ECHA note: Options to assess persistence of volatile substances in regulatory PBT assessment

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Contents

1. Introduction

When assessing the persistence of substances, higher tier biodegradation tests in soil, sediment and/or surface water systems are required using standard OECD 307, 308 and 309 Test Guidelines (TGs), respectively. In these tests it is crucial to differentiate disappearance of the test substance due to degradation and other dissipation processes. Volatilisation of the test substance makes the interpretation of the study more difficult and increases uncertainty of the persistence assessment.

In OECD 307, 308 and 309 TGs it is stated that the tests are suitable for non-volatile and slightly volatile substances. However, simulation tests can also be performed with volatile substances if proper care and measures are taken to minimise possible loss of the test substance from the test system through volatilisation. For example, OECD TG 308 tests have been performed for highly volatile substances, e.g. decamethylcyclopentasiloxane (EC 208-764-9) (Unnamed, 2010), and the results have been found suitable for regulatory persistence assessment as can be seen in ECHA (2018).

According to *ECHA Guidance on Information requirements and Chemical Safety assessment* (IR&CSA), *Chapter R.11*, simulation testing of volatile substances (or any substances) "not technically feasible" means that it has been impossible, with allocation of reasonable efforts, to develop suitable analytical methods and other test procedures to accomplish testing so that reliable results can be generated. Appropriate analytical methods should have a suitable sensitivity and be able to detect relevant changes in concentration (including that of degradation products).

According to the fourth introductory paragraph to REACH Annex XIII, the PBT assessment of substances must be based on data obtained under 'relevant conditions'. 'Real environmental conditions' can vary widely across the European Union, depending on where and when a substance is being used and the use(s) in question. 'Relevant conditions' means conditions that allow for an objective assessment of the PBT/vPvB properties of a substance instead of the PBT/vPvB properties of a substance in particular environmental conditions[1].

According to the *ECHA Guidance on IR&CSA, Chapter R.11: PBT/vPvB assessment* (ECHA, 2017), a conclusion on persistence needs to be derived for all environmental compartments. Exclusion of certain environmental compartments from the P/vP assessment based on absence of exposure may be acceptable only if justified. This is because if a substance is (very) persistent and (very) bioaccumulative, even low emissions/exposure can lead to accumulation of the substance in the environment in the long run. Therefore, if environmental exposure cannot be excluded, adequate methods to avoid volatilisation during simulation testing, without compromising the validity of the test, should be applied to conclude on persistency.

In the current document, different aspects related to degradation testing of volatile substances are discussed, e.g. how to assess persistence when volatilisation may be a problem in simulation testing, considerations for test set-up and test design, and data treatment. Provided examples illustrate approaches taken in regulatory assessment.

The present ECHA note is based on the discussions taking place in the PBT Expert Group and is intended to inform duty holders about available approaches to improve persistence assessment of volatile substances.

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[1] Board of Appeal decisions: Case A-013-2014, BASF, Decision of the Board of Appeal of 7 December 2016, paragraph 113; and Case A-004-2017, 3v Sigma S.p.A., Decision of the Board of Appeal of 15 January 2019, paragraph 57.

2. Indicators for volatility

2.1. Indicator values for volatility (Henry's Law constant and vapour pressure)

Volatility of a substance is defined as its tendency to vaporise from solids and liquids. The important key parameters describing this tendency are vapour pressure, which gives the equilibrium between the condensed and vapour phases of the substances, and boiling point (temperature where vapour pressure equals the ambient pressure).

In the VOC Solvents Directive (1999/13/EC), the EU defines volatile organic compounds (VOC) as "any organic compound having an initial boiling point less than or equal to 250 °C (482 °F) measured at a standard atmospheric pressure of 101.3 kPa". However, in simulation studies, substances with a lower boiling point may also volatilise.

Another important parameter describing the volatility of the substance is the Henry's Law constant (HLC), which offers an indication on the transfer of a substance from the liquid phase to the gas phase. It is a ratio of a substance's partial pressure in air to the concentration of the substance in solution at a given temperature, which is expressed in units of pascals (or atmospheres) for air to moles per cubic meter for water ($Pa.m^3/mol$). The tendency of substances to volatilise increases as this ratio gets higher.

In OECD 307 and 308 TGs, no clear cut-off value for volatility is provided while the OECD TG 309 indicates that substances with a Henry's Law constant (HLC) of <1 Pa m^3/mol can be considered non-volatile and a threshold of <100 Pa m^3/mol is indicated for slightly volatile substances.

Many OECD guidelines make reference to volatile substances but no harmonised threshold values for volatility are indicated among them (see Table 1). Additionally, in OECD TGs 220, 222, 232 and 226, it is stated that other factors such as high water solubility or high adsorption to soil, which may limit the volatilisation potential, should also be taken into account when deciding whether or not the test substance can be tested.

Based on the OECD TGs (OECD 220, 222, 232 and 226), the OECD soil toxicity test should not be associated with substances with VP ≥300 Pa or $K_{air/soil}$[2] partition coefficient >1. Furthermore, according to studies by Thomas (1982) and Mackay (1985, 1992) (cited in ECETOC, 1996), an HLC of >0.1 Pa m^3/mol can give rise to a loss of substance at rates that are important relative to the length of typical short-term ecotoxicity tests, although it is dependent on the test design. These threshold values can be used as an indication of potential volatility in simulation tests, but it should be noted that they cannot be extrapolated directly to simulation tests as the conditions in soil toxicity tests and simulation tests differ.

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[2] Air/soil partition coefficient refers to the wet soil.

Table 1. Several volatility limit values with regard to Henry's Law constant (HLC) and vapour pressure (VP) mentioned in different guidelines.

Reference			HLC		VP
			Atm m3/mol	Pa m3/mol	Pa
		K_{aw} ratio			
OECD 309 (2004)	Applicable to non-volatile substances (in open flasks)	-	10^{-5}	<1	-
	Applicable to slightly volatile substances (in closed flasks with headspace).	-	10^{-3}	$<10^2$	-
OECD 310[3] (2006)	Applicable to volatile substances	-	-	< 50	-
		K_{as} ratio			
OECD, 220[4] (2016)	Not applicable to volatile substances	>1	-	-	>300 (at 25ºC)
OECD, 222[4] (2016)		>1	-	-	>300 at 25ºC)
OECD, 232[4] (2016)		>1	-	-	>300 (at 25ºC)
OECD, 226[4] (2016)		> 1	-	-	>300 (at 25ºC)
EFSA, (2014)	Not applicable to volatile substances (in soil field studies)	-	-	-	$\geq 10^{-4}$

K_{aw}, partition ratio between air and water; K_{as}, partition ratio between air and soil. HLC, Henry´s Law constant which offers an indication on the transfer of a substance from the aqueous phase to the gas phase; VP, vapour pressure.

It would seem that the most significant physical property regarding substance losses to the air in non-aquatic systems is its vapour pressure (VP). The higher the vapour pressure of a substance, the more volatile it would be. However, in aquatic systems it should be noted that for substances with similar VPs and molecular weights, higher water solubility would lead to a lower transfer from water to air. Similarly, testing of substances with higher VP in combination with high Koc may be possible, which indicates that dissipation from soil does not only depend on VP. Therefore, thresholds for partition coefficients or vapour pressure solely does not allow for a prediction of the volatilisation rates because other factors can affect the long-term volatilisation tendency.

2.2. Distribution models

Distribution modelling, which provides an estimation on the fate of the substance in the relevant environmental compartments: air, water, soil or sediment, can also be used to assess whether there may be potential issues with volatilisation during simulation testing. With this objective, the following distribution models are referred: SimpleTreat and Level I Fugacity model.

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[3] Using the recommended headspace to liquid volume ratio of 1:2, volatile substances with a Henry's law constant of up to 50 Pa.m3.mol-1 can be tested as the proportion of test substance in the headspace will not exceed 1%.

[4] According to OECD 220 Enchytraeid reproduction test, OECD 222 Earthworm Reproduction Test, OECD 232 Collembolan Reproduction Test in Soil and OECD 226 Mite reproduction test guidelines, the tests may not be applicable to substances for which the air/soil partition coefficient is greater than one, or to substances with vapour pressure exceeding 300 Pa at 25°C.

2.2.1. SimpleTreat[5] (ST)

SimpleTreat v4.0 is an assessment tool for the fate of substances in wastewater treatment plants. The model estimates the extent to which environmental compartments (soil, water and air) are exposed to substances discharged into the sewer.

The tool predicts the fate and emission of substances in wastewater treatment plants and considers the most important processes like volatilisation, mixing, adsorption and degradation. Depending on the test results for ready and/or inherent biodegradability of a substance, specific first order biodegradation rate constants are assigned to the substance.

A process for volatilisation from the aeration tank (Mikkelsen, 1995) is incorporated in the current version and elimination due to volatilisation from the sewage system is also estimated. The model includes rules for the equilibrium partitioning of ionised substances (organic acids and bases) which are applied to sewage and activated sludge. Ionisable organics are present as neutral and charged species in fraction according to input substance parameters such as apparent octanol-water partition coefficient (Dow) and acid dissociation constant (pKa) at environmental characteristic pH.

2.2.2. Level I Fugacity model[6]

The **Level I calculation** is a steady-state calculation with no inflow, outflow, nor intermedia transport. Degrading reactions are not considered either. This type of model predicts the fate and environmental distribution of neutral substances based on calculations considering melting point (MP), VP, HLC, water solubility (WS), log Kow and Koc. The results indicate where a substance is likely to partition and in which environmental media the concentrations are likely to be highest (i.e. the fugacity capacity is largest). Hence, Level I models could be suitable for predicting partitioning in a closed system like the closed bottles of simulation tests.

2.2.3. Examples with models' estimations

The four example substances and their physico-chemical properties are shown in Table 2. In this section the volatility in variable experimental setups (Table 3) are compared with distributions of these substances estimated by SimpleTreat, Level I and EPISUITE (Level III) models (Table 4).

Following model conditions have been considered when running the models:
- Selected SimpleTreat considerations
    - neutral substance;
    - surface aeration instead of bubbling to diminish volatilisation as considered appropriate for volatile substances if HCL exceeds 250 Pa·m3·mol-1 (KAW = 0.1) (Struijs, 2014);
    - minimum allowed wind speed (1m/s) applied; and
    - degradation equal to 0 to consider a higher concentration for distribution.
- Level I fugacity model with default parameters provided by the model; and
- Level III fugacity model (EPISUITE)
    - steady state, but not equilibrium, conditions are assumed.

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[5] https://www.rivm.nl/en/soil-and-water/simpletreat
[6] Spreadsheets for fugacity models can be downloaded at
https://www.trentu.ca/cemc/resources-and-models

The EPISUITE Level III fugacity model predicts partitioning between air, soil, sediment and water using a combination of default parameters and various input parameters that may be user defined or estimated by other programs within EPISUITE. Calculations with EPISUITE have also been included for comparability purposes.

Level I estimates the fate of a substance based on minimum endpoints to define neutral substances. SimpleTreat estimates the fate of a substance in a sewage treatment plant and the extent to which environmental compartments (soil, water and air) are exposed to substances discharged into the sewer, while Level III of EPISUITE estimates the distribution of emissions in environmental compartments. Therefore, the processes and conditions considered in the three models differ, and consequently, higher differences in the predicted distributions can be expected, especially regarding Level III.

Table 3 summarises the observations on volatility made during the experimental tests with variable test set-ups. Environmental distributions calculated using Level I, Level III and SimpleTreat models are included in Table 4**Error! Reference source not found.**. Highest similarities in percentages of modelled compartment distribution are observed between Level I and SimpleTreat models.

Cassiffix (EC 422-040-1) was considered a moderately volatile substance and therefore the guidance document OECD TA 23 was recommended to be consulted to help achieve and maintain the required exposure concentration in the aquatic toxicity test requested in the same ECHA decision on substance evaluation as the simulation test. However, no measures to minimise volatilisation were taken in the simulation test.

The observed volatilisation collected in the polyurethane foam (PUF) traps (40 %) is similar to the average estimated partitioning by level I and ST in the air compartment (40.6 %). Recovery in the sterile controls was c.a 70 % of applied radioactivity (AR), and thus, c.a. 30 % AR was dissipated out of the system. Between 30 % and 50 % of the substance is predicted to end up in the air by SimpleTreat and Level I, respectively. However, SimpleTreat estimates the highest percentage of the substance (50 %) in the water compartment, probably weighting the high WS of Cassiffix (11.1 mg/L) over the HLC (20 Pa m^3/mol at 12ºC).

For water soluble and highly adsorptive substances, the use of *Kow* as an input in the SimpleTreat model may lead to an overestimation of the aquatic exposure concentration (ECHA, 2016). This could explain the discrepancy observed between the high partitioning to the aquatic compartment when estimating the distribution of Cassiffix with SimpleTreat and the high volatilisation observed in the simulation test.

For HMDS (EC 203-492-7) and DHNP (EC 202-046-9), both the SimpleTreat and Level I model predict high volatilisation and a significant fraction of the substance ending up in the atmospheric compartment (c.a. 100 %). In both cases, those endpoints that indicate potential volatilisation (VP and HLC) showed high values while other endpoints did not indicate a big strength in counteracting volatilisation, i.e. low WS (<1 mg/L), and low absorptivity (log Koc c.a. 3). For DHNP, although the application of measures to minimise volatility seemed to be effective and useful to reduce the expected volatilisation (silicone oil carrier), reservations are raised since by dosing the test substance in silicone oil, a third phase, where the test substance would preferentially partition into, was introduced into the test system of the OECD TG 309 study. The amount of DHNP partitioned into silicone oil would be less available for biodegradation compared to the amount partitioned in the water phase if the supply of the test substance to the water phase is slower than the biodegradation capacity of the microorganisms.

Table 2. Substances used in comparison and physico-chem properties.

EC	422-040-1	203-492-7	202-046-9	229-782-3	435-790-1
name	Cassiffix	Hexamethyldisilosane (HMDS)	Decahydronaphthalene (DHNP)	Trigonox 29	HFE 7500
MP	-25	-68.2	-40	-30	-19.75
BP	301.5	100.5	187	60[7]	121.8
MW	234.377	162.377	138.25	302.45	414.114
log Kow	4.72	5.06	4.2	6.9	6
Vp (Pa)	1.5	5500	106.4	0.009	847
WS (mg/L)	11.1	0.93	0.889	0.093	0.021
HLC (Pa m3/mol)[8]	41.71(25º)/20(12º) 30	606000	10740	38.54(25º)/18.47 (12º)	16702598.00
log Koc	3.34	2.53	3.19	5.4	4.88
Requested OECD	309	308	309	309	308
	CC3=CC[C@H]([C@]12 CCCC(C)(C1)OC2)C3(C)C	[Si](O[Si](C)(C)C)(C)(C)C	C1CCC2CCCCC2C1	CC1CC(C)(C)CC(C1)(OOC(C)(C)C)OOC(C)(C)C	CCOC(C(C(F)(F)F)(C(F)(F)F)F)(C(C(C(F)(F)F)(F)F)(F)F)F

Table 3. Data on volatilisation observed in the simulation tests.

	Cassifix	HMDS	DHNP	Trigonox 29		
Requested in SEV	OECD 309 [a]	308 [b]	309 [c]	pre 309 [a]	pre 308 [a]	def 308 [a]
concen. Used	20-100 µg/l	0.2 µg/g sed	1-10 µg/l	44-88 µg/l	44-88 µg/l	0.4 µg/g
%PUF (%dissi)	40% (+≈30%)	33-50%	c.a. 0%	≈50%	≈35%	≈6% (+≈25%)

%PUF: radioactivity measured in volatile traps; %dissipation by volatilisation:
a) coloured in reddish, no measures to minimise volatility applied;
b) in greenish, measures applied: minimises the headspace volume; spiking solvent and use of a method to minimise volatilisation of the test substance during the test procedure;
c) in greenish, measures applied: reduced headspace:liquid ratio and silicone oil carrier.

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[7] Substance decomposes before boiling. Self-Accelerating Decomposition Temperature (SADT) of the substances is 60 °C.
[8] Estimated by EUSES at environmental temperature (12ºC).

Table 4. Partitioning prediction by applying distribution models.

		Cassifix	HMDS	DHNP	Trigonox 29	HFE-7500
	Requested in SEV	309	308	309	308	308
	Distribution	%	%	%	%	%
AIR	Level I	52	100	99.8	0.7	100
	EPISUITE	0.075	48.5	10.5	0.3	4.23
	mult	0.108	48.5	11.7	0.3	4.23
	single	0.313	15.1	3.51	0.21	0.89
	mult	0.108	48.5	11.7	0.3	4.23
	single	0.015	96.8	14.8	0.06	91.2
	Simple Treat	29.27	92.68	83.92	0.87	48.65
WATER	Level I	8	0	0.04	0.18	0
	EPISUITE	7.71	50.1	63	1.92	5.35
	mult	7.42	50.1	59.1	1.92	5.35
	single	65.8	83.7	91.7	5.11	5.54
	mult	7.42	50.1	59.1	1.92	5.35
	single	0.04	0	0.107	0	0
	Simple Treat	49.88	4.35	4.27	4.17	2.56
SEDIMENT	Level I	1	0	0.15	97	0
	EPISUITE	3.97	0.718	2.34	35.6	90.3
	mult	3.82	0.718	3.06	35.6	90.3
	single	33.8	1.2	4.75	0.04	0
	mult	3.82	0.718	3.06	35.6	90.3
	single	0.022	0	0	0.08	0
	Simple Trreat	5.68	0.08	0.38	29.89	2.45
SOIL	Level I	39	0	0	2.2	0
	EPISUITE	88.2	0.657	24.2	62.2	0.162
	mult	88.7	0.657	26.1	62.2	0.162
	single	0.019	0	0	94.6	93.6
	mult	88.7	0.657	26.1	62.2	0.162
	single	99.9	3.2	85.1	99.9	8.75
	Simple Treat	.	.	.	.	.

EPISUITE: white rows-emission values equally for air water and soil, blue rows-100 % emission in water and brown rows-100 % emission in soil. Single: single level III output runs the fugacity model once per EPI using as environmental emission rates 1000 kg/h to soil, water and air; Mult: multiple level III outputs run the fugacity model 7 times per EPI run using permutations of air, water and soil rates as either 0 or 1000 kg/hr.

In the case of Trigonox 29, both SimpleTreat and Level I models indicate a very low potential for volatilisation. The low volatilisation estimated based on the physico-chemical properties (c.a. 0.7 %) does not support the high volatilisation, between 35 and 50 % of AR, observed in the simulation tests (preliminary OECD TG 309 and 308 studies and definitive OECD TG 308 study). In the preliminary test, the substance was applied via the water phase and no measures to address volatilisation were applied. In the definitive test, the test substance was spiked in the sediment, but the test resulted in high volatilisation: only ca. 3.5-5 % AR was collected in the volatile traps, ca. 6-7 % AR in the foam bungs and ca. 18-29 % AR was extracted from the vessel head, rubber seal and tubing system. The radioactivity recovered in the tubing extracts suggests that the test item was volatilised from the water and was absorbed by the plastic tubing and foam bung.

It is interpreted that the level I model and ST gave a higher weight to the adsorption capacity (log Koc 5.4) against HLC (18 Pa m^3/mol at 12ºC). For substances with low water solubility, which is the case of Trigonox 29, adsorption onto sludge may be a significant elimination mechanism and it could explain the overestimation of the distribution in the soil/sediment compartments.

For Cassiffix and Trigonox 29, high volatility was not expected since their HLCs are relatively low (HLC <100 Pa·m^3/mol), but above the value to regard substances as non-volatiles in practice (HLC less than about 1 Pa·m^3/mol), according to the OECD 309 guideline (OECD, 2004). Therefore, some volatility could be expected. Adsorptive properties could have also been relevant since the extraction of the vessel head, down tube, rubber seal and tubing recovered c.a. 25 % AR. Volatilisation and adsorption made the results difficult to interpret.

In the case of HFE-7500, no simulation study is yet available (an OECD 308 study requested in a ECHA decision on substance evaluation[9]) and therefore no comparison with experimentally observed volatilisation has been made. Measures to minimise losses of the test substance due to volatilisation were requested in the decision.

In conclusion:

- For practical purposes, an HLC ≥0.1 Pa m^3/mol can be considered as an indication of potential volatility in simulation tests, since volatility cannot be excluded, and can give rise to a loss of a substance at rates that are important relative to the length of typical ecotoxicity tests (Thomas, 1982). Substances with an HLC >1.0 Pa m^3/mol or VP above 300 Pa (ECHA, 2012) will partition preferentially into the gas phase resulting in volatility and, therefore, should be treated and considered as volatile substances.

- Regarding estimations with distribution models: Level I and SimpleTreat distribution models give an indication of affinity and distribution of substances based on intrinsic physico-chemical properties of the substance. However, volatilisation in a simulation test cannot always be excluded even in those cases where distribution to air is predicted to be low by the models. For example, for some substances with high WS and/or high Koc, the models may overestimate the partitioning to the aquatic and sediment compartment (ECHA, 2016), and adsorption to the soil compartment (Cooke, 2003, Voustas et al., 2005), and consequently, may underestimate the partitioning to air. In some cases, salts, surfactants and humic acids, which are present in soil pore-water, can affect the HLC for a given compound and lead to lower volatilisation from the soil than what is predicted by distribution models based on physico-chemical properties (Baker et al., 1996).

- Simple compartment models, such as the Level I fugacity model, can be an important first step in conducting a multimedia distribution and therefore exposure assessment. Such models can provide useful information on the fate of substances to determine the need for further considerations when testing.

- Henry's Constant can be measured experimentally but typically it is estimated as the ratio of the concentrations in air and water (i.e. vapour pressure and water solubility). However, surface active substances adversely interfere with, or even preclude the determination of, water solubility and, in particular, the octanol/water partition coefficient. SimpleTreat v4.0, includes rules for the equilibrium partitioning of ionised substances (organic acids and bases).

- All parameters used in distribution modelling are temperature dependent. The simulation studies should normally be performed at environmentally relevant temperatures of 12 °C. However, most vapour pressure values are measured or estimated at 20 or 25°C, which can result in an overestimation of the volatility.

- Experience and information from other existing studies, e.g. volatility observed in ecotoxicity tests, can be useful to assess whether there may be potential issues with volatilisation.

- Considering the above issues, no strict thresholds are defined to decide on the applicability of simulation tests (OECD 307, 308 and/or 309), or to decide whether measures to limit volatilisation are needed. Instead, a case-by-case assessment of potential volatilisation is recommended, taking into account vapour pressure,

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[9] https://echa.europa.eu/documents/10162/7ce1da82-63ba-dd94-71b8-2115f4ea36b3

HLC, distribution modelling and additional factors such as water solubility, phase partitioning and adsorption.

- The volatilisation process may be significant in the aquatic compartment if HLC of a substance is >1 Pa.m^3/mole. The recommendation is therefore that for these volatile substances with a Henry's Law Constant >1.0, measures to minimise volatilisation should be applied to maintain >70 % of mass balance. If after applying all reasonable measures to minimise volatilisation in the pre-test, the parent substance cannot be available to microorganisms or within the test system due to volatilisation processes, testing should be considered not technically feasible in a case-by-case approach.

3. Options to address volatile substances

Due to inherent substance properties, the standard OECD test designs would be not applicable in the case of volatile substances since test conditions could promote the dissipation of the test item out of the test system. For that reason, modifications in the standard test should be applied to set-up reliable test designs and results, avoiding a very rapid dissipation due to volatilisation of the substance which would also affect the real substance concentration.

OECD TGs 307, 308 and 309 recommend applying a static biometer-type system for testing slightly volatile substances. Recent degradation studies with soil (OECD TG 307) (OECD, 2002b) and with an aqueous medium (OECD TGs 308 and 309 (OECD, 2002a, 2004), where volatilisation can be more significant, suggest improved closed test set-ups which makes it possible to generate reliable degradation kinetics data also for highly volatile substances (Shrestha et al., 2019; Shrestha et al, 2020; Birch et al., 2017).

In the following, some options to address volatile substances in simulation tests are described. However, as a general rule, whenever the test system is handled or modified, consistencies with the OECD TG conditions should be kept, e.g. avoid disturbance of the sediment layer and ensuring sufficient dissolved oxygen (DO) concentration in the aqueous phase in the case of OECD TG 308.

3.1. Pre-testing (OECD TGs 307, 308 and 309)

Only OECD TG 308 indicates that an optional preliminary test may be considered appropriate to establish a duration and sampling regime of the definitive test. In the case of volatile substances, before the definitive test, a pre-test or pilot study could be necessary to determine what modifications to the guideline are necessary and feasible (e.g. biometer type or closed system).

To develop an appropriate biodegradation test set-up for a volatile substance, a series of preliminary tests with slight modifications are recommended to assure a complete mass balance in the definitive test. Regardless of the physico-chemical properties, as long as the pre-test indicates volatilisation of the substance from the test system, biometer-type systems should be preferred.

Results from the preliminary test as a basis for the selected relevant experimental conditions should be reported. The homogeneity and stability of the exposure concentrations, or any other endpoints measured, e.g. dissolved oxygen (DO) concentrations, should also be quantitatively monitored in the definitive study to ensure confirmation of relevant test conditions and to avoid uncertainty on the results.

3.2. Test vessel and design

In many cases, low recovery rates in the simulation test data result in high uncertainty and complicate the use and interpretation of results including kinetic modelling. Therefore, whenever volatility is suspected, biometer-type flasks should be used to ensure adequately high recovery rates.

The following considerations have been found regarding the biometer-type flask design to obtain a full mass balance in simulation tests:

Closed vessels minimising headspace (OECD TGs 307, 308 and 309).
OECD TG 309 indicates that using closed flasks with a headspace, it is possible to test slightly volatile substances without losses from the test system. However, too much headspace might lead to higher mass distribution into the headspace volume leading to an initial reduced fraction in the water, sediment or soil available for degradation, and a corresponding effect on degradation kinetics. The headspace can be reduced but should not be completely eliminated to be sure of maintaining aerobic conditions. It should be noted, that when a test substance partitions to the headspace, it can, on one hand, be dissipated from the test system if it adsorbs to caps, tubes etc. or leaks out. On the other hand, if degradation of the substance occurs in the water/soil phase, diffusion of the substance from gas back to water/soil increases due to the increased concentration gradient between the phases.

Modified set-ups comprised of smaller test vessels ranging from 100-125 ml have been used in some studies (Shrestha et al, 2019; Unnamed, 2017 (decahydronaphthalene EC 202-046-9)).

Maintaining aerobic conditions in closed test systems (OECD TGs 307, 308 and 309).
For closed systems, OECD TG 308 states that a regular exchange of the headspace gas is required in the aerobic test to compensate for the oxygen consumption by the biomass, but a detailed description of the system is not provided (OECD, 2002a). The TG recommends that when testing slightly volatile substances in a biometer-type system, gentle stirring of the water surface should be applied, at the same time avoiding as far as possible any disturbance of the sediment phase.

Shrestha et al. (2020) mentioned additional measures that could be applied to enhance the aeration of the water phase in closed set-ups, e.g. overhead stirring of the water surface, which was considered as a promising option. In another study, Shrestha et al. (2021), investigated the influence of different test set-ups and agitation techniques on the degradation, distribution and non-extractable residue (NER) formation of 14C labelled phenanthrene to determine an appropriate closed set-up for conducting OECD 308 tests. The influence of shaking at 80rpm/40rpm and overhead stirring of water phase was compared. It was observed that shaking with 80rpm resulted in a more stable oxygen concentration in the water phase and showed no influence in abiotic NER formation. On the other hand, shaking with 40 rpm resulted in lower NER formation but a reduction of 65 % of mineralisation.

These measures will promote oxygen diffusion but will also likely affect diffusion and partitioning of the test substance throughout the water and sediment phases (Shrestha et al., 2016 and 2021; Honti et al., 2016). Furthermore, depending on the intensity of stirring or shaking they could also introduce changes in oxygen conditions of the sediment layer. Especially shaking could influence the potential stratification of the sediment and maintenance of the anaerobic layer. OECD TG 308 mentions that

the aerobic test simulates an aerobic water column over an anaerobic sediment layer that will be underlain with an anaerobic gradient. This might very likely not be the case anymore if rigorous shaking is applied. It is noted that shaking at approximately 100 rpm is recommended to maintain the suspension of particles and the aerobic conditions in OECD TG 309. This raises concerns that shaking at 80rpm, as was done in Shrestha *et al*. (2020), in an OECD TG 308 test could lead to conditions resembling more those of a surface water test with suspended solids/sediment. These factors are all likely to have an influence on the degradation process (Shrestha *et al*, 2021) and thus, on the study outcomes.

Therefore, based on the above information, in the case of OECD TG 308, stirring with an overhead stirrer without disturbing the sediment layer and avoiding sediment resuspension can also be considered. However, in the case of OECD TGs 308/309, if aeration of the water phase is done, a gentle aeration of the surface water is preferred. It should be taken into account that in OECD TG 308, the acclimation period allows the system to reach a reasonable stability and it is indicated that should be carried out under exactly the same conditions as the test incubation. It is also important to quantify the amount of parent substance and relevant transformation products if removed from the test system in the outlet gas during the aeration events. An adequate report of the aeration process should be provided to ensure a proper evaluation of the reliability and adequacy of the study results.

For transformation OECD TG 307 tests under aerobic conditions, the soil moisture content should be adjusted to maintain adequate aeration and nutrition of soil microflora. The soil moisture content is expressed as mass of water per mass of dry soil and should be regularly controlled (e.g. in 2 week intervals) by weighing the incubation flasks and water losses compensated by adding water. The OECD TG 307 states that care should be given to prevent or minimise losses of test substance and/or transformation products by volatilisation during moisture addition.

It is noted that moisture loss may be more relevant for tests performed under flow-through conditions than for tests with biometer-type or closed flasks, which are used for volatile substances. However, if moisture addition would be needed in a test with volatile substances, there is no recommendation in the guideline on how to do it in a way that minimises losses of the test substance and/or transformation products. One option could potentially be to add water through a septum using a syringe with accompanying capture of the displaced gas phase. However, it is not clear whether this would be feasible in practice and whether even distribution of moisture would be obtained. It seems that further experience on this issue is needed.

System geometry (OECD TGs 308 and 309).
Another promising option to promote the oxygenation of the system would be to use a system geometry with a larger diameter to increase surface area at the air-water and water-sediment interfaces providing a larger contact area in the water-sediment and water-air interfaces (Shrestha *et al*., 2016). However, system geometries, in particular headspace volume and height of the water and sediment columns, are important factors influencing the partitioning of volatile substances within the test system, which will also influence degradation (Hennecke *et al*., 2014; Shrestha *et al*., 2016).

It should be taken into account that modified systems in OECD TG 308, with a thinner sediment layer, and OECD TG 309, with a higher suspended sediment content, in terms of the amount of sediments and redox conditions, can result in statistically significant higher mineralisation compared to the standard OECD systems (Shrestha *et al.,* 2016). Larger fraction of oxic sediment in the modified OECD TG 308 system caused by the large interfacial area, the stirred water phase and the thinner sediment layer led to an enhanced degradation in a study by Shrestha *et al*. (2016).

Shrestha *et al*. (2020) demonstrates that the choice of system geometry and sediment:water ratio influences the partitioning of the test substances between different compartments (water, sediment and headspace) and can therefore affect their degradation.

Regarding the volatilisation process itself, the surface area in a closed system is only of influence on the time to equilibrium, not on the amount of substance volatilised. The amount volatilised is mainly determined by the ratios of the different phases. This can be controlled by minimising the head-space. Considering the potentially significant effect of system geometry on biodegradation of test chemicals, the test design and the modifications proposed should be consistent with the standard conditions defined in the respective OECD test guideline to clarify if the substance meets the criteria for persistency according to Annex XIII to REACH and allow comparison of modified OECD TG 308 or 309 tests with other simulation test results performed under standard conditions.

Influence of the organic carbon (OC) content (OECD TG 307, 308 and 309)
In general, higher volatilisation of a test substance is expected for systems with low organic carbon (OC) content (Shrestha *et al*., 2019) and for sediment systems with high OC greater and more rapid partitioning to sediment. Higher mineralisation and degradation of test substance in sediment with high OC content was also attributed to higher microbial biomass in the sediment. Similarly, soils with lower OC content may have greater volatilisation of test substances than soils with a higher OC (Shrestha *et al*., 2019). Sorption of the test substance to organic matter in the soil reduces its volatilisation (Burkhard and Guth, 1981; Chiou and Shoup, 1985; Alvarez-Benedi *et al*., 1999) and, with the extended residence time, the extent of biodegradation may increase in a closed flask test set-up whenever it does not preclude bioavailability to microorganisms.

According to the *ECHA [Guidance on IR&CSA](...), Chapter R.7b*, the amount of suspended solids in the water phase of the 309 pelagic tests should be representative of the level of suspended solids in the EU surface water. The concentration of the suspended solids in the surface water sample used in the OECD TG 309 pelagic test should therefore be approximately 15 mg dw/L. Testing natural surface waters containing between 10 and 20 mg SPM dw/L is considered acceptable in OECD TG 309 (pelagic test). Furthermore, if reporting non-extractable residues (NER) in the test results, the extraction procedure and solvent used to obtain a quantitative measure of NER should be explained and scientifically justified.

Organic carbon is usually the dominant retention mechanism for chemicals in soil, although clay surfaces can play an important role as well. OECD TG 307 recommends using a soil with organic carbon content in the range of 0.5-2.5 % to determine the transformation pathway and variable organic carbon contents in the additional three soils used to determine transformation rate, but no ranges for organic carbon content are defined for these three soils. In the Final Report of the OECD Workshop on Selection of Soils/Sediments (OECD, 1995), which is referenced in OECD TG 307, seven soils with varying organic carbon content (and other characteristics) are recommended for soil adsorption/desorption studies, and the highest organic carbon content range for these soils is >10 %. Therefore, in the case of testing volatile substances in an OECD TG 307 study, it could be recommended that the types of tested soils include at least one soil with high organic carbon content, e.g. >10 %.

Absorbing surfaces (all TGs)
Minimising tubing, plastic and rubber components or other absorbing surfaces in respirometer systems
These components have been observed to lead to increased losses of volatile substances from test systems (Unnamed, 2019; Brown *et al*., 2020) resulting in lower

bioavailability and lower biodegradation observed as well as lower mass balance recoveries. However, the part adsorbed to parts within the water phase, such as bottle walls, may be available for biodegradation and it could be possible to quantify the adsorbed fraction (even from the parts in the headspace) by using appropriate solvent. Therefore, special attention should be paid to components located in the headspace. Stainless steel, free-plastic bottle caps and lock systems (Swagelok connections, Hamlet valves and fitting) were used to completely close the test setup (Shrestha *et al.*, 2019, 2020).

3.3. Sampling

Increased number of samplings to monitor the concentrations in water, sediment/soil and air.

According to OECD TG 308, the number of sampling times should be at least six (including zero time). However, additional sampling points during the initial period of the study may be needed to determine the rate of distribution between the different phases of the test system (water, sediment or soil and air) for a successful kinetic modelling. This is a necessary provision for a successful kinetic modelling when performing the data evaluation because it may be necessary to re-calculate the test concentration and to adequately identify the point in time to use as the starting point for calculation of the half-life. Therefore, initial loss of a substance is crucial for the calculations.

3.4. Preparation of test solutions

Co-solvents (All TGs)

Volatile hydrophobic substances are especially difficult to test. OECD TG 308 recommends the addition of a test substance via aqueous solution, which however is not possible for hydrophobic volatile chemicals due to their volatilisation losses and low solubility. The combination of hydrophobicity and volatility makes it challenging to dissolve a substance in water and avoid evaporative losses. In terms of the necessity to use a solvent, application of such a hydrophobic volatile substance is almost impossible without a co-solvent as the test substance volatilises immediately while preparing the application solution in water. For a non-volatile test substance, the co-solvent should be evaporated after the test substance application but during the application of volatile substances the evaporation step has to be avoided as loss of the test substance may occur along with the solvent, particularly if the test substance is more volatile than the solvent.

The selection of a proper organic solvents is a critical step. The use of a co-solvent can affect the oxygen levels, which in turn affects microbial activity and likely also the degradation of the test substance. A decrease in oxygen concentration in the water phase was attributed to increased oxygen consumption by microorganisms degrading the solvent (Shrestha *et al.,*2020). Therefore, the use of a co-solvent should be avoided or minimised as much as possible and, if needed, a solvent with slow degradation should be chosen. The solubility and density of the co-solvent would seem relevant, together with the way of incubation (static, different types of mixing). Properties of some co-solvents and their relationships to properties of analytes are given in Kästner *et al.* (2018). The study by Kim *et al*. (2010) has information on different mixing methods in degradation studies.

If a co-solvent is used, it should be noted that the test substance needs to be diffused from the solvent phase to water phase before biodegradation can start (although literature reports also that direct uptake from the solvent phase may be sometimes possible). Hence, depending on the rate of diffusion/partitioning, this may potentially limit the biodegradation rate. However, it should be noted that if biodegradation occurs it will usually "drive" the diffusion to the water phase due to the increasing

concentration gradient. Additionally, microbial attachment and growth on the surface of the solvent phase might be relevant too and is probably time dependent. Therefore, sterile controls must be added with measurements from the solvent phase and the water phase, if possible, to indicate the distribution kinetics under abiotic conditions. Research regarding biodegradation related to non-aqueous phase liquids (NAPLs) (Alexander, 1999) and potential microbial attachment and growth on the surface of the solvent phase may be relevant in the context of using co-solvents in biodegradation studies.

If unavoidable, the use of low amounts of water miscible solvents (such as acetone, ethanol) is permitted for application and distribution of the test substance, but this should not exceed 1 % v/v and should not have adverse effects on microbial activity of the test system (OECD, 2002a). An additional complication to consider is that the use of lower solvent:water ratios could cause the test substance to either precipitate or volatilise from the application solution and this has to be avoided (Shrestha *et al*., 2020).

Passive dosing (OECD TGs 308 and 309)
Alternative methods, such as passive dosing, have been indicated in some studies. Birch *et al*., (2017) used this approach to generate an aqueous stock solution in primary biodegradation experiments. However, further experience on the applicability of this method for preparing test solutions in simulation tests is needed.

Individual sample preparation
According to OECD GD 23, in the case of volatile substances, test vessels should be sealed during preparation and exposure and the headspace kept to a minimum or eliminated. Test concentrations should, where possible, be prepared individually by addition of a test substance directly to the test vessels rather than by dilution of a stock solution. Additionally, when samples are collected for analysis they should be placed in zero headspace vials (OECD 2020) to avoid losses during analytical procedure. Reporting of procedural recovery for sample processing steps is therefore recommended and should be considered in data interpretation, especially when dealing with volatile substances.

3.5. Monitoring oxygen levels in headspace

Oxygen depletion is expected to be an issue in closed flask test set-ups, especially when a co-solvent without an evaporation step is used. This might considerably change the test conditions during the incubation period and also the degradation of the test substance. So, the aerobic conditions inside the flasks need to be monitored, preferably both in the headspace and water phase in the case of an OECD TG 308 test.

External oxygen measurements are recommended to minimise test substance losses due to opening of the vessel. Oxygen saturation in the headspace and water phase can be measured with a Fibre optic oxygen meter (Firesting O2, Pyroscience) without the need to open the vessel (Shrestha *et al.,* 2019). However, if it is possible to reliably demonstrate that O_2 concentration remains sufficiently high until the end of the study, then it is not necessary to have constant monitoring of O_2. Solvent control may indicate that at least for the period of degradation of the reference substance, the O_2 was sufficient (assuming that ThOD of the added reference substance and test substance are similar) even in the presence of a solvent although it would not inform on the measured acceptable O_2 level at the end of the study.

3.6. Adsorption traps

In flow-through systems, the set-up should be connected to a series of absorption traps containing different trapping solutions, e.g. NaOH and ethylene glycol or tenax flasks to trap mineralised $^{14}CO_2$ and volatile parent/degradation products, respectively. An adsorption tube can be permanently connected to the flask in order to passively trap the volatilised fraction (Shrestha *et al.*, 2019).

Other methods such as using purge and trap gas chromatography/mass spectrometry with evaluation of mass balance at each time point may also be applicable for volatile substances, but it should be confirmed whether potential adsorption of hydrophobic substances on sediments or soils may prevent recovery, and whether potential less volatile transformation/degradation products can be reliably measured with the selected method.

3.7. Methods of application

The method of spiking may be adapted to avoid losses due to volatilisation during spiking or equilibration. In the case of volatile substances, the application solution will be spiked directly on the matrix and the biometer-flask is then immediately closed using an insert cap. Direct application of the test substance into the sediment (spiking) is currently not mentioned in OECD TG 308, but such application techniques have been developed for sediment toxicity testing and have been suggested as potential solutions for testing challenging substances in the context of OECD TG 308 studies (ECHA *Guidance on IR&CSA*, Chapter R.11).

3.8. Sterile controls

Sterile controls are always recommended to be included in the test system to determine to what extent the test substance decrease is due to biotransformation or to potential abiotic losses (e.g. adsorption to caps, leaking out of the system, formation of non-extractable residues (NER)). Test conditions and operational conditions in sterile controls should be the same as that in active vessels (see sections on "Sterile controls" in ECHA *Guidance on IR&CSA*, Section R.7.9.4.1 in Chapter R.7b and Section R.11.4.1.1.3 in Chapter R.11).

3.9. ^{14}C radio-labelled material

The simulation study must be performed preferably using a radio-labelled test material and a mass balance has to be included in reporting. Radio-labelling shall be applied at the most stable part of the molecule. Use of a ^{14}C labelled test substance facilitates the monitoring of the test substance and degradation kinetics. It also allows the complete mass balance and differentiating mineralisation, NER formation and volatilisation to be obtained. So, it will facilitate the interpretation of the results. However, if a parent substance or transformation/degradation products are part of the volatile fraction, additional substance-specific analyses should be applied for kinetic modelling.

If radio-labelling is not feasible, a systematic and reliable monitoring of the test substance and transformation/degradation products should be performed in all compartments of the test system (water/sediment or soil and air) and reported during the whole simulation study. However, it should be noted that radio-labelling is often costly and not always offer the most cost-effective option to address volatility of a test substance in simulation testing, for example, when compared with information obtained from sterile controls.

3.10. Sample processing and chemical analysis.

Other aspects of sample processing of volatile substances are not well described within the test guidelines. For example, losses of a parent substance during sample processing may bias the results and is not addressed. To minimise losses of the substance during a degradation test, passive dosing and sampling with silicone rod at the end of the experiment has been applied by Birch *et al*. (2017). However, uncertainties have been referred based on the efficacy of different partitioning donors and silicones for passive dosing and sampling (Gilbert *et al*., 2016).

4. Data treatment and interpretation issues

In simulation tests, it is crucial to differentiate degradation and losses due to the dissipation of the test substance from the test system. When calculating DT50 values based on the disappearance of the test substance from the test system (or one compartment of the system), both degradation and other dissipation processes may have affected the disappearance.

In such cases, the DT50 value is not representative of the DegT50 value. If partitioning of the test substance to headspace occurs and it acts as a sink, the reduction in the bioavailability of the substance can influence the degradation kinetics. Therefore, adequate data treatment methods are needed for generating meaningful degradation kinetics in tests where volatilisation of the test substance occurs.

In this section, three different volatilisation correction approaches for the kinetic analysis of simulation studies are presented. In all three correction procedures, it is assumed that the volatile losses of the parent are adequately identified and quantified in the volatile trapping systems. If part of the volatilised test substance was not trapped and was lost from the test system, the results of the kinetic analysis have more uncertainty. Losses through other processes, such as adsorption, increase uncertainties in the kinetic analyses. Furthermore, if repartitioning from the headspace to water (or water-sediment) or soil phase may occur, e.g. if the test substance is not trapped during the test and is lost from the test system only when the test bottle is acidified and purged for CO_2 analysis, the volatilisation correction approaches presented below should not be used as they consider the volatilised fraction as a sink.

A. Separate fitting of data on total dissipation and volatile traps (Verbruggen, E., personal communication, 16 March 2022) –
instead of Appendix 11 of FOCUS guidance (2014)

B. Simultaneous fitting of data on parent in water-sediment and volatile traps to SFO kinetics in OECD 308 using ModelMaker (Jene, 2007b in Annex I of the CLP report on EC 254-938-2 (2018))[10]

C. Simultaneous fitting of data on parent in soil and volatile traps to SFO kinetics in OECD 307 using CAKE (Shrestha *et al*., 2019)

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[10] Annex I to the CLH report (EC 254-938-2)
https://echa.europa.eu/documents/10162/17218/clh_rep_annex_pendimethalin_en.pdf/76b2244 3b-b1a3-802f-1b0d-ab04f42ff9e8

A. Separate fitting of data on total dissipation and volatile traps

Appendix 11 of the FOCUS Generic guidance for Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration (2014) includes correction procedures to account for volatilisation in the kinetic analysis. However, this approach where kvol is derived from the fit of the amount accumulated in the volatile traps is incorrect since kvol should give a result close to the ktot fitted from the dissipation of the substance from the test system.

Deriving kvol from the amount accumulated in the volatile traps may lead to cases where kinetics calculations end up with even slightly negative degradation rates, because the overall dissipation rate fitted in one way is subtracted from the overall dissipation rate fitted in another way. Therefore, a proposal to substitute the FOCUS Guidance Appendix 11 regarding estimation of kDEG is presented in this section. The approach applies for cases where both the total dissipation and volatilisation follow first order kinetics.

In a simulation test, the decrease in concentration of the parent substance in the test system reflects the overall dissipation of the test substance in the test system. From the concentrations of parent substance in the test system, no distinction can be made between the different processes that have caused this dissipation if the processes occur concurrently. The pattern of decrease thus follows the overall kinetics of the simultaneous processes.

If the two simultaneous processes (degradation and volatilisation) are occurring with the same kinetic pattern (e.g. first-order degradation of the parent substance will be accompanied by a first-order volatilisation of the parent substance), the amount dissipated due to one of the two processes is a constant fraction of the total dissipation. This also holds true for the amount accumulated in the volatile traps. The amount of volatiles trapped at any time is assumed to be a constant fraction of the amount dissipated from the test system. This means that the accumulated amount follows the overall dissipation. However, data should be checked to verify that this assumption is met.

Hence, for first-order kinetics, if ktot = kvol + kdeg, the pattern of volatilisation accumulated in the volatile traps thus follows ktot and not kvol. That is because the amount that is degraded is also not available anymore for volatilisation. Therefore, it is not correct to derive kvol from the fit of the data of the volatile traps.

In the case of first-order kinetics, the formula for the total overall dissipation losses can be expressed, as:

$k_{tot} = k_{deg} + k_{vol}$

with

k_{tot} = first-order overall dissipation rate
k_{vol} = first-order overall volatilisation rate
k_{deg} = first-order overall degradation rate

If the starting mass in the test system is m_{tot}, then in principle with a complete mass balance (e.g. for radioactivity), the amount of parent substance degraded if all the substance has dissipated is:

$m_{tot} = m_{deg}(\infty) + m_{vol}(\infty)$
$m_{deg}(\infty) = m_{tot} - m_{vol}(\infty)$

Where:

$m_{deg}(\infty)$ = the mass degraded in the test system when all the substance has dissipated;
m_{tot} = the starting mass in the test system; and
$m_{vol}(\infty)$ = mass volatilised and accumulated in the traps when all the substance has dissipated.

The mass of the parent substance volatilised and accumulated in the gas trap can be fitted as:

$$m_{vol}(t) = m_{vol}(\infty)*(1-exp(-k_{tot}*t)$$

Thus, the amount $m_{vol}(\infty)$ can be derived from the fit of the volatilised mass in the gas traps. Because m_{tot} and $m_{vol}(\infty)$ are known, $m_{deg}(\infty)$ can be calculated.

Because $k_{deg} / k_{tot} = m_{deg}(\infty) / m_{tot}$, then k_{deg} can be calculated as:

$$k_{deg} = k_{tot} * (m_{tot} - m_{vol}(\infty)) / m_{tot}$$

In summary, to be able to estimate k_{deg}, what is needed is the overall dissipation rate constant (k_{tot}), the starting mass of radioactivity (m_{tot}) and the fitted amount of volatilised radioactivity if all the substance has dissipated ($m_{vol}(\infty)$).

For additional clarification, intermediate equations and explanations are provided in Annex I to this document.

B. Simultaneous fitting of data on parent in water-sediment and volatile traps to SFO kinetics in OECD 308 using ModelMaker

In the OECD TG 308 study (STUDY CA 7.2.2.3/4) included in the CLH report of pendimethalin, ModelMaker (v3.0.4) was used to simultaneously fit the total residue data of the whole system and the cumulative volatilisation data to derive DegT50 that described the volatilisation corrected total degradation of the substance.

A compartment model was set up to describe the total dissipation indicated by the total dissipation rate kTOT as the sum of the degradation and the volatilisation indicated by the degradation rate kDEG and the volatilisation rate kVOL. A schematic diagram of the model is shown below (Figure 1). The model was implemented in ModelMaker (v4.0) and the Chi[2] error level was calculated using the FOCUS kinetics tool FOCUS_DEGKIN_v2.
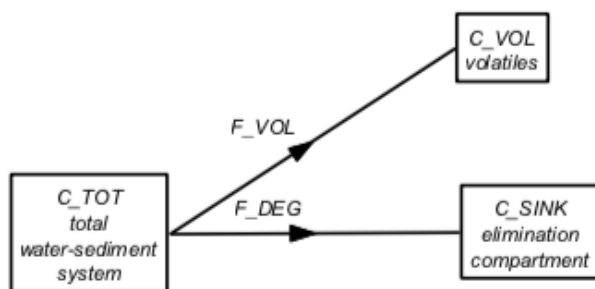

Figure 1. Compartment model for the parent in total water-sediment system (C_TOT) including volatilisation (C_VOL) and sink (elimination compartment) implemented in ModelMaker (Figure 4.1.4.1-6 in the Annex I of the CLP report on EC 254-938-2).

The underlying differential equation system is given by:

$$\frac{\partial}{\partial t} C_{TOT} = -k_{DEG}\, C_{TOT} - k_{VOL}\, C_{TOT} = -k_{TOT}\, C_{TOT}$$

$$\frac{\partial}{\partial t} C_{VOL} = +k_{VOL}\, C_{TOT}$$

$$\frac{\partial}{\partial t} C_{SINK} = +k_{DEG}\, C_{TOT}$$

Where:
C_{TOT} = total measured concentration in water-sediment system
C_{VOL} = cumulative volatilisation
C_{SINK} = cumulative degradation products (and other elimination processes, e.g. NER)
k_{DEG} = degradation rate of the system
k_{VOL} = volatilisation rate of the system
k_{TOT} = total dissipation rate of the system

C. Simultaneous fitting of data on parent in soil and volatile traps to SFO kinetics in OECD 307 using CAKE

Shrestha *et al*. (2019) presented an extended kinetic modelling to enable volatilisation to be considered in the modelling of degradation kinetics in OECD TG 307 tests. In the model, the volatilisation losses are considered as an additional product that neither decline nor repartition into the soil. The volatilisation is thus treated as a separate sink for the parent substance, and it is considered to occur in parallel to the biodegradation. Therefore, in this extended model the degradation and the volatilisation of the substance were considered as two processes and separated so that individual rate constants could be calculated for the volatilisation process as well as the degradation process (Figure 2).
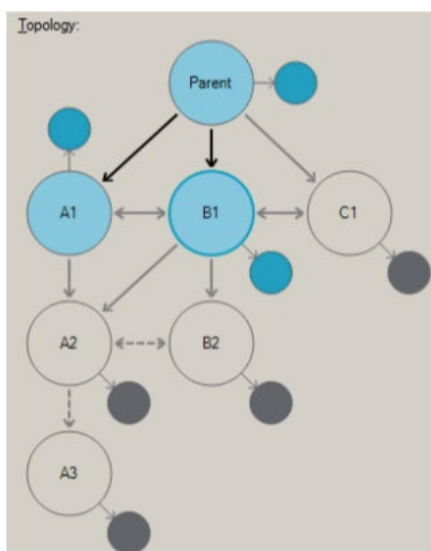

Figure 2. Structure of the model used in the CAKE tool. Parent: concentration of parent measured in soil; A1: sum of extractable metabolites; B1: volatilised parent.

In general, the model assumes first-order kinetics with k as an overall dissipation rate and c the concentration of the test substance according to following equation:

$$\frac{dc}{dt} = -kc \qquad \text{(Equation 3)}$$

However, for the extended model, it is assumed that k consists of two rate constants kV (volatilisation rate) and kT (transformation rate):

k = kT + kV   (Equation 4)

To describe the ratio of the two parallel processes, the model internally uses "fractions" FV (volatilisation fraction) and FT (transformation fraction) which can be calculated based on the individual rates for volatilisation and transformation together with the overall decline rate as follows:

$$F^T = \frac{k^T}{k} \qquad \text{(Equation 5)}$$
$$F^V = \frac{k^V}{k} \qquad \text{(Equation 6)}$$

When the model is run in CAKE, the values for the two fractions (FT and FV) are estimated by the tool. They describe how the optimisation tool evaluates the importance of the respective processes, transformation and volatilisation, in the experiment. Based on the fraction for volatilisation and the overall DT50 estimated by CAKE, half-lives for volatilisation (DT50,vol: half-life due to the volatilisation of the substance) and for transformation (DegT50: half-life due to all (primary) transformation processes) can be calculated using the following equations:

$$DT^{50,vol} = \frac{DT^{50}}{F^V} \quad \text{(Equation 7)}$$
$$DegT^{50} = \frac{DT^{50}}{1-F^V} \quad \text{(Equation 8)}$$

It is noted that this DegT50 does not only include the formation of degradation products but also other processes (e.g. formation of NER or loss of the substance through adsorption to test vessel, leakage etc).

Volatility correction - conclusions
The above volatilisation correction approaches A, B and C assume first-order kinetics, and hence, it is essential to have a good fit to the SFO kinetics. Otherwise volatilisation corrections based on these approaches have a high uncertainty.

According to the FOCUS Guidance Appendix 11, volatilisation correction is most straightforward when SFO kinetics apply but it can also be used with FOMC, HS and DFOP kinetics if certain assumptions apply to the data. However, no studies were found where bi-phasic kinetics would have been applied in volatilisation correction.

If the mass balance decreases towards the end of the study, it could suggest possible losses of the test substance or volatile transformation products from the test system by volatilisation, and in those cases it is important to also have information on sterile controls. Other possible reasons for a decreased mass balance include e.g. losses due to adsorption or during sampling/sample treatment. In the data treatment, it should be considered to normalise (correct) the results for a decreasing mass balance if it is not known what caused this decrease. However, any test that does not meet the quality criteria for recovery should be treated cautiously.

A comparison of the current FOCUS and approaches B and C applied in the calculation of DegT50 has been performed for two case studies, one with high volatilisation (^{14}C-Cassiffix, EC 422-040-1 with OECD TG 309) and one with medium volatilisation (^{14}C-Tefluthrin, CAS 79538-32-2 with OECD TG 308) (Uotila and Vega, 2021).

The proposal A for substituting Appendix 11 of FOCUS (2014) could explain some negative results in kDEG after the application of the current FOCUS approach. It was concluded that the two approaches B and C developed for water-sediment and soil studies, can also be used in surface water studies as the underlying assumptions apply similarly to all three systems (environmental compartments). If the data has a

good fit to SFO, the simultaneous modelling approaches (approaches B and C above) seem to give similar results. It is not known what caused the small differences in the results of approaches B and C. It could potentially be related to the model optimisation parameters. This was suggested to explain the small differences observed between ModelMaker and other tools in Ranke *et al*. (2018).

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Annex I. Proposal for substituting Appendix 11 of FOCUS guidance, by using separate fitting of data on total dissipation and volatile traps.

Intermediate equations and additional explanations.

In the case of first-order kinetics, the formula for the dissipation losses can be expressed by the following differential equations:

- total dissipation: $dm/dt = -k_{tot} \, m(t)$ eq [1]
- losses from volatilisation: $dm_{vol}/dt = k_{vol} \, m(t)$ eq [2]
- losses from degradation: $dm_{deg}/dt = k_{deg} \, m(t)$ eq [3]

with

k_{tot} , the first-order overall dissipation rate
k_{vol} , the first-order overall volatilisation rate
k_{deg} , the first-order overall degradation rate

and $k_{tot} = k_{deg} + k_{vol}$ eq [4]

$m(t)$ = the mass of the parent substance in the test system at time t
$m_{vol}(t)$ = the mass volatilised and accumulated in the traps at time t
$m_{deg}(t)$ = the mass degraded in the test system at time t

If the starting mass in the test system is m_{tot}, then in principle with a complete mass balance (e.g. for radioactivity), the amount of parent substance degraded if all the substance has dissipated is:

$m_{tot} = m_{deg}(\infty) + m_{vol}(\infty)$ eq [5]

Therefore, $m_{deg}(\infty) = m_{tot} - m_{vol}(\infty)$ eq [6]

Where:
m_{tot} = the starting mass in the test system
$m_{deg}(\infty)$ = the mass degraded in the test system when all the substance has dissipated
$m_{vol}(\infty)$ = the mass volatilised and accumulated in the traps when all the substance has dissipated

It can be assumed that the amount of volatiles trapped at any time is a constant fraction (noted α is the following equations) of the amount dissipated from the test system[11]:

$m_{vol}(t) = \alpha \, (m_{tot} - m(t))$ eq [7]

Therefore:

$m(t) = m_{tot} - m_{vol}(t)/\alpha$ eq [8]

By combining eq [2] and eq [8], one gets:

$dm_{vol}/dt = -k_{vol}/\alpha \cdot m_{vol}(t) + (k_{vol} \, m_{tot})$ eq [9]

The solution of this differential equation is:

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[11] This assumption must be verified. This can easily be done from the raw results of the experiment.

$m_{vol}(t) = a\, m_{tot} + x\, \exp(-k_{vol}/a\, t)$ eq [10]

where x is a constant

At t=0, $m_{vol}(t) = 0$. Therefore $x = -a\, m_{tot}$ and eq [10] can be rewritten as:

$m_{vol}(t) = a\, m_{tot} (1 - \exp(-k_{vol}/a\, t))$ eq [11]

At t=∞, by definition, $m_{vol}(t) = m_{vol}(\infty)$ and from eq [7] or eq [11], $m_{vol}(t) = a\, m_{tot}$,

therefore: $m_{vol}(\infty) = a\, m_{tot}$ eq [12]

Since the solution of differential equation [eq 1] is:

$m(t) = m_{tot} \exp(-k_{tot}\, t)$ eq [13]

then by combining eq [8] and eq [13]:

$m_{vol}(t) = a\, m_{tot} (1 - \exp(-k_{tot}\, t))$ eq [14]

By combining eq [11] and eq [14], one gets:

$k_{tot} = k_{vol}/a$ eq [15]

Therefore, from eq [11], eq [12] and eq [15]:

$m_{vol}(t) = m_{vol}(\infty) (1 - \exp(-k_{tot}\, t))$ eq [16]

and

$a = m_{vol}(\infty) / m_{tot} = k_{vol} / k_{tot}$ eq [17]

Using eq [16], the mass of the parent substance volatilised and accumulated in the gas trap can be fitted to derive parameter $m_{vol}(\infty)$.

As for degradation, it can similarly be assumed that the amount of substance degraded at any time is a constant fraction β of the amount dissipated from the test system:

$m_{deg}(t) = \beta\, (m_{tot} - m(t))$ eq [18]

Using the same reasoning as above, one gets:

$\beta = m_{deg}(\infty) / m_{tot} = k_{deg} / k_{tot}$ eq [19]

Combining eq [6] and eq [19], k_{deg} can be calculated as:

$k_{deg} = k_{tot} (m_{tot} - m_{vol}(\infty)) / m_{tot}$ eq [20]

m_{tot} is known,
$m_{vol}(\infty)$ can be estimated by fitting eq [16],
k_{tot} can be estimated by fitting eq [13] or eq [16].

In summary, to be able to estimate k_{deg}, what is needed is the overall dissipation rate constant (k_{tot}), the starting mass of radioactivity (m_{tot}) and the fitted amount of volatilised radioactivity if all substance has dissipated ($m_{vol}(\infty)$)