

ECOTOXICITY TESTING FOR ENVIRONMENTAL RISK ASSESSMENT INCLUDING BIOACCUMULATION AND TOXICITY CRITERIA

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Overview

- Environmental hazard assessment under REACH (as covered in ANNEX's VII to X).
- Bioaccumulation and Toxicity as part of PBT assessment.
- Methods for reducing 'B' testing using 'in vitro' methods and / or a reduced 'in vivo' test.



Environmental Testing Effects

ENVIRONMENTAL HAZARD ASSESSMENT

- Required for classification and labelling
- Derivation of the PNEC

PBT ASSESSMENT



Environmental Hazard Assessment

- Aquatic compartment (including sediment)
- Terrestrial compartment
- *Atmospheric compartment*
- *Microbiological activity in sewage treatment systems*



Summary

| | ≥1 | ≥10 | ≥100 | ≥1000 |
|--|----|-----|------|-------|
| Short-term toxicity test, invertebrates (<i>Daphnia</i>) | X | X | X | X |
| Growth inhibition aquatic plant (Algae) | X | X | X | X |
| Short-term toxicity test, fish | - | X | X | X |
| Long-term toxicity test, invertebrates (<i>Daphnia</i>) | - | - | X | X |
| Long-term toxicity test, fish | - | - | X | X |
| Bioaccumulation in aquatic species (fish) | - | - | X | X |
| Terrestrial short-term toxicity test, invertebrate | - | - | X | X |
| Terrestrial effects on soil micro-organism | - | - | X | X |
| Terrestrial short-term toxicity test, plant | - | - | X | X |
| Terrestrial long-term toxicity test, invertebrate | - | - | - | X |
| Terrestrial long-term toxicity test, plant | - | - | - | X |
| Long-term toxicity to sediment organisms | - | - | - | X |
| Long term/reproductive toxicity to birds | - | - | - | X |



Environmental Testing Effects

What data might be available

- Acute effects to algae, daphnids, fish
- Chronic exposures (reproduction, development, life-cycle)
- Terrestrial (including birds and bees)



Environmental Testing

Effects

Minimum requirement (key data)

Acute testing in one aquatic species

- Daphnia and algae cheap - easy and non vertebrate
- Demonstrates if there is a biological effect
- Reference point for read across
- Can make a rough PNEC estimate



Environmental Testing

Effects

- PNEC based on effects to organisms from laboratory testing
- Safety factors applied depending on the type and duration of test data used
 - I.e: 1000 if only one short-term L(E)C50 available
 - I.e: 50 if two long-term results (EC10 or NOECs) from species representing two trophic levels (fish and/or Daphnids and/or algae)



Higher Tier Effects Testing

- Further testing may be required depending on exposure scenarios (e.g. sediments or marine)
- PNECs for the marine environment and sediments can be estimated from aquatic data
- Specific data on marine or sediment dwelling species may reduce safety factors



Higher Tier Effects Testing - Endocrine Disruptors

- Further testing may be required if there is evidence an endocrine mode of action
- No fully validated guidelines for EDC's but a 21 day fish screening assay and a fish sexual development test are currently being validated by the OECD
- Advise on appropriate testing based on the expected mode of action of a chemical



(P)BT Criteria - REACH

Bioaccumulation

Toxicity

REACH
PBT

BCF >2000

Chronic NOEC <0.01 mg/l for marine or freshwater organisms

Substance is classified as CMR (category 1 and 2 for C and M or category 1, 2 and 3 for reproduction)

Other evidence of chronic toxicity, as identified by classifications T, R48, or Xn, R48 according to directive 67/548/EEC

REACH
vPvB

BCF >5000

Not applicable

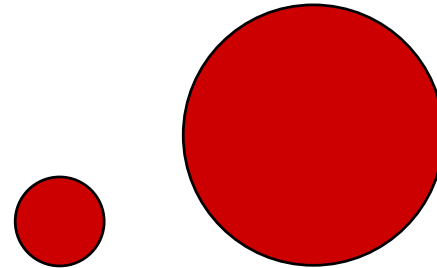
CMR: Carcinogenic, mutagenic or toxic to reproduction



Bioaccumulation Assessment

Physiochemical Properties

- Molecular size and weight



- Log Kow **4.5**

- Read across with other substances



Bioaccumulation Assessment Mammalian

- Chronic data
- Toxicokinetic studies with mammals

Uptake

Metabolism

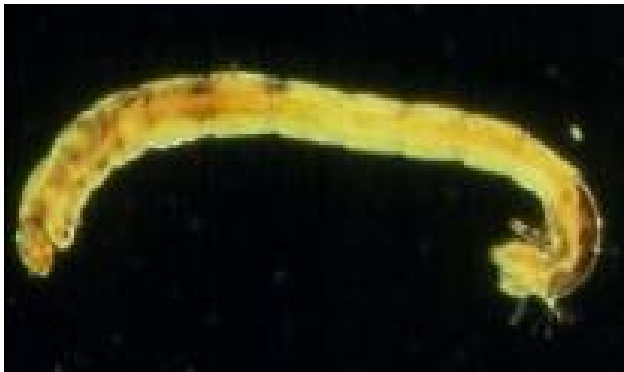
Excretion



Bioaccumulation Assessment

Aquatic

- **Field data and biomagnification**
BMF or TMF values greater than 1
- **Bioconcentration with benthic & invertebrate species**



Bioaccumulation Assessment Aquatic

- Experimental data



OECD 305



ASTM E1022-94



Increasing complexity of hepatic system

In vivo



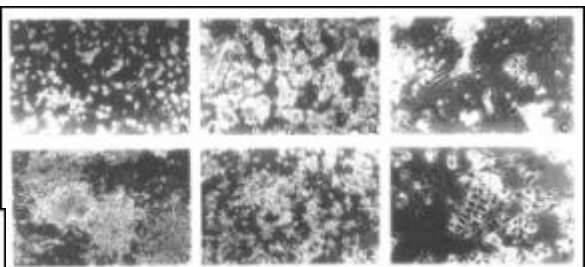
In situ



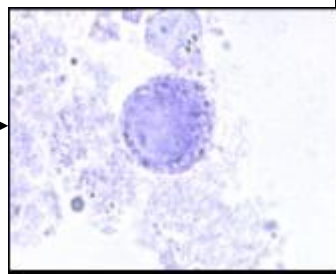
Slices



Culture



Suspension



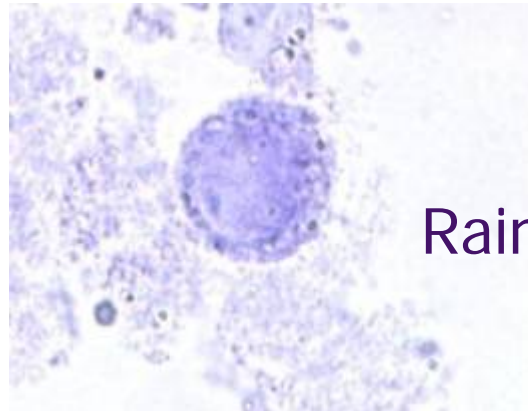
S9



Bioaccumulation Assessment In Vitro



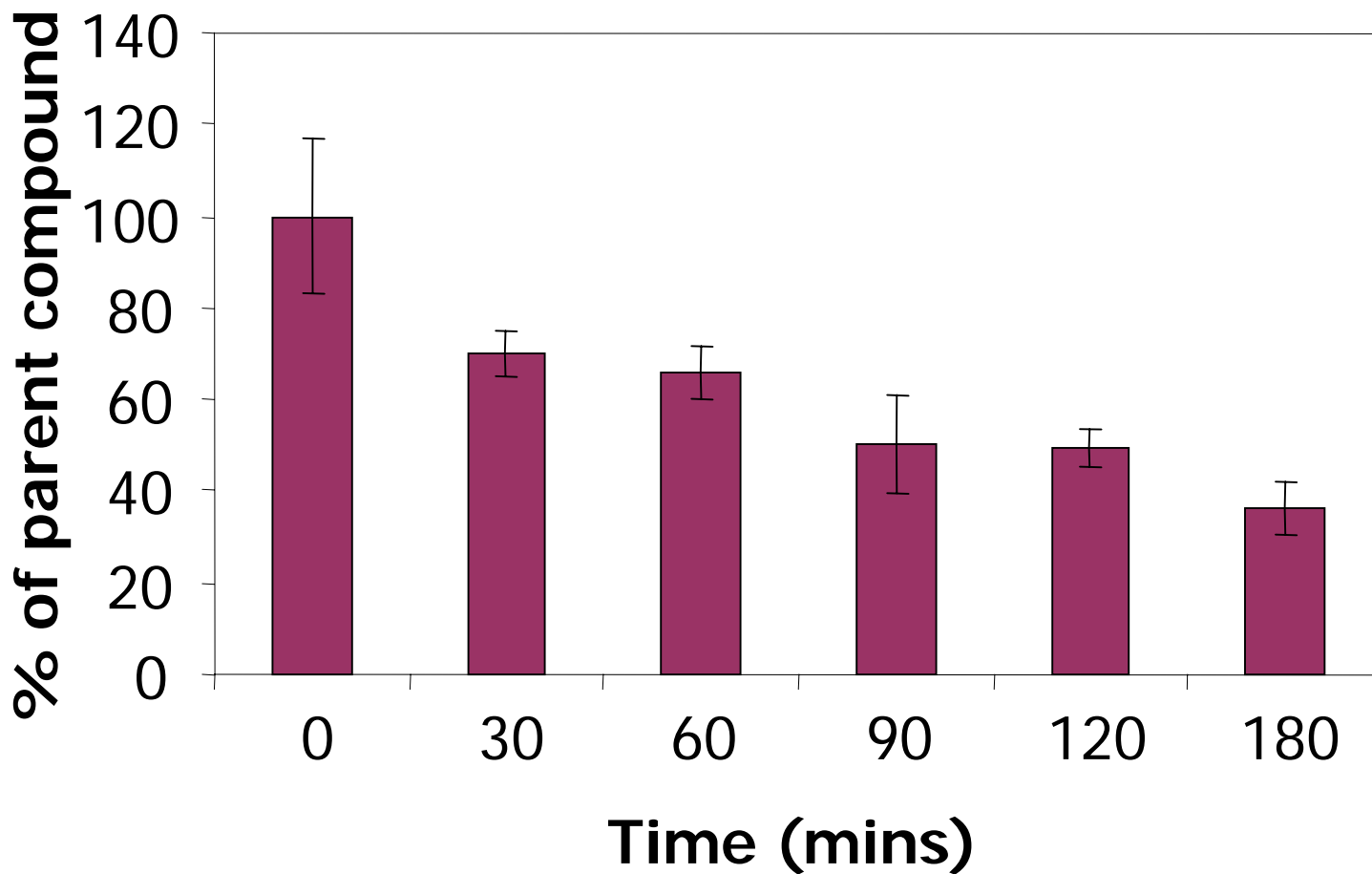
S9



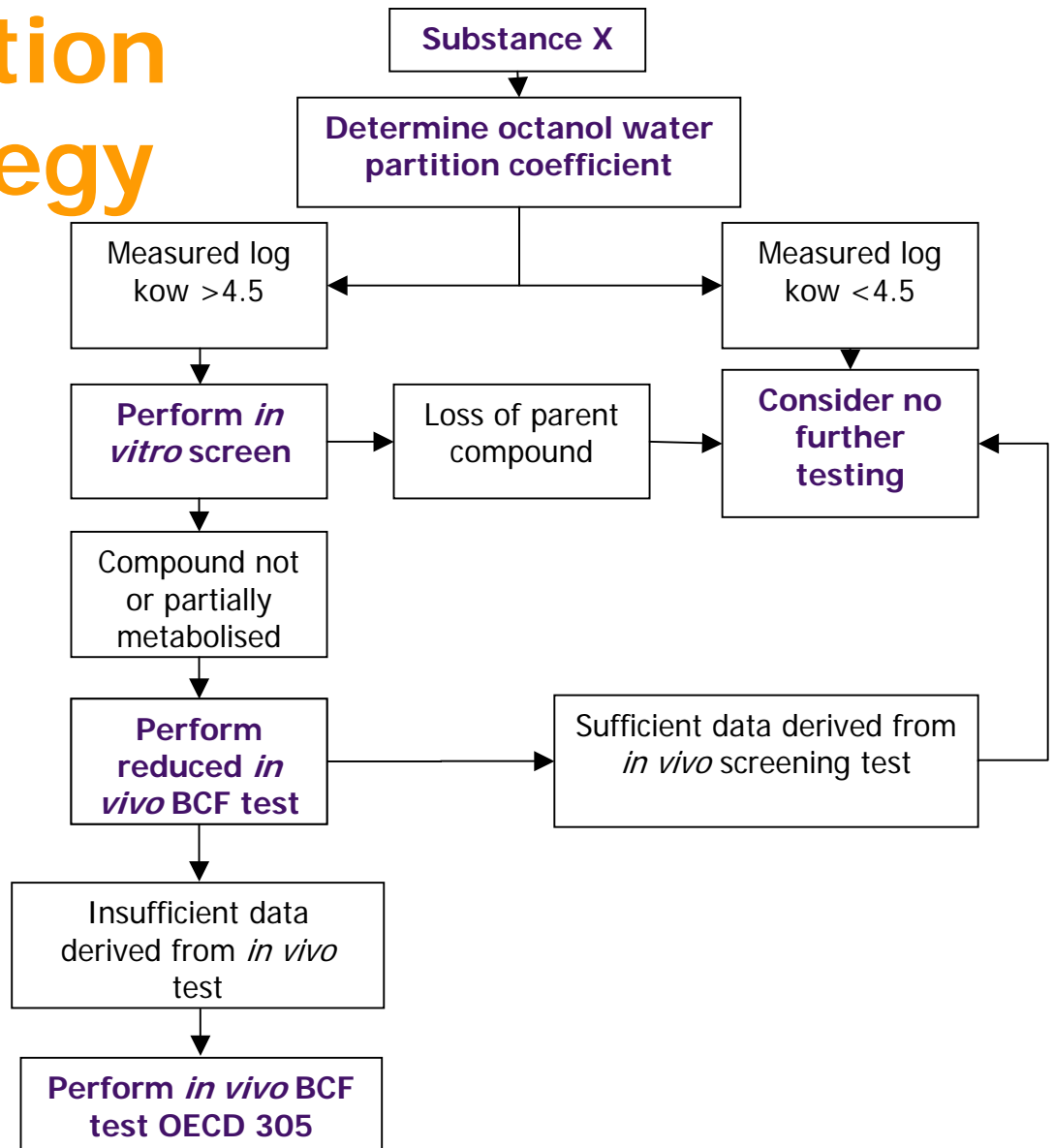
Rainbow trout hepatocyte



Incubation of Chemical Z With Trout S9 Pooled Fish (1 μM , 15°C)



Bioconcentration Testing Strategy



Summary of "B "

- Physiochemical properties: MW, size, read across, log Kow
- Mammalian data: Chronic, toxicokinetic (uptake, metabolism, excretion)
- *In vivo* data: Field, biomagnification, invertebrate, sediment, reduced *in vivo* test
- *In vitro* data: trout S9, trout hepatocyte assays
- Definitive data: ASTM E1022-94, OECD 305



Toxicity Assessment

Definitive Criteria

- Long-term NOEC for marine or freshwater organisms is <0.01 mg/l
- Substance classified as carcinogenic (category 1 and 2), mutagenic (category 1 and 2) or toxic for reproduction (category 1, 2 or 3)
- Evidence of chronic toxicity as identified by classifications: T, R48 or Xn, R48 according to Directive 67/538/EEC



Toxicity Assessment Screening Data

| Type of data | Criterion | Screening assignment | Definitive assignment |
|-------------------------------|--|----------------------|-----------------------|
| Short-term aquatic toxicity* | $EC_{50}/LC_{50} \geq 0.1 \text{ mg l}^{-1}$ | Presumably not T | |
| Short-term aquatic toxicity* | $EC_{50}/LC_{50} < 0.1 \text{ mg l}^{-1}$ | Potentially T | |
| Short-term aquatic toxicity** | $EC_{50}/LC_{50} < 0.01 \text{ mg l}^{-1}$ | | T |

* From acute tests or validated QSARs

** From acute tests

Screening assignments should always be considered together for P, B and T to decide if substance a potential PBT or vPvB



Toxicity Assessment Definitive Data



Summary of "T"

- Mammalian data: CMR, T, R48, Xn R48 = T. No testing required
If not, assess acute T data
- Acute T data: any $EC_{50} < 0.1 \text{ mg/l}$ = Potential T, assess chronic data
all $EC_{50} \geq 0.1 \text{ mg/l}$ confirm not false -ve, screen for P, B
- Consider P, B assessment: If P, B confirmed assess chronic T data
If confirmed not P or not B, stop
- Chronic T data: (non-vertebrate species first, unless fish most sensitive species):
NOEC $< 0.01 \text{ mg/l}$ PBT confirmed
NOEC $\geq 0.01 \text{ mg/l}$, not T, stop





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