance assessed			
Oxacycloheptadecan-2-one (109-29-5)		not applicable	not applicable
Potential additional reference standard			
Decachlorobiphenyl (2051-24-3)		10 – 20%	16.36%
Benzo[k]fluoranthene (207-08-9)		20 – 30%	24.78%
Nonylbenzene (1081-77-2)		30 – 40%	30.11%
3,3,5-Trimethylcyclohexyl salicylate (118-56-9)		40 – 50%	45.28%
Dodecane (112-40-3)		50 – 60%	53.85%
Hexadecanoic acid (57-10-3)		80 – 90%	88.00%
Eicosanoic acid methyl ester (1120-28-1)		90 – 100%	94.59%

[a] No substance had a structural similarity score in the ranges of 0 – 10%, 60 – 70% or 70 – 80%.

Solubility in the mobile phase and compatibility with detectors should also be accounted for when assessing the suitability of reference substances for calibration, as well as the potential requirement for non-linear calibration due to the range of log K_{OW} values that need to be encompassed. As an initial assessment the water solubility of the potential

reference substances with log K_{OW} values > 6 and the proof-of-concept substances were obtained (as detailed in the 'Solubility assessment' spreadsheet that accompanies this report). Fifteen of the additional reference substances had water solubility values ≤ 0.005 mg/L and 11 more were ≤ 0.1 mg/L, with the total range being 1.13×10^{-5} mg/L to 44 mg/L. This is not surprising since substances with high log K_{OW} values are typically very hydrophobic. Information is not available to assess the solubility of these substances in organic solvents therefore, in practice, a solubility assessment in the mobile phase to be used should be performed prior to selection of the reference substance.

4.3 Summary

The proof-of-concept approach has shown that structural similarity between test substances and reference substances can be accounted for using free-to-use software. However, additional factors such as the requirement of different mobile phases, or non-linear calibration, also need to be considered.

5 Conclusions and recommendations

We have identified 32 substances with confirmed log K_{ow} values greater than or equal to 6, which could act as potential reference standards for measuring log K_{ow} values in the range of 6.1 to 10.2 using OECD TG 117. They represent a variety of chemical structures.

A definitive log K_{ow} was 'not fully confirmed' for a further 30 substances (Table A 1 in the Appendix), and the experimental log K_{ow} 'could not be confirmed' for another 28 substances (Table A 2 in the Appendix). An additional 147 substances have undergone either OECD TG 117 or 123 studies and were determined to be 'potentially reliable with restrictions'.

Using a proof-of-concept approach for 2 mono-constituent and 2 multi-constituent substances, it has been shown that publicly accessible software (OECD QSAR Toolbox) can aid the selection of reference substances with log K_{ow} values > 4. There are a number of different profilers available, and in this case only the Dice measure with the PubChem molecular features profiler has been used, except for one substance where the Dice and Tanimoto measures with the PubChem molecular features profiler were used.

As this project was purely desk-based and did not include any practical experimentation, an area for future work is an investigation of the reference standards in practice to assess the compatibility of the substances in providing reliable calibration regression lines for determination of log K_{ow} values > 6. A practical study could also investigate whether the interaction between the different reference substances affects the operating conditions of the HPLC.

Recommendations for further work on the basis of the findings of this project are:

- To perform ring testing for substances that fall into the 'not fully confirmed' or 'could not be confirmed' categories during the confirmation of experimental data assessment to increase the amount of experimental (or peer-reviewed and widely accepted) log K_{ow} values. This information could then be used to assign sufficient confidence in the reported result to be able to meet the 'confirmed' criteria. This testing may take the form of additional OECD TG 117 studies, or other guideline studies as appropriate. There is currently a lack of halogenated (in particular brominated, substances) in the potential additional reference substances, and therefore this may be a focus for further work.
- To assess how the 32 substances identified as potential reference substances interact in practice in the HPLC column and whether a correlation coefficient between the capacity factor (log k) and log K_{ow} of 0.9 can be met, as recommended by the guideline (OECD, 2022b).
- To consider the potential requirement for different mobile phases, or non-linear calibration when using the potential additional reference substances. Future work may involve utilising some of the proposed reference standards in practice to assess their overall suitability.

- To perform structural similarity assessments using other profilers may also prove useful, to assess differences between them, and to determine the most suitable method to use (which may be different for different substance types.

Additionally, based on the findings of the structural similarity assessment, and to avoid the need for setting an acceptability threshold for the degree of structural similarity between test and reference substances, and in the absence of any existing guidance on its use, it is recommended that the degree of structural similarity of each standard used to the test substance is reported. This approach could equally be applied to tests conducted within the normal applicability range of OECD TG 117.

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List of abbreviations

Term	Meaning
CAS	Chemical abstracts service
CRO	Contract research organisation
DDT	Dichlorodiphenyltrichloroethane
ECETOC	The European Centre for Ecotoxicology and Toxicology of Chemicals
ECHA	European Chemicals Agency
EC number	European Community number
EU	European Union
GLP	Good laboratory practice
HPLC	High performance liquid chromatography
J-CHECK	Japan Chemicals Collaborative Knowledge database
k	Capacity factor
K_{OW}	Octanol-water partition coefficient
K_d	Adsorption coefficient for the substance to food/particulates from water.
L	Litre
Log	Logarithmic value
mg	Milligram
NIH	National Institute of Health
NITE	National Institute of Technology and Evaluation
OECD	Organisation for Economic Co-operation and Development
PAH	Polycyclic aromatic hydrocarbon
PBT	Persistent, Bioaccumulative and Toxic
QSAR	Quantitative structure–activity relationship

REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
SIDS	Screening information dataset
SMILES	Simplified molecular-input line-entry system
TG	Test guideline
UK	United Kingdom
US EPA	United Stated Environmental Protection Agency
UVCB	Substances of unknown or variable composition, complex reaction products or biological materials.

Appendix: Substances ‘not fully confirmed’ or ‘not confirmed’

Table A 1 Substances with ‘not fully confirmed’ experimental log K_{OW} values > 6

Substance	CAS number	Method	Log K_{OW}
2,4,6-Tri-tert-butylphenol	732-26-3	OECD TG 117	7.1
(1S,3aR,9aR,9bS,11aS)-9a,11a-Dimethyl-1-(2-methyl-1,3-dioxolan-2-yl)-1,2,3,3a,6,8,9,9a,9b,10,11,11a-dodecahydrospiro[cyclopenta[a]phenanthrene-7,2'-[1,3]dioxolane]	19592-55-3	OECD TG 117	6.7
1,4-Bis[(4-methylphenyl)amino]-9,10-anthraquinone	128-80-3	OECD TG 117	6.407
1,4-Bis[(4-methylphenyl)amino]-9,10-anthraquinone	128-80-3	OECD TG 117	8.16
4-Ethoxy-2,3-difluoro-4'-propyl-1,1'-biphenyl	157248-24-3	OECD TG 117	6.3
2,4-Dichlorobenzoyl 2,4-dichlorobenzene-1-carboperoxoate	133-14-2	OECD TG 117	6
4-Bromo-4'-ethyl-2-fluoro-1,1'-biphenyl	116713-40-7	OECD TG 117	6.3
Cyclohexadecanone	2550-52-9	OECD TG 117	7.77
(3S,6E)-3,7,11-Trimethyldodeca-6,10-dienal	194934-66-2	OECD TG 117	6.21
2,4,4-Trimethylpentan-2-yl 2-ethylhexaneperoxoate	22288-43-3	OECD TG 117	6.2
1,1'-(Hydrazine-1,2-diylidenedimethylylidene)di(2-naphthol)	2387-03-3	OECD TG 117	6.269
3-Ethoxyandrosta-3,5-dien-17-one	972-46-3	OECD TG 117	6.4
1,2,4-Tributyl benzene-1,2,4-tricarboxylate	1726-23-4	OECD TG 117	6.17
2-(2-{2-[(2-Ethylhexanoyl)oxy]ethoxy}ethoxy)ethyl 2-ethylhexanoate	94-28-0	OECD TG 117	6.1
Bis(2-ethylhexyl)amine	106-20-7	OECD TG 117	7.3
{3-Fluoro-4'-pentyl-[1,1'-biphenyl]-4-yl}boronic acid	936-618-0	OECD TG 117	6

Substance	CAS number	Method	Log K_{OW}
2-Ethylhexyl benzoate	5444-75-7	OECD TG 117	6.21
1-Bromoheptadecafluorooctane	423-55-2	OECD TG 117	6.1
Butyl 4,4-bis(tert-butylperoxy)pentanoate	995-33-5	OECD TG 117	6.34
Phenol, paraalkylation products with C10-15 branched olefins (C12 rich) derived from propene oligomerization, calcium salts, sulfurized, including distillates (petroleum), hydrotreated, solvent-refined, solvent-dewaxed, or catalyc dewaxed, light or heavy paraffinic C15-C50	701-249-4	OECD TG 117	9.8
2,4,6,8-Tetramethyl-2,4,6,8-tetravinyl-1,3,5,7,2,4,6,8-tetroxatetrasilocane	2554-06-5	OECD TG 117	6.47
2-[(2-Hexyldecyl)oxy]benzamide	202483-62-3	OECD TG 117	7.38
3-(Dodecylsulfanyl)-1-[(1S,2R)-2,6,6-trimethylcyclohex-3-en-1-yl]butan-1-one	878665-13-5	OECD TG 117	9.4; 9.6; >9.2
Tris(2-ethylhexyl) benzene-1,2,4-tricarboxylate	3319-31-1	OECD TG 123	8
1,2-Benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich	68515-48-0	OECD TG 123	8.8
4-(3,4,5,6-Tetramethyloctan-2-yl)phenol	121158-58-5	OECD TG 123	7.14
(1R,4E,9S)-4,11,11-Trimethyl-8-methylidenebicyclo[7.2.0]undec-4-ene	87-44-5	OECD TG 123	6.23
Isodecyl diphenyl phosphate	29761-21-5	OECD TG 123	6.11
4,4'-Propane-2,2-diylbis(2,6-dibromophenol)	79-94-7	OECD TG 123	>= 6.24 <= 6.42
4-(Dodecylsulfanyl)-4-methylpentan-2-one	855737-35-8	OECD TG 123	7.1

Table A 2 Substances with 'not confirmed' experimental log K_{OW} values > 6

Substance	CAS number	Method	Log K_{OW}
(17alpha)-3-Ethoxy-19-norpregna-3,5-dien-20-yn-17-ol	96487-85-3	OECD TG 117	6.1
(Propan-2-yl)cyclohexane	696-29-7	OECD TG 117	6
8,9,10,11-Tetrachloro-12H-isoindolo[2,1-a]perimidin-12-one	20749-68-2	OECD TG 117	7.1
Tris[4-(dimethylamino)phenyl]methanol	467-63-0	OECD TG 117	6.093
1-Phenyldecane-1,3-dione	68892-13-7	OECD TG 117	6.5
(Octan-2-yl)benzene	31047-57-1	OECD TG 117	7; 7.1; 7.2
Bis[C5-(linear and branched)-alkyl] benzene-1,4-dicarboxylate	2097734-13-7	OECD TG 117	6.6
(11Z)-N,N-bis(2-hydroxyethyl)icos-11-enamide; (13Z)-N,N-bis(2-hydroxyethyl)docos-13-enamide; (9E)-N,N-bis(2-hydroxyethyl)octadec-9-enamide; (9E,12E)-N,N-bis(2-hydroxyethyl)octadeca-9,12-dienamide; (9E,12E,15E)-N,N-bis(2-hydroxyethyl)octadeca-9,12,15-trienamide	68187-80-4	OECD TG 117	6.75
2-tert-Butyl-4-[1-(5-tert-butyl-4-hydroxy-2-methylphenyl)butyl]-5-methylphenol	85-60-9	OECD TG 117	6.4
1,2-Bis(7-methyloctyl) benzene-1,2-dicarboxylate	28553-12-0	OECD TG 117	7.4
[(4-{6-tert-Butyl-7-chloro-1H-pyrazolo[1,5-b][1,2,4]triazol-2-yl}phenyl)carbamoyl]methyl 2-hexyldecanoate	379268-96-9	OECD TG 117	8.45
4,8-Dicyclohexyl-6-hydroxy-2,10-dimethyl-12H-dibenzo[d,g][1,3,2]dioxaphosphocin	73912-21-7	OECD TG 117	7.1
2,4,6,8,10-Pentamethyl-1,3,5,7,9,2,4,6,8,10-pentaoxapentasilecane	6166-86-5	OECD TG 117	6.33
Isopentyl p-methoxycinnamate	71617-10-2	OECD TG 123	6.242
2-[(2-Hydroxypropyl)(C16-18 sat. C18 unsat. alkyl)amino]propan-1-ol	1309955-79-0	OECD TG 123	6.2
N-[2-(Piperazin-1-yl)ethyl]C18-unsatured-alkylamide	1228186-18-2	OECD TG 123	6.7 6.1
Distillates (coal tar), high-temperature, heavy oils	140203-21-0	OECD TG 123	6.11 6.13 >= 4.6 <= 6.2 6.22

Substance	CAS number	Method	Log K_{OW}
Zinc bis[O,O-bis(8-methylnonyl) dithiophosphate]	25103-54-2	OECD TG 123	6
3-Methylcyclopentadecan-1-one	541-91-3	OECD TG 123	6.06
Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene	68411-46-1	OECD TG 123	6.66
Trihexadecyl citrate	4560-68-3	OECD TG 123	7.27
N-C16-C18(even numbered)-Alkyl-N,N-dimethyl-C16-C18(even numbered)-alkyl-1-aminium chloride	92129-33-4	OECD TG 123	8.2 8.4 8.4
Cyclododeca-1,5,9-triene	4904-61-4	OECD TG 123	6.8
{2-[(2-Methylundec-1-en-1-yl)oxy]ethyl}benzene	2489743-82-8	OECD TG 123	8.146
(9E)-N-[3-(Dimethylamino)propyl]octadec-9-enamide	1379524-06-7	OECD TG 123	6.1
Fatty acids, C16-18(even numbered) and C18-unsatd., butyl esters	84988-74-9	OECD TG 123	6.01
3-(Dodecylsulfanyl)-1-[(1S,2R)-2,6,6-trimethylcyclohex-3-en-1-yl]butan-1-one	878665-13-5	OECD TG 123	> 9.2
Fatty acids, palm-oil (C16-C18), Me esters, chlorinated (35-45 % w/w)	95009-45-3	OECD TG 123	≥ 5.4 ≤ 6
Octadecenamide	124-26-5	Unknown*	6.5

* Data taken from study record where this value is quoted as being used for octadecenamide as a reference material.

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