How to bring your registration dossier in compliance with REACH – Tips and Hints Part 4

Biodegradation II –

How to choose the appropriate method for Ready Biodegradability testing?

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11 September 2013
11:00 - 13:00 Helsinki Time (EEST, GMT +3)

Tips and hints for concluding on ready biodegradability

Biodegradation

Webinar Part 3, 14 May 2013

- Instructions for reporting
  - Used Test Guideline
  - Results and the interpretation
  - QSARs
  - Details on inoculum used
  - Adaptation

Biodegradation II

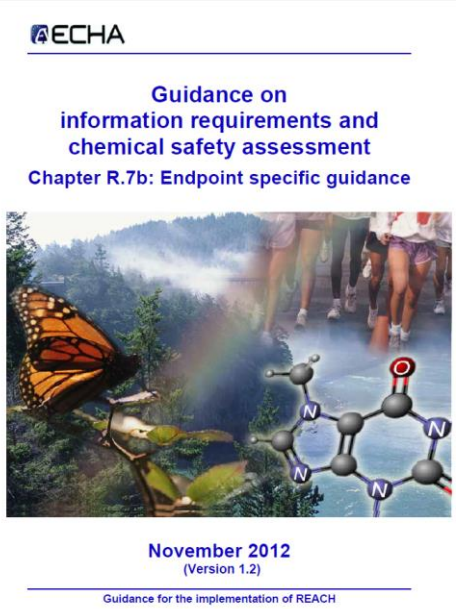
Webinar Part 4, 11 September 2013

- Applicability of the ready biodegradation Test Guidelines
- Instructions for reporting
  - Substance properties
  - Study design
  - Validity criteria

REACH Information requirements

	Column 1	**Column 2**
Annex VII	9.2.1.1. Ready biodegradability	9.2.1.1. The study does not need to be conducted if the substance is inorganic.

Choose appropriate Test Guideline to conclude on ready biodegradability for your substance



OECD 301* and 310 Ready Biodegradability

- 301 A: DOC Die-Away
- 301 B: CO2 Evolution (Modified Sturm Test)
- 301 C: MITI (I) (Ministry of International Trade and Industry, Japan)
- 301 D: Closed Bottle
- 301 E: Modified OECD Screening
- 301 F: Manometric Respirometry
- 310 Ready biodegradability - CO2 in sealed vessels (Headspace Test)

* and corresponding Guidelines

(ECHA Guidance R.7b, Appendix 7.9-1 e.g. EU C.4, ISO)

PASS LEVEL after 28 days (with 10–day window)

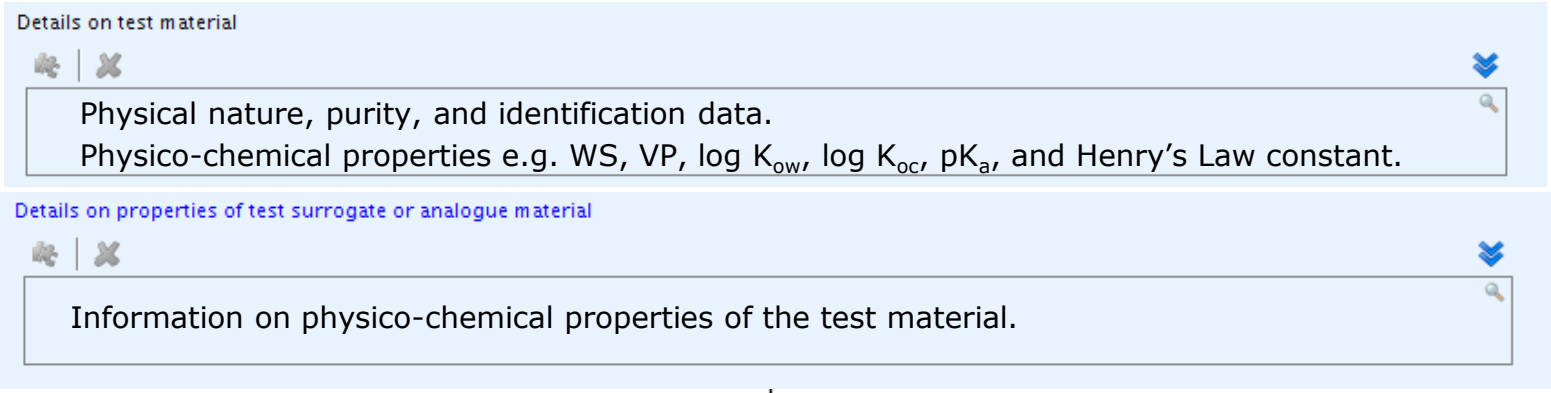
- **60% $ThCO_2$** - Theoretical carbon dioxide production
- **60% ThOD -** Theoretical oxygen demand
- 70% DOC - Dissolved organic carbon removal

- 10 day window begins when the degree of biodegradation has reached 10 %.

Substance properties and biodegradation testing

- Substance properties influence the applicability of specific Test Guidelines
- Information on physico-chemical properties enables identification of the most appropriate Test Guideline
  - Vapour pressure
  - Water solubility
  - Adsorption- desorption
  - Partition coefficient
  - Dissociation constant in water

In IUCLID 5.2.1 Biodegradation in water: screening tests



OECD 301 Applicability of test methods; ready biodegradability

Test	Analytical method	Suitability for compounds which are: Poorly water soluble	Volatile	Adsorbing
DOC Die-Away (301 A)	Dissolved organic carbon	-	-	+/-
CO_2 Evolution (301 B)	Respirometry: CO_2 evolution	+	-	+
MITI (I) (301 C)	Respirometry: oxygen consumption	+	-/+	+
Closed Bottle (301 D)	Respirometry: dissolved oxygen	+/-	+	+
Modified OECD Screening (301 E)	Dissolved organic carbon	-	-	+/-
Manometric Respirometry (301F)	Oxygen consumption	+	+/-	+

Consider the applicability of the Test Guideline (I)

If any deviations in the test conditions described in the Test Guideline

→ e.g. corresponding Test Guideline is used

- Concentration of the test substance
- Concentration of the inoculum
- Concentration of the elements in mineral medium
- pH
- Temperature

→ JUSTIFY possible deviations

Consider the applicability of the Test Guideline (II)

OECD Ready biodegradability tests (301 series and 310)

- All test applicable when
  - Water solubility is at least 100 mg/l
  - Substance is non-volatile
  - Substance is non-adsorptive

- OECD 301 Annex III Evaluation of the biodegradability of poorly water soluble compounds
- ECHA Guidance on information requirements and chemical safety assessment R.7B Appendix 7.9-3 Testing the Biodegradation of poorly Water Soluble Substances

Specific applicability criteria

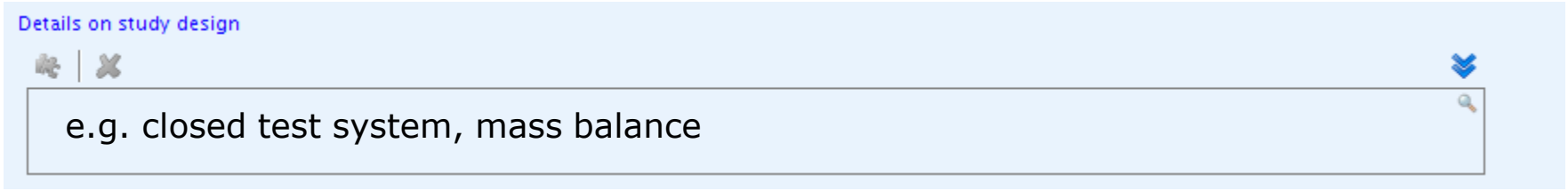
OECD 301A and 301E

- Not suitable for volatile substances
- Not suitable for poorly water soluble substances (< 100 mg/L)
- Carbon content and, preferably, the purity or relative proportions of major components should be known

OECD 301B

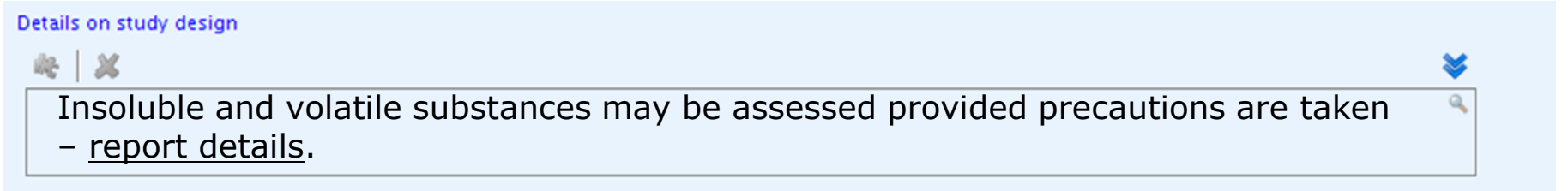
- Not suitable for volatile substances
- Carbon content and, preferably, the purity or relative proportions of major components should be known

Details on how the substance properties have been taken into account may be reported under IUCLID 5.2.1 Study design:



Specific applicability criteria

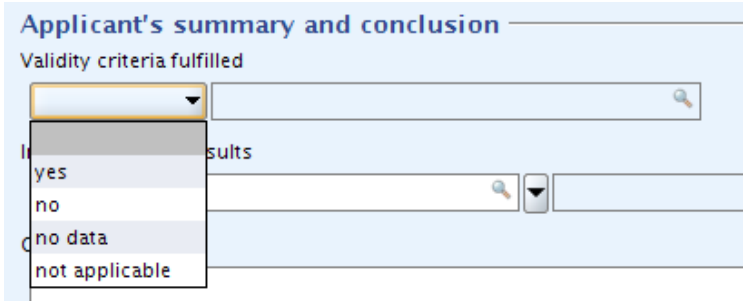
OECD 301C, 301D and 301F



- Insoluble substances;
  - Report dispersion and agitation procedures (OECD 301C and 301D)
  - Do not use solvents or emulsifying agents (OECD 301C)
- Volatile substances
  - Keep the "dead " gas space to a minimum (OECD 301C)
- When ThOD can not be determined (OECD 301D and 301F)
  - Determine COD (chemical oxygen demand)
  - If the test substance is not completely oxidised in the COD test, degradation may be overestimated
  - ➢ Report how taken into account

Report if validity criteria are met (OECD 301 series)

- Report that validity criteria of the test applied is met



- General validity criteria for OECD 301 series
  - Difference of extremes of replicate values of the test chemical at the plateau, at the end of the tests or at the end of the 10-d window is less than 20 %
  - Percentage degradation of the reference compound has reached the pass levels by day 14
  - If toxicity control (test substance + reference compound) degrades less than 35 % (DOC) or less than 25 % (ThOD or ThCO2) within 14 days, lower concentration of the test substrate and/or a higher inoculum concentration should be used

Report if validity criteria are met (OECD 301 series)

- Additional specific test dependent validity criteria

Test Guideline	Validity criteria
OECD 301B	• IC content of the test substance suspension at the beginning of the test must be less than 5 % of the TC. • Total CO_2 evolution in the inoculum blank at the end of the test is greater than 70 mg CO_2/l, results should be examined critically.
OECD 301C and OECD 301F	• The oxygen uptake of the inoculum blank should not be greater than 60 mg O_2/l in 28 days. • If the pH is outside the range 6-8.5 and oxygen consumption by the test substance is < 60 %, lower test concentration could be used.
OECD 301C	• Oxygen consumption of the reference compound exceeds 40 % after 7 days or 65 % after 14 days
OECD 301D	• Oxygen depletion in the inoculum blank should not exceed 1.5 mg dissolved oxygen /l. • Oxygen concentration in the test bottles should not fall below 0.5 mg/l.

OECD 310 Ready Biodegradability – CO2 in sealed vessels (Headspace test)

- Applicability
  - Suitable for water soluble and insoluble substances
    - Ensure good dispersion
  - Applicable for volatile substances up to 50 Pa.m3.mol-1 with headspace to liquid volume ratio of 1:2
  - If test is performed with smaller headspace consider the bioavailability and sufficient oxygen concentration – report details
- Validation criteria
  - The mean percentage degradation of the reference substrate is > 60% in the 14th day of incubation
  - The mean amount of TIC in blank controls at the end of the test is < 3 mg C/l

Links to relevant Guidance documents

- Guidance on information requirements and chemical safety assessment
  - Chapter R.7b: Endpoint specific guidance / R.7.9 Degradation/biodegradation

  http://echa.europa.eu/documents/10162/13632/information_requirements_r7b_en.pdf
- Practical Guides
  - How to report weight of evidence, data waiving, (Q)SARs…

  http://echa.europa.eu/practical-guides

Thank you!

http://echa.europa.eu/contact/helpdesk-contact-form