Integrated Testing Strategies for REACH: 3Rs & Environmental Testing

In vitro & optimized *in vivo* testing to assess environmental risks

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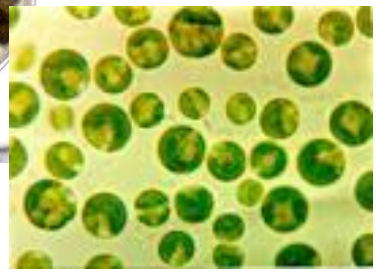

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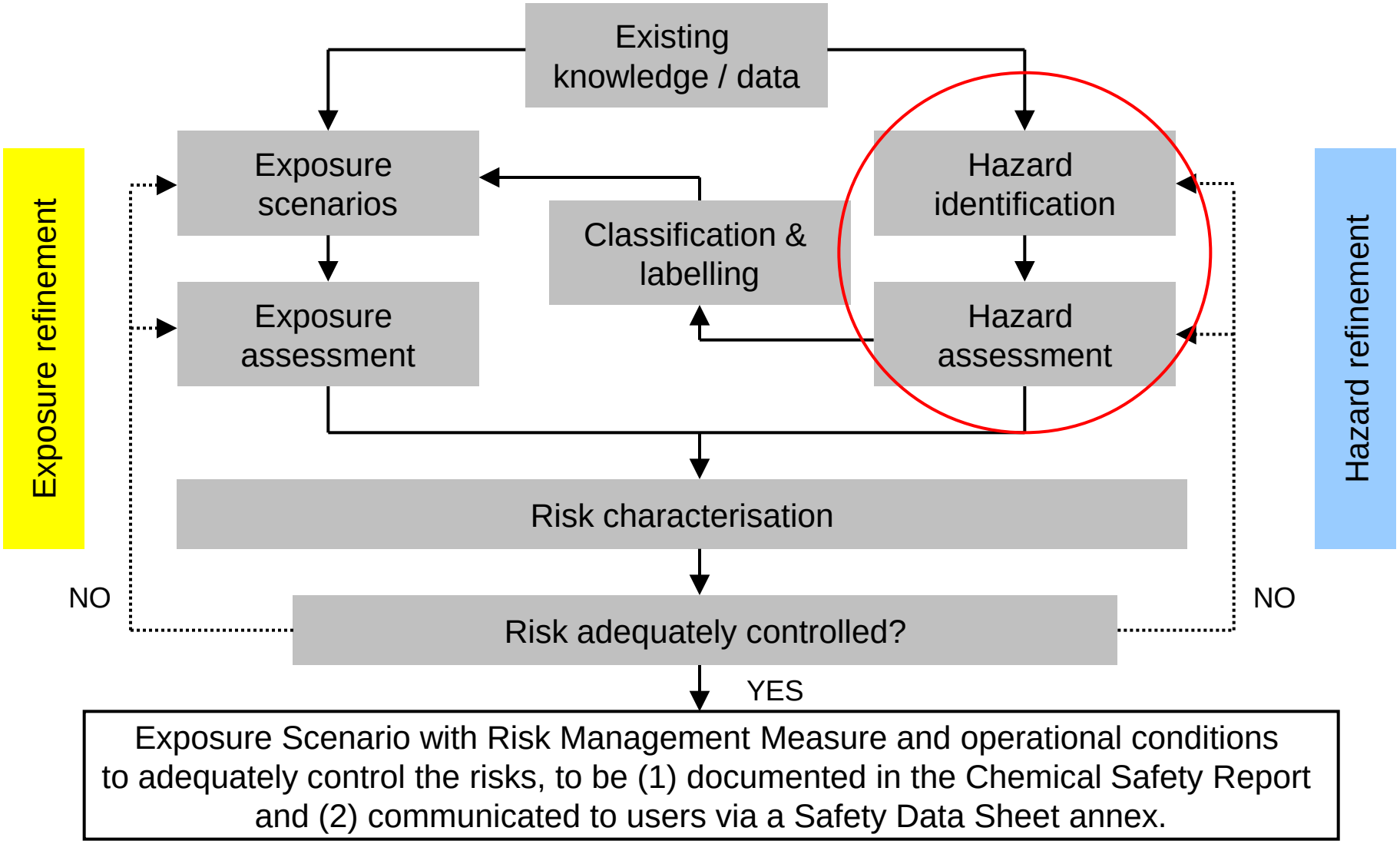
Overview

- Aquatic hazard assessment
  - acute effects testing
  - chronic effects testing
  - bioaccumulation
- Mode of action and 3Rs

Acknowledgements

- Members of ECETOC (2007) Task Force on Intelligent Testing Strategies in Ecotoxicology
- Bram Versonnen et al., SETAC Europe (2006) course organisers “On the borders of REACH”
- ECVAM Task Force on Ecotoxicology

Chemical Safety Assessment Overview



Source: Versonnen et al (2006)

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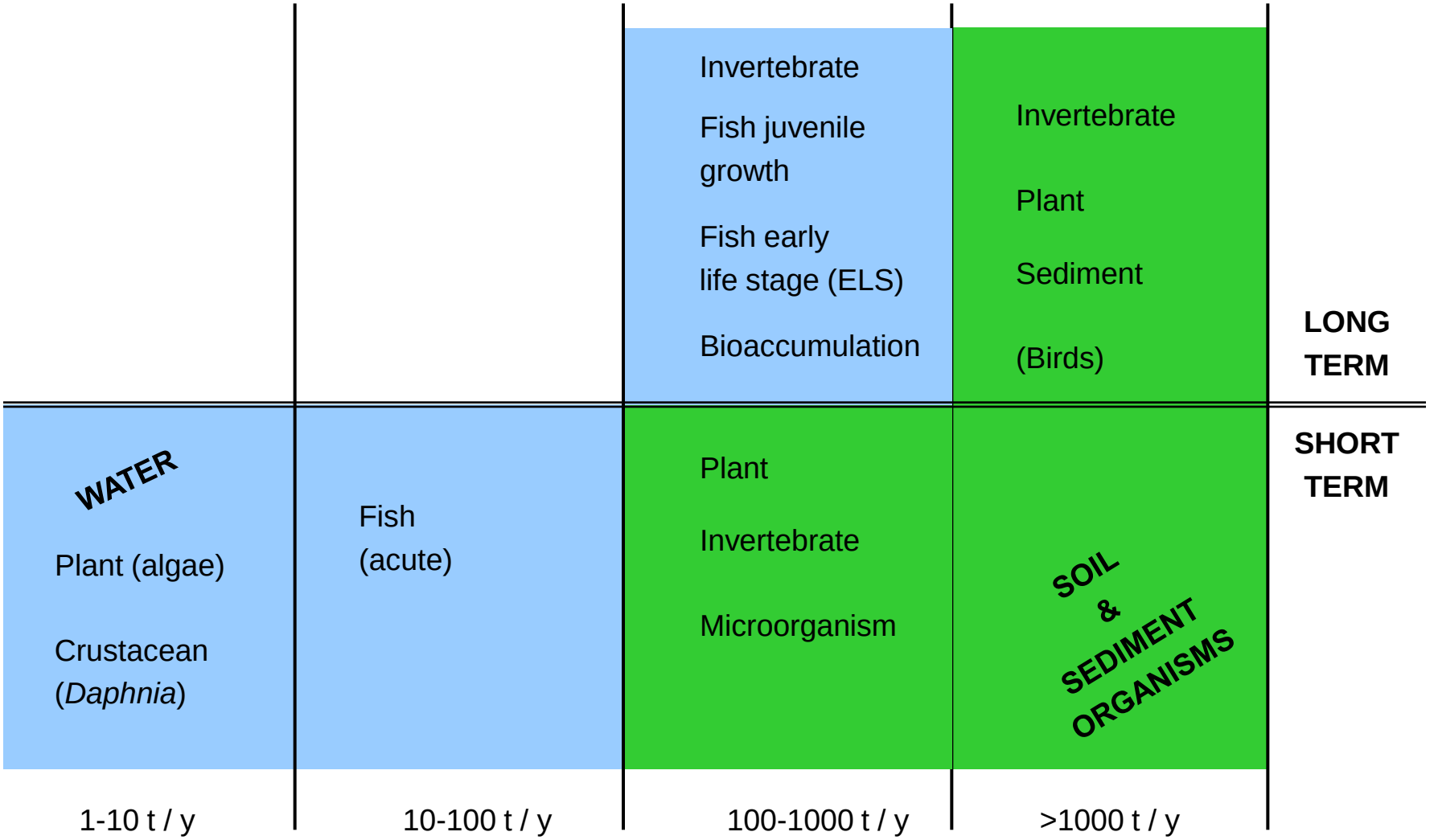
Ecotoxicity Testing Requirements



Source: Versonnen et al (2006)

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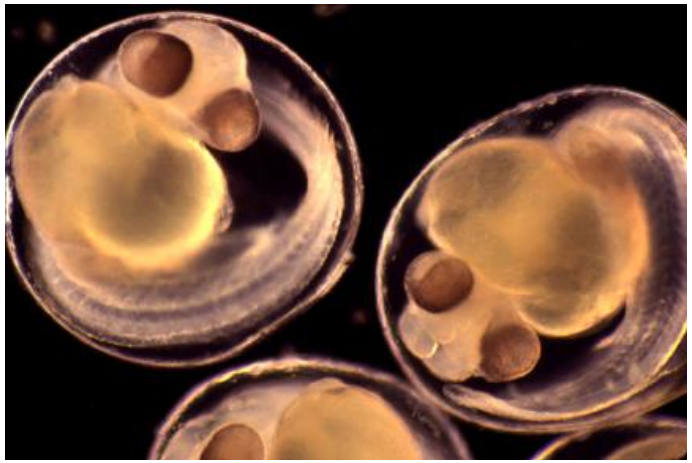
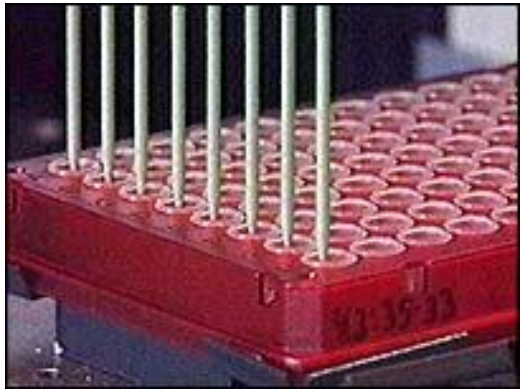
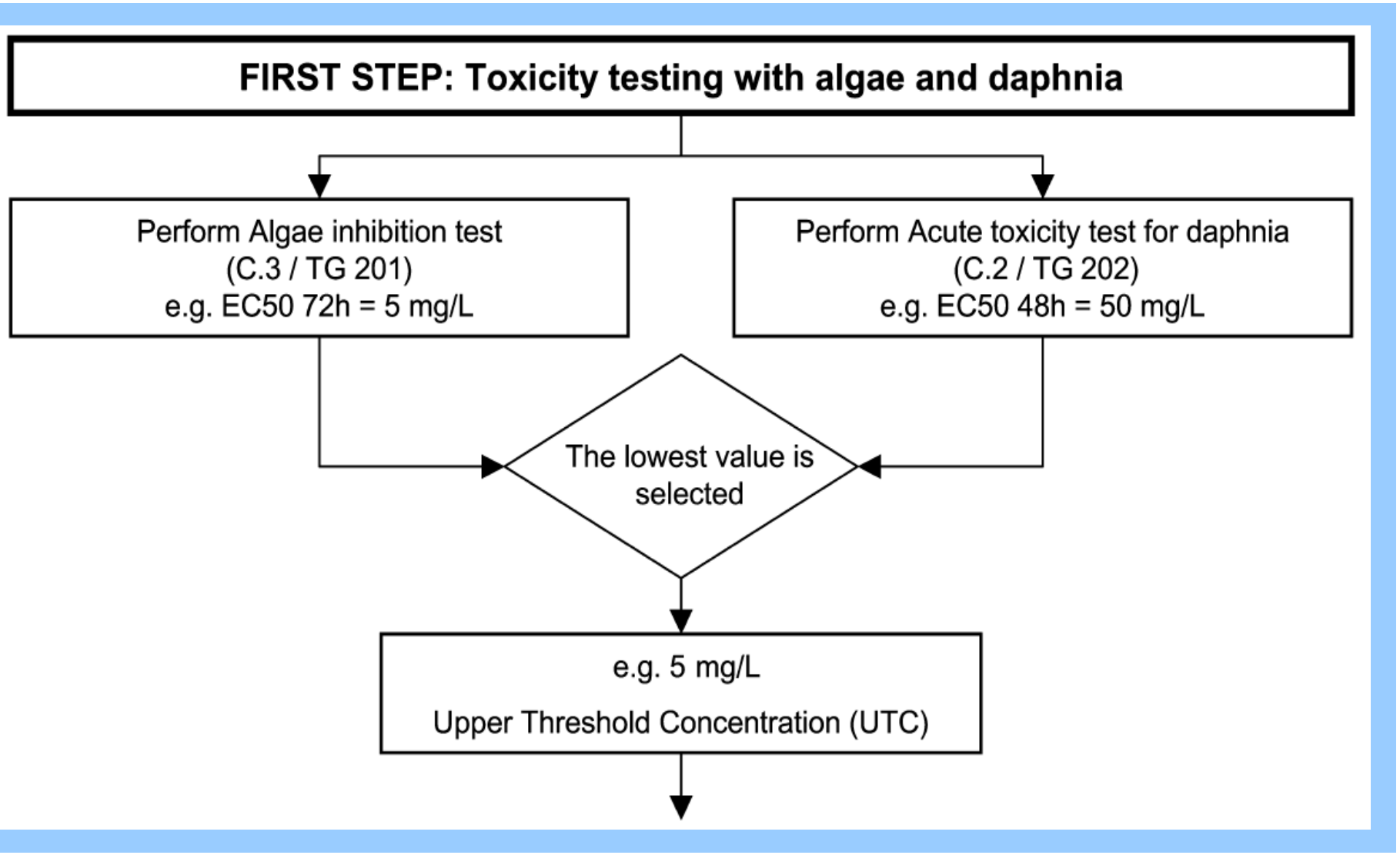
Fish acute & chronic testing & 3Rs?





Reduction in fish acute testing (Step 1)



Ref: Jeram et al (2005) Reg Tox Pharmacol 42: 218-224

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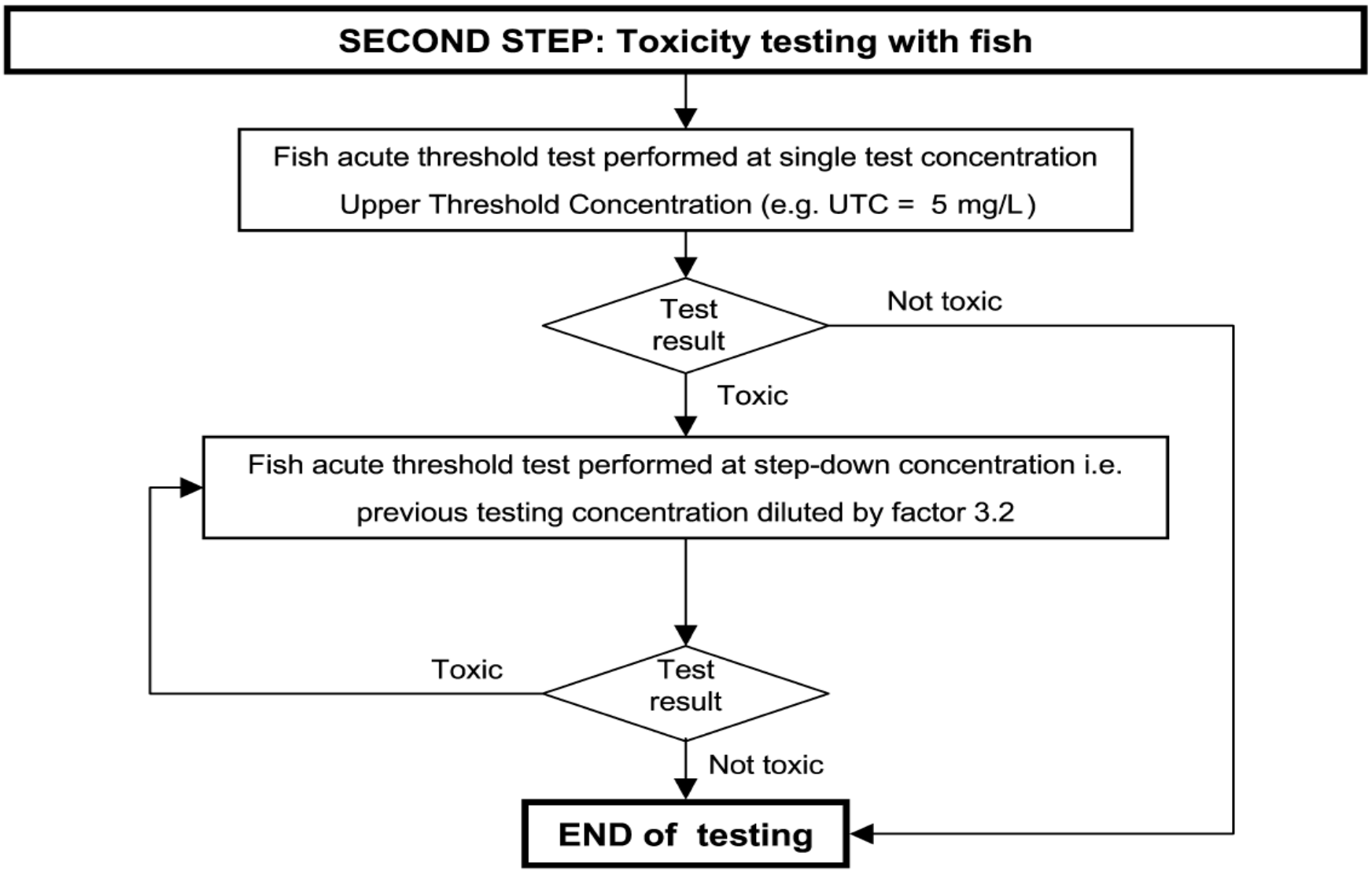
Reduction in fish acute testing (Step 2)



Ref: Jeram et al (2005) Reg Tox Pharmacol 42: 218-224

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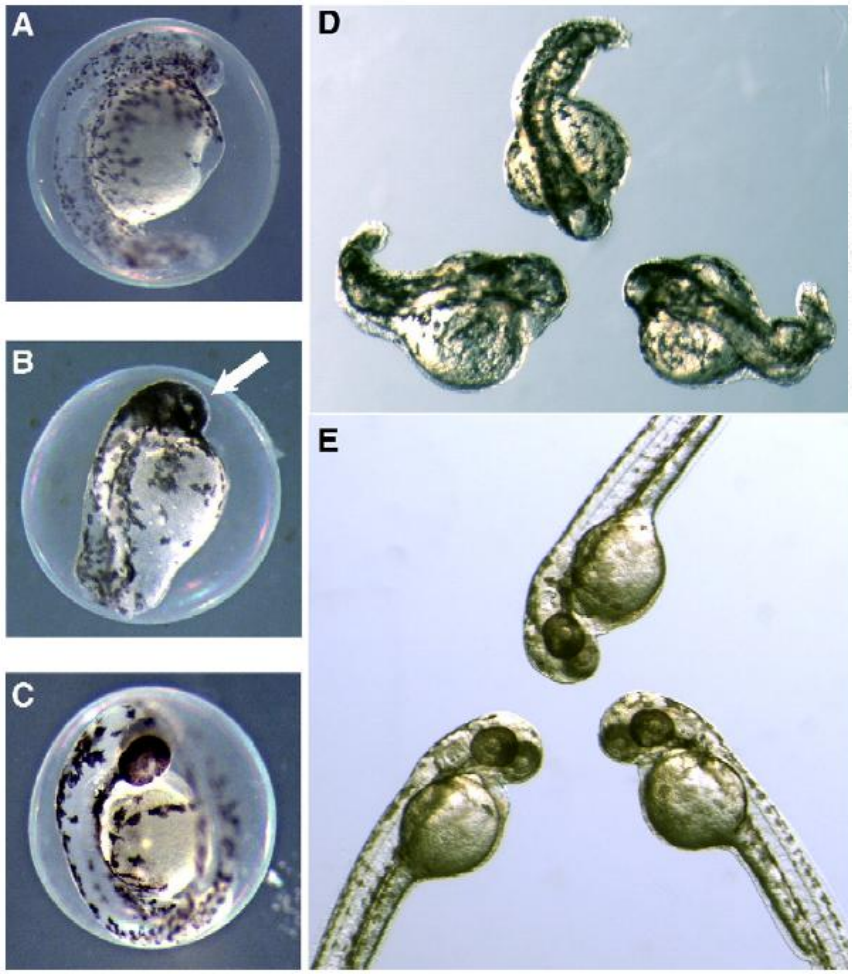
Fish acute threshold (step-down) test

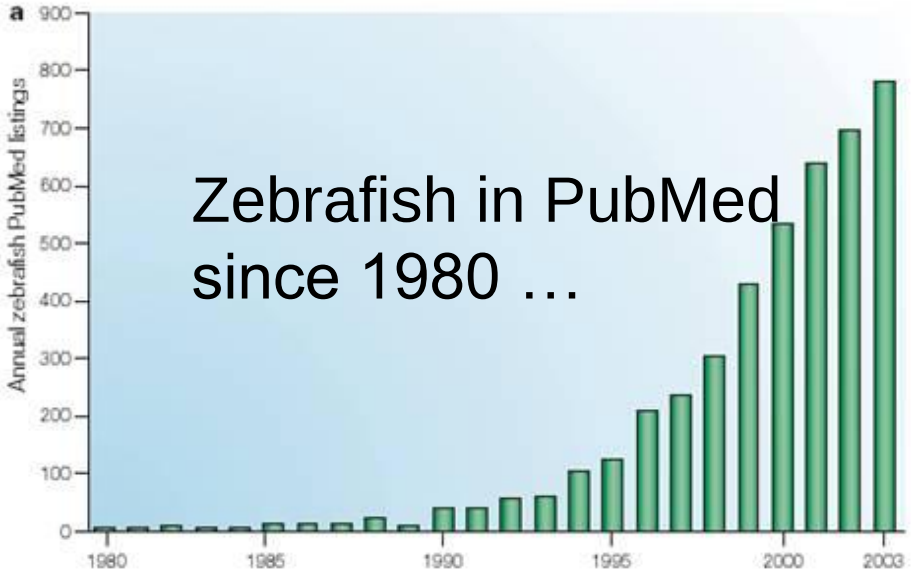
	Acute fish toxicity test	Acute threshold (step-down) test
Species	Standard freshwater and marine fish species	Standard freshwater and marine fish species
Age of test organisms	Adults or juveniles	Adults or juveniles
No. of fish per test vessel	Minimum of 7	5
No. of test concentrations	5 minimum plus appropriate controls	1 minimum plus appropriate controls
No. of replicate test vessels per test concentration	1	1
No. of fish per study	Typically 42 (35 exposed + 7 in control)	Proposed 10 (5 exposed + 5 in control)
Dilution series	1.0, 1.8, 3.2, 5.6, 10…	Not applicable
Sequential step down series	Not applicable	Factor of 3.2 step-down
Chemical exposure duration	96 h	96 h (or less)
Endpoints	Mortality within 96 h	Mortality or severe morbidity within 96 h
Test acceptability—controls	Mortality: 1 out of 7 fish	Mortality or severe morbidity: 1 out of 5 fish
Calculation of LC_{50}	Probit, Moving Average Angle or Binomial	If required, estimated by binomial method of in

Estimated 54 – 71% reduction in number of fish required

Ref: Hutchinson et al (2003) Environ Toxicol Chem 22: 3031-3036

Fish embryos as alternatives





- New OECD Test Guideline Fish Embryo Toxicity (FET) Test as an alternative to OECD TG 203 (fish acute test)
- 48h exposure with four endpoints:
  - (i) coagulation of fertilized eggs
  - (ii) lack of somite formation
  - (iii) detachment of tail-bud from yolk sac
  - (iv) lack of heart-beat.

Ref: Berry et al (2007) Comp Biochem Physiol 145C: 61-72

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ToxCast™ Research Programme

Chemicals
1
2
3
etc …

Physico-chemical indicators

Computational indicators

Biochemical indicators

Cellular indicators

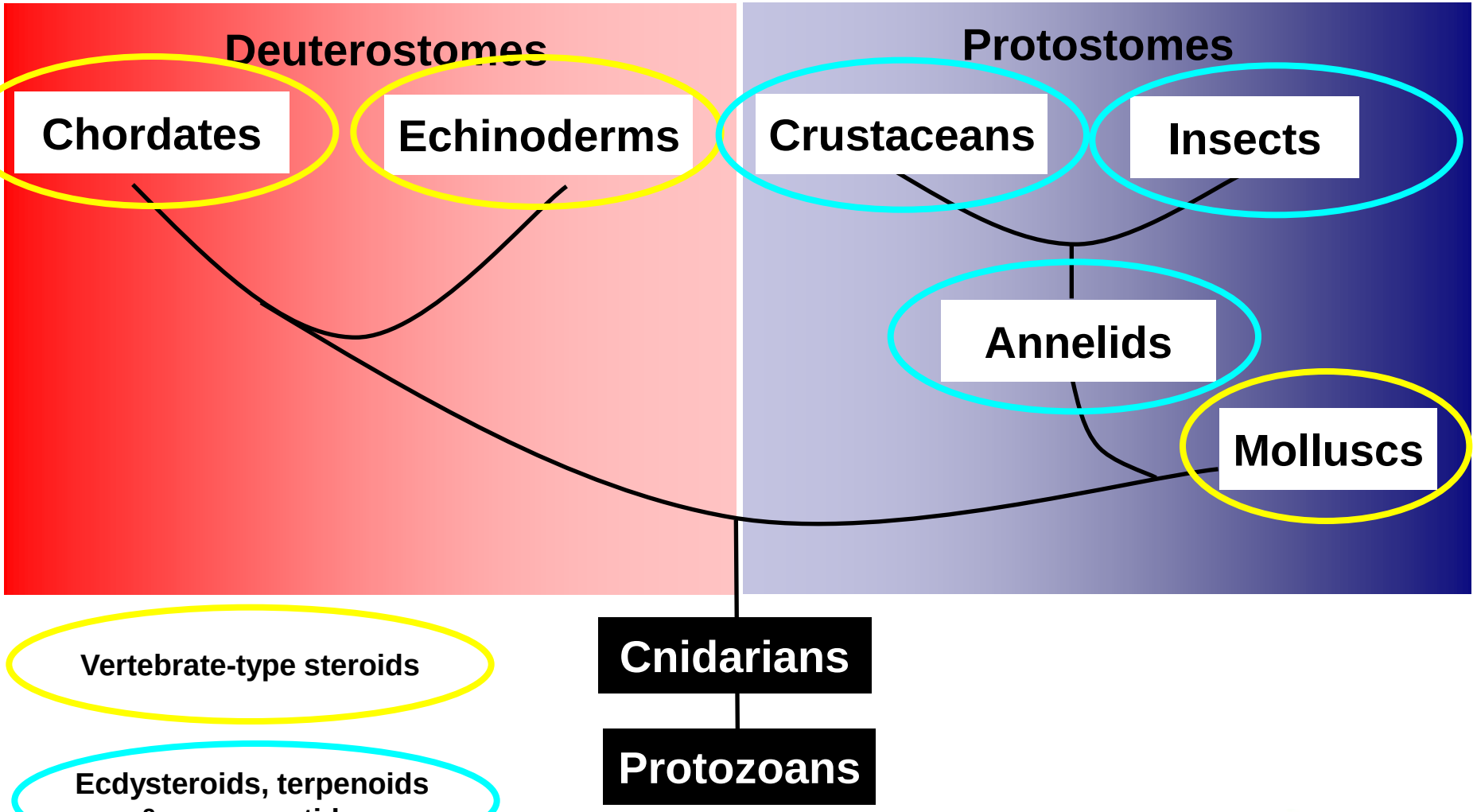
In vitro molecular studies

Model organism studies
Zebrafish
Chemical 1
Chemical 2
Chemical 3
etc …

Ref: Dix et al (2007) Toxicol Sci 95: 5-12

Aquatic testing & alerts over endocrine activity (*in vitro* or mammalian)?

Example - endocrine disrupter MOA



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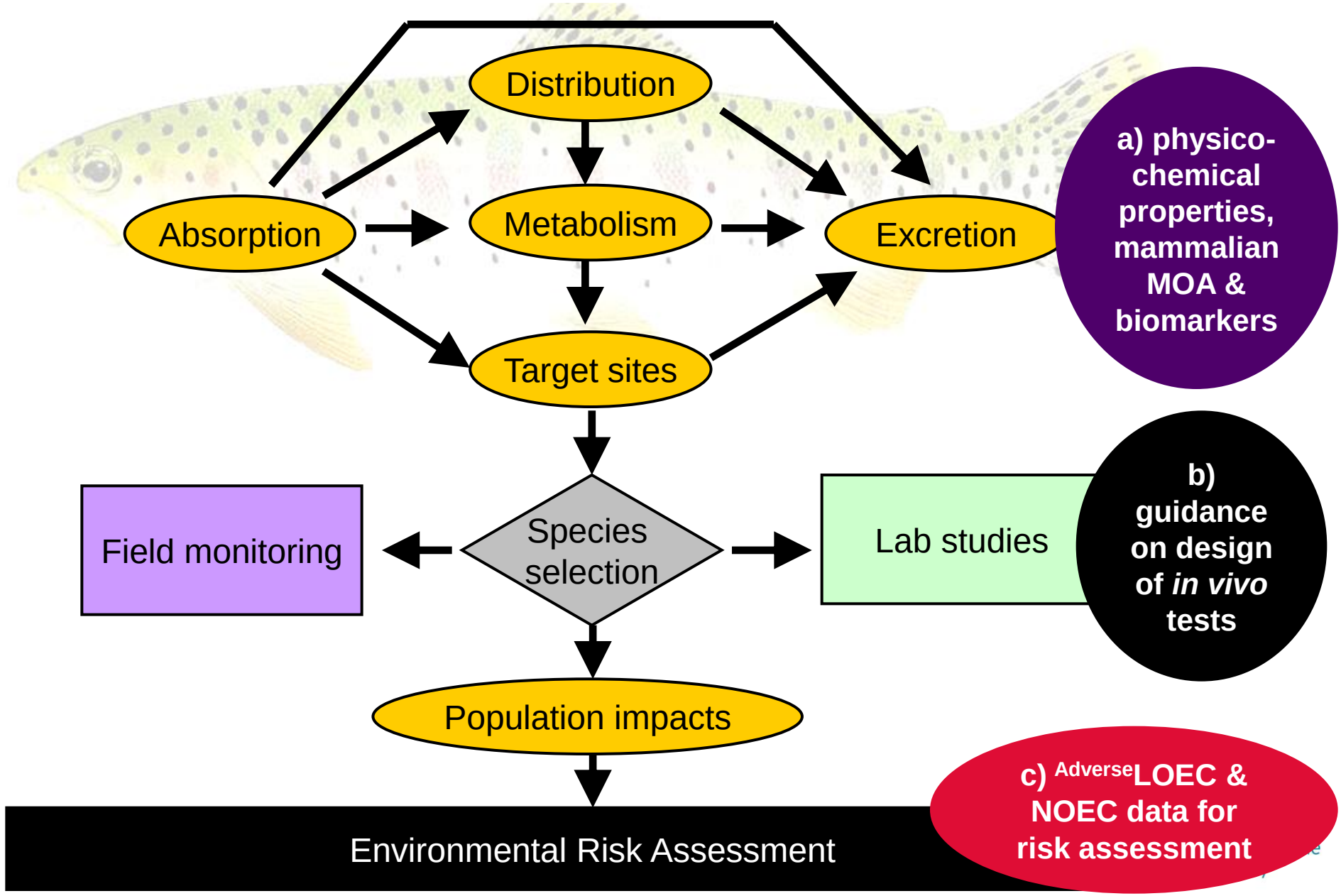
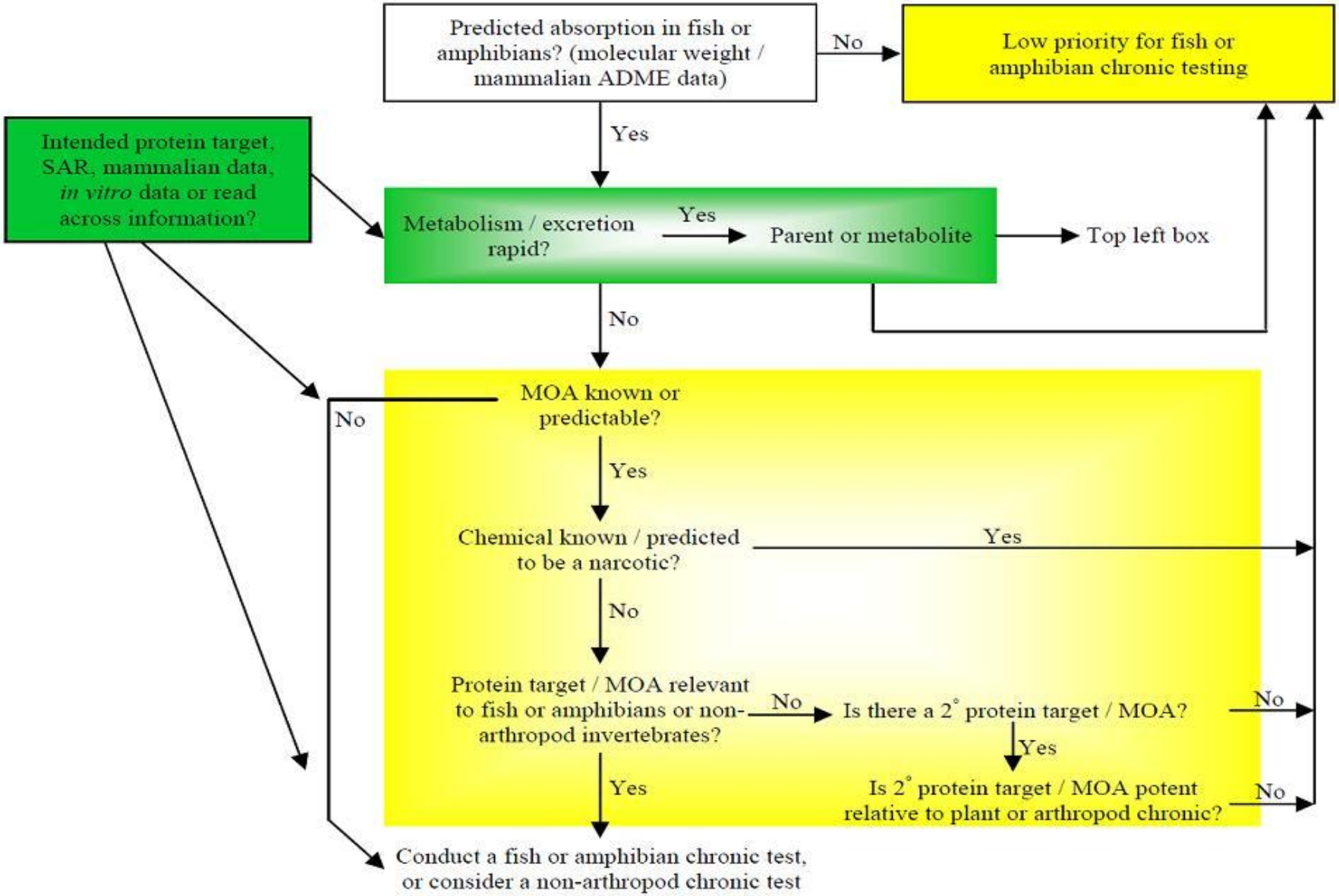
Hazard id versus assessment



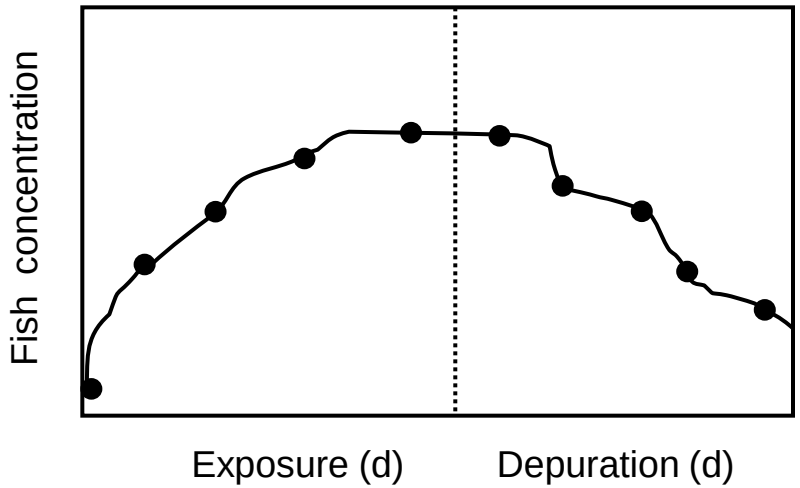
ECETOC (2007) ITS Approach



Bioconcentration & 3Rs?

OECD Fish Bioconcentration Test

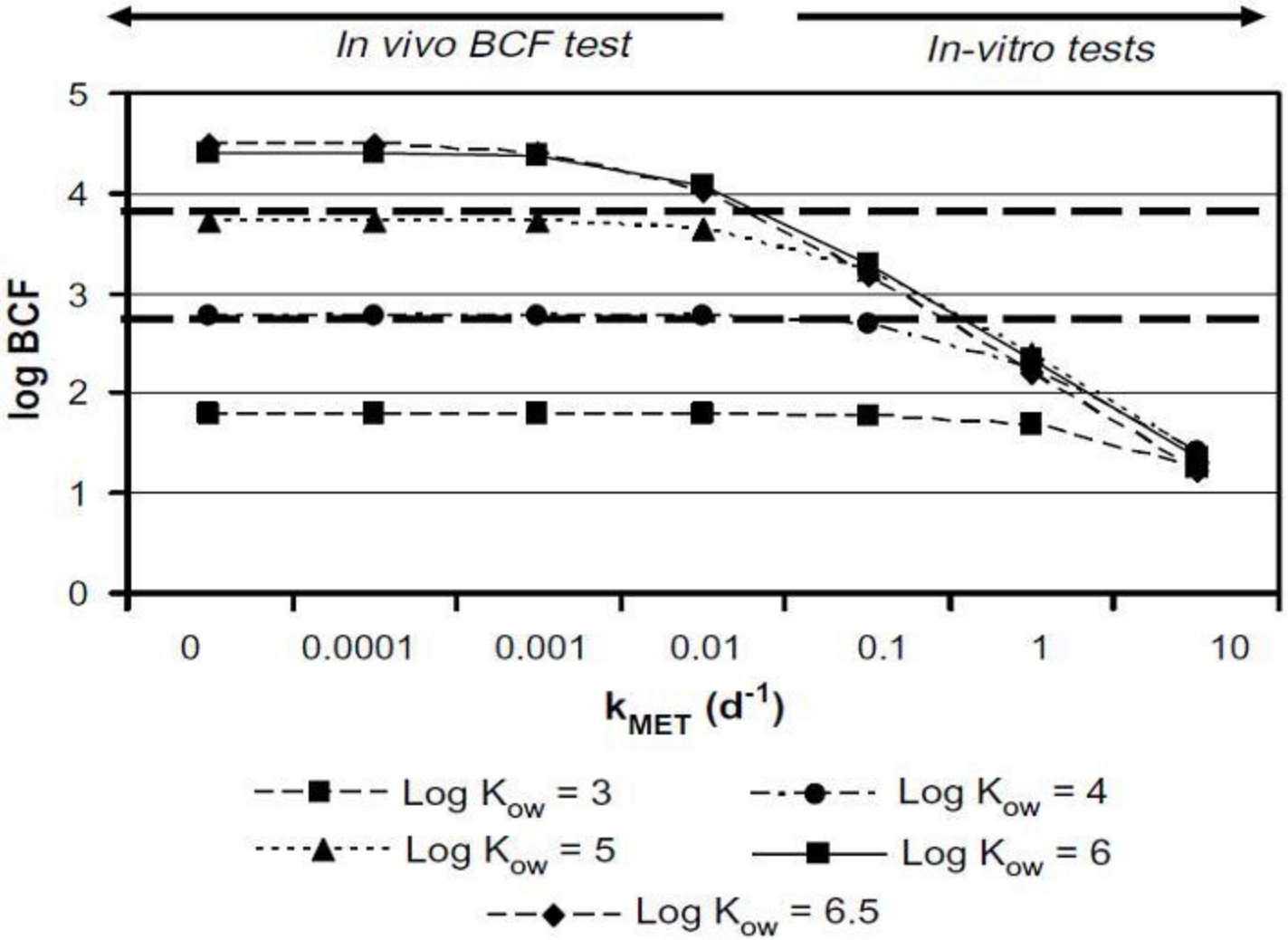
- OECD Test Guideline 305:
  - typically >42d duration
  - minimum of 108 fish per study



- Scope to:
  - reduce number of animals
  - shorten timeframe
  - remove elimination phase, calculate depuration

Source: Versonnen et al (2006)

BCF predictions for chemicals linked to whole-body elimination rates (k_{MET})



BCF 5000 (log BCF 3.7)

BCF 500 (log BCF 2.7)

Ref: Cowan-Ellsberry et al (2008) Chemosphere 70: 1804-1817

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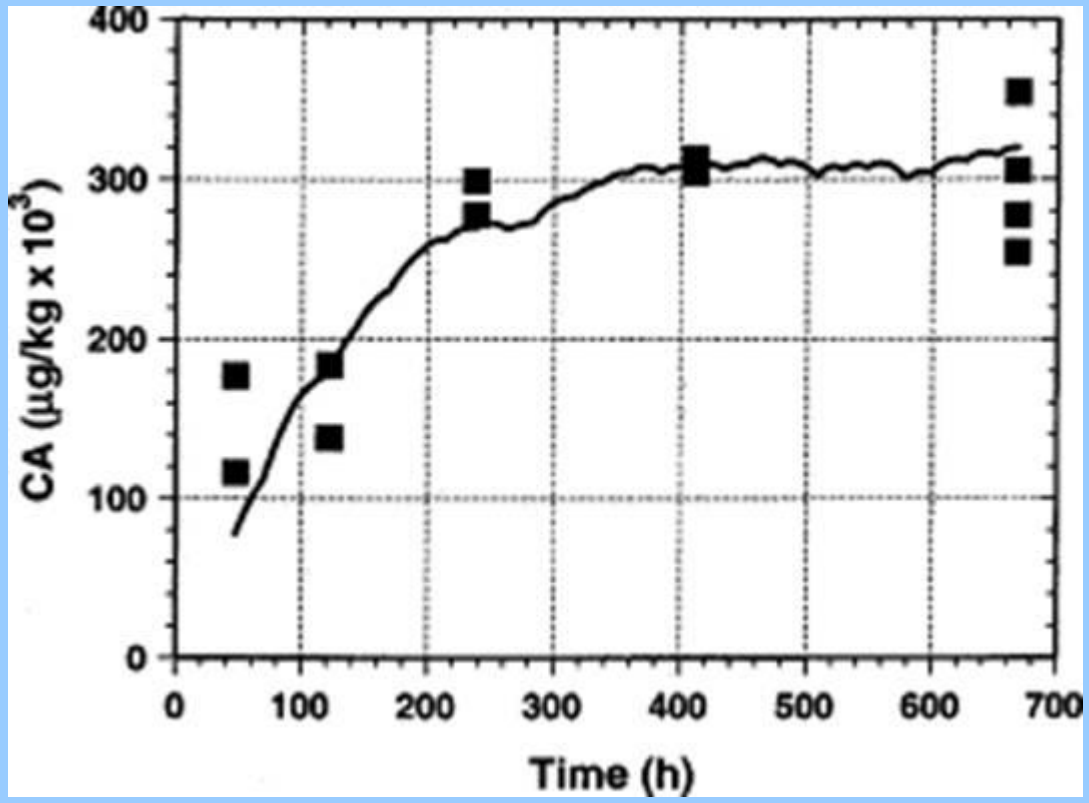
Bioconcentration in invertebrates

Example: bioaccumulation of naphthalene (676 µg l^{-1}) in crustacean



Crustacean: *Diporeia* sp.

Ref: Landrum et al (2003) Chemosphere 51: 481-489

Ecological importance of invertebrates

Organism	Tissue	Total lipids (mean % of dry weight)
Amphipod	Whole animal	18.3 - 45 23 - 30
Annelids	Whole animal	2.6 - 6.5
Barnacles (2 species)	Whole animal	6.5 - 11.8
Copepods	Whole animal	58
Crab	Hepatopancreas	15.4
Crab	Muscle	4.0
Echinoderms	Soft tissues	11.1 - 14.4
Fish (cod)	Eggs Larvae	13.5 10.2 - 21.2
Fish (herring)	Eggs Larvae	11.4 15.2 - 19.4
Fish (turbot)	Larvae	18.2 - 26.0
Fish (zebrafish)	Eggs Larvae Juveniles	10.3 19.6 11.2

Ref: ECETOC (2007) TF Intelligent Testing Strategies in Ecotoxicology

Conclusions

- Significant opportunity for reducing fish testing through *in vivo*, *in vitro* & *in silico* approaches
- Fish acute testing can be reduced by 54-71% through UTC approach
- Mode of action may be a useful guide for 'read across' between *in vitro* and *in vivo* (mammals – fish)
- Fish bioconcentration assessments can be improved through inclusion of *in vitro* k_{MET} data to BCF modelling or using invertebrate models as an ecologically relevant alternative