ECHA
EUROPEAN CHEMICALS AGENCY

How to report robust study summaries
Practical Guide 3

Version 2.0 – November 2012

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Version	Changes	
Version 1	First edition	March 2010
Version 2	Revision of the Practical guide addressing structure and content in relation to the updated sub-chapter R.7.1 'Physicochemical properties' within the 'Guidance on information requirements and chemical safety assessment R.7a: Endpoint specific guidance' and new or revised OECD Test Guidelines. The update includes the following: • To synchronise Section 3: 'Endpoint specific information for physicochemical endpoints' within this Practical Guide, with updated sub-chapter R.7.1 'Physicochemical properties'. The update of R.7.1 was necessary because the criteria in Article 14 of the REACH Regulation for determining whether a Chemical Safety Assessment needs to be carried out, have been amended to refer to the CLP Regulation rather than the Dangerous Substances Directive. • To update Section 4 and 5 on the endpoint specific information for environmental and human health endpoints, respectively; with new and revised OECD Test Guidelines, e.g. OECD TG 305 Bioaccumulation in Fish: Aqueous and Dietary Exposure; OECD TG 443 Extended One-Generation Reproductive Toxicity Study; OECD TG 405 Acute Eye Irritation/Corrosion. • To revise Attachment 1 by adding additional supporting text under 'Rationale for reliability incl. deficiencies'.	November 2012

Practical Guide 3: How to report robust study summaries

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PREFACE

The purpose of this practical guide is to assist registrants in preparing the Robust Study Summaries for all endpoints that need to be included in the IUCLID registration dossier depending on the information requirements under REACH Regulation.

The information given in this practical guide does not describe the requirements to pass the technical completeness check which are illustrated in the Dossier Submission Manual (No. 05 - How to complete a technical dossier for registrations and PPORD notifications). They should be seen as guide for preparation of robust study summaries that contain advice to allow thorough evaluation and derivation of conclusions for classification and labelling and/or risk assessment.

1. Introduction

In order to demonstrate the safe use of substances, registrants need to fulfil the information requirements as stipulated in Articles 10 and 12 in conjunction with the Annexes VII-X and XI to the REACH Regulation 1907/2006/EC.

Full study reports for each endpoint should not be added in the technical dossier, but **robust study summaries** (**RSS**) or **study summaries** must be provided.

A RSS is a detailed summary of the objectives, methods, results and conclusions of a full study report providing sufficient information to make an independent assessment of the study minimising the need to consult the full study report (Article 3 (28) of REACH). A study summary is a summary of the objectives, methods, results and conclusions of a full study report providing sufficient information to make an assessment of the relevance of the study (Article 3 (29) of REACH).

The aim of this manual is to assist registrants in preparing the individual RSS of the technical dossier for the following sections of the IUCLID file:

IUCLID section 4: Physical and chemical properties

IUCLID section 5: Environmental fate and pathways

IUCLID section 6: Ecotoxicological information

IUCLID section 7: Toxicological information

IUCLID section 8: Analytical methods

This practical guide describes in detail which study characteristics should be reported for the individual endpoints listed in the IUCLID sections described above. The endpoints described in this practical guide are structured according to the IUCLID section numbering and cover all the standard information requirements listed in the REACH Annexes VII to X.

1.1. When to provide a Robust Study Summary (RSS) or Study Summary

For the preparation of the registration dossier registrants are required by the REACH Regulation to evaluate all available information. This process includes the evaluation of the quality of data (relevance, adequacy and reliability), the selection of key study(ies) for each endpoint and the drafting of the relevant RSSs or study summaries as described in the Guidance on Registration.

Article 14 (1) in conjunction with Annex I and Article 10 (a)(vii) of the REACH Regulation require the provision of RSSs for information derived from the application of Annexes VII to XI for substances above 10 tonnes per year. Annex I (1.1.4 & 3.1.5) describes the conditions under which RSS shall be prepared and submitted. Normally, the study or studies giving rise to the highest concern and that are used to derive conclusions in the chemical safety assessment shall be subject to RSS. In general, for all studies providing data that are used in the hazard assessment, a RSS is recommended.

Furthermore, the Guidance on Registration (p. 92) recommends registrants to provide RSS in the technical dossier for all key studies, including for substances manufactured and/or imported at less than 10 tonnes per year. This would facilitate the evaluation conducted by the Agency and the Member States, as well as save resources of the registrant in case of a tonnage update. For the same reason, it is recommended that registrants use RSS also for covering physicochemical endpoints under section 4 of the IUCLID file.

In case a weight of evidence (WoE) approach is used, it is highly recommended to provide a RSS for all studies that are provided. Especially in case of conflicting data, good RSS ensure a transparent assessment of the data adequacy, relevance and reliability. In certain cases, several key studies might be available. In this case, RSS are required for all key studies.

In addition, other studies may also require detailed descriptions if they are of possible relevance. In particular, for studies that are flawed, but indicate critical results, robust study summaries highlighting the weaknesses of the studies need to be prepared as well. Such studies flagged as 'disregarded study' in field 'Purpose flag' in IUCLID.

For all other available studies, used as supporting information in the assessment of the substance, only a study summary needs to be provided in the technical dossier as for these studies less details are necessary. For technical details on study summaries please consult section 6 of this practical guide.

For further background information, please consult the Guidance on Registration, which contains additional information on this subject in relation to REACH obligations in section "*5.2.4 Information requirements on intrinsic properties (Annexes VII to X)*".

2. General aspects related to the preparation of a robust study summary (RSS)

2.1 General instruction

In order to prepare a complete RSS, detailed information on the applied methodology, test materials, the study results and conclusions have to be provided in the structured fields of IUCLID 5. It should be also demonstrated whether specific validity, quality, or repeatability criteria for the study have been met as specified in the description of the corresponding (EU or OECD) test method. In the "Applicant's summary and conclusions" field of the study record endpoint it should be clear 1) whether or not the validity criteria have been fulfilled and 2) which conclusions were derived from the underlying data.

The following issues listed below may hamper the evaluation of the adequacy and/or the relevance of the study including lack of sufficient information on:

- any missing administrative data (e.g. purpose flag, study result type, reliability, etc.),
- any non justified deviation from the test protocol chosen,
- whether the study was conducted according to GLP (Please note that this point is strictly required for all endpoints apart from the physicochemical endpoints where it is recommended),
- reference of the study,
- test substance such as name of test material, its form and physical state, composition, purity, impurities, accuracy, etc.,
- test organism including information on the species, its source, age at study initiation, size and weight, method of breeding, feeding, and acclimatisation (this point is relevant only to the eco-toxicity and toxicity endpoints)
- study design,
- detailed description of test conditions,
- results and discussion, etc.

Missing information may raise questions on the validity of the study and the conclusions drawn regarding classification and labelling and/or risk assessment and finally may lead to a data gap of the information required by REACH.

2.2 General aspects related to information common for all endpoints

In order to report a RSS in IUCLID 5 the option of 'all fields' in the header of the endpoint study record has to be selected. To fill-in the proper IUCLID fields, the registrant should follow the guidance provided in the IUCLID 5 End User manual[1] which is available in 22 different EU languages.

In IUCLID, a RSS for each endpoint is composed of the common general part and the endpoint specific part, dependent on the applied methodology and characteristic for each endpoint. The RSS requirements on general information relevant for ALL endpoints related to (non-) testing methods are listed in the table below and presented in more detail in the sub-chapters below.

Administrative data
- Purpose flag (pick-list)
- Robust study summary (checkbox)
- Study result type (pick-list)
- Reliability (pick-list)
- Rationale for reliability

Data source
- Complete reference
- Data access (pick-list)
- Data protection claimed (pick-list)

Materials and methods
- Method/guideline followed (pick-list or description if different from the pick-list)
- Principles of method if other than guideline
- GLP compliance

Test materials
- Identity of test material same as for substance defined in section 1 (if not read-across)
- Test material identity
- Details on test material (if other than submission substance)
- Details on properties of test surrogate or analogue material

Conclusions
- Detail relevant observations and dose response relationship
- Report on any unusual results or observations

2.2.1 General aspects related to the administrative data

The main aim of this part of the RSS is to identify the purpose of the record (e.g., "key study"), the type of result (e.g., "experimental study"), data waiving indication (if any), reliability indication, and flags for indicating the regulatory purpose envisaged and/or any confidentiality restrictions. This kind of data characterises the relevance of a RSS and are therefore valid and repeated for each endpoint. In order to fulfil requirements related to the administrative data the following should be addressed:

1 http://guidance.echa.europa.eu/docs/guidance_document/iuclid_en.pdf

- Purpose flag (pick-list)
- Robust study summary (checkbox)
- Study result type (pick-list)
- Reliability (pick-list)
- Rationale for reliability incl. deficiencies

2.2.2 General aspects related to data source

The data source information is mainly related to the full reference of the study. For assessing the reliability of the study, it is necessary to know the correct and complete bibliographic reference of the study report or publication the study summary is based on. Therefore the information related to the data source section of RSS should comprise:

- Complete reference (including the year when the study was performed)
- Data access (pick-list)
- Data protection claimed (pick-list)

Note: The IUCLID 5 CSR plug-in captures the fields "Author" and "Year" for specifying the bibliographic citations in the overview tables. To avoid any manual intervention, it is recommended to complete these fields in the relevant endpoint study records. If no individuals are cited as authors, enter name of company or organisation or 'Anon.' as appropriate.

2.2.3 General aspects related to materials and methods

The information related to the materials and methods should comprise:

- Method/guideline followed (pick-list or description if different from the pick-list)
- Principles of method if other than guideline
- GLP compliance

Note that all deviations from guideline methods should be described, identified and reported. Moreover, if no guideline was followed, it is necessary to include a description of the principles of the test protocol or estimated method used in the study. Details should be entered in appropriate distinct fields of section MATERIALS AND METHODS if available. It is also required to provide a justification for using this method if appropriate.

If an estimation method was used it is necessary to state the equation(s) and/or computer software or other methods applied to calculate the value(s).

2.2.4 General aspects related to test materials

The description of the test material should give detailed information on the tested substance and is comprised of the following issues:

- Information on whether the identity of the test material is the same as for the substance defined in IUCLID section 1 (if not read-across). In case read-across is used select “no” in the drop down menu "Test material same as for substance defined in section 1 (if not read-across)”.
- Information on test material identity.
- Details on test material (if other than submission substance).

- Details on properties of test surrogate or analogue material.

It is important to note that any deviations of the registered substance should be listed (e.g. amount of impurities). Moreover, all possible effects of the deviation from the registered substance to the obtained test results should be analysed and reported in the RSS.

2.2.5 General aspects related to results and discussions and to applicants summary and conclusions

In this part of the RSS the results and the conclusions should be reported. The summary of all observations and when relevant any concentration/dose response relationship should be presented preferably in the tabular form. Furthermore, a summary on how any effects observed in the study are relevant for classification and labelling and how they can be used in risk assessment should be provided.

The discussion on any significant deviations from the guideline should be reported including anything unusual about the test and other relevant information which might have influenced the results.

The validity (or quality/repeatability) criteria of the applied testing method should be fulfilled and this should be clearly stated and their fulfilment should be conclusive from the details included in the RSS following the OECD or EC test guidelines as required by REACH.

Note: In case the CSR is generated by the IUCLID 5 CSR plug-in it should be taken into account that only selected IUCLID 5 fields are captured by this tool. In general, you are requested to provide results in the IUCLID "Result repeatable block fields" for each endpoint study record. It will allow you to transfer automatically information from these result fields to the CSR when the IUCLID 5 CSR plug-in is used. The list of fields to be filled in the "Results and discussion" block will vary depending on the endpoint. Therefore we recommend to consult the Data Submission Manual 5 "How to complete a technical dossier for registrations and PPORD notifications" available in the ECHA website at

http://echa.europa.eu/web/guest/support/dossier-submission-tools/reach-it/data-submission-industry-user-manuals , for instructions on how to fill the results.

In addition it is advised to create **endpoint summaries** for each endpoint, where relevant, where under "Discussion" you can include the overall summary on how any effects observed in the study(ies) are relevant for classification and labelling and how they can be used in risk assessment taking into account all the studies available for this endpoint. This information can then be automatically transferred in the CSR when the IUCLID 5 CSR plug-in is used.

For further information on the CSR Tool plug-in, please refer to the corresponding User Manual for more information:

http://iuclid.echa.europa.eu/index.php?fuseaction=home.documentation&type=public

3. Endpoint specific information for Physicochemical endpoints

In IUCLID, a RSS for each physicochemical endpoint is composed of the common general parts described in detail in section 2 and the endpoint specific parts, dependent on the applied methodology and characteristic for each endpoint.

The general aspects described in section 2 should be applied for all endpoints described below. In addition detailed information for each physicochemical endpoint necessary in order to build a complete RSS is listed in the endpoint boxes in the sub-chapters below.

All endpoint specific characteristics should be described in such a way that the RSS allow an independent assessment of the endpoints' reliability and completeness. The objectives, methods, results and conclusions of the full study report should be reported in a transparent manner as described for all other endpoints in this practical guide.

3.1 State of the substance at 20°C and 101,3 kPa (appearance / physical state / colour)

Materials and methods

- temperature (°C) (if under non-standard conditions)
- pressure value and unit

Results and discussion

- physical state (gaseous, liquid or solid)
- form (e.g. compact, crystalline, fibre, filaments, flakes, particulates, paste, pellets, powder, or viscous liquid etc.)
- colour
- odour
- other remarks related to physical state, appearance or colour

Reference to other ECHA Guidance Documents

Further detailed guidance physical state of the substance can be found in:

- IUCLID 5 End User Manual in the following chapter:

IUCLID Section	REACH Annex	Endpoint title	IUCLID End User Manual Chapter
4.1	VII.7.1	Appearance/physical state/colour	E.4.2

3.2 Melting point/ freezing point

Materials and methods

- type of method or reference to the standard or the test method applied.

Results and discussion

- melting point value (°C) as measured;
- rate of temperature increase if available;
- decomposition or sublimation temperature (if applicable);
- measurement uncertainty if available;
- if testing is waived, the reasons for waiving must be documented in the dossier.

Any deviation from the guideline method used (and reasons for it) or any other special consideration should be reported. In cases where there is more than one source of data, the endpoint summary under results and discussion should provide a justification for the selection of the key study chapter.

Reference to other ECHA Guidance Documents

Further detailed guidance on melting point/ freezing point can be found in:

- Guidance on information requirements and chemical safety assessment: Chapter R7a, section: R.7.1.2.
- IUCLID 5 End User Manual in the following chapters:

IUCLID Section	REACH Annex	Endpoint title	IUCLID End User Manual Chapter
4.2	VII 7.2	Melting point/freezing point	E.4.3

3.3 Boiling point

Materials and methods

- type of method or reference to the standard or the test method applied

Results and discussion

- boiling point value (°C) as measured;
- pressure value and unit;
- rate of temperature increase if available;
- decomposition (if applicable);
- measurement uncertainty if available;
- boiling/melting point value in ºC (corrected to standard pressure, except where the boiling point has been determined at specified reduced pressures) (as above, but in a separate block of fields);
- if testing is waived, the reasons for waiving must be documented in the dossier.

Note: In cases where the boiling point is determined at reduced pressure a determination at ambient pressure is obviously not possible. A boiling point at standard pressure could then only be derived by extrapolation of the vapour pressure curve in cases where a vapour pressure curve is known. Even in such cases this corrected/extrapolated boiling point could only be nominal one and would be potentially misleading because it is not possible to determine it at ambient pressure.

Any deviation from the guideline method used (and reasons for it) or any other special consideration should be reported. In cases where there is more than one source of data, the endpoint summary under results and discussion should provide a justification for the selection of the key study chapter.

Reference to other ECHA Guidance Documents

Further detailed guidance on boiling point can be found in:

- Guidance on information requirements and chemical safety assessment: Chapter R7a, section: R.7.1.3.
- IUCLID 5 End User Manual in the following chapters:

IUCLID Section	REACH Annex	Endpoint title	IUCLID End User Manual Chapter
4.3	VII 7.3	Boiling point	E.4.4

3.4 Density (relative density)

Materials and methods

- type of method or reference to the standard or the test method applied.

Results and discussion

- temperature (°C);
- relative (for gases)/ absolute (for liquids and solids) density value (dimensionless);
- measurement uncertainty if available;
- if testing is waived, the reasons for waiving must be documented in the dossier.

Any deviation from the guideline method used (and reasons for it) or any other special consideration should be reported. In cases where there is more than one source of data, the endpoint summary under results and discussion should provide a justification for the selection of the key study chapter.

Reference to other ECHA Guidance Documents

Further detailed guidance on relative density can be found in:

- Guidance on information requirements and chemical safety assessment: Chapter R7a, section: R.7.1.4.
- IUCLID 5 End User Manual in the following chapter:

IUCLID Section	REACH Annex	Endpoint title	IUCLID End User Manual Chapter
4.4	VII 7.4	Density	E.4.5

3.5 Particle size distribution (Granulometry)

Material and methods

- sample preparation, such as any sonication, grinding, or addition of dispersion agents (if any);
- if a suspending medium is used (e.g. sedimentation test): indicate type of medium, temperature, pH, concentration and solubility of the substance in the suspending medium;
- the type of method used.

Results and discussion

- in the particle size field: mean and standard deviation;
- in the particle size distribution at different passages field: size and distribution;
- approximate information on particle shape (e.g. spherical, plate like, needle shaped) if available;
- for fibres: indicate both length and diameter of fibres.

Any deviation from the guideline method used (and reasons for it) or any other special consideration should be reported. In cases where there is more than one source of data, the endpoint summary under results and discussion should provide a justification for the selection of the key study chapter.

Reference to other ECHA Guidance Documents

Further detailed guidance on particle size distribution (Granulometry) can be found in:

- Guidance on information requirements and chemical safety assessment: Chapter R7a, section R.7.1.14.
- IUCLID 5 End User Manual in the following chapter:

IUCLID Section	REACH Annex	Endpoint title	IUCLID End User Manual Chapter
4.5	VII 7.14	Particle size distribution (Granulometry)	E.4.6

3.6 Vapour pressure

Materials and methods

- type of method or description of the apparatus or reference to the standard or the test method applied.

Results and discussion

- measured value of the vapour pressure for at least two temperatures
- temperature (°C)

estimate of the vapour pressure at 20 or 25 ºC (if not measured at these temperatures);

- if a transition (change of state, decomposition) is observed, the following should be noted:
  - nature of change;
  - temperature at which change occurs.

- if testing is waived, the reasons for waiving must be documented in the dossier.

Any deviation from the guideline method used (and reasons for it) or any other special consideration should be reported. In cases where there is more than one source of data, the endpoint summary under results and discussion should provide a justification for the selection of the key study chapter.

Reference to other ECHA Guidance Documents

Further detailed guidance on vapour pressure can be found in:

- Guidance on information requirements and chemical safety assessment: Chapter R7a, section: R.7.1.5.
- IUCLID 5 End User Manual in the following chapter:

IUCLID Section	REACH Annex	Endpoint title	IUCLID End User Manual Chapter
4.6	VII 7.5	Vapour pressure	E.4.7

3.7 Partition coefficient (n-octanol/water)

Materials and methods

Shake-flask method (EU A.8/ OECD TG 107):
- equilibrium concentrations of the test substance in both phases;
- relative volumes of the two phases;
- analytical method(s).

Calculation method (EU A.8):
- identification of the method;
- working principle of the method;
- reference to the method;
- information on source chosen to justify K_{ow} values of fragments being manipulated;
- applicability of the method.

HPLC method (EU A.8/OECD TG 117):
- column(s) used;
- mobile phase (composition, buffer, pH);
- reference substances with respective Kow values from the literature;
- concentrations measured.

Slow-stirring method (OECD TG 123):
- label purity of labelled chemicals and molar activity (where appropriate);
- sampling times;
- description of the test vessels and stirring conditions;
- number of replicates;
- temperature during the experiment;
- volumes of 1-octanol and water at the beginning, during and remaining after the test;
- determined concentrations of the test substance in 1-octanol and water as a function of time;
- description of the test vessels and stirring conditions (geometry of the stirring bar and of the test vessel, vortex height in mm, and when available: stirring rate) used;
- analytical methods used to determine the test substance (its repeatability and sensitivity) and the method limit of quantification;
- sampling times;

- pH of the aqueous phase and of the buffers used, when pH is adjusted for ionisable molecules;
- number of replicates;
- demonstration of mass balance;
- temperature and standard deviation or the range of temperature during the experiment;
- the regression of concentration ratio against time.

Results and discussion

- final value for log Kow;
- Kow values and their mean;
- standard deviations of individual Kow values;
- theoretical value when it has been calculated;
- temperature of the test solutions (°C);
- pH value(s) of the aqueous solution(s);
- composition and concentration of buffers;
- concentration of the stock solution;
- if testing is waived, the reasons for waiving must be documented in the dossier.

Any deviation from the guideline method used (and reasons for it) and reasons for it or any other special consideration should be reported. In cases where there is more than one source of data, the endpoint summary under results and discussion should provide a justification for the selection of the key study chapter.

Reference to other ECHA Guidance Documents

Further detailed guidance on partition coefficient can be found in:

- Guidance on information requirements and chemical safety assessment: Chapter R7a, section R.7.1.8.
- IUCLID 5 End User Manual in the following chapter:

IUCLID Section	REACH Annex	Endpoint title	IUCLID End User Manual Chapter
4.7	VII 7.8	Partition coefficient	E.4.8

3.8 Water solubility

Materials and methods

- description of the apparatus and dimensions or reference to the standard or the test method applied;
- results from preliminary test (if any);
- chemical identity and impurities (preliminary purification step, if any);
- water temperature during saturation process;
- analytical method employed;
- any evidence of chemical instability;
- all information relevant for the interpretation of the results.

If Column Elution method:

- concentrations, flow rates and pH for each sample;
- mean and standard deviations of five samples at least;
- average for each of two successive runs at least;
- nature and loading of support material;
- solvent used.

If Flask method:

- pH of each sample;
- individual analytical determinations and the average;
- average of the values for different flasks.

Results and discussion

- water solubility in (mg/L) at temperature (°C);
- pH value and concentration of test substance;
- description of solubility (if relevant);
- if testing is waived, the reasons for waiving must be documented in the dossier.

Any deviation from the guideline method used (and reasons for it) or any other special consideration should be reported. In cases where there is more than one source of data, the endpoint summary under results and discussion should provide a justification for the selection of the key study chapter.

Reference to other ECHA Guidance Documents

Further detailed guidance on water solubility can be found in:

- Guidance on information requirements and chemical safety assessment: Chapter R7a, section R.7.1.7.
- IUCLID 5 End User Manual in the following chapter:

IUCLID Section	REACH Annex	Endpoint title	IUCLID End User Manual Chapter
4.8	VII 7.7	Water solubility	E.4.9

3.9 Surface tension

Materials and methods

- description of the apparatus and dimensions or reference to the standard or the test method applied;
- test material identity: apart from general issues, if surface tension of active impurities affects results, it should be noted.

Results and discussion

- surface tension value and unit (preferably mN/m or N/m but other units are also acceptable);
- concentration of the solution[2];
- age of solution[1];
- type of water or solution used[1];
- results from repeated measures with varied equilibrium time (of the solution);
- several measurement results should be provided to assess the possible time-dependency of the measurement. Equilibration times may vary from minutes to hours. Measurements should be sufficient to prove that a constant surface tension was reached;
- if testing is waived, the reasons for waiving must be documented in the dossier.

Any deviation from the guideline method used (and reasons for it) or any other special consideration should be reported. In cases where there is more than one source of data, the endpoint summary under results and discussion should provide a justification for the selection of the key study chapter.

Reference to other ECHA Guidance Documents

Further detailed guidance on surface tension can be found in:

- Guidance on information requirements and chemical safety assessment: Chapter R7a, section R.7.1.6.
- IUCLID 5 End User Manual in the following chapter:

IUCLID Section	REACH Annex	Endpoint title	IUCLID End User Manual Chapter
4.10	VII 7.6	Surface tension	E.4.11

3.10 Flash point

Materials and methods

- reference to the standard or the test method applied;
- open cup or closed cup (for classification purposes only the closed cup methods are allowed);
- equilibrium or non-equilibrium method.

Results and discussion

- corrected flashpoint and unit;
- data on repeatability and reproducibility as given in the method;
- if testing is waived, the reasons for waiving must be documented in the dossier.

[2] As indicated in test A.5. Surface tension described in Council Regulation (EC) No 440/2008

Any deviation from the guideline method used (and reasons for it) or any other special consideration should be reported. In cases where there is more than one source of data, the endpoint summary under results and discussion should provide a justification for the selection of the key study chapter.

Reference to other ECHA Guidance Documents

Further detailed guidance on flash point can be found in:

- Guidance on information requirements and chemical safety assessment: Chapter R7a, section: R.7.1.9.
- IUCLID 5 End User Manual in the following chapter:

IUCLID Section	REACH Annex	Endpoint title	IUCLID End User Manual Chapter
4.11	VII 7.9	Flash point	E.4.12

3.11 Auto flammability (self-ignition temperature)

3.11.1 For gases and liquids (Auto-ignition)

Material and methods

- description of the apparatus or reference to the standard or the test method applied;
- quantity of sample used.

Results and discussion

- the value or the range of the auto-ignition temperature;
- if testing is waived, the reasons for waiving must be documented in the dossier.

For liquids/gases: observations (e.g decomposition with air, reactions with moisture, etc.)
For solids see the below chapter 3.11.2.

Any deviation from the guideline method used (and reasons for it) or any other special consideration should be reported. In cases where there is more than one source of data, the endpoint summary under results and discussion should provide a justification for the selection of the key study chapter.

Reference to other ECHA Guidance Documents

Further detailed guidance on auto flammability (self-ignition temperature) can be found in:

- Guidance on information requirements and chemical safety assessment: Chapter R7a, section R.7.1.12.
- IUCLID 5 End User Manual in the following chapter:

IUCLID Section	REACH Annex	Endpoint title	IUCLID End User Manual Chapter
4.12	VII 7.12	Auto flammability	E.4.13

3.11.2 For solids and liquids adsorbed on a large surface (Self-heating)

See section 3.12.7 of this guidance document for further details and information.

3.12 Flammability

3.12.1 Flammable gases

Material and methods

- description of the apparatus and dimensions or reference to the standard or the test method applied;
- test temperature;
- tested concentrations.

Results and discussion

- indicate lower and upper explosion limits in % volume;
- if testing is waived, the reasons for waiving must be documented in the dossier.

Any deviation from the guideline method used (and reasons for it) or any other special consideration should be reported. In cases where there is more than one source of data, the endpoint summary under results and discussion should provide a justification for the selection of the key study chapter.

Reference to other ECHA Guidance Documents

Further detailed guidance on flammability can be found in:

- Guidance on information requirements and chemical safety assessment: Chapter R7a, section R.7.1.10.1.
- IUCLID 5 End User Manual in the following chapter:

IUCLID Section	REACH Annex	Endpoint title	IUCLID End User Manual Chapter
4.13	VII 7.10	Flammability	E.4.14

3.12.2 Flammable liquids

Material and methods

See chapter 3.10 Flash point and chapter 3.3 Boiling point.

Results and discussion

- for solids: indicate burning time
- corrected flashpoint and unit;
- data on repeatability and reproducibility as given in the method;
- boiling point value (°C) as measured;
- pressure value and unit;
- rate of temperature increase;

- decomposition (if applicable);
- measurement uncertainty if available;
- boiling point value in ºC (corrected to standard pressure, except where the boiling point was determined at reduced pressures) (as above, but in a separate block of fields);
- if available explosion limits;
- if testing is waived, the reasons for waiving must be documented in the dossier.

Any deviation from the guideline method used (and reasons for it) or any other special consideration should be reported. In cases where there is more than one source of data, the endpoint summary under results and discussion should provide a justification for the selection of the key study chapter.

Reference to other ECHA Guidance Documents

Further detailed guidance on flammability can be found in:

- Guidance on information requirements and chemical safety assessment: Chapter R7a, section R.7.1.10.2.
- IUCLID 5 End User Manual in the following chapter:

IUCLID Section	REACH Annex	Endpoint title	IUCLID End User Manual Chapter
4.13	VII 7.10	Flammability	E.4.14

3.12.3 Flammable solids

Material and methods

- description of the apparatus and dimensions or reference to the standard or the test method applied**;**
- indicate if preliminary and/or main test performed;
- moisture content;
- particle size and distribution (if available) (see chapter 3.5 Particle size distribution (Granulometry)).

Results and discussion

- indicate burning time
- pass/non pass of the wetted zone (in the case of the UN Test N.1);
- if testing is waived, the reasons for waiving must be documented in the dossier.

Any deviation from the guideline method used (and reasons for it) or any other special consideration should be reported. In cases where there is more than one source of data, the endpoint summary under results and discussion should provide a justification for the selection of the key study chapter.

Reference to other ECHA Guidance Documents

Further detailed guidance on flammability can be found in:

- Guidance on information requirements and chemical safety assessment: Chapter R7a, section R.7.1.10.3.

- IUCLID 5 End User Manual in the following chapter:

IUCLID Section	REACH Annex	Endpoint title	IUCLID End User Manual Chapter
4.13	VII 7.10	Flammability	E.4.14

3.12.4 Self-reactive substances and mixtures

Material and methods

- See UN Recommendations on the Transport of Dangerous Goods, Manual of Test and Criteria, Part II, classification procedures and test series A-H.

Results and discussion

- type of self-reactive substance;
- decomposition energy (value and method of determination);
- SADT (Self accelerating decomposition temperature) together with the volume the SADT relates to;
- detonation properties (Yes/Partial/No);
- deflagration properties (Yes rapidly/Yes slowly/No);
- effect of heating under confinement (Violent/Medium/Low/No);
- explosive power if applicable (Not low/Low/None).

For assigning the type of self-reactive substance, the list of currently assigned self-reactive substances according to the 2.4.2.3.2.3 of the UN Recommendations on the Transport of Dangerous Goods, Model Regulations can be used, in cases where the assignment was based on test(s) according to the UN Manual of Tests and Criteria. The relevant underlying test data may be collected from the respective UN documents from the UN Committee of experts on the transport of dangerous goods, from test reports produced by competent authorities or industry, or from other reliable sources.

Any deviation from the guideline method used (and reasons for it) or any other special consideration should be reported. In cases where there is more than one source of data, the endpoint summary under results and discussion should provide a justification for the selection of the key study chapter.

An example on how the data mentioned above could be documented in the chemical safety report (CSR) is presented in IR/CSA Chapter R.7a: Endpoint specific guidance, Figure R.7.1-2.

Reference to other ECHA Guidance Documents

A template data set does not currently exist in IUCLID for the hazard class “self-reactive substances”. As long as there is no specific section available in IUCLID the test results in IUCLID section 4.23 “Additional physico-chemical information” under the endpoint title “Self-reactive substances” should be inserted. In the CSR the information should be included under flammability.

3.12.5 Pyrophoric liquids

Material and methods

- Description of the apparatus and dimensions or reference to the standard or the test method applied.

Note that in this case the experience in handling may be sufficient.

Results and discussion

- whether ignition occurs when poured or whether the filter paper is charred;
- if testing is waived, the reasons for waiving must be documented in the dossier.

Any deviation from the guideline method used (and reasons for it) or any other special consideration should be reported. In cases where there is more than one source of data, the endpoint summary under results and discussion should provide a justification for the selection of the key study chapter.

Reference to other ECHA Guidance Documents

Further detailed guidance on flammability can be found in:

- Guidance on information requirements and chemical safety assessment: Chapter R7a, section R.7.1.10.5.
- IUCLID 5 End User Manual in the following chapter:

IUCLID Section	REACH Annex	Endpoint title	IUCLID End User Manual Chapter
4.13	VII 7.10	Flammability	E.4.14

3.12.6 Pyrophoric solids

Material and methods

- description of the apparatus and dimensions or reference to the standard or the test method applied;
- particle size and distribution (if practicable);

Note that in this case experience in handling may be sufficient.

Results and discussion

- whether ignition occurs when poured;
- if testing is waived, the reasons for waiving must be documented in the dossier.

Any deviation from the guideline method used (and reasons for it) or any other special consideration should be reported. In cases where there is more than one source of data, the endpoint summary under results and discussion should provide a justification for the selection of the key study chapter.

Reference to other ECHA Guidance Documents

Further detailed guidance on flammability can be found in:

- Guidance on information requirements and chemical safety assessment: Chapter R7a, section R.7.1.10.6.
- IUCLID 5 End User Manual in the following chapter:

IUCLID Section	REACH Annex	Endpoint title	IUCLID End User Manual Chapter
4.13	VII 7.10	Flammability	E.4.14

3.12.7 Self-heating substances and mixtures

Material and methods

- description of the apparatus and dimensions or reference to the standard or the test method applied;
- indicate if preliminary and/or main test performed;
- moisture content;
- particle size and distribution (if available).

Results and discussion

- indicate temperature rise obtained for the individual tests and classification result.

Any deviation from the guideline method used (and reasons for it) or any other special consideration should be reported. In cases where there is more than one source of data, the endpoint summary under results and discussion should provide a justification for the selection of the key study chapter.

Reference to other ECHA Guidance Documents

Further detailed guidance on flammability can be found in:

- Guidance on information requirements and chemical safety assessment: Chapter R7a, section R.7.1.10.7.
- IUCLID 5 End User Manual in the following chapter:

IUCLID Section	REACH Annex	Endpoint title	IUCLID End User Manual Chapter
4.13	VII 7.10	Flammability	E.4.14

3.12.8 Substances and mixtures which in contact with water emit flammable gases

Material and methods

- description of the apparatus and dimensions or reference to the standard or the test method applied;
- partice size and distribution.

Results and discussion

- indicate whether full test was performed or whether it was terminated at a particular

step/stage;
• substance identity of evolved gas;
• indicate whether the gas evolved ignites spontaneously;
• rate of gas evolution (unless the test has been terminated);
• if testing is waived, the reasons for waiving must be documented in the dossier.

Any deviation from the guideline method used (and reasons for it) or any other special consideration should be reported. In cases where there is more than one source of data, the endpoint summary under results and discussion should provide a justification for the selection of the key study chapter.

Reference to other ECHA Guidance Documents

Further detailed guidance on flammability can be found in:

- Guidance on information requirements and chemical safety assessment: Chapter R7a, section R.7.1.10.8.
- IUCLID 5 End User Manual in the following chapter:

IUCLID Section	REACH Annex	Endpoint title	IUCLID End User Manual Chapter
4.13	VII 7.10	Flammability	E.4.14

3.12.9 Organic peroxides

Material and methods

- See UN Recommendations on the Transport of Dangerous Goods, Manual of Test and Criteria, Part II, classification procedures and test series A-H.

Results and discussion

• if testing is waived, the reasons for waiving must be documented in the dossier;
• type of organic peroxide;
• SADT (Self accelerating decomposition temperature) together with the volume the SADT related to;
• detonation properties (Yes/Partial/No);
• deflagration properties (Yes rapidly/Yes slowly/No);
• effect of heating under confinement (Violent/Medium/Low/No);
• explosive power, if applicable (Not low/Low/None).

For assigning the Type of organic peroxide, the list of currently assigned organic peroxides according 2.5.3.2.4 of the UN RTDG can be used, in case the assignment was based on a test according to the UN MTC. The relevant underlying test data may be collected from the respective UN documents from the UN Committee of experts on the transport of dangerous goods, from test reports produced by either competent authorities or industry, or from other reliable sources (such as e.g. the dedicated database 'DATATOP').

Any deviation from the guideline method used (and reasons for it) or any other special consideration should be reported. In cases where there is more than one source of data, the endpoint summary under results and discussion should provide a justification for the selection of the key study chapter.

An example on how the data mentioned above could be documented in the chemical safety report (CSR) is presented in IR/CSA Chapter R.7a: Endpoint specific guidance, Figure R.7.1-3.

Reference to other ECHA Guidance Documents

Further detailed guidance on flammability can be found in:

- Guidance on information requirements and chemical safety assessment: Chapter R7a, section R.7.1.10.9.
- IUCLID 5 End User Manual in the following chapter:

IUCLID Section	REACH Annex	Endpoint title	IUCLID End User Manual Chapter
4.13	VII 7.10	Flammability	E.4.14

3.13 Explosiveness (explosive properties)

Material and methods

- Reference to the standard and the test method applied;
- Description of the substance that was tested.

Results and discussion

- if testing is waived, the reasons for waiving must be documented in the dossier;
- if testing is not waived then the tests done according to the UN Recommendations on the Transport of Dangerous Goods, Manual of Tests and Criteria and the outcome (explosive or not explosive) must be documented in the dossier. The mechanical sensitivity test according to UN Test Series 3a and 3b must be done and documented if UN Test Series 1 or 2 give a positive result. If data according to test A.14 are available, then the results can be used instead of UN Test series 3a and 3b.

Any deviation from the guideline method used (and reasons for it) or any other special consideration should be reported. In cases where there is more than one source of data, the endpoint summary under results and discussion should provide a justification for the selection of the key study chapter.

Reference to other ECHA Guidance Documents

Further detailed guidance on explosive properties can be found in:

- Guidance on information requirements and chemical safety assessment: Chapter R7a, section R.7.1.11.
- IUCLID 5 End User Manual in the following chapter:

IUCLID Section	REACH Annex	Endpoint title	IUCLID End User Manual Chapter
4.14	VII 7.11	Explosiveness	E.4.15

3.14 Oxidising properties

3.14.1 Oxidising properties: oxidising gases

Material and methods

- reference to the standard applied.

Results and discussion

- if the test is positive indicate that the gas is ‘oxidising’;
- if testing is waived, the reasons for waiving must be documented in the dossier.

Any deviation from the guideline method used (and reasons for it) or any other special consideration should be reported. In cases where there is more than one source of data, the endpoint summary under results and discussion should provide a justification for the selection of the key study chapter.

Reference to other ECHA Guidance Documents

Further detailed guidance on oxidising properties can be found in:

- Guidance on information requirements and chemical safety assessment: Chapter R7a, section: R.7.1.13.1.
- IUCLID 5 End User Manual in the following chapter:

IUCLID Section	REACH Annex	Endpoint title	IUCLID End User Manual Chapter
4.15	VII 7.13	Oxidising properties	E.4.16

3.14.2 Oxidising properties: oxidising liquids

Material and methods

- description of the apparatus and dimensions or reference to the standard or the test method applied.

Results and discussion

- indicate the results of the spontaneous ignition test;
- indicate the mean pressure rise time for the test substance;
- indicate the mean pressure rise time for the reference substance(s);
- interpretation of results;
- if testing is waived, the reasons for waiving must be documented in the dossier.

Any deviation from the guideline method used (and reasons for it) or any other special consideration should be reported. In cases where there is more than one source of data, the endpoint summary under results and discussion should provide a justification for the selection of the key study chapter.

Reference to other ECHA Guidance Documents

Further detailed guidance on oxidising properties can be found in:

- Guidance on information requirements and chemical safety assessment: Chapter R7a, section: R.7.1.13.2.
- IUCLID 5 End User Manual in the following chapter:

IUCLID Section	REACH Annex	Endpoint title	IUCLID End User Manual Chapter
4.15	VII 7.13	Oxidising properties	E.4.16

3.14.3 Oxidising properties: oxidizing solids

Material and methods

- description of the apparatus and dimensions or reference to the standard or the test method applied;
- particle size and distribution.

Results and discussion

- if testing is waived, the reasons for waiving must be documented in the dossier;

If the UN Test O.1 was used:
indicate if a vigorous reaction was observed;
indicate the maximum burning time for the test mixture;
indicate the maximum burning time for the reference mixtures;
interpretation of results, including any relevant special observations;
estimated accuracy of the result (including bias and precision).

If A.17 test method was used:
indicate if in the preliminary test, a vigorous reaction was observed;
indicate the maximum burning rate for the test mixture;
indicate the maximum burning rate for the reference mixture;
interpretation of results, including any relevant special observations;
estimated accuracy of the result (including bias and precision).

Any deviation from the guideline method used (and reasons for it) or any other special consideration should be reported. In cases where there is more than one source of data, the endpoint summary under results and discussion should provide a justification for the selection of the key study chapter.

Reference to other ECHA Guidance Documents

Further detailed guidance on oxidising properties can be found in:

- Guidance on information requirements and chemical safety assessment: Chapter R7a, section: R.7.1.13.3.
- IUCLID 5 End User Manual in the following chapter:

IUCLID Section	REACH Annex	Endpoint title	IUCLID End User Manual Chapter
4.15	VII 7.13	Oxidising properties	E.4.16

3.15 Stability in organic solvents and identity of relevant degradation products

This endpoint needs to be fulfilled on a case by case basis. As several different methods can be used to document this intrinsic property, we recommend the same strategy for drafting RSS as described for the other endpoints. The general aspects described in section 2 should also be applied for this endpoint. All endpoint specific characteristics should be described in such a way that the RSS allows an independent assessment of the endpoints reliability and completeness. The objectives, methods, results and conclusions of the full study report should be reported in a transparent manner as described for all other endpoints in this practical guide.

Any deviation from the guideline method used (and reasons for it) or any other special consideration should be reported. In cases where there is more than one source of data, the endpoint summary under results and discussion should provide a justification for the selection of the key study chapter.

Reference to other ECHA Guidance Documents

Further detailed guidance on stability in organic solvents can be found in:

- Guidance on information requirements and chemical safety assessment: Chapter R7a, section: R.7.1.16.
- IUCLID 5 End User Manual in the following chapter:

IUCLID Section	REACH Annex	Endpoint title	IUCLID End User Manual Chapter
4.17	IX 7.15	Stability in organic solvents and identity of relevant degradation products	E.4.18

3.16 Dissociation constant

Materials and methods

- type of method;
- test guideline followed.

Test Materials

- test material identity.

Results and discussion

- concentration of the substance;
- test results as pKa-value(s);
- temperature of the test medium (ºC);
- if testing is waived, the reasons for waiving must be documented in the dossier.

Any deviation from the guideline method used (and reasons for it) or any other special consideration should be reported. In cases where there is more than one source of data, the endpoint summary under results and discussion should provide a justification for the selection of the key study chapter.

Reference to other ECHA Guidance Documents

Further detailed guidance on dissociation constant can be found in:

- Guidance on information requirements and chemical safety assessment: Chapter R7a, section: R.7.1.17.
- IUCLID 5 End User Manual in the following chapter:

IUCLID Section	REACH Annex	Endpoint title	IUCLID End User Manual Chapter
4.21	IX 7.16	Dissociation constant	E.4.22

3.17 Viscosity

Materials and methods

- type of method;
- test guideline followed.

Results and discussion

- viscosity value and unit according to the used test method;
- preferred units are m Pa·s (for dynamic viscosity) and mm^2/s (for static viscosity) but other units are also accepted;
- each measured value should be accompanied with temperature (in °C). Usually two values are needed. Preferably one value is measured in app. 20°C and another in app. 20°C higher temperature. Two determinations of viscosity should be measured for each temperature;
- for non-Newtonian liquids, the results obtained are preferred in the form of flow curves, which should be interpreted;
- individual and mean values should be provided at each temperature.[3]

Any deviation from the guideline method used (and reasons for it) or any other special consideration should be reported. In cases where there is more than one source of data, the endpoint summary under results and discussion should provide a justification for the selection of the key study chapter.

Reference to other ECHA Guidance Documents

Further detailed guidance on viscosity can be found in:

- Guidance on information requirements and chemical safety assessment: Chapter R7a, section: R.7.1.18.
- IUCLID 5 End User Manual in the following chapter:

IUCLID Section	REACH Annex	Endpoint title	IUCLID End User Manual Chapter
4.22	IX 7.17	Viscosity	E.4.23

[3] From OECD Guideline 114 ”Viscosity of liquids”

4. Endpoint specific information for Environmental endpoints

In IUCLID, a RSS for each environmental endpoint is composed of the common general parts described in detail in the section 2 and the endpoint specific parts, dependent on the applied methodology and characteristic for each endpoint.

The general aspects described in section 2 should be applied for all of the endpoints described below. In addition detailed information for each environmental endpoint necessary in order to build a complete RSS is listed in the following sub-chapters.

All endpoint specific characteristics should be described in such a way that the RSS allows an independent assessment of the endpoints reliability and completeness. The objectives, methods, results and conclusions of the full study report should be reported in a transparent manner as described for all other endpoints in this practical guide.

4.1 Endpoint specific information related to the environmental fate

The information necessary to prepare a RSS for each environmental fate endpoint is listed in the sub-chapters below. An example on the IUCLID RSS for biodegradation in water can be found in Annex 1.

4.1.1 Stability (Hydrolysis as function of pH)

Materials and methods

- test conditions: pH and temperature; description of the incubation system used; test duration;
- test design: sampling times; number of replicates; volume of buffered test substance solutions incubated;
- details on buffer solutions (i.e. pHs and reagents used);
- details on the test substance adherence to the equipment used;
- amount of test substance applied;
- solvents (type and amount) used for application of the test substance;
- method(s) of extraction;
- methods for quantification and identification of the test substance and its hydrolysis products; repeatability and sensitivity of the analytical methods;

Results and discussion

- half-life or DT50 for the different pHs and temperatures tested
- recoveries;
- mass balance during and at the end of the studies (when labelled test substance is used);
- results of preliminary test;
- identity of degradation products (if any).

Reference to other ECHA Guidance Documents

Further detailed guidance on stability can be found in:

- Guidance on information requirements and chemical safety assessment, Volume 5: Chapter R7b, section: R.7.9.

- IUCLID 5 End User Manual in the following chapters:

IUCLID Section	REACH Annex	Endpoint title	IUCLID End User Manual Chapter
5.1.2	VIII 9.2.2.1	Hydrolysis	E.5.2.3
5.1.1	X 9.3.4	Phototransformation in air	E.5.2.2
5.1.3	X 9.3.4	Phototransformation in water	E.5.2.4
5.1.4	X 9.3.4	Phototransformation in soil	E.5.2.5

4.1.2 Biodegradation

For IUCLID example on robust study summary for biodegradation screening test please see Annex 1.

Screening test

Materials and methods

- details on inoculum (nature and sampling site(s), concentration and any pre-conditioning treatment – any adaptation to be mentioned specifically)
- duration of test
- details on test conditions (composition of medium, test temperature, pH, CEC (meq/100g), continuous darkness: yes/no, etc).
- oxygen conditions (if relevant, the oxygen uptake of the inoculum blank (mg 0_2/l) after 28 d or oxygen depletion in the inoculum blank after 28 d and the residual concentration of oxygen in the test bottles)
- initial test substance concentration, vehicle used, pre-acclimatisation
- information on controls and blank system used
- details on sampling: (frequency, method and sterility)
- details on analytical method to measure biodegradation
- identity of reference substance(s) used
- parameter followed for degradation estimation
- method of calculating measured concentrations (arithmetic mean, geometric mean, etc.)

Results and discussion& Applicant's Summary and conclusion (interpretation of results)

- degradation % after time, comparison with threshold criterion for the specific guideline, including the result at the end of a 10-day window (does not apply to the MITI method, see the test method for the definition of the 10-day window)
- degradation results presented preferably with graphs of percentage degradation against time for the test and reference substances, the lag phase, degradation phase, the 10-d window and slope; if no graph then at least indication of the duration of the lag phase, the degradation phase and location of the 10-d window within the test period
- replicate values of the degradation % of the test chemical at the degradation rate at the plateau, in the end of test, and/or after 10-d window, as appropriate
- degradation % of the reference compound by day 14 (if relevant also after 7 days)
- degradation % within 14 days in a toxicity test containing both the test substance and a reference compound
- specific chemical analytical data, if available
- assessment of inhibition and, if observed, quantitative estimation of the effect(s) observed at

different concentrations
- any inhibition phenomena or unusual observations or other information affecting the results
- breakdown products: yes/no, if yes description of breakdown products and the information whether they are transient or stable
- if relevant, inorganic carbon (IC) content of the test substance suspension in the mineral medium at the beginning of the test and total carbon (TC) content
- if relevant, total $C0_2$ evolution in the inoculum blank at the end of the test.

Simulation tests (water, soil, sediment)

Materials and methods
- details on water/soil/sediment sample (e.g. location and description of sampling site including, if possible, contamination history; if relevant: organic C, clay content and soil texture, Cation Exchange Capacity and pH)
- duration of test
- details on test conditions (test temperature, pH, continuous darkness: yes/no, etc.)
- oxygen conditions
- amount of test substance applied, test concentration and reference substance concentration, solubilising agent if relevant
- information on controls and blank system used
- details on sampling: (e.g. frequency, method and sterility)
- repeatability and sensitivity of the analytical methods used including the limit of detection
- (LOD) and the limit of quantification (LOQ), recovery %
- identity of reference substance(s) used

Results and discussion
- half-life or DT50 , DT75 and DT90 for the test substance and, where appropriate, for major transformation products including confidence limits,
- averages of the results observed in individual replicates, for example length of lag phase, degradation rate constant and degradation half-life
- the results of the final mass balance check
- where appropriate, identification, molar concentration and percentage of applied of major transformation products, a proposed pathway of transformation
- where applicable, an assessment of transformation kinetics for the test substance and characterisation of non-extractable (bound) radioactivity or residues in soil
- where applicable, degradation % and time interval of degradation of the reference compound

Reference to other ECHA Guidance Documents

Further detailed guidance on biodegradation can be found in:

- Guidance on information requirements and chemical safety assessment, Volume 5: Chapter R7b, section R.7.9.
- IUCLID 5 End User Manual on the following chapter:

IUCLID Section	REACH Annex	Endpoint title	IUCLID End User Manual Chapter
5.2.1	VII 9.2.1.1	Biodegradation in water: screening tests	E.5.3.2

5.2.2	IX 9.2.1.2; IX 9.2.1.4	Biodegradation in water and sediment: simulation test	E.5.3.3
5.2.3	IX 9.2.1.3	Biodegradation in soil	E.5.3.4
5.2.4	X 9.3.4	Mode of degradation in actual use	E.5.3.5

4.1.3 Bioaccumulation

Fish Test (OECD TG 305)

(Can be applied for relevant parts also to a RSS on bioaccumulation by sediment organisms or soil organisms but in these cases the RSS will include also some specific additional information.)

Materials and methods

Aqueous exposure test:

- chemical identification data, such as IUPAC or CAS name, CAS number, and appropriate information on substance ID and the analytical methods in the case of UVCB
- test species and origin; include the scientific name of the species (if the name has changed, include both the new name and the name specified in the test report)
- test procedure and test design, nominal test concentrations, the means of the measured values and their standard deviations
- water source and quality within test vessels, detailed information on feeding, selected feeding rate, amount given and frequency
- information on the treatment of fish and water samples, methods used for treatment randomization and assignment of fish to test vessels
- date of introduction of test organisms to test solutions and test duration
- description of range-finding tests and results, if available

Dietary exposure test:

- all the above as for aqueous exposure
- any information on stability of the test substance in prepared food
- substance nominal concentration in food, spiking technique, amount of (lipid) vehicle used in food spiking process (if used), test substance concentration measurements in spiked diet for each analysis (at least in triplicate before study start and at end of uptake) and mean values
- if used, type and quality of carrier oil or solvent (grade, supplier, etc) used for food spiking
- food type employed (proximate analysis, grade or quality, supplier, etc.), feeding rate during uptake phase, amount of food administered and frequency (including any adjustments based on sampled fish weight)
- time at which fish were collected and euthanized for chemical analysis for each sample point (e.g. one hour before the following day's feeding)

Results and discussion

- results from any preliminary study performed
- information on any adverse effects observed
- complete description of all chemical analysis procedures employed including limits of detection and quantification, variability and recovery
- tabulated fish weight (and length) data, linked to individual fish chemical concentrations (and lipid content, if applicable), both for control and exposure groups (for example using unique identifiers for each sampled fish) and calculations for derived growth rate constant(s)
- tabulated test substance concentration data in fish (Cf, linked to individual fish) and water (Cw) (with mean values for test group and control, standard deviation and range, if appropriate) for all sampling times
- curves (including all measured data), showing growth, i.e. fish weight vs. time, uptake and depuration of the test chemical in the fish
- natural logarithm transformed concentration vs. uptake time (including the derived uptake rate constant k1)

Aqueous exposure test:

- mortality of the control fish and the fish in each exposure chamber and any observed abnormal behaviour
- the lipid content of the fish, including the method used, and if derived, lipid normalisation factor (Ln, factor to express results relative to fish lipid content of 5%)
- steady-state and kinetic bioconcentration factor and derived uptake and depuration rate constants k1 and k2, together with the variances in k2 (slope and intercept) if sequential fitting is used
- the summary table on depuration rate constants and BCFs indicated in the guideline

Dietary exposure test:

- measured lipid concentrations in food (spiked and control diet), individual, mean values and standard deviations
- tabulated fish lipid contents data, mean values for test group and control at test start, end of uptake and end of depuration
- raw dietary BMF, lipid and growth-corrected BMF, tissue-specific results if applicable. Metabolites and their accumulation pattern
- the summary table on depuration rate constants and BMFs indicated in the guideline

Reference to other ECHA Guidance Documents

Further detailed guidance on bioaccumulation can be found in:

- Guidance on information requirements and chemical safety assessment, Volume 6: Chapter R7c, section: R.7.10.
- IUCLID 5 End User Manual in the following chapter:

IUCLID Section	REACH Annex	Endpoint title	IUCLID End User Manual Chapter
5.3.1	IX 9.3.2	Bioaccumulation: aquatic / sediment	E.5.4.2
5.3.2		Bioaccumulation: terrestrial	E.5.4.3

4.1.4 Transport and distribution

HPLC method (OECD TG 121, EU C.19)

Materials and methods

- description of the HPLC equipment and operating conditions (column, mobile phase, means of detection, temperature);
- dead time and method used for its determination;
- reference substances (identity, purity, Koc, retention times) with results of at least 6 measurements with at least one of them above and one below the expected value for the test substance;
- quantities of test and reference substances introduced in the column.

Results and discussion

- average retention data and estimated d log Koc value for test compound
- all values of log Koc derived from individual measurements

Batch equilibrium method (OECD TG 106, EU C.18)

Materials and methods

- details on soil types (nature and sampling site(s), organic C, clay content and soil texture, and pH, if relevant Cation Exchange Capacity)
- information on the test substance (nominal and analytical test concentrations, stability and adsorption on the surface of the test vessel, solubilising agent if relevant (and justification for its use), radiochemical purity if relevant)
- details on test conditions (e.g. soil/solution ratio, number of replicates and controls, sterility, test temperature, and pH of the aqueous phase before and after contact with the soil)
- details on sampling (e.g. frequency, method)
- details on the analytical methods used for determination of the substance (detection limit, recovery %)

Results and discussion

- soil dry mass, total volume of aqueous phase, concentration of test substance in solution and/or soil after agitation and centrifugation, equilibration time, Koc and Kd, if appropriate mass balance
- explanations of corrections made in the calculations, if relevant (e.g. blank run)

Leaching in soil columns (OECD TG 312)

Materials and methods

- details on soil types (nature and sampling site(s), organic C, clay content and soil texture, Cation Exchange Capacity, bulk density (for disturbed soil), water holding capacity and pH
- information on the test substance (amount of test substance and, if appropriate, reference substance applied, solubilising agent if relevant (and justification for its use), radiochemical purity if relevant)
- details on test conditions (number of replicates and controls, test temperature, amount, frequency and duration of application of artificial rain)
- details on the analytical methods used for determination of the substance (detection limit, recovery %)
- reference substance used

Results and discussion

- Koc, tables of results expressed as concentrations and as % of applied dose for soil segments and leachates
- mass balance, if appropriate
- leachate volumes
- leaching distances and, where appropriate, relative mobility factors

Adsorption control within an inherent biodegradability test (OECD TG 302B)

Materials and methods

- details on inoculum
- information on the test substance (toxicity to bacteria, test concentration)
- details on test conditions (blank controls used, inoculum and test compound ratio (as DOC))
- details on sampling (frequency)
- details on the analytical methods used for determination of the DOC or COD
- reference substance

Results and discussion

- estimate of the extent of adsorption to STP sludge made from the elimination level in this Zahn-Wellens inherent biodegradation test, based on the 3-hour value if possible
- values beyond 24 hours should not normally be used but where data is not available for adsorption up to 24 hours, data from time scales beyond this can only be used if adsorption is the only removal mechanism, with an upper limit of 7 days
- if relevant results of testing of inhibition of biodegradation

Simulation test/field measurement (OECD Guidance Document 22)

Materials and methods

- details on soil types (nature and sampling site(s); if relevant: organic C, clay content and soil texture, Cation Exchange Capacity and pH;
- details on lysimeter or field conditions
- information on the test substance (nominal and analytical test concentrations, solubilising agent if relevant (and justification for its use), radiochemical purity if relevant)
- details on test climate conditions (e.g. air temperature, solar radiation, humidity, potential evaporation or rate of artificial rainfall), soil temperature and soil moisture and duration of the study
- details on sampling (frequency, method) and statistical analysis
- details on the analytical methods used for determination of the test substance (detection limit, recovery %)

Results and discussion

- concentration of test substance in soil layers; Koc and Kd, if appropriate mass balance and concentrations and as % of applied dose for soil segments and leachates
- explanations of corrections made in the calculations, if relevant (e.g. blank run)
- main results including assumptions in the interpretation of the results and conclusions

Distribution modelling

Materials and methods

- model name and version
- date of the model development
- model type description e.g. steady-state, dynamic, fugacity, Gaussian, Level I-IV, etc.
- environmental compartments which the model covers
- information on model segmentation and environmental properties
- input parameters (minimum information required for assessing the partitioning and degradation behaviour):
    - vapour pressure
    - water solubility
    - molecular weight
    - octanol-water partition coefficient
    - information on ready biodegradability
    - for inorganic chemicals: it is recommended to have information on the partition coefficients and possible abiotic transformation products
- temperature effect

Results and discussion

- key exposure routes and distribution of the substance among them
- uncertainty assessment
- main results including assumptions in the interpretation of the results and conclusions

Reference to other ECHA Guidance Documents

Further detailed guidance on transport and distribution can be found in:

- Guidance on information requirements and chemical safety assessment, Volume 5: Chapter R7b, section R.7.1.15.

- IUCLID 5 End User Manual in the following chapters:

IUCLID Section	REACH Annex	Endpoint title	IUCLID End User Manual Chapter
5.4.1	VIII 9.3.1	Adsorption / desorption	E.5.5.2
5.4.2		Henry's Law constant	E.5.5.3
5.4.3	X 9.3.4	Distribution modelling	E.5.5.4
5.4.4	X 9.3.4	Other distribution data	E.5.5.5
5.5		Environmental data	E.5.6
5.5.1		Monitoring data	E.5.6.2
5.5.2		Field studies	E.5.6.3
5.6		Additional information on environmental fate and behaviour	E.5.7

4.2 Ecotoxicity - Endpoint specific information

The information necessary to prepare a RSS for each ecotoxicity endpoint is listed in the sub-chapters below. An example on the IUCLID RSS for the short term toxicity to fish can be found in Annex 2.

Several Test Guidelines have been subject to update(s), thus it is of utmost importance to specify the version (year) of the Test Guideline used to carry out the test when reporting the test results in the RSS. The most up-to-date version should also be specified, highlighting the differences with the Guideline used for testing, if applicable.

4.2.1 Aquatic toxicity

Short-term toxicity on fish

Materials and methods

- test species and origin; include the scientific name of the species (if the name has changed, include both the new name and the name specified in the test report)
- acclimation period
- size and age of fish
- test conditions (dissolved oxygen, pH, hardness, type of water, temperature, lighting, test system[4], flow-rate/renewal time[5], solubilising agent, etc.)

[4] Static, semi-static, flow-through
[5] If semi-static: renewal time, if flow-through: flow rate or renewal time

- test duration/total exposure duration
- test design (test concentrations throughout the test, number of controls, number of replicates, number of animals per replicate and loading, etc.)
- preliminary test, if conducted
- mortality in the controls

Results and discussion

- observations in the controls (mortality, etc.)
- observations (number of dead fish, abnormal appearance and behaviour)
- monitoring of test concentrations
- other measurements throughout the test (dissolved oxygen, pH, temperature, etc.)
- LC50 at 24, 48, 72 and 96 hours, dose-response relationships (including LC0 and LC100), description of statistical analysis performed

Long-term toxicity on fish:
Fish early-life stage (FELS) toxicity test

Materials and methods

- test species and origin; include the scientific name of the species (if the name has changed, include both the new name and the name specified in the test report)
- acclimation period
- size and age of fish
- test conditions (dissolved oxygen, pH, hardness, type of water, temperature, lighting, feeding, test system[6], solubilising agent and its effects, etc.)
- preliminary test
- test duration/total exposure duration
- test design (test concentrations, number of controls, number of replicates, number of eggs, per replicate and loading, etc.)

Results and discussion

- observations in the controls (survival of the fertilised eggs, etc.)
- observations (hatching success and post-hatch survival, abnormal appearance and behaviour, individual weights at the end of the test, etc.)
- monitoring of test concentrations
- other measurements throughout the test (dissolved oxygen, pH, hardness, temperature, etc.)
- expression of results: cumulative mortality; number of healthy fish at the end of the test; time to start of hatching and end of hatching; numbers of larvae hatching each day; number and description of morphological abnormalities; number and description of behavioural effects; length and weight of surviving animals
- EC10 or NOEC and LOEC, dose-response relationships, description of statistical analysis performed

[6] Static, semi-static, flow-through

Fish short term toxicity test on embryo and sac-fry stages

Materials and methods

- test species and origin; include the scientific name of the species (if the name has changed, include both the new name and the name specified in the test report)
- acclimation period
- test conditions (dissolved oxygen, pH, hardness, type of water, temperature, lighting, test system[7], solubilising agent, etc.)
- preliminary test
- test duration/total exposure duration
- test design (test concentrations, number of controls, number of replicates, loading, etc.)

Results and discussion

- observations in the controls (survival of the fertilised eggs, etc)
- observations (hatching success and post-hatch survival, abnormal appearance and behaviour, individual weights at the end of the test, etc.)
- monitoring of test concentrations
- other measurements throughout the test (dissolved oxygen, pH, hardness, temperature, etc.)
- expression of results: cumulative mortality; number of healthy larvae at the end of the test; time to start of hatching and end of hatching; numbers of larvae hatching each day; number and description of morphological abnormalities; number and description of behavioural effects; length and weight of surviving animals
- EC10 or NOEC and LOEC, dose-response relationships, description of statistical analysis performed

Aquatic Toxicity – Fish, juvenile growth test

Materials and methods

- test species and origin; include the scientific name of the species (if the name has changed, include both the new name and the name specified in the test report)
- acclimation period
- weight of fish at the beginning of the test
- test conditions (dissolved oxygen, pH, hardness, type of water, temperature, lighting, feeding, test system[8], solubilising agent, etc.)
- preliminary test
- test duration/total exposure duration
- test design (test concentrations, number of controls, number of replicates, loading, etc.)

Results and discussion

- observations in the controls: (mortality, growth rate of control organisms, etc.)
- observations: growth (weight), any abnormalities (e.g. mortality, appearance, behaviour)
- monitoring of test concentrations
- other measurements throughout the test (dissolved oxygen, pH, hardness, temperature, etc.)
- expression of results: growth rate, observations on mortality or abnormalities
- EC10 or NOEC and LOEC, dose-response relationships, description of statistical analysis performed

[7] Static, semi-static, flow-through
[8] Static, semi-static, flow-through

Short-term toxicity on aquatic invertebrates

Materials and methods

- test species and origin; include the scientific name of the species (if the name has changed, include both the new name and the name specified in the test report)
- species life stage
- test conditions (dissolved oxygen, pH, hardness, type of water, temperature, lighting, test system[9], solubilising agent, etc.)
- test duration/total exposure duration
- acclimation period
- test design (test concentrations, number of controls, number of replicates, number of animals per vessel, feeding pattern, reference substance used for the organisms sensitivity check, etc.)

Results and discussion

- observations in the controls (e.g. immobilised organisms)
- observations (mobility/survival)
- monitoring of test concentrations
- other measurements throughout the test (e.g. dissolved oxygen, pH, temperature)
- EC50, IC50 or LC50 at each 24h interval, dose-response relationships (including LC0 and LC100), description of statistical analysis performed

Long-term toxicity on aquatic invertebrates

Materials and methods

- test species and origin; include the scientific name of the species (if the name has changed, include both the new name and the name specified in the test report)
- acclimation period
- species lifestage
- test conditions (dissolved oxygen, pH, hardness, TOC, type of water, temperature, lighting, feeding, test system[10], solubilising agent, etc.)
- preliminary test
- test duration
- test design (test concentrations, number of controls, number of replicates, number of animals, etc.)

Results and discussion

- observations in the controls: (number of juveniles per parent, presence of living males, ephippia produced, etc.)
- observations: number of offspring (daily count), number of dead parents (daily count), any other observed effects (e.g. growth of parents)
- monitoring of test concentrations
- other measurements throughout the test (dissolved oxygen, pH, hardness, temperature)
- expression of results: i.e. total number of living offspring produced per parent animal alive at the end of the test (including control)
- EC10 or NOEC and LOEC, dose-response relationships, description of statistical analysis performed

[9] Static, semi-static, flow-through
[10] Static, semi-static, flow-through

Algal growth inhibition test

Materials and methods

- test species; include the scientific name of the species (if the name has changed, include both the new name and the name specified in the test report)
- initial cell concentration
- test conditions (temperature, lighting, test medium, pH, test system, solubilising agent, etc.)
- test duration/total exposure duration
- test design (e.g. test concentrations, number of controls, number of replicates)
- controls conditions (pH, etc.)

Results and discussion

- observations in the controls (increase in biomass, growth rate, etc.)
- details on the determination of algal biomass (method for cell counting, cell density, chlorophyll, etc.)
- determination of growth rates
- growth curves (evidence of exponential growth in the controls, growth rate evolution throughout the test in the test vessels, etc.)
- other effects (microscopic appearance of algal cells, changes in size, shape or colour, percent mortality of cells, etc.)
- monitoring of test concentrations
- other measurements throughout the test (temperature, pH, etc.)
- EC50, EC10 at 24h intervals or NOEC and LOEC for both growth rate and biomass, dose-response relationships, description of statistical analysis performed

Aquatic plants growth inhibition test

Materials and methods

- test species; include the scientific name of the species (if the name has changed, include both the new name and the name specified in the test report)
- initial frond number
- test conditions (temperature, lighting, test medium, pH, test system, solubilising agent, etc.)
- test duration/total exposure duration
- test design (test concentrations, number of controls, number of replicates, etc.)

Results and discussion

- observations in the controls
- observations (e.g. frond number, frond area, dry or fresh weight, chlorophyll-a)
- determination of growth rates
- other effects (frond and root size and appearance, necrosis, chlorosis, gibbosity, loss of buoyancy, etc.)
- monitoring of test concentrations
- other measurements throughout the test (pH, light intensity, temperature, etc.)
- EC50, EC10 or NOEC and LOEC at the different reporting timings, dose-response relationships, description of statistical analysis performed

Toxicity to micro-organisms

Materials and methods

- test organisms or details on incolulum
- test conditions (temperature, reference substance used, etc.)
- test duration/total exposure duration
- test design (test concentrations, description of microbial innoculum including pre-treatment if any, number of controls, number of replicates, etc.)

Results and discussion

- results of control respiration rates or the measured endpoint
- abiotic oxygen uptake
- all measured data including the EC50 of the reference substance
- inhibition curve and method for the EC50
- EC50, EC10 or NOEC and LOEC and, if possible, 95 per cent confidence limit, description of statistical analysis performed
- all observations and any deviations from the test guideline which could have influenced the result

Reference to other ECHA Guidance Documents

Further detailed guidance on aquatic toxicity can be found in:

- Guidance on information requirements and chemical safety assessment, Volume 5: Chapter R7b.
- IUCLID 5 End User Manual in the following chapters:

IUCLID Section	REACH Annex	Endpoint title	IUCLID End User Manual Chapter
6.1.1	VII 9.1.3	Short-term toxicity to fish	E.6.2.2
6.1.2	IX 9.1.6	Long-term toxicity to fish	E.6.2.3
6.1.3	VIII 9.1.1	Short-term toxicity to aquatic invertebrates	E.6.2.4
6.1.4	IX 9.1.5	Long-term toxicity to aquatic invertebrates	E.6.2.5
6.1.5	VII 9.1.2	Toxicity to aquatic algae and cyanobacteria	E.6.2.6
6.1.6	VII 9.1.2	Toxicity to aquatic plants other than algae	E.6.2.7
6.1.7	VIII 9.1.4	Toxicity to microorganisms	E.6.2.8
6.1.8	IX 9.1	Toxicity to other aquatic organisms	E.6.2.9

4.2.2 Sediment toxicity

Materials and methods

- test organisms (species, age, pre-treatment, etc.); include the scientific name of the species (if the name has changed, include both the new name and the name specified in the test report)
- test conditions:
    - sediment – composition of formulated sediment (also pH, organic carbon content, information on possible chemical contamination of sediment components) or origin of natural sediments (also pH, organic carbon content, recommended by C/N ratio and granulometry); conditions of preconditioning of natural sediments; sediment surface area; depth of sediment layer and the ratio of it to the depth of the overlying water
    - water used (pH, total hardness, ammonium concentration, oxygen content, etc.)
    - solvents or dispersants used for preparation of stock solution
    - food and feeding of test organisms and exposure duration
    - incubation conditions (aeration, temperature, photoperiod and light intensity)
    - method of spiking and equilibrium between water-phase and sediment-phase period
    - data on measured concentrations of test substance in the overlying water, the pore water and the sediment at the start and at the end of the test at the highest concentration and the lower one
    - type of system used (e.g. static)
- test design (test concentrations, number of controls, number of replicates, number of organisms per replicate, analytical method, etc.)
- test duration/total exposure duration
- data to assess the validity of performed test

Results and discussion

- observations in the controls (the emergence in the controls at the end of the test, etc.)
- observations on toxicological effects (delayed hatching, instar development etc.)
- OECD TG 218, 219:
    - number of emerged male and female midges per vessel and per day
    - number of larvae which failed to emerge as midges per vessel
    - mean individual dry weight of larvae per vessel, and per instar, if appropriate
    - development rate of fully emerged midges per replicate and treatment rate
    - % emergence rate per replicate and test concentration
- OECD TG 233:
    - number of emerged male and female midges per vessel and per day for the 1st and 2nd generation
    - characteristics of each egg rope (size, shape and fertility)
    - fecundity – total number of egg ropes per total number of females added to the breeding cage
    - fertility – total number of fertile egg ropes per total number of females added to the breeding cage
- OECD TG 225:
    - number of worms per replicate at the beginning and end of the test
    - abnormal behaviour if any
    - dry weight of the worms of each test chamber at the end of the test
    - total number, and if determined, number of complete and incomplete worms
- measured test concentrations
- estimates of the toxic endpoint(s) (e.g. ECx and confidence intervals, NOEC and LOEC) dose-response relationships, description of statistical analysis performed

Reference to other ECHA Guidance Documents

Further detailed guidance on sediment toxicity can be found in:

- Guidance on information requirements and chemical safety assessment, Volume 5: Chapter R7b, sections: R.7.8.7 – R.7.8.11.
- IUCLID 5 End User Manual in the following chapter:

IUCLID section	REACH Annex	Endpoint title	IUCLID End User Manual Chapter
6.2	X 9.5.1	Sediment toxicity	E.6.3

4.2.3 Terrestrial toxicity

Short term toxicity on terrestrial invertebrates

Materials and methods

- test species and origin; include the scientific name of the species (if the name has changed, include both the new name and the name specified in the test report)
- breeding conditions
- age, size (mass) range of test organisms
- substrate type: preparation of the test medium, maximum water holding capacity (when applicable), when natural soil also its suitability for testing
- test conditions: method and auxiliary substances used for application of test substance, temperature, and (where applicable) pH value at start of test, light intensity, feeding regime, moisture content of soil at start and at end of test
- test duration/total exposure duration
- test design: test concentrations, number of controls, number of replicates, number of animals and quantity of test medium per replicate and per control

Results and discussion

- observations in the controls (mortality, etc.)
- observations: average live weight, number of live and dead animals, obvious physical or pathological symptoms or distinct changes in behaviour
- mortality with reference substance
- LC50 value and method used to determine it, dose-response relationships (including LC0 and LC100), description of statistical analysis performed

Long term toxicity on terrestrial invertebrates

Materials and methods

- test species and origin; include the scientific name of the species (if the name has changed, include both the new name and the name specified in the test report)
- breeding conditions
- age, size (mass) range of test organisms
- substrate type: preparation of the test medium, maximum water holding capacity, when natural soil also its suitability for testing,
- test conditions: method and auxiliary substances used for application of test substance, temperature, duration of light-dark cycles, light intensity, feeding regime, pH and moisture content of soil at start and at end of test
- test duration/total exposure duration
- test design: test concentrations, number of controls, number of replicates, number of animals and loading (per dry mass) per replicate and per control

Results and discussion

- observations in the controls (number of juveniles, mortality, etc.)
- observations: % adult mortality, % changes in body weight and average live weight of live adults (where applicable) at the end of the adult exposure period of the test, number of juveniles at end of test, obvious or pathological symptoms or distinct changes in behaviour
- results obtained with the reference test substance
- the LC50, the NOEC and LOEC and (recommended) ECx (e.g. EC50, EC10) for reproduction and other endpoints, dose-response relationships, description of statistical analysis performed

Long term toxicity on terrestrial arthropods

Materials and methods

- test species and origin; include the scientific name of the species (if the name has changed, include both the new name and the name specified in the test report)
- culturing conditions
- age range of test organisms
- substrate type: preparation of the test medium, maximum water holding capacity, when natural soil also its suitability for testing
- test conditions: method and auxiliary substances used for application of test substance, temperature, duration of light-dark cycles, light intensity, feeding regime, pH and moisture content of soil at start and at end of test
- test duration/total exposure duration
- test design: test concentrations, number of controls, number of replicates, number of animals and dry mass of test medium per replicate and per control, description of the extraction method

Results and discussion

- observations in the controls (mortality, etc.)
- observations: number of adult females and % adult mortality, number of juveniles, obvious or pathological symptoms or distinct changes in behaviour
- results obtained with the reference test substance
- LC50, NOEC and LOEC and (recommended) ECx (e.g. EC50, EC10) for reproduction and other endpoints, dose-response relationships, description of statistical analysis performed

Short term and long term toxicity to terrestrial plants

Materials and methods

- test species/variety and criteria for selecting the species, plant families, scientific and common names, source and history of the seeds
- rationale for selection of mono- and di-cotyledon species tested
- seed storage, treatment and maintenance
- substrate type: soil/substrate characteristics (e.g. texture, pH), when natural soil also its suitability for testing, nutrient medium if used
- test conditions: test facility and test system (e.g. pot dimension, amount of soil), application of test substance (e.g. method/equipment/calibration for methods, auxiliary substances used), growth conditions (e.g. light intensity, photoperiod, max/min temperatures, watering schedule and method, fertilization, pollination when included)
- test duration/total exposure duration
- test design: test concentrations/exposure rates including chemical verification, number of seeds per pot, of plants per dose, of replicates (pots) per exposure rates, type and number of controls, stage of plant development at the start of test

Results and discussion

- table of all endpoints for each replicate, test rate/concentration and species
- observations of endpoints (mortality, emergence, biomass measurements, shoot height, etc.) as a percentage of the controls,
- percent, qualitative and quantitative description of visual injury (also description of rating scale if used)
- EC50, ER50, E(R)C10, NOEC and LOEC (necessary for long term), dose-response relationships, description of statistical analysis performed

Toxicity to soil microorganisms - Nitrogen transformation test

Materials and methods

- nitrogen content of the test substance (where relevant)
- complete identification of the soil used (origin, sand/silt/clay content, pH, organic carbon content, nitrogen content, initial nitrate concentration, CEC, microbial mass, moisture content, etc.)
- details of amendment and type of soil with organic substrate (source, composition, carbon content, nitrogen content, sieve size)
- test conditions (moisture, temperature, lighting)
- test duration, sampling times
- test system (e.g. sealed containers)
- test design (concentrations tested, number of controls, number of replicates, etc.)
- method for the application of the tests substance to soil (use of carrier?)
- method for extraction of nitrate from soil
- analytical procedure and equipment used to analyse nitrate

Results and discussion

- observations: nitrate production (mg nitrate/ kg dry weight soil/day) (preferably in a tabular form), variation between the replicates in treated and control samples
- EC50, EC25 or EC10 values with the confidence interval, the dose response curve and data on statistical treatment of the results

Toxicity to soil microorganisms – Carbon transformation test

Materials and methods

- complete identification of the soil used (e.g. origin, sand/silt/clay content, pH, organic carbon content, nitrogen content, CEC, microbial mass, moisture content)
- details of the amendment of soil with organic substrate
- test conditions (moisture, temperature, lighting)
- test duration, sampling times
- test system (e.g. sealed containers)
- test design (concentrations tested, number of controls, number of replicates, etc.)
- method for the application of the tests substance to soil (use of carrier?)
- method for measuring the respiration rate (e.g. either mean CO2 released or mean O2 consumed)

Results and discussion

- observations: respiration rate (mg CO2/ kg dry weight soil/h or mg O2/ kg dry weight soil/h) (preferably mean and individual and in a tabular form), variation between the replicates in treated and control samples
- EC50, EC25 or EC10 values with the confidence interval, dose-response relationships, description of statistical analysis performed

Toxicity to birds - Avian reproduction test

Materials and methods

- test species and origin (+ justification if other than recommended in guidelines)
- acclimation condition (e.g. period, food)
- age
- conditions during the test, incubation and breeding conditions (birds per pen, replicates, temperature, humidity, lighting regime, test facilities, feeding, egg storage, incubation, hatching, turning frequency, ventilation, etc.)
- method for contaminating the food with the test substance
- test diets: method of preparation, number of concentrations used, nominal and (where determined) measured dietary concentration of test substance at each level, assay method used to determine actual concentrations, frequency of mixing and renewal, carrier (if used), storage conditions, method of application
- test duration/total exposure duration
- test design: test concentrations, number of controls, number of replicates, loading
- description of the basal diet, including source, composition, manufacturer's nutrient analysis (protein, carbohydrate, fat, calcium, phosphorus, etc.) and any supplements and carriers used

Results and discussion

- Observations (for all test concentrations and the controls):
- mortality of adults
- body weight of adults at the start of the exposure period, prior to onset of egg laying, and at the termination of the study
- food consumption of adults: one or 2-week intervals throughout the study
- frequency, duration, and description of signs of toxicity, along with severity, numbers affected and any remissions
- egg production (i.e. number of eggs laid per hen after 10 weeks)
- percentage of cracked eggs (not incubated)
- egg viability. (eggs set for incubation only)
- hatchability (i.e. percentage of hatchlings that survive to 14 days)
- eggshell thickness (preferably in tabular form)
- survival of young
- body weight of young
- food consumption of young: 1st and 2nd week after hatching
- details of gross pathological examinations
- results of residue analysis (if performed)
- monitoring of the test concentrations in the food throughout the test period and the analytical method used
- method of statistical analysis, results expressed as NOEC and – if relevant – the rationale to pass from NOAEL to NOEC, dose-response relationships, description of statistical analysis performed

Reference to other ECHA Guidance Documents

Further detailed guidance can be found in:

- Guidance on information requirements and chemical safety assessment, Volume 6: Chapter R7c.
- IUCLID 5 End User Manual in the following chapters:

IUCLID section	REACH Annex	Endpoint title	IUCLID End User Manual Chapter
6.3.1	IX 9.4.1, X 9.4.4	Toxicity to soil macroorganisms except arthropods	E.6.4.2
6.3.2	IX 9.4.1, X 9.4.4	Toxicity to terrestrial arthropods	E.6.4.3
6.3.3	IX 9.4.3, X 9.4.6	Toxicity to terrestrial plants	E.6.4.4
6.3.4	IX 9.4.2	Toxicity to soil micro-organisms	E.6.4.5
6.3.5	X 9.6.1	Toxicity to birds	E.6.4.6
6.3.6	X 9.4	Toxicity to other above-ground organisms	E.6.4.7
6.4		Biological effects monitoring	E.6.5
6.5		Biotransformation and kinetics	E.6.6
6.6		Additional ecotoxicological information	E.6.7

5. Endpoint specific information for Human Health endpoints

5.1 Acute toxicity – oral, inhalation, dermal

Materials and methods

Test type

Test animals

- species/strain/sex
- no. of animals per sex per dose
- age and weight at the study initiation

Administration / exposure

- route of administration – oral (gavage, other), dermal, inhalation (aerosol, vapour, gas, particulate), other
- duration of test/exposure period
- doses/concentration levels, rationale for dose level selection
- post exposure observation period
- control group and treatment
- vehicle: identification, concentration and volume used, justification of choice of vehicle (if other than water)

  for inhalation studies
  - type of inhalation exposure and test conditions (e.g.: exposure apparatus,
  - method of exposure ("whole body", "oro-nasal", or "head only"), exposure data)
  - analytical verification of test atmosphere concentrations
  - particle size (for studies with aerosols, indicate mass median aerodynamic diameter and geometric standard deviation or give other specifications)
  - type or preparation of particles (for studies with aerosols)

  for dermal studies
  - area covered (e.g. 10% of body surface)
  - occlusion (e.g. semi-occlusive)
  - total volume applied
  - removal of test substance (e.g. water or solvent)
- statistical methods

Results and discussion

- deaths to be given in tabular form showing sex/dose given/no of animals/no of deaths should be those considered to be due to the test substance. Information on any other deaths should be given under other remarks.
- value (LD50 or LC50) with confidence limits if calculated
- number of deaths at each dose level
- provide additional information that may be needed to adequately assess data for reliability and use, including the following, if available:
  - time of death (provide individual animal time if less than 24 hours after dosing).
  - clinical signs: description, severity, reversibility, time of onset and duration at
  - each dose level
  - necropsy findings, including doses affected, severity and number of animals affected
  - potential target organs (if identified in the report)
  - other findings
  - if both sexes tested, results should be compared

Additional

- doses (OECD guidelines 401 and 425 do not provide dose levels, so these must be described in detail)

Overall remarks, attachments

Provide toxicological evaluation of all the findings of the study (adverse and non adverse effects, reversible and irreversible effects) explaining also the biological relevance of the effects observed in animals and if needed address human relevance. Include, if relevant, the impact of confounding factors in the effects observed in the study.
Discuss any significant deviations from the guideline.

Applicant's summary and conclusions

Provide information related to classification and labelling under *interpretation of results*, and the conclusion of the study under *conclusions*.

Reference to other ECHA Guidance Documents

Further detailed guidance on acute toxicity can be found in:

- Guidance on information requirements and chemical safety assessment; Volume 4: Chapter 7a, Section R.7.4
- IUCLID 5 End User Manual in the following chapters:

IUCLID Section	REACH Annex	Endpoint title	IUCLID End User Manual Chapter
7.2		Acute toxicity	E.7.3
7.2.1	VII 8.5.1	Acute toxicity: oral	E.7.3.2
7.2.2	VIII 8.5.2	Acute toxicity: inhalation	E.7.3.3
7.2.3	VIII 8.5.3	Acute toxicity: dermal	E.7.3.4.
7.2.4		Acute toxicity: other routes	E.7.3.5

5.2 Irritation/Corrosion

NOTE: For *in vitro* studies, see the specific EU test method and/or OECD Test Guideline section "data and reporting" for the endpoint specific information needed.

5.2.1 Skin irritation/Corrosion

Materials and methods

- type of the method: *in vivo / in vitro*
- cell type or line for *in vitro* test

Test animals

- species/strain/sex
- no. of animals per sex per dose
- age and weight at the study initiation

Administration/exposure

- pH of the test material
- duration of exposure: length of time test material is in contact with animal/cell
- total dose: amount/concentration of test material applied to skin in mg/ml
- post exposure observation period
- control group and treatment
- vehicle: identification, concentration and volume used, justification of choice of vehicle (if other than water)
- time points at which grading/scoring took place, (e.g. 1, 4 24, 48, 72 hours, 14 days, etc)
- grading scale: specify/name of the grading/system used
- preparation of the test site, area covered (e.g. 10% of body surface), shave or not, abraded or not, pre-treatment of site, patch type: occlusive/semi-occlusive
- removal of test substance (e.g. water or solvent)
- statistical methods

Results and discussion

- irritant/corrosive response data: cumulative total and percent responders, preferably in tabular form for each individual animal for each observation time period:
  - numerical skin grades at 1, 4, 24, 48 and 72 hours
  - delayed grading scores at 7 to 14 days
  - whether the effects observed were reversible
- description of all lesions: erythema/oedema findings, other dermal lesions and/or systemic effects.
- overall irritation score

Overall remarks, attachments

Provide toxicological evaluation of the findings of the study and if relevant include a summary of confounding factors that may affect the results of the study.
Discuss any significant deviations from the guideline.

Applicant's summary and conclusions

Provide information related to classification and labelling under *interpretation of results*, and the conclusion of the study under *conclusions*.

Reference to other ECHA Guidance Documents

Further detailed guidance on skin irritation/corrosion can be found in:

- Guidance on information requirements and chemical safety assessment; Volume 4: Chapter 7a, Section R.7.2

- IUCLID 5 End User Manual in the following chapters:

IUCLID Section	REACH Annex	Endpoint title	IUCLID End User Manual Chapter
7.3		Irritation/corrosion	E.7.4.
7.3.1	VII 8.1, VIII 8.1.1	Skin irritation/corrosion	E.7.4.2.

5.2.2 Eye irritation / Corrosion

NOTE: For *in* vitro/ex vivo studies see the specific EU test method and/or OECD Test Guideline section "data and reporting" for the endpoint specific information needed.

Materials and methods
- test type: *in vivo*/*in vitro*
- cell line: if *in vitro* method, list cell type/line

Test animals
- species/strain/sex
- no. of animals per sex per dose
- age and weight at the study initiation

Administration / exposure
- pH of the test substance
- time points at which grading/scoring took place (e.g. 1 hour, 24, 48, 72 hours, 14 days etc.)
- name of the scoring method used to score irritation
- tool used to asses score: hand-slit lamp, biomicroscope, fluorescein, other
- duration of test/exposure period
- doses/concentration levels
- post exposure observation period
- vehicle: identification, concentration and volume used, justification of choice of vehicle (if other than water)
- removal of test substance (e.g. water or solvent)
- statistical methods

Results and discussion
- irritant/corrosive response data: preferably in tabular form for each individual animal for each observation time period (e.g. 1, 24, 48 and 72 hours)
- description of serious lesions if observed
- narrative description of the degree and nature of irritation/corrosion observed
- description of any non-ocular topical effects observed
- number of animals affected
- recovery/irreversibility of the effects (up to 21 days)
- overall irritation score

Overall remarks, attachments

Provide toxicological evaluation of the findings of the study and if relevant include a summary of confounding factors that may affect the results of the study.
Discuss any significant deviations from the guideline.

Applicant's summary and conclusions

Provide information related to classification and labelling under *interpretation of results*, and the conclusion of the study under *conclusions*.

Reference to other ECHA Guidance Documents

Further detailed guidance on eye irritation can be found in:

- Guidance on information requirements and chemical safety assessment; Volume 4: Chapter 7a, Section R.7.2
- IUCLID 5 End User Manual in the following chapters:

IUCLID Section	REACH Annex	Endpoint title	IUCLID End User Manual Chapter
7.3		Irritation/corrosion	E.7.4.
7.3.2	VII 8.2, VIII 8.2.1	Eye irritation	E.7.4.3.

5.2.3 Skin sensitisation

Materials and methods

- test type: traditional sensitisation test, LLNA, other

Test substance and control test substances:

- identification data (e.g. CAS number, if available; source; purity; known impurities; lot number)
- physical nature and physicochemical properties (e.g. volatility, stability, solubility)
- if formulation, composition and relative percentages of components

Test animals

- species/strain/sex
- no. of animals per sex per dose
- age and weight at the study initiation
- control group and treatment
- for LLNA source of CBA mice

Administration / exposure

- justification for choice of vehicle
- route of induction and challenge administration:
  - injection/topical
  - with/without occluded patch
  - type of patch used
- induction:
  - concentration(s) of test substance
  - induction vehicle (identification, concentration and volume used)
  - note whether more than one dose was given
  - the spacing between doses
  - mention any pre-treatment that may have been conducted
- challenge:
  - concentration (if applicable)
  - note whether more than one dose was given
  - vehicle (if applicable)
- grading system used (traditional tests); for other tests (i.e., LLNA), identify the endpoint to measure effect (e.g., proliferation of lymph nodes)
- statistical methods

For LLNA test conditions:

- justification for dose selection (including results from pre-screen test, if conducted)
- details of treatment and sampling schedules

- methods for measurement of toxicity
- criteria for considering studies as positive or negative
- details of any protocol deviations and an explanation on how the deviation affects the study design and results

Reliability check:
- a summary of results of latest reliability check, including information on test substance
- concentration and vehicle used
- concurrent and/or historical positive controls (PC) and concurrent negative (solvent/vehicle) control data for testing laboratory
- if a concurrent PC was not included, the date and laboratory report for the most recent

periodic PC and a report detailing the historical PC data for the laboratory justifying the basis for not conducting a concurrent PC

Results and discussion
- conclude whether the test substance is positive, negative or equivocal
- data should be summarised in tabular form, showing for each animal the skin reactions at each observation point (e.g. number of animals with skin grades of 0, 1, 2, and 3 at each observation time)
- narrative description of the nature and degree of effects observed
- any histopathological findings
- provide additional information that may be needed to adequately assess data for reliability and use, including the following, if available:
    - whether the substance was a skin irritant at the tested concentrations
    - incidence of skin scores greater than 1 for test and control groups
    - sensitisation ratio (maximisation test)
    - description, severity, time of onset and duration of clinical signs and/or lesions at the site of contact at each dose level
    - results of rechallenge

For the LLNA study results, provide the following additional information:
- time course of onset and signs of toxicity, including dermal irritation at site of administration, if any, for each animal
- time of animal sacrifice and time of ATP measurement for each animal
- a table of individual mouse BrdU or RLU values and SI values for each dose treatment group
- mean and associated error term (e.g. SD, SEM) for BrdU labelling index/mouse or RLU/mouse for each treatment group and the results of outlier analysis for each treatment group
- calculated SI and an appropriate measure of variability that takes into account the interanimal variability in both the test substance and control groups
- dose response relationship
- statistical analyses, where appropriate

Overall remarks, attachments
Provide toxicological evaluation of the findings of the study, their biological relevance and if needed human relevance. If relevant, include a summary of confounding factors that may affect the results of the study.
Discuss any significant deviations from the guideline.

Applicant's summary and conclusions
Give a brief commentary on the results, with a dose-response analysis and a conclusion as to

whether the test substance should be considered a skin sensitiser. Provide information related to classification and labelling under *interpretation of results*, and the conclusion of the study under *conclusions*.

Reference to other ECHA Guidance Documents

Further detailed guidance on skin and respiratory sensitisation can be found in:

- Guidance on information requirements and chemical safety assessment; Volume 4: Chapter 7a, Section R.7.3
- Chapter R.7.3
- IUCLID 5 End User Manual in the following chapters:

IUCLID Section	REACH Annex	Endpoint title	IUCLID End User Manual Chapter
7.4		Sensitisation	E.7.5
7.4.1	VII 8.3	Skin sensitisation	E.7.5.2
7.4.2		Respiratory sensitisation	E.7.5.3

5.3 Repeated Dose Toxicity

Materials and methods

Test Type

Test animals

- species/strain/sex
- no. of animals per sex per dose
- age and weight at the study initiation

Administration / exposure

- route of administration – oral (gavage, drinking water, feed), dermal, inhalation (aerosol, vapour, gas, particulate), other
- duration and frequency of test/exposure period
- doses/concentration levels, rationale for dose level selection
- post exposure observation period
- vehicle: identification, concentration and volume used, justification of choice of vehicle (if other than water)
- control group and treatment
- test substance formulation/diet preparation, achieved concentration, stability and homogeneity of the preparation
- actual doses (mg/kg bw/day) and conversion factor from diet/drinking water test substance concentration (ppm) to the actual dose, if applicable
- satellite groups and reasons they were added

  for inhalation studies
  - type of inhalation exposure and test conditions (e.g. exposure apparatus)
  - method of exposure (“whole body”, “oro-nasal”, or “head only”), exposure data
  - analytical verification of test atmosphere concentrations
  - particle size (for studies with aerosols, indicate mass median aerodynamic diameter and geometric standard deviation or give other specifications)
  - type or preparation of particles (for studies with aerosols)

  for dermal studies
  - area covered (e.g. 10% of body surface)
  - occlusion (e.g. semi-occlusive)
  - total volume applied
  - removal of test substance (e.g. water or solvent)
- statistical methods

Results and discussion

Describe the relevant findings. If no effects occurred, explicitly note "No effects"

- NOAEL(C) (NOEL)
- LOAEL(C) (LOEL)
- actual dose received by dose level by sex, if known
- details on analytical verification of doses or concentrations
- toxic response/effects by sex and dose level
- provide data preferably in tabular form where applicable
- provide additional information that may be needed to adequately assess data for reliability and use including the following if available. Provide at a minimum qualitative descriptions of elements where dose effect related observations were seen:
  - body weight and body weight changes
  - food/water consumption
  - description, severity, time of onset and duration of clinical signs (whether reversible or

not)
- sensory activity, grip strength and motor activity assessments (when available)
- ophthalmologic findings: incidence and severity
- haematological findings: incidence and severity
- clinical biochemistry findings: incidence and severity
- mortality and time to death
- gross pathology findings: incidence and severity
- terminal organ weights and organ/body weight ratios
- histopathology findings: incidence and severity

- statistical treatment of results, where appropriate

Overall remarks, attachments

Provide toxicological evaluation of all the findings of the study (adverse and non adverse effects, reversible and irreversible effects) explaining also the biological relevance of the effects observed in animals and if needed address human relevance.
Discuss any significant deviations from the guideline.

Applicant's summary and conclusions

Provide information related to classification and labelling under *interpretation of results*, and the conclusion of the study under *conclusions*.

Reference to other ECHA Guidance Documents

Further detailed guidance on repeated dose toxicity can be found in:

- Guidance on information requirements and chemical safety assessment; Volume 4: Chapter 7a, Section R.7.5
- IUCLID 5 End User Manual in the following chapters:

IUCLID Section	REACH Annex	Endpoint title	IUCLID End User Manual Chapter
7.5		Repeated dose toxicity	E.7.6
7.5.1	VIII, IX 8.6, X 8.6.3	Repeated dose toxicity: oral	E.7.6.2.
7.5.2	VIII, IX 8.6, X 8.6.3	Repeated dose toxicity: dermal	E.7.6.3
7.5.3	VIII, IX 8.6, X 8.6.3	Repeated dose toxicity: inhalation	E.7.6.4
7.5.4		Repeated dose toxicity: other routes	E.7.6.5

5.4 Genetic toxicity

5.4.1 Genetic toxicity in vitro

Note: reporting may vary depending on the test

Materials and methods

- type of genotoxicity, type of study (e.g. bacterial reverse mutation test, mammalian cell gene mutation test, *in vitro* mammalian chromosome aberration test, etc.)
- strain or cell type or cell line, target gene if applicable
- type and composition of metabolic activation system:
    - species and cell type
    - quantity
    - induced or not induced
    - chemicals used for induction
    - co-factors used
- test concentrations, and reasoning for selection of doses if applicable
- vehicle: identification, concentration and volume used, justification of choice of vehicle (if other than water)
- statistical methods
- test design
    - number of replicates
    - number of doses, justification of dose selection
    - positive and negative control groups and treatment
    - details on slide preparation
    - number of metaphases analyzed
    - justification for choice of vehicle
    - solubility and stability of the test substance in vehicle if known
    - description of follow up repeat study
    - criteria for evaluating results (e.g. cell evaluated per dose group, criteria for scoring aberrations)

Results and discussion

- data should be presented preferably in tabular form
- justification should be given for choice of tested dose levels (e.g. dose-finding studies)
- cytotoxic concentrations with and without metabolic activation
- genotoxic effects (e.g. positive, negative, unconfirmed, dose-response, equivocal) with and without metabolic activation
- concurrent negative (solvent/vehicle) and positive control data
- indicate test-specific confounding factors such as pH, osmolarity, whether substance is volatile, water soluble, precipitated, etc., particularly if they affect the selection of test concentrations or interpretation of the results
- statistical results
- provide additional information that may be needed to adequately assess data for reliability and use including the following if available. Provide at a minimum qualitative descriptions of elements where dose effect related observations were seen:
    - frequency of reversions/mutations/aberrations, polyploidy
    - mean number of revertant colonies per plate and standard deviation, number of cells with chromosome aberrations and type of chromosome aberrations given separately for each treated and control culture,

- precipitation concentration if applicable
- mitotic index

Overall remarks, attachments

Provide toxicological evaluation of the findings of the study. If relevant include a summary of confounding factors that may affect the results of the study and analysis of equivocal results. Discuss any significant deviations from the guideline.

Applicant's summary and conclusions

Provide information related to classification and labelling under *interpretation of results*, and the conclusion of the study under *conclusions*.

Reference to other ECHA Guidance Documents

Further detailed guidance on genetic toxicity can be found in:

- Guidance on information requirements and chemical safety assessment; Volume 4: Chapter 7a, Section R.7.5
- IUCLID 5 End User Manual in the following chapters:

IUCLID Section	REACH Annex	Endpoint title	IUCLID End User Manual Chapter
7.6		Genetic toxicity	E.7.7
7.6.1	VII 8.4.1, VIII 8.4.2, 8.4.3,	Genetic toxicity *in vitro*	E.7.7.2

5.4.2 Genetic Toxicity in vivo

Note: reporting may vary depending on the test

Materials and methods

- type of genotoxicity, type of study (in vivo mammalian chromosome aberration test etc.)

Test animals

- species/strain/sex
- no. of animals per sex per dose
- age and weight at the study initiation

Administration / exposure

- doses/concentration levels, vehicle, rational for dose selection
- vehicle: identification, concentration and volume used, justification of choice of vehicle (if other than water)
- details on test system and conditions, and rationale on route of administration, exposure
- actual doses (mg/kg bw/day) and conversion factor from diet/drinking water test substance concentration (ppm) to the actual dose, if applicable
- duration of study, frequency of treatment, sampling times and number of samples
- control groups and treatment
- positive and negative (vehicle/solvent) control data
- methods of slide preparation
- criteria for scoring and number of cells analysed per animal
- statistical methods

For transgenic rodent somatic and germ cell gene mutation assay also report:

- data from the range-finding study
- details of the administration of the test substance
- methods for measurement of animal toxicity, including, where available, histopathological or hematological analyses and the frequency with which animal observations and body weights were taken
- methods for verifying that the test substance reached the target tissue, or general circulation, if negative results are obtained
- actual dose (mg/kg body weight/day) calculated from diet/drinking water test substance concentration (ppm) and consumption, if applicable
- detailed description of treatment and sampling schedules and justifications for the choices
- method of euthanasia
- procedures for isolating and preserving tissues
- methods for isolation of rodent genomic DNA, rescuing the transgene from genomic DNA, and transferring transgenic DNA to a bacterial host
- source and lot numbers of all cells, kits and reagents (where applicable)
- methods for enumeration of mutants
- methods for molecular analysis of mutants and use in correcting for clonality and/or calculating mutation frequencies, if applicable

Results and discussion

- effect on mitotic index or PCE/NCE ratio by dose level by sex
- genotoxic effects (positive, negative, unconfirmed, dose-response, equivocal)
- concurrent positive and concurrent and historical negative control data
- NOAEL(NOEL) (C)/LOAEL(LOEL) (C)
- statistical results
- describe additional information that may be needed to adequately assess data for reliability

and use, including the following, if available: Mortality at each dose level by sex:

- mutant/aberration/mPCE/polyploidy frequency
- description, severity, time of onset and duration of clinical signs at each dose level and sex
- body weight changes by dose and sex
- food/water consumption changes by dose and sex

For transgenic rodent somatic and germ cell gene mutation assay also report:

- animal condition prior to and throughout the test period, including signs of toxicity
- body and organ weights at sacrifice
- for each tissue/animal, the number of mutants, number of plaques or colonies evaluated, mutant frequency
- for each tissue/animal group, number of packaging reactions per DNA sample, total number of mutants, mean mutant frequency, standard deviation
- dose-response relationship, where possible
- for each tissue/animal, the number of independent mutants and mean mutation frequency, where molecular analysis of mutations was performed
- concurrent and historical negative control data with ranges, means and standard deviations

If ambiguous results are presented; the registrant should not only describe in detail the methods and results but should also attempt to explain why different results are observed in different tests and the basis of the final conclusions; it has to be reminded that it needs to be concluded whether the substance is genotoxic or not.
Discuss if it can be verified that the test substance reached the general circulation or target tissue, if applicable.

Overall remarks, attachments

Provide toxicological evaluation of the findings of the study explaining also the biological relevance of the effects observed in animals and if needed address human relevance. If relevant include a summary of confounding factors that may affect the results of the study.
Discuss any significant deviations from the guideline.

Applicant's summary and conclusions

Provide information related to classification and labelling under *interpretation of results*, and the conclusion of the study under *conclusions*.

Reference to other ECHA Guidance Documents

Further detailed guidance on genetic toxicity can be found in:

- Guidance on information requirements and chemical safety assessment; Volume 4: Chapter 7.a, Section R.7.7.1
- IUCLID 5 End User Manual in the following chapters:

IUCLID Section	REACH Annex	Endpoint title	IUCLID End User Manual Chapter
7.6		Genetic toxicity	E.7.7
7.6.2	VIII, X 8.4.	Genetic toxicity *in vivo*	E.7.7.3

5.5 Toxicity to Reproduction / Fertility

Materials and methods

- test type (one generation, two generation, extended one-generation reproductive toxicity study (EOGRTS), screening, combined, other)
- depending on the study type, the RSS should include the following information obtained in the present study from P, F_1 animals and F_2 animals (where relevant)

Test substance:

- all relevant available information on the substance, toxicokinetic and toxicodynamic properties of the test substance
- identification data
- purity

Test animals

- species/strain/sex
- number of animals per sex per dose
- age and weight at the study initiation

Pre-test data from EOGRTS:

• vaginal smear data for P females before initiation of treatment (if data are collected at that time)

• P generation pairing records indicating male and female partner of a mating and mating success

• litter of origin records for adult F1 generation animals

Test conditions

- rationale for dose level selection
- details of test substance formulation/diet preparation, achieved concentrations
- details (rationale, etc.) of the administration of the test substance
- conversion from diet/drinking water test substance concentration (ppm) to the achieved dose (mg/kg body weight/day), if applicable
- details of food and water quality (including diet composition, if available)

Additionally for inhalation studies

- type of inhalation exposure and test conditions (e.g.: exposure apparatus)
- method of exposure ("whole body", "oro-nasal" (nose-only), or "head only", exposure data)
- analytical verification of test atmosphere concentrations
- particle size (for studies with aerosols, indicate mass median aerodynamic diameter and geometric standard deviation or give other specifications)
- type or preparation of particles (for studies with aerosols)

Additionally for dermal studies

- area covered (e.g. 10% of body surface)
- occlusion (e.g. semi-occlusive)
- total volume applied
- removal of test substance (e.g. water or solvent)

- statistical methods

Results and discussion

Describe the relevant findings. If no effects occurred, explicitly note "No effects"

- NOAEL/NOAEC (NOEL/NOEC) and LOAEL/LOAEC (LOEL/LOEC) for both males and females of P, F1 and F2 generations, as appropriate
- the lowest relevant NOAEL/NOAEC (NOEL/NOEC and LOAEL/LOAEC LOEL/LOEC for parental systemic toxicity, reproduction (fertility effects) and offspring effects
- actual dose received by dose level by sex if known
- present results preferably in tabular form by sex and generation for each test group with statistical results (as appropriate):

The following results should be reported if assessed:

- food consumption, water consumption if available, food efficiency (body weight gain per gram of food consumed, except for the period of cohabitation and during lactation), and test material consumption (for dietary/drinking water administration) for P and F1 animals
- absorption data (if available)
- body weight data for P animals
- body weight data for the selected F1 animals postweaning
- time of death during the study or whether animals survived to termination
- nature, severity and duration of clinical observations (whether reversible or not, organs examined at necropsy, others (e.g. anogenital distance)
- haematology, urinalysis and clinical chemistry data including TSH and T4
- phenotypic analysis of spleen cells (T-, B-, NK-cells)
- bone marrow cellularity
- toxic response data
- number of P and F1 females with normal or abnormal oestrous cycle and cycle duration
- time to mating (precoital interval, the number of days between pairing and mating)
- toxic or other effects on reproduction, including numbers and percentages of animals that accomplished mating, pregnancy, parturition and lactation, of males inducing pregnancy, of females with signs of dystocia/prolonged or difficult parturition
- duration of pregnancy and, if available, parturition
- numbers of implantations, litter size and percentage of male pups
- number and percent of post-implantation loss, live births and stillbirths
- litter weight and pup weight data (males, females and combined), the number of runts if determined
- number of pups with grossly visible abnormalities
- toxic or other effects on offspring, postnatal growth, viability, etc.
- data on physical landmarks in pups and other postnatal developmental data
- data on sexual maturation of F1 animals
- data on functional observations in pups and adults, as applicable
- body weight at sacrifice and absolute and relative organ weight data for the P and adult F1 animals
- necropsy findings
- detailed description of all histopathological findings
- total cauda epididymal sperm number, percent progressively motile sperm, percent morphologically normal sperm, and percent of sperm with each identified abnormality for P and F1 males
- numbers and maturational stages of follicles contained in the ovaries of P and F1 females, where applicable
- enumeration of corpora lutea in the ovaries of F1 females

- statistical treatment of results, where appropriate
- haematology, urinalysis and clinical chemistry data including TSH and T4
- phenotypic analysis of spleen cells (T-, B-, NK-cells)
- bone marrow cellularity
- toxic response data

Cohort 2 parameters (if assessed in EOGRTS):
- detailed description of the procedures used to standardize observations and procedures as well as operational definitions for scoring observations
- list of all test procedures used, and justification for their use
- details of the behavioural/functional, neuropathological and morphometric procedures used
- short justification explaining any decisions involving professional judgment
- detailed description of all behavioural/functional, neuropathological and morphometric findings by sex and dose group, including both increases and decreases from controls
- brain weight
- any diagnoses derived from neurological signs and lesions, including naturally-occurring diseases or conditions
- images of exemplar findings
- low-power images to assess homology of sections used for morphometry
- statistical treatment of results, including statistical models used to analyze the data, and the results, regardless of whether they were significant or not
- relationship of any other toxic effects to a conclusion about the neurotoxic potential of the test chemical, by sex and dose group
- impact of any toxicokinetic information on the conclusions

Cohort 3 parameters (if assessed in EOGRTS):
- serum IgM antibody titres (sensitization to SRBC or KLH), or splenic IgM PFC units (sensitization to SRBC)
- performance of the TDAR method should be confirmed as part of the optimisation process by laboratory setting up the assay for the first time, and periodically (e.g. yearly) by all laboratories

Overall remarks, attachments

Provide toxicological evaluation of the findings of the study explaining also the biological relevance of the effects observed in animals and if needed address human relevance.
If relevant include a summary of confounding factors that may affect the results of the study.
Discuss any significant deviations from the guideline.

Applicant's summary and conclusions

Provide information of the reproductive and offspring toxicity in relation to parental toxicity and (proposal of) classification for reproduction (fertility) under *interpretation of results*, and the conclusion of the study under *conclusions*. Provide all information not obtained during the study, but useful for the interpretation of the results, e.g., similarities of effects to any known neurotoxicants. For EOGRTS studies with cohort 2, include a discussion of the overall interpretation of the data based on the results, including a conclusion of whether or not the chemical caused developmental neurotoxicity and the NOAEL. For EOGRTS studies with cohort 3, include a discussion of the overall interpretation of the data based on the results, including a conclusion of whether or not the chemical caused developmental immunotoxicity and the NOAEL.

Reference to other ECHA Guidance Documents

Further detailed guidance on reproductive toxicity can be found in:

- Guidance on information requirements and chemical safety assessment; Volume 4: Chapter 7.a, Section R.7.6
- IUCLID 5 End User Manual in the following chapters:

IUCLID Section	REACH Annex	Endpoint title	IUCLID End User Manual Chapter
7.8		Toxicity to reproduction	E.7.9
7.8.1	VIII, IX and X 8.7	Toxicity to reproduction	E.7.9.2

5.6 Developmental Toxicity / Teratogenicity

Materials and methods

Test type (developmental toxicity, screening, combined, other)

Test animals

- species/strain/sex
- no. of animals per sex per dose
- age and weight at the study initiation

Administration / exposure

- route of administration – oral (gavage, drinking water, feed), dermal, inhalation (aerosol, vapour, gas, particulate), other
- duration of test/exposure period
- doses/concentration levels, rationale for dose level selection
- duration and frequency of test/exposure period
- control group and treatment
- vehicle: identification, concentration and volume used, justification of choice of vehicle (if other than water)
- test substance formulation/diet preparation, achieved concentration, stability and homogeneity of the preparation
- actual doses (mg/kg bw/day) and conversion factor from diet/drinking water test substance concentration (ppm) to the actual dose, if applicable
- details on mating procedure or insemination
- historical control data if available

for inhalation studies

- type of inhalation exposure and test conditions (e.g.: exposure apparatus,
- method of exposure ("whole body", "oro-nasal", or "head only"), exposure data
- analytical verification of test atmosphere concentrations
- particle size (for studies with aerosols, indicate mass median aerodynamic diameter and geometric standard deviation or give other specifications)
- type or preparation of particles (for studies with aerosols)

for dermal studies

- area covered (e.g. 10% of body surface)
- occlusion (e.g. semi-occlusive)
- total volume applied
- removal of test substance (e.g. water or solvent)

- statistical methods

Results and discussion

Describe the relevant findings. If no effects occurred, explicitly note "No effects"

- NOAEL (NOEL) (C)and LOAEL (LOEL) (C) maternal toxicity
- NOAEL (NOEL) and LOAEL (LOEL) developmental toxicity
- actual dose received by dose level by sex if available
- present maternal and fetal (or offspring) data with dose levels preferable in tabular form for each test group with statistical results (as appropriate):

for dams (per dose)

- number of pregnant and non-pregnant dams
- number of dams with abortions, early deliveries, stillbirths, resorptions and/or dead fetuses
- mortality and day of death
- clinical signs: description, severity, time of onset and duration

- haematological and clinical biochemistry findings if available
- mean number of implantations, live fetuses (pups), resorptions (early and late), dead fetuses, abortions and stillbirths per litter (with implants)
- pre and post implantation loss: number and percent
- number of corpora lutea
- duration of pregnancy
- body weight, body weight change and gravid uterine weight, including optionally, body weight change corrected for gravid uterine weight
- other organ weight changes if available
- histopathological findings: nature and severity
- necropsy findings including uterine weight

for fetuses/offspring (per dose)

- mean number and percent of live offspring
- sex ratio
- mean fetal/pup body weight by sex and with sexes combined
- external, soft tissue and skeletal malformations and other relevant alterations
- number and percent of fetuses and litters with malformations (including runts) and/or variations as well as description and incidences of malformations and main variations (and/or retardations)

- criteria for categorisation of external, soft tissue, and skeletal malformations and other relevant alterations

In addition, provide data on any dose-related observations.

Overall remarks, attachments

Provide toxicological evaluation of the findings of the study explaining also the biological relevance of the effects observed in animals and if needed address human relevance. If relevant include a summary of confounding factors that may affect the results of the study.
Discuss any significant deviations from the guideline.

Applicant's summary and conclusions

Provide information of the reproductive and offspring toxicity in relation to parental toxicity and (proposal of) classification for reproduction (fertility) under *interpretation of results*, and the conclusion of the study under *conclusions*.

Reference to other ECHA Guidance Documents

Further detailed guidance on developmental toxicity can be found in:

- Guidance on information requirements and chemical safety assessment; Volume 4: Chapter 7.a, Section R.7.6
- IUCLID 5 End User Manual in the following chapters:

IUCLID Section	REACH Annex	Endpoint title	IUCLID End User Manual Chapter
7.8		Toxicity to reproduction	E.7.9
7.8.2	IX and X 8.7.2	Developmental toxicity/teratogenicity	E.7.9.3

5.7 Carcinogenicity

Materials and methods

- Test type (e.g. lifelong bioassay, initiation/promotion, transgenic, neonatal mouse or other)

Test animals

- species/strain/sex
- no. of animals per sex per dose
- age and weight at the study initiation

Administration / exposure

- route of administration – oral (gavage, drinking water, feed), dermal, inhalation (aerosol, vapour, gas, particulate), other
- duration of test/exposure period
- doses/concentration levels, rationale for dose level selection
- frequency of treatment
- control group and treatment
- post exposure observation period
- vehicle: identification, concentration and volume used, justification of choice of vehicle (if other than water)
- test substance formulation/diet preparation, achieved concentration, stability and homogeneity of the preparation
- actual doses (mg/kg bw/day) and conversion factor from diet/drinking water test substance concentration (ppm) to the actual dose, if applicable
- satellite groups and reasons they were added

  for inhalation studies
  - type of inhalation exposure and and test conditions (e.g.: exposure apparatus,
  - method of exposure (“whole body”, “oro-nasal”, or “head only”), exposure data)
  - analytical verification of test atmosphere concentrations
  - particle size (for studies with aerosols, indicate mass median aerodynamic diameter and geometric standard deviation or give other specifications)
  - type or preparation of particles (for studies with aerosols)

  for dermal studies
  - area covered (e.g. 10% of body surface)
  - occlusion (e.g. semi-occlusive)
  - total volume applied
  - removal of test substance (e.g. water or solvent)
- statistical methods

Results and discussion

Describe the relevant findings. If no effects occurred, explicitly note "No effects"

Results shall be presented preferably in tabular form where appropriate:

- mortality and time to death (indicate number died per sex per dose and time to death)
- clinical signs
- body weight gain
- food/water consumption
- ophthalmoscopic examination
- clinical chemistry
- haematology
- urinalysis
- organ weights
- necropsy findings: nature and severity

- histopathological findings: nature and severity
    - non neoplastic histopathological findings,
    - neoplastic histopathological findings,
- tumour incidence data by sex, dose and tumour type
- toxic response data by sex and dose
- time to tumours (for dermal route and skin tumours: give mean time until appearance of tumour or time until appearance of first tumour or other measure)
- statistical results (unless already described with specific test results above)

Overall remarks, attachments

Provide toxicological evaluation of the findings of the study explaining also the biological relevance of the effects observed in animals and if needed address human relevance. If relevant include a summary of confounding factors that may affect the results of the study.
Discussion can also include:

- any modelling approaches;
- dose-response relationships;
- historical control data;
- consideration of any mode of action information;
- BMD, NOAEL or LOAEL determination;

Applicant's summary and conclusions

Provide information related to classification and labelling under *interpretation of results*, and the conclusion of the study under *conclusions*.

Reference to other ECHA Guidance Documents

Further detailed guidance on carcinogenicity can be found in:

- Guidance on information requirements and chemical safety assessment; Volume 4: Chapter 7.a, Section R.7.7.8
- IUCLID 5 End User Manual in the following chapters:

IUCLID Section	REACH Annex	Endpoint title	IUCLID End User Manual Chapter
7.7	X, 8.9.1	Carcinogenicity	E.7.8

5.8 Toxicokinetics

A toxicokinetic assessment based on the available data is required for substances manufactured/imported at quantities above 10 t/a.

The toxicokinetic assessment can either be based on (i) information from a toxicokinetic study if already available or (ii) a theoretical estimation taking into account/based on the physicochemical properties of the substance and the data from *in vivo* and *in vitro* studies available as well as other relevant information on analogue substances. The information provided in this section is very important for the interpretation of observations made in the repeat dose toxicity tests and for the risk assessment when estimates of dermal and oral exposure are required.

If a toxicokinetic study is available registrants are advised to follow the Robust study summary template for pharmacokinetics as described in chapter 2 of the Manual of investigation of HPV chemicals at: http://www.oecd.org/dataoecd/13/17/36045066.pdf. In addition the relevant parts of the IUCLID template shall be filled with as much detail as possible.

In general, for toxicological studies the following should be reported:

Methods

- species, age and strain
- number and sex of animal
- housing and feeding condition
- information on the test substance
- justification for any modification of route of exposure and exposure conditions, if applicable
- justification for selection of dose levels
- type of solvent or vehicle, if any, used
- number of treatment groups and number of animals per group
- dosage levels and volume (and specific activity of the dose when radioactivity is used)
- route(s) and methods of administration
- frequency of dosing
- total radioactivity per animal
- sample collection and handling
- analytical methods used for separation, quantitation and identification of metabolites
- limit of detection for the employed methods
- statistical analysis

Results

- quantity and percent recovery of radioactivity in urine, faeces, expired air, and urine and faeces cage wash.
    - for dermal studies, also include data on substance recovery from treated skin, skin washes, and residual radioactivity in the skin covering apparatus and metabolic unit as well as results of the dermal washing study;
    - for inhalation studies, also include data on recovery of test substance from lungs and nasal tissues;
- tissue distribution reported as percent of administered dose and concentration (microgram equivalents per gram of tissue), and tissue-to-blood or tissue-to-plasma ratios
- material balance developed from each study involving the assay of body tissues and excreta

- plasma concentrations and toxicokinetic parameters (bioavailability, AUC, Cmax, Tmax, clearance, half-life) after administration by the relevant route(s) of exposure
- rate and extent of absorption of the test substance after administration by the relevant route(s) of exposure
- quantities of the test substance and metabolites (reported as percent of the administered dose) collected in excreta
- a figure with the proposed metabolic pathways and the molecular structures of the metabolites.

Discussion and Conclusions

This section should contain:

- provide a proposed metabolic pathway based on the results of the metabolism and disposition of the test substance
- discussion on any potential species and sex differences regarding the disposition and/or biotransformation of the test substance
- table and discussion on the identification and magnitude of metabolites, rates of clearance, bioaccumulation potential, and level of tissue residues of parent, and/or metabolite(s), as well as possible dose-dependent changes in TK parameters, as appropriate
- any relevant TK data obtained in the course of conducting toxicity studies
- a conclusion that can be supported by the findings of the study.

If a toxicokinetic study is not available, include consideration of chemical structure, molecular weight, physical form, particle size, vapour pressure, water solubility, LogP, and information on hydrolysis. Evidence from structure activity relationships (SAR) and information about analogous structures may also provide useful information, i.e. what is known about absorption, distribution, metabolism and excretion of similar substances.

Observations of local and systemic effects in toxicity studies should be considered and differences in toxicity for different routes of exposure should be taken into account. Consider also the potential for bioaccumulation and the influence of metabolic activation on the activity of the substance as observed in *in vitro* mutagenicity assays.

Reference to other ECHA Guidance Documents

- Further detailed guidance on toxicokinetics can be found in:
- Guidance on information requirements and chemical safety assessment; Volume 4: Chapter 7.a, Section R.7.12
- IUCLID 5 End User Manual in the following chapters:

IUCLID Section	REACH Annex	Endpoint title	IUCLID End User Manual Chapter
7.1.1	VIII 8.8	Basic toxicokinetics	E.7.2.2
7.1.2	VIII 8.8	Dermal absorption	E.7.2.3

6. General aspects related to THE preparation of study summary

The level of detail to be used for the description of supporting studies is usually to be decided on a case-by-case basis.

For instance, detailed descriptions can be sensible if these supporting studies are used to defend the key study identified against conflicting results from less valid studies. In this case the study shall be flagged as “disregarded” and in order to prepare the endpoint study record, detailed information on the applied methodology, test materials, the study results and conclusions have to be provided in the relevant fields of IUCLID. It should be also demonstrated whether the specific validity, quality, or repeatability criteria for the study have been met as specified in the description of the corresponding (EU or OECD) test method. In the “Applicant’s summary and conclusions” field of the study record endpoint it should be clear 1) whether or not the validity criteria have been fulfilled and 2) which conclusions were derived from the underlying data.

In order to report a Study Summary in IUCLID 5 the option of ‘basic fields’ in the header of the endpoint study record has to be selected. To fill-in the proper IUCLID fields, the registrant should follow the guidance provided in the IUCLID End User manual[11].

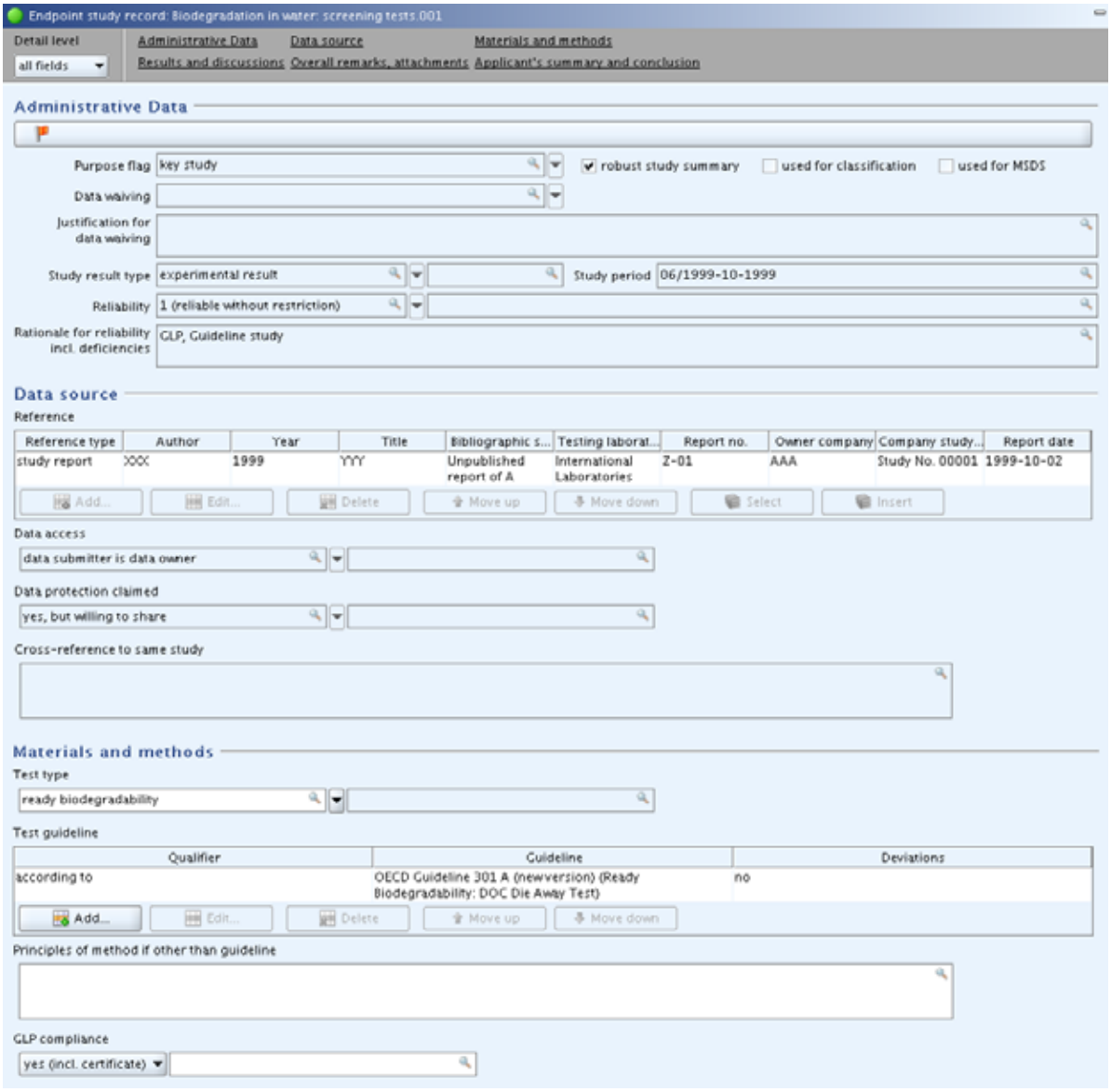
Please note that although the template for Study Summary (basic fields) contains less fields to be filled in comparison to the template for Robust Study Summary (all fields), the information must still be provided in sufficient detail to allow a technically qualified person to make an assessment of the relevance of the study without having to go back to the full study report.

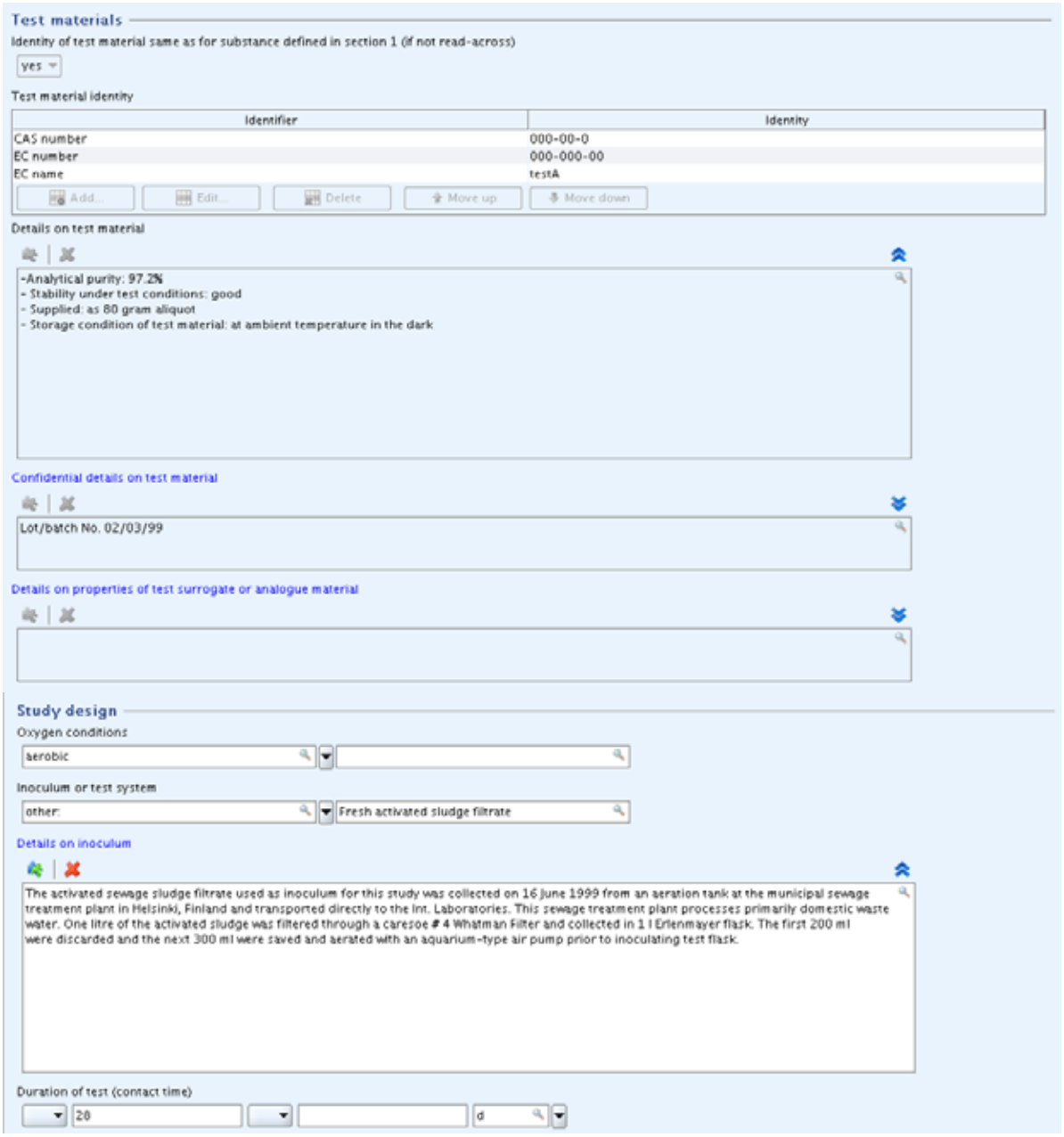
[11] http://guidance.echa.europa.eu/docs/guidance_document/iuclid_en.pdf

ATTACHMENTS

Attachment 1: IUCLID example on RSS for Biodegradation

An example of the Robust Study Summary for the ready biodegradability in water: screening test for substance testA (CAS: 000-00-0, EC: 000-000-00).




Test materials
Identity of test material same as for substance defined in section 1 (if not read-across)
yes
Test material identity
Identifier
Identity
CAS number
000-00-0
EC number
000-000-00
EC name
testA
Add...
Edit...
Delete
Move up
Move down
Details on test material
-Analytical purity: 97.2%
- Stability under test conditions: good
- Supplied: as 80 gram aliquot
- Storage condition of test material: at ambient temperature in the dark
Confidential details on test material
Lot/batch No. 02/03/99
Details on properties of test surrogate or analogue material
Study design
Oxygen conditions
aerobic
Inoculum or test system
other:
Fresh activated sludge filtrate
Details on inoculum
The activated sewage sludge filtrate used as inoculum for this study was collected on 16 June 1999 from an aeration tank at the municipal sewage treatment plant in Helsinki, Finland and transported directly to the lnt. Laboratories. This sewage treatment plant processes primarily domestic waste water. One litre of the activated sludge was filtered through a caresoe # 4 Whatman Filter and collected in 1 l Erlenmayer flask. The first 200 ml were discarded and the next 300 ml were saved and aerated with an aquarium-type air pump prior to inoculating test flask.
Duration of test (contact time)
20
d

Initial test substance concentration

Initial conc.	Based on
20 mg/L	DOC
30 mg/L	test mat.

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Parameter followed for biodegradation estimation

Parameter followed for biodegradation estimation
DOC removal

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Details on analytical methods

Degradation of testA was monitored by assessing the removal of DOC by the inoculum. DOC was analysed in duplicate at each time point using Dorh mann DC-80 Carbon Analyser. Degradation was calculated by subtracting the amount of DOC in the negative (inoculum only) control from that in the test material or positive control sample at any given time point and then dividing it by the initial DOC concentration at time 0.

Details on study design

TestA was incubated for 28 days in continuously agitated 2 litre open beakers (in duplicate) in the dark with an inoculum originally collected from the local municipal STP. The incubation temperature was 19 - 23 oC, pH was 7.6 and was steady during the test duration, O2 concentration measured wit h the Oxygen Meter (model XXX) ranged from 8.00 - 8.15 mg/L over the study. The concentration of inoculum was 1 ml of inoculum per litre of test s olution. The concentration of testA corresponded to 20 mg/L (30 mg testA/litre).
Controls included:
- reference compound + inoculum
- inoculum only (inoculum blank)
- test substance + reference substance + inoculum (toxicity control)
- test substance + sterilising agent (abiotic sterile control)
- test substance + sterilising agent + inoculum (adsorption control)

Reference substance

benzoic acid, sodium salt

Any other information on materials and methods incl. tables

Results and discussions

Preliminary study

no data

Test performance

not applicable

% Degradation of test substance

%Degr.	St. dev.	Parameter	Sampling time	Remarks
15		DOC removal	7 d	
89.5		DOC removal	14 d	
90.5		DOC removal	21 d	
89.9		DOC removal	27 d	
90		DOC removal	28 d	

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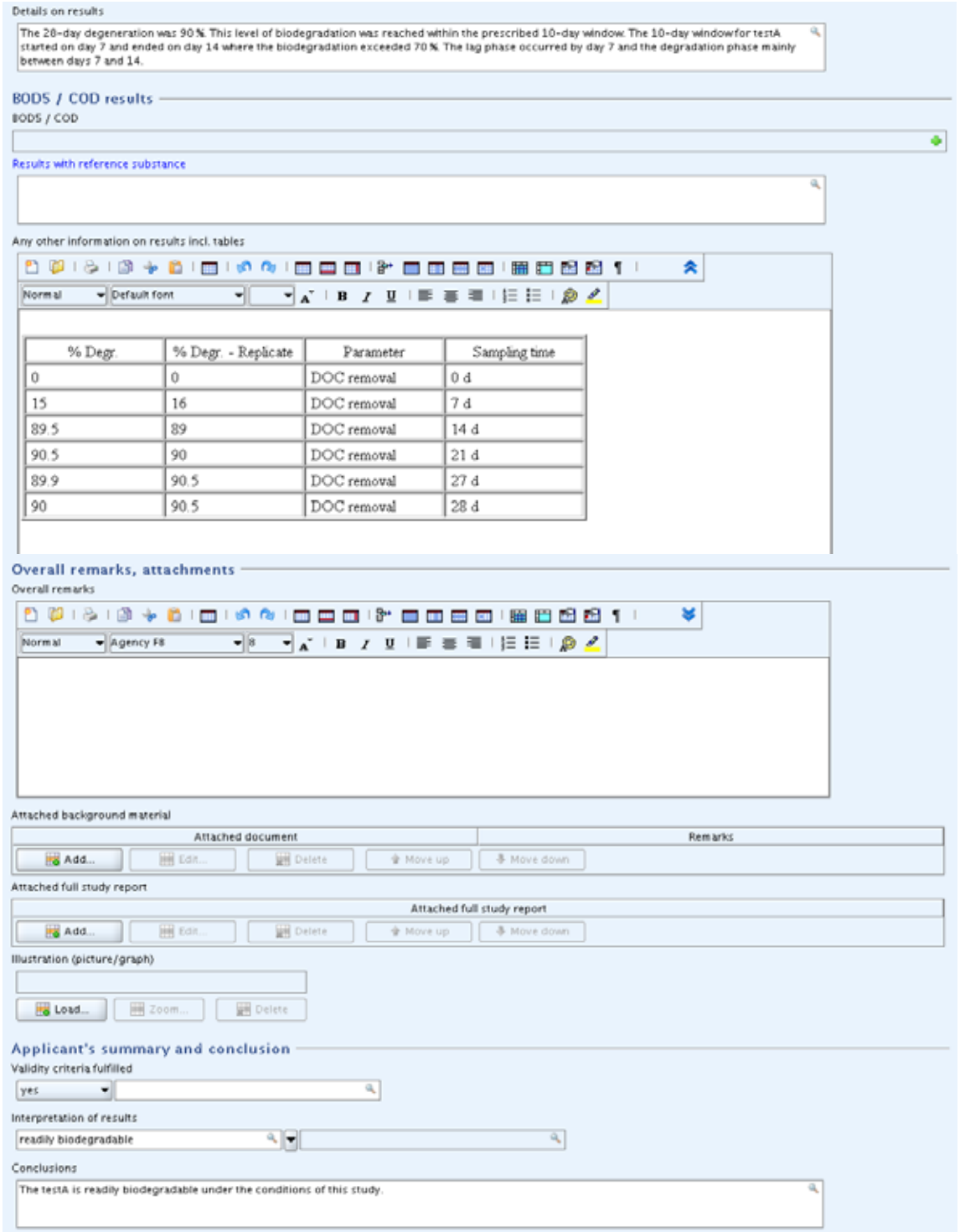

Details on results

The 28-day degeneration was 90 %. This level of biodegradation was reached within the prescribed 10-day window. The 10-day windowfor testA started on day 7 and ended on day 14 where the biodegradation exceeded 70 %. The lag phase occurred by day 7 and the degradation phase mainly between days 7 and 14.

BOD5 / COD results

BOD5 / COD

Results with reference substance

Any other information on results incl. tables

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% Degr.	% Degr. - Replicate	Parameter	Sampling time
0	0	DOC removal	0 d
15	16	DOC removal	7 d
89.5	89	DOC removal	14 d
90.5	90	DOC removal	21 d
89.9	90.5	DOC removal	27 d
90	90.5	DOC removal	28 d

Overall remarks, attachments

Overall remarks

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Attached background material

Attached document	Remarks

Add... | Edit... | Delete | Move up | Move down

Attached full study report

Attached full study report

Add... | Edit... | Delete | Move up | Move down

Illustration (picture/graph)

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Applicant's summary and conclusion

Validity criteria fulfilled

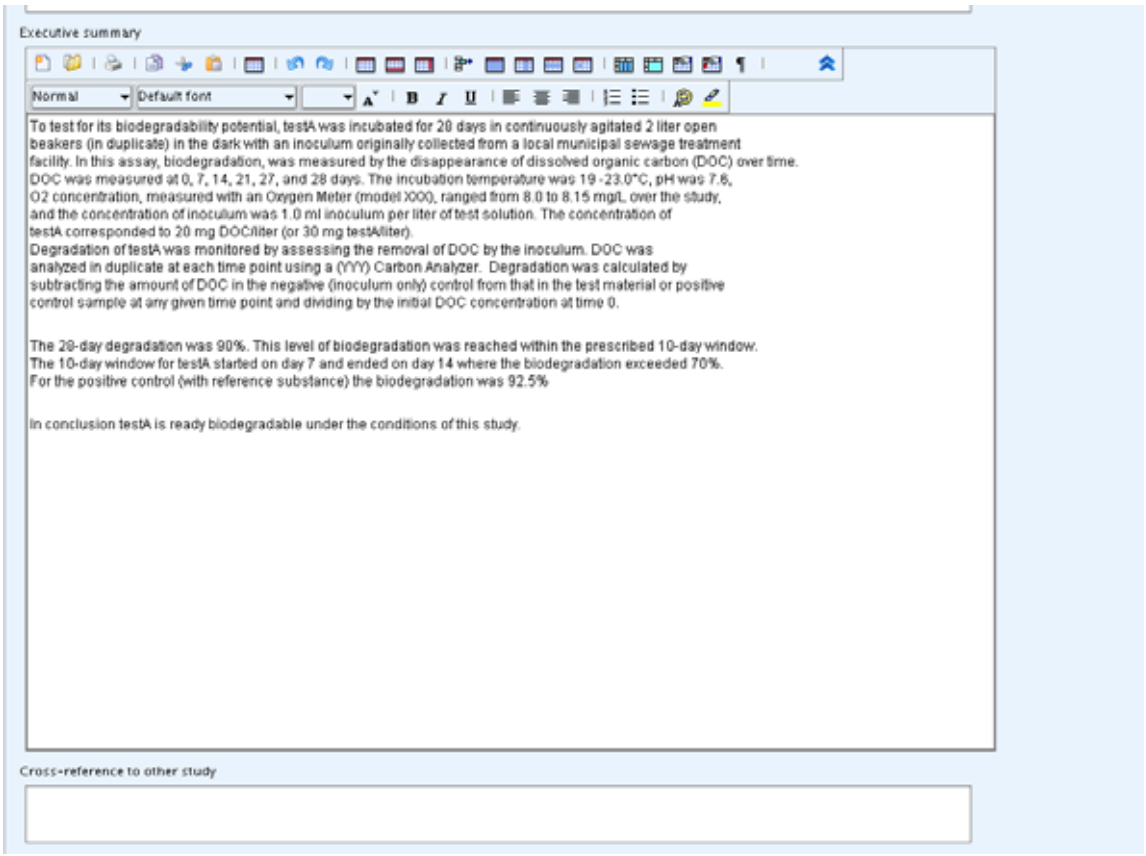
yes

Interpretation of results

readily biodegradable

Conclusions

The testA is readily biodegradable under the conditions of this study.


Executive summary
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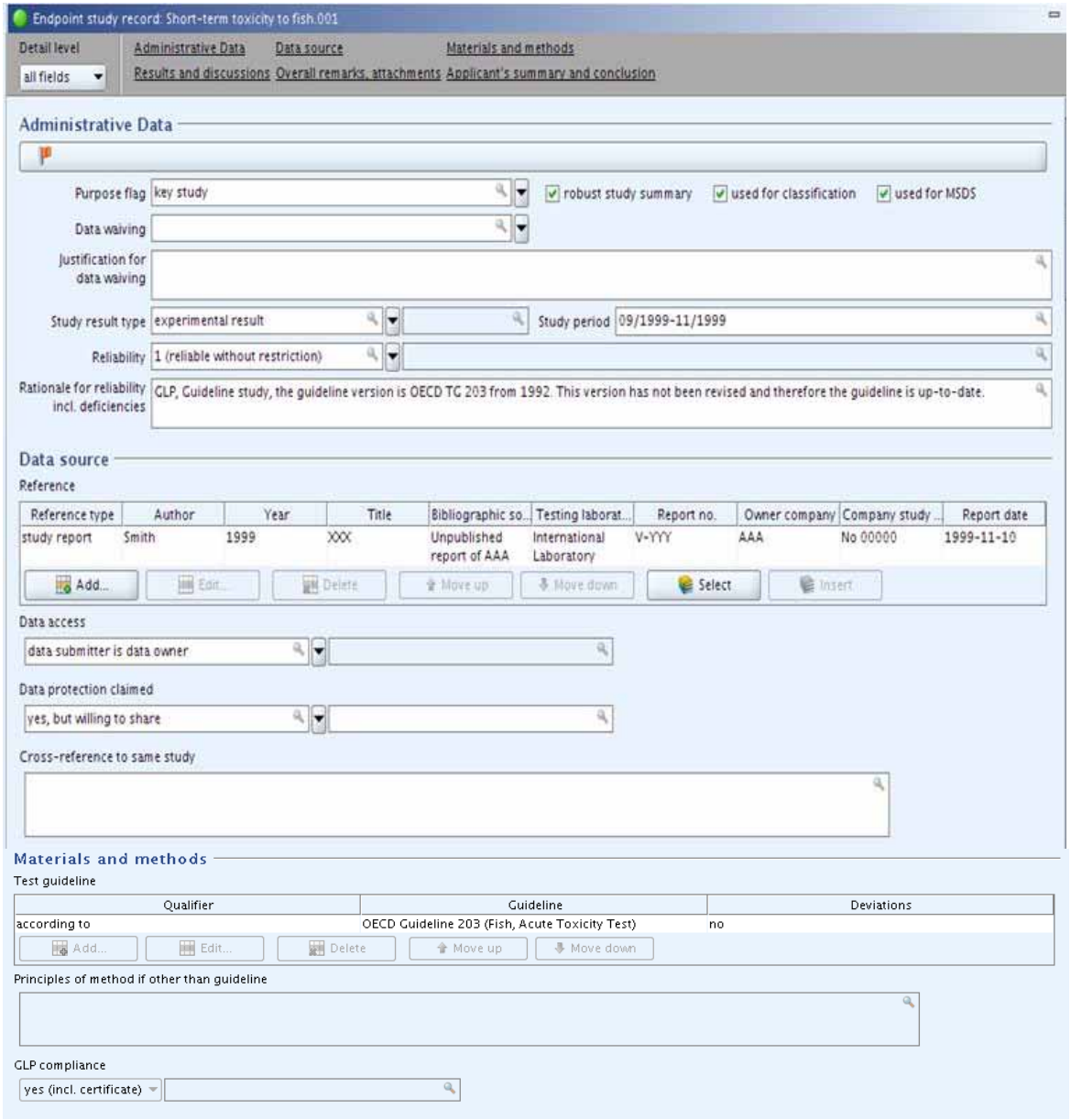
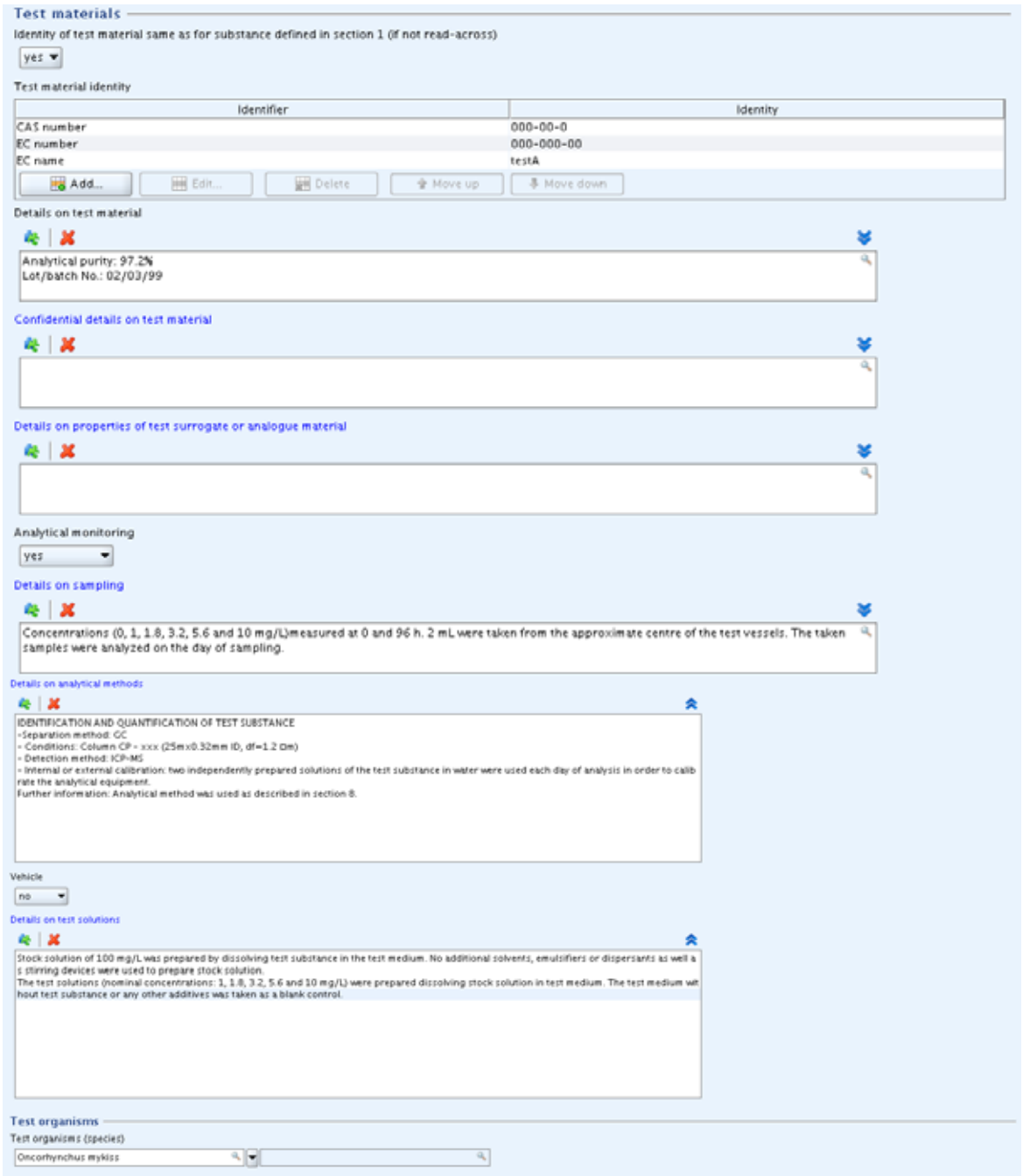
To test for its biodegradability potential, testA was incubated for 28 days in continuously agitated 2 liter open beakers (in duplicate) in the dark with an inoculum originally collected from a local municipal sewage treatment facility. In this assay, biodegradation, was measured by the disappearance of dissolved organic carbon (DOC) over time. DOC was measured at 0, 7, 14, 21, 27, and 28 days. The incubation temperature was 19 -23.0°C, pH was 7.6, O2 concentration, measured with an Oxygen Meter (model XXX), ranged from 8.0 to 8.15 mg/L over the study, and the concentration of inoculum was 1.0 ml inoculum per liter of test solution. The concentration of testA corresponded to 20 mg DOC/liter (or 30 mg testA/liter).
Degradation of testA was monitored by assessing the removal of DOC by the inoculum. DOC was analyzed in duplicate at each time point using a (YYY) Carbon Analyzer. Degradation was calculated by subtracting the amount of DOC in the negative (inoculum only) control from that in the test material or positive control sample at any given time point and dividing by the initial DOC concentration at time 0.
The 28-day degradation was 90%. This level of biodegradation was reached within the prescribed 10-day window.
The 10-day window for testA started on day 7 and ended on day 14 where the biodegradation exceeded 70%.
For the positive control (with reference substance) the biodegradation was 92.5%
In conclusion testA is ready biodegradable under the conditions of this study.
Cross-reference to other study

Attachment 2: IUCLID example on RSS for short term toxicity to fish

An example of the Robust Study Summary for the short term toxicity to fish for substance test A (CAS: 000-00-0, EC: 000-000-00)





Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test material identity

Identifier	Identity
CAS number	000-00-0
EC number	000-000-00
EC name	testA

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Details on test material

Analytical purity: 97.2%
Lot/batch No.: 02/03/99

Confidential details on test material

Details on properties of test surrogate or analogue material

Analytical monitoring

yes

Details on sampling

Concentrations (0, 1, 1.8, 3.2, 5.6 and 10 mg/L)measured at 0 and 96 h. 2 mL were taken from the approximate centre of the test vessels. The taken samples were analyzed on the day of sampling.

Details on analytical methods

IDENTIFICATION AND QUANTIFICATION OF TEST SUBSTANCE
-Separation method: GC
- Conditions: Column CP = xxx (25m x0.32mm ID, df=1.2 Dm)
- Detection method: ICP-MS
- Internal or external calibration: two independently prepared solutions of the test substance in water were used each day of analysis in order to calib rate the analytical equipment.
Further information: Analytical method was used as described in section 8.

Vehicle

no

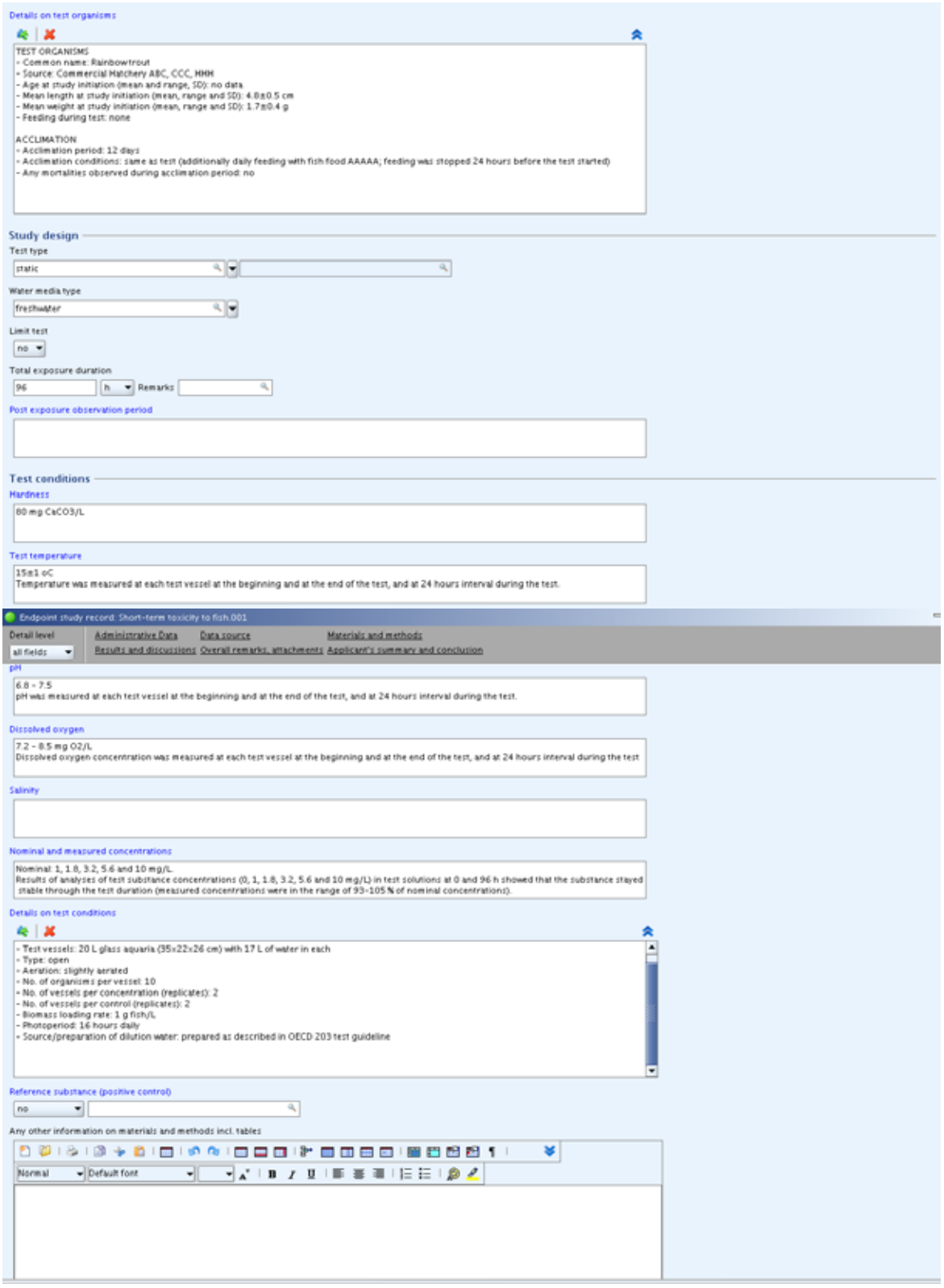
Details on test solutions

Stock solution of 100 mg/L was prepared by dissolving test substance in the test medium. No additional solvents, emulsifiers or dispersants as well a s stirring devices were used to prepare stock solution.
The test solutions (nominal concentrations: 1, 1.8, 3.2, 5.6 and 10 mg/L) were prepared dissolving stock solution in test medium. The test medium wit hout test substance or any other additives was taken as a blank control.

Test organisms

Test organisms (species)

Oncorhynchus mykiss


Details on test organisms
TEST ORGANISMS
- Common name: Rainbowtrout
- Source: Commercial Hatchery ABC, CCC, HHH
- Age at study initiation (mean and range, SD): no data
- Mean length at study initiation (mean, range and SD): 4.8±0.5 cm
- Mean weight at study initiation (mean, range and SD): 1.7±0.4 g
- Feeding during test: none
ACCLIMATION
- Acclimation period: 12 days
- Acclimation conditions: same as test (additionally daily feeding with fish food AAAAA; feeding was stopped 24 hours before the test started)
- Any mortalities observed during acclimation period: no
Study design
Test type
static
Water media type
freshwater
Limit test
no
Total exposure duration
96
h
Remarks
Post exposure observation period
Test conditions
Hardness
80 mg CaCO3/L
Test temperature
15±1 oC
Temperature was measured at each test vessel at the beginning and at the end of the test, and at 24 hours interval during the test.
Endpoint study record: Short-term toxicity to fish.001
Detail level
all fields
Administrative Data
Data source
Materials and methods
Results and discussions
Overall remarks, attachments
Applicant's summary and conclusion
pH
6.8 - 7.5
pH was measured at each test vessel at the beginning and at the end of the test, and at 24 hours interval during the test.
Dissolved oxygen
7.2 - 8.5 mg O2/L
Dissolved oxygen concentration was measured at each test vessel at the beginning and at the end of the test, and at 24 hours interval during the test
Salinity
Nominal and measured concentrations
Nominal: 1, 1.8, 3.2, 5.6 and 10 mg/L.
Results of analyses of test substance concentrations (0, 1, 1.8, 3.2, 5.6 and 10 mg/L) in test solutions at 0 and 96 h showed that the substance stayed stable through the test duration (measured concentrations were in the range of 93-105 % of nominal concentrations).
Details on test conditions
- Test vessels: 20 L glass aquaria (35x22x26 cm) with 17 L of water in each
- Type: open
- Aeration: slightly aerated
- No. of organisms per vessel: 10
- No. of vessels per concentration (replicates): 2
- No. of vessels per control (replicates): 2
- Biomass loading rate: 1 g fish/L
- Photoperiod: 16 hours daily
- Source/preparation of dilution water: prepared as described in OECD 203 test guideline
Reference substance (positive control)
no
Any other information on materials and methods incl. tables
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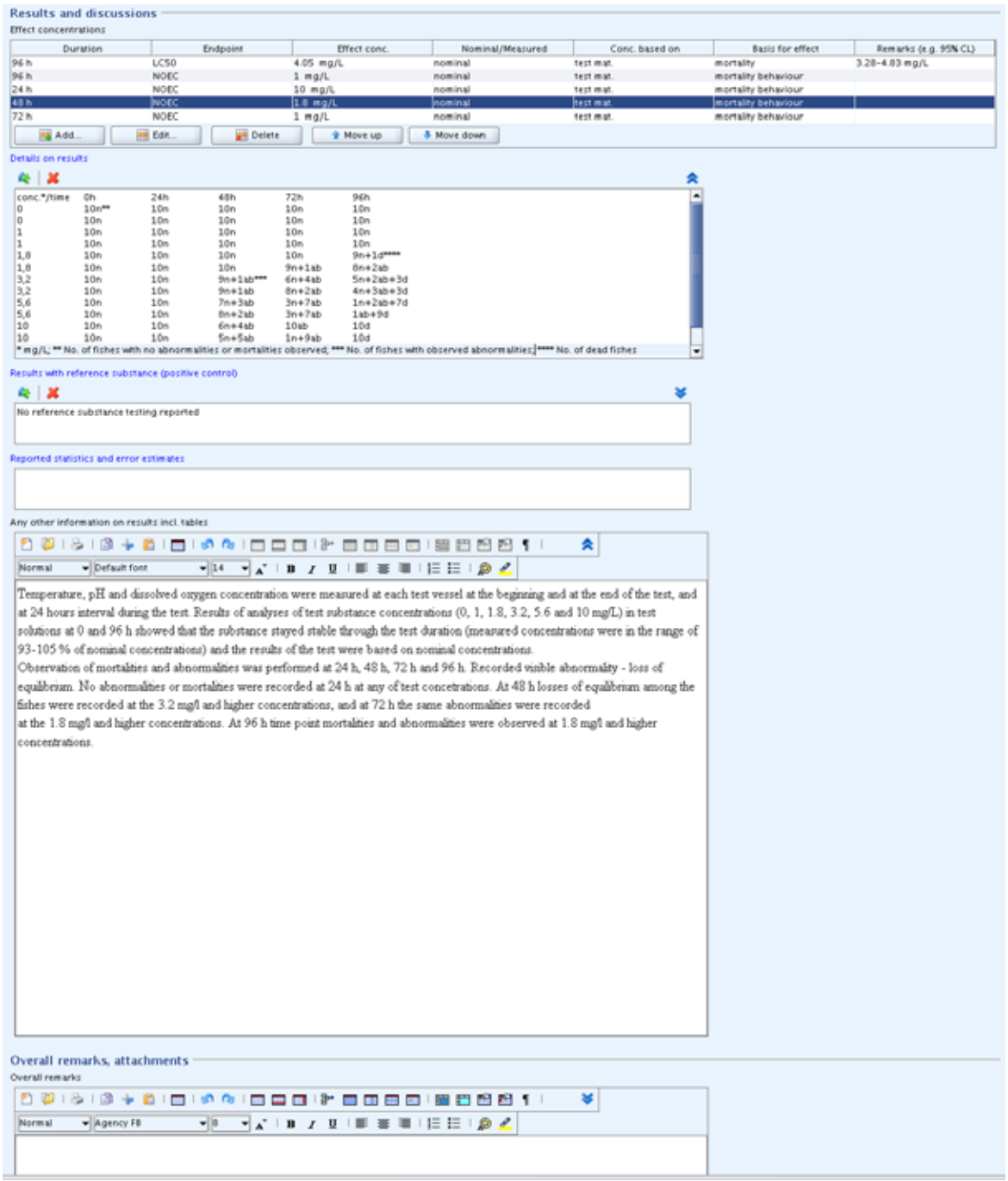

Results and discussions

Effect concentrations

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
96 h	LC50	4.05 mg/L	nominal	test mat.	mortality	3.28-4.83 mg/L
96 h	NOEC	1 mg/L	nominal	test mat.	mortality behaviour	
24 h	NOEC	10 mg/L	nominal	test mat.	mortality behaviour	
48 h	NOEC	1.8 mg/L	nominal	test mat.	mortality behaviour	
72 h	NOEC	1 mg/L	nominal	test mat.	mortality behaviour	

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Details on results

conc.*/time	0h	24h	48h	72h	96h
0	10n**	10n	10n	10n	10n
0	10n	10n	10n	10n	10n
1	10n	10n	10n	10n	10n
1	10n	10n	10n	10n	10n
1,8	10n	10n	10n	10n	9n+1d*****
1,8	10n	10n	10n	9n+1ab	8n+2ab
3,2	10n	10n	9n+1ab***	6n+4ab	5n+2ab+3d
3,2	10n	10n	9n+1ab	8n+2ab	4n+3ab+3d
5,6	10n	10n	7n+3ab	3n+7ab	1n+2ab+7d
5,6	10n	10n	8n+2ab	3n+7ab	1ab+9d
10	10n	10n	6n+4ab	10ab	10d
10	10n	10n	5n+5ab	1n+9ab	10d

* mg/L; ** No. of fishes with no abnormalities or mortalities observed; *** No. of fishes with observed abnormalities; **** No. of dead fishes

Results with reference substance (positive control)

No reference substance testing reported

Reported statistics and error estimates

Any other information on results incl. tables

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Temperature, pH and dissolved oxygen concentration were measured at each test vessel at the beginning and at the end of the test, and at 24 hours interval during the test. Results of analyses of test substance concentrations (0, 1, 1.8, 3.2, 5.6 and 10 mg/L) in test solutions at 0 and 96 h showed that the substance stayed stable through the test duration (measured concentrations were in the range of 93-105 % of nominal concentrations) and the results of the test were based on nominal concentrations.
Observation of mortalities and abnormalities was performed at 24 h, 48 h, 72 h and 96 h. Recorded visible abnormality - loss of equilibrium. No abnormalities or mortalities were recorded at 24 h at any of test concetrations. At 48 h losses of equilibrium among the fishes were recorded at the 3.2 mg/l and higher concentrations, and at 72 h the same abnormalities were recorded
at the 1.8 mg/l and higher concentrations. At 96 h time point mortalities and abnormalities were observed at 1.8 mg/l and higher concentrations.

Overall remarks, attachments

Overall remarks

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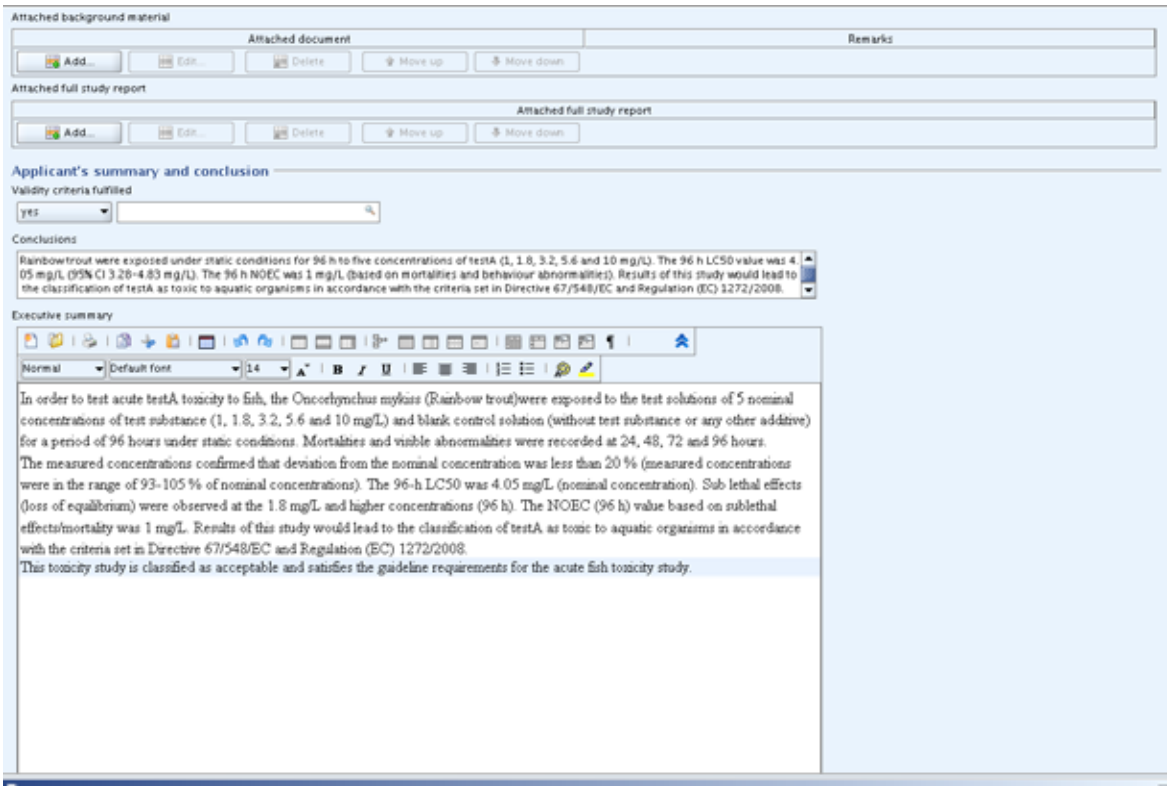
Attached background material
Attached document
Remarks
Add...
Edit...
Delete
Move up
Move down
Attached full study report
Attached full study report
Add...
Edit...
Delete
Move up
Move down
Applicant's summary and conclusion
Validity criteria fulfilled
yes
Conclusions
Rainbow trout were exposed under static conditions for 96 h to five concentrations of testA (1, 1.8, 3.2, 5.6 and 10 mg/L). The 96 h LC50 value was 4.05 mg/L (95% CI 3.28-4.83 mg/L). The 96 h NOEC was 1 mg/L (based on mortalities and behaviour abnormalities). Results of this study would lead to the classification of testA as toxic to aquatic organisms in accordance with the criteria set in Directive 67/548/EC and Regulation (EC) 1272/2008.
Executive summary
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In order to test acute testA toxicity to fish, the Oncorhynchus mykiss (Rainbow trout)were exposed to the test solutions of 5 nominal concentrations of test substance (1, 1.8, 3.2, 5.6 and 10 mg/L) and blank control solution (without test substance or any other additive) for a period of 96 hours under static conditions. Mortalities and visible abnormalities were recorded at 24, 48, 72 and 96 hours.
The measured concentrations confirmed that deviation from the nominal concentration was less than 20 % (measured concentrations were in the range of 93-105 % of nominal concentrations). The 96-h LC50 was 4.05 mg/L (nominal concentration). Sub lethal effects (loss of equilibrium) were observed at the 1.8 mg/L and higher concentrations (96 h). The NOEC (96 h) value based on sublethal effects/mortality was 1 mg/L. Results of this study would lead to the classification of testA as toxic to aquatic organisms in accordance with the criteria set in Directive 67/548/EC and Regulation (EC) 1272/2008.
This toxicity study is classified as acceptable and satisfies the guideline requirements for the acute fish toxicity study.

EUROPEAN CHEMICALS AGENCY
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