Current state of the art on guidance and information on Integrated Testing Strategies (ITS)

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Overview

- Purpose
- Context
- The Framework of Information Gathering
- Information requirements
- Reliability, relevance and adequacy of information
- Animal testing and REACH
- Adaptation possibilities
  - QSAR
  - In vitro
  - Categories
- Guidance - examples
- Information flow
- Conclusions
Toxicity Profiles - “Information Requirements”

- **Purpose** - Provide information for a Chemical Safety Assessment
  
  - Classification and Labelling - each endpoint
  - PBT / vPvB assessment
  - No (Minimal) Effect Levels to derive DNEL / DMEL
    - Appropriate routes and durations
  - [Priority setting]

- **Guidance** - “Information Requirements and Chemical Safety Assessment”
  
  - how to fulfill the information requirements on intrinsic properties
  - how to use all information and testing in an optimal way (reducing animal usage) for decision-making under REACH
Guidance on information requirements and chemical safety assessment

Part A: Introduction to the Guidance Document
The Basic REACH Process – per Substance

- Identified Uses
- Exposure data Models
- Hazard Assessment Tox / Env
- Risk Characterisation
- Use Controlled?
- Yes: Downstream eSDS How to handle...
- No: CSA
  - Effect levels Classified or PBT?
  - No: CSA
  - Yes: ECHA - Registration Dossier + CSR

Required Information

ECHA - Registration Dossier + CSR
Framework for Information Generation - stepwise

1. Gather and Share Existing Information
   Intrinsic Properties

2. Consider Information needs
   Tonnage / Registration type

3. Identify Information Gaps
   vs. Annex VII-X

4. Generate new data / propose testing
1. Gather and Share Existing Information
   Intrinsic Properties

• **All** available relevant information
  o Irrespective on Annex VII- X requirement tonnage level.
  + **Test data**
    + In vitro / in vivo
  + **Non test data**
    + (Q)SAR models
    + Grouping of Substances
    + Read across, weight of evidence
    + Exposure considerations

  + **Physicochemical data**
  + Non-guideline studies, + Human data, incl. Epidemiology
  + Any other data that may assist in identifying the presence or absence of hazardous properties of the substance

• **Data collection strategies**
  • Competent Existing Reviews as a starting point
  • Inclusive Search strategies
  • Proprietary data - though SIEFs

Annex Xi adaptations
Assessment of reliability, relevance and adequacy of information

- **Reliability**
  - Was the study well performed?
  - Endpoint specific – Ranking tools
    - Klimisch score - 1 to 4

- **Relevance**
  - Fit for the endpoint?

- **Adequacy**
  - Ability to meet the information requirements and allow a conclusion to be formed
    - I.e. effect levels, C&L, PBT
  - Weight of evidence (WoE)
    - Holistic view of the data
2. Consider Information needs

Tonnage / Registration type

<table>
<thead>
<tr>
<th></th>
<th>&lt;1</th>
<th>1-10 tpa*</th>
<th>10-100</th>
<th>100-1000</th>
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<tbody>
<tr>
<td>Non Isolated Intermediate</td>
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<tr>
<td>On-site Isolated Intermediate under strict control</td>
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<tr>
<td>Transported Isolated Intermediate under strict control</td>
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<tr>
<td>Polymer</td>
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1 Registration to include the following:
Identity of manufacturer and intermediate, Classification, any available phys-chem, health or environmental data. Brief description of use, details of risk management measures
* Tonnes per annum
## Annex VII

<table>
<thead>
<tr>
<th>State of the substance at 20°C and 101.3 kPa</th>
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</thead>
<tbody>
<tr>
<td>Melting/freezing point</td>
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<tr>
<td>Boiling point</td>
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<tr>
<td>Relative density</td>
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<td>Vapour pressure</td>
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<td>Surface tension</td>
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<tr>
<td>Water solubility</td>
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<tr>
<td>Partition coefficient n-octanol/water, flask shake method</td>
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<tr>
<td>Flash-point</td>
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<tr>
<td>Flammability, liquids</td>
</tr>
<tr>
<td>Explosive properties</td>
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<tr>
<td>Self-ignition temperature for liquids and gases</td>
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<tr>
<td>Oxidising properties</td>
</tr>
<tr>
<td>Granulometry (particle size distribution)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Toxicity Testing and Biodegradability</th>
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</thead>
<tbody>
<tr>
<td>Short-term toxicity testing on Daphnia</td>
</tr>
<tr>
<td>Growth inhibition study on algae</td>
</tr>
<tr>
<td>Ready biodegradability - Modified Sturm test</td>
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<tr>
<td>Ready biodegradability - Closed bottle test</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Skin and Eye Irritation</th>
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</thead>
<tbody>
<tr>
<td>Skin irritation (indicate if in vitro)</td>
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<tr>
<td>Eye irritation (indicate if in vitro)</td>
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<tr>
<td>Skin sensitisation</td>
</tr>
<tr>
<td>In vitro gene mutation study in bacteria</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Acute Toxicity</th>
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<tbody>
<tr>
<td>Acute toxicity, oral route (OECD 420, 423 or 425)</td>
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</tbody>
</table>
## Annex VIII

<table>
<thead>
<tr>
<th>In vitro cytogenicity in mammalian cells</th>
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<tbody>
<tr>
<td>In vitro gene mutation study in mammalian cells</td>
</tr>
<tr>
<td>Other in vivo mutagenicity test: micronucleus test (OECD 474) or UDS assay (OECD 486)</td>
</tr>
<tr>
<td>Acute toxicity, inhalation</td>
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<tr>
<td>Acute toxicity, dermal route</td>
</tr>
<tr>
<td>Short-term repeated dose toxicity in rats (28 days), oral/dermal/inhalation</td>
</tr>
<tr>
<td>Screening for reproduction/development toxicity, rats</td>
</tr>
<tr>
<td>Assessment of toxicokinetic behaviour (based on required studies)</td>
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</tbody>
</table>

- Short-term toxicity testing on fish
- Activated sludge respiration inhibition testing
- Hydrolysis as a function of pH and identification of degradation products
- Adsorption/desorption screening study (HPLC method)
## Annex IX

<table>
<thead>
<tr>
<th>Test Type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish early-life stage (FELS) toxicity test</td>
<td></td>
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<tr>
<td>Fish short-term toxicity test on embryo and sac-fry stages</td>
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<tr>
<td>Fish, juvenile growth test</td>
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<tr>
<td>Simulation testing on ultimate degradation in surface water</td>
<td></td>
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<tr>
<td>Soil simulation testing (for substances adsorbing to soil)</td>
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<tr>
<td>Soil simulation testing (for substances adsorbing to sediment)</td>
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<tr>
<td>Identification of degradation products</td>
<td></td>
</tr>
<tr>
<td>Bioconcentration in (one) aquatic species, preferably fish</td>
<td></td>
</tr>
<tr>
<td>Further studies on adsorption/desorption</td>
<td></td>
</tr>
<tr>
<td>Short-term toxicity to invertebrates</td>
<td></td>
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<tr>
<td>Effects on soil micro-organisms</td>
<td></td>
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<tr>
<td>Short-term toxicity to plants</td>
<td></td>
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<tr>
<td>Long-term toxicity testing on Daphnia, 21 days</td>
<td></td>
</tr>
<tr>
<td>Sub-chronic toxicity study (90-day) in rats, oral/dermal/inhalation</td>
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<tr>
<td>Development toxicity study, rats</td>
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<tr>
<td>Development toxicity study, rabbits (depends on 1st result)</td>
<td></td>
</tr>
<tr>
<td>One-generation reproduction study (enhanced)</td>
<td></td>
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<tr>
<td>Two-generation reproduction toxicity study</td>
<td></td>
</tr>
</tbody>
</table>
### Annex X

| Stability in organic solvents and identity of relevant degradation products |
| Dissociation constant |
| Viscosity |

| Further environmental fate and behavioural studies |
| Long-term toxicity testing on invertebrates (unless in Annex IX) |
| Long-term toxicity testing on higher plants (unless in Annex IX) |
| Long-term toxicity to sediment organisms |
| Long-term or reproductive toxicity to birds |

| Chronic toxicity (12 months or longer), rats (exposure/use driven) |
| Carcinogenicity study/combined chronic toxicity, rats (exposure/use driven) |
| Other studies (to be listed below) |

| Confirmatory testing on biodegradation rates (aerobic and/or anaerobic) |
| Long-term toxicity testing on soil invertebrates other than earthworms |
| Emissions to water |
| Emissions to land |
| Emissions to air |
| Occupational exposure in manufacture |
| Occupational exposure in use |
| Consumer exposure |
| End of life |

| Analytical methods (may be requested or lack of availability justified) |
3. Identify Information Gaps
   vs. Annex VII- X

- Comparison of information requirements against availability
- Use Adaptation possibilities - Annex XI
  - Testing does not appear scientifically necessary
  - Use of existing data
    - Data on physical-chemical properties from experiments not carried out according to GLP or the test methods referred to in guidance docs
    - Data on human health and environmental properties from experiments not carried out according to GLP or the test methods referred to in Article 13(3)
    - Historical human data
  - Weight of evidence
  - Qualitative or Quantitative structure-activity relationship (Q)SAR
  - In vitro methods
  - Grouping of substances and read-across approach
  - Testing is technically not possible
  - Substance-tailored exposure-driven testing
3. Identify Information Gaps - 2

vs. Annex VII- X

- Comparison of information requirements against availability
- Use Adaptation possibilities

... Features other considerations that can influence information requirements:

- Toxicokinetics - not specifically required under REACH but can inform testing strategies and category approaches.

- Non-standard approaches necessary for evaluating certain classes of substance e.g. metals, inorganics, petroleum substances etc.
REACH & balancing Animal Testing

- **Directive 86/609/EC** –
  - "where a validated alternative method of testing is available, the animal test should not be carried out".
- Scientific and Political pressure to reduce the numbers of animals used in toxicity testing for REACH
  - One Substance One Registration concept - data sharing
- 30,000 substances in REACH - potential animal usage - 2.6 - 3.9M
  - > 50% from reproductive and developmental toxicity testing
- REACH 2nd Parliamentary Reading
  - Several Amendments proposed were designed to reduce animal usage through three R's principle and promote alternatives
- Preregistration and the SIEFs
  - mechanisms to ensure sharing of vertebrate animal data
- Further animal testing as a last resort

*Pedersen et al. (2003)*
In vitro data

- Suitable in vitro test methods
  - Defined - validation criteria
  - Limited number of assays
  - May fully or partly replace an animal test
- Other suitable methods – not fully validated
  - Well developed - may be used (Annex XI - 1.4)
    - Justification required
- Potentially useful to
  - Provide mechanistic insight
- All - Judge data on relevance, reliability, and completeness
(Q)SAR

- OK if...
- results are derived from a (Q)SAR model whose scientific validity has been established
- the substance falls within the applicability domain of the (Q)SAR model
- results are adequate for the purpose of classification and labelling and/or risk assessment
- adequate and reliable documentation of the applied method is provided
  - OECD toolbox – links many current methodologies
  - Presence or absence of an effect
    - supports grouping
  - (Q)SAR – potentially useful in WoE approaches
Grouping Approaches (Categories) - Concepts

- Structural similarity - similarity characteristics – allows read across
  - Physico-chemical, human health and/or environmental toxicological / fate properties
- Does not necessarily imply the same response - Trends

<table>
<thead>
<tr>
<th>Substance</th>
<th>Substance</th>
<th>Substance</th>
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</thead>
<tbody>
<tr>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>Endpoint 1</td>
<td>Information</td>
<td>Infer</td>
</tr>
<tr>
<td>Endpoint 2</td>
<td>Infer</td>
<td>Information</td>
</tr>
</tbody>
</table>

- Used to indicate either the presence or the absence of an effect.
- Avoid the need to test all members of the group for all endpoints of interest
- Individual substance - assessed on the basis of the evaluation of the category
- Linear series of effects where applicability domain applies
- Possibility for subcategories - or domain specific grouping
- Requires competent justification that works for each endpoint separately
  - Extended from both US EPA HPV and OECD
- UVCBs - unknown or variable composition
  - Linear concepts do not hold – overall data set.
Animal Models & *In vitro* Approaches – Issues

- Animal Models
- Read Across, QSAR Categories
- *In vitro*
- Quantitative
- Qualitative
- Fit for purpose?
- Risk
- Hazard
**Information Requirements – Guidance**

- **IR & CSA Reference Chapter 7 a b c**

**Guidance for each endpoint includes:**

- **Endpoint-specific information requirements**; guidance on the information prescribed by REACH for each tonnage band
- **Information on the endpoint and its sources** gives guidance on identifying information sources and how to ensure the reliability of the used information
- **Evaluation of available information**; gives guidance on when and how to use *in vitro* and non-test information e.g. QSAR, read across and grouping, including guidance on what is ‘adequate information’
- **Conclusions for the endpoint** provide adequate and relevant information for registration sufficient for:
  - Classification & Labelling;
  - carrying out a Chemical Safety Assessment;
  - assessing whether a substance is PBT or vPvB
- **When information is missing, formulate guidance and an Integrated testing strategy for the specified endpoint.**
Part 2 - Physico-chem and Human Health

- Endpoint specific guidance
  7.1 Physico-chemical properties; adsorption desorption
  7.2 Skin & Eye Irritation/corrosion & respiratory irritation
  7.3 Skin & respiratory sensitisation
  7.4 Acute toxicity
  7.5 Repeated dose toxicity
  7.6 Reproductive & Developmental Toxicity
  7.7 Mutagenicity and Carcinogenicity
Part 3 – Environmental Fate and Effects

- Endpoint specific guidance
  7.8 Aquatic pelagic toxicity
  7.9 Degradation/Biodegradation
  7.10 Aquatic bioaccumulation
  7.11 Effects on Terrestrial Organisms
  7.12 Guidance on Toxicokinetics
  7.13 Substances requiring special consideration

- Appendices include
  8.1 Threshold of Toxicological Concern (TTC)
Example approach - Irritation and corrosion

Figure R.7.2-1 Overview of the Integrated Testing Strategy for irritation/corrosion

*Generation of new testing data according to Annex VII to VIII and with due observation of the rules for adaptation of the standard testing regime laid down in Annex XI.*
Figure R.7.3-1 Integrated testing strategy for skin sensitisation

Gather and evaluate existing information (human, animal, in vitro, (Q)SAR, read across and chemical category data) on skin sensitisation according to Annex VI, step 1.

Does available information indicate that:
- The substance should be classified for corrosivity?, or
- The substance is a strong acid (pH<2.0) or base (pH>11.5)?; or
- The substance is self-flammable in air at room temperature?

no

Consider required information needs (Annex VII: 8.3) and make an overall weight of evidence assessment.

Does available information provide sound conclusive evidence indicating that the substance is a skin sensitisier or non-sensitisier?

no

Consider classification for skin sensitisation or justify if no classification is considered necessary based on conclusive data.

yes

Provide justification for no further in vivo testing.

Are there in vitro tests available that can generate relevant data?

yes

Perform the in vitro tests (see guidance text R.7.3.3.1)

no

Perform a LLNA or a reduced LLNA, (see guidance text 7.3.4.1) or provide justification for and conduct another appropriate in vivo test.
Figure R.7.7-1 Flow chart of the mutagenicity testing strategy
Figure R.7.7-1 Flow chart of the mutagenicity testing strategy

REACH Annexes IX and X

consider whether in vivo test is required
check bioavailability
check available data
consider proper in vivo (follow up) test
consider integration into other toxicity tests

For evidence of clastogenicity, a micronucleus test, a chromosome aberration test or a Comet assay whereas for evidence of gene mutations a gene mutations test with transgenic mice, an unscheduled DNA synthesis test or a Comet assay would be the appropriate follow up test.
Seek expert advice

The 2nd in vivo test should only be performed if it is required to make a conclusion on the genotoxicity of the substance under investigation.

1st in vivo test

2nd in vivo test

testing complete

not genotoxic

check available data
check for information on a genotoxic hazard to germ cells

insufficient

sufficient

germ cell genotoxicity test

testing complete genotoxic in somatic and germ cells

testing complete genotoxic in somatic cells

Evidence of genotoxicity is an indicator of potential carcinogenicity; see guidance on carcinogenicity
Information flow

All available information

Key Studies
Supporting data
Test proposals

Registration dossier

Record Conclusions on
• No effect levels
• C&L
The Basic REACH Process – per Substance

- Identified Uses
- Exposure data
- Models
- Hazard Assessment
  - Tox / Env
- Risk Characterisation
- Effect levels
  - Classified or PBT?
- CSA
- Use Controlled?
- No
- Yes
  - Downstream
e-SDS
- How to handle...
- No
- Yes
  - ECHA - Registration Dossier + CSR

Required Information

- ECHA
  - Registration Dossier + CSR

SETAC Brussels, September 2008
Conclusions

- Implementation of the Information Requirements of REACH requires a logical stepwise approach
  - Collection of data
  - Sharing
  - Evaluation of Existing data
  - Data gaps and the development of testing strategies - limits use of vertebrates
  - Building tox profiles for REACH
- Clear requirement to avoid animal testing - but information needs to be fit for purpose i.e.
  - C&L
  - No effect levels
- Conceptually simple
  - But complex processes surround and complex techniques
- Resource intensive