# Aquatic laboratory toxicity tests and the Tier-1 effect assessment procedure

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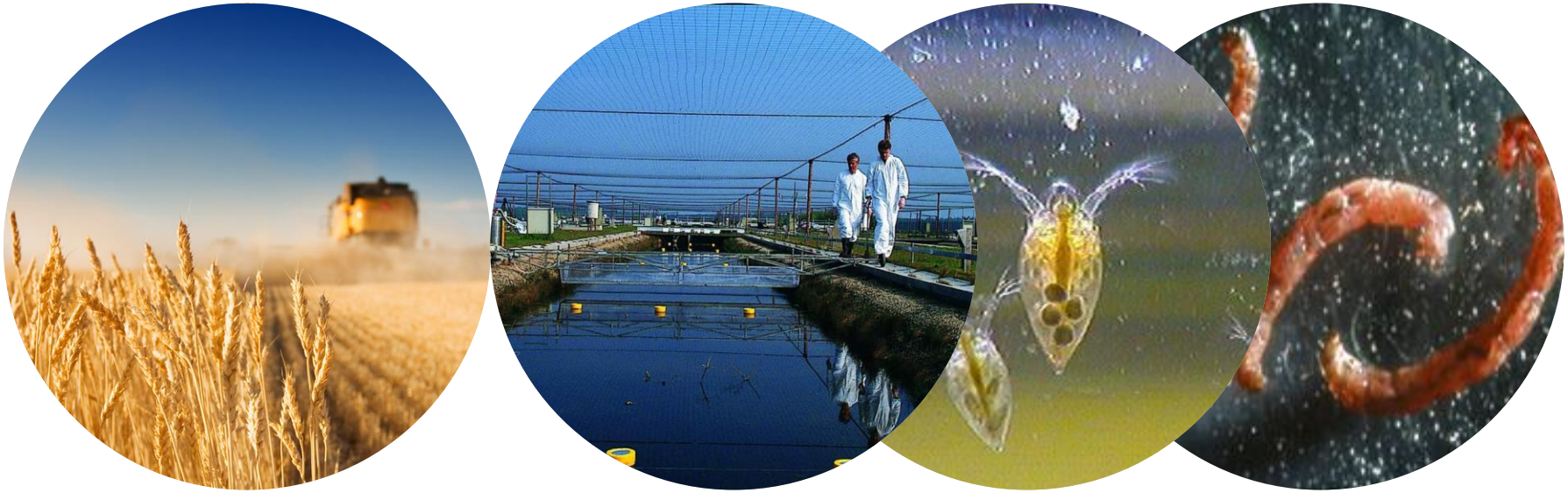

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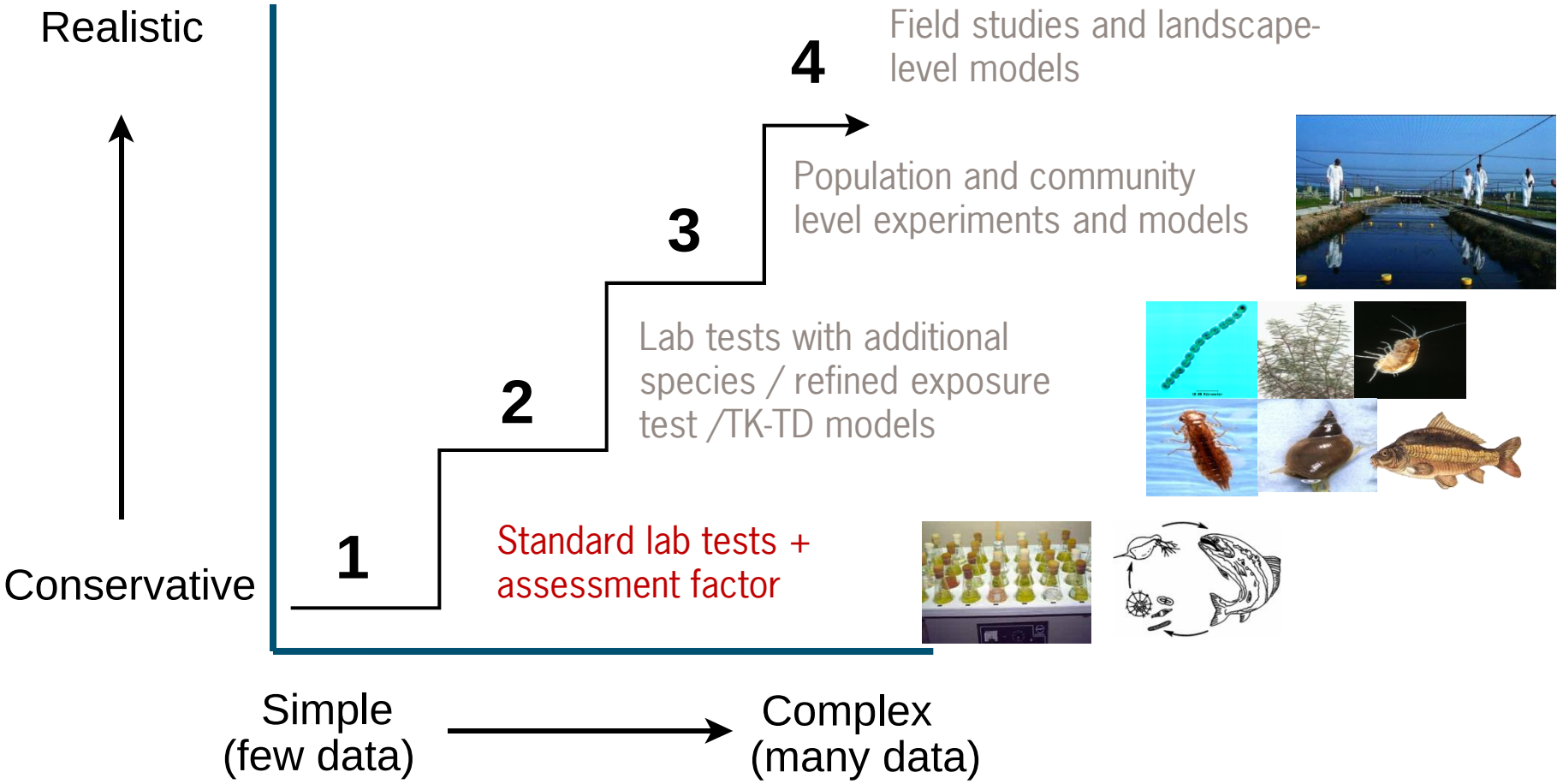
# Tiered approach in effect assessment



# Laboratory toxicity testing

Which species?
- sensitive, important (ecologically, economically), indigenous?

Which life-stage?
- juvenile, adult?

What exposure duration?
- acute, chronic?

What end-point?
- lethal, sublethal?

What exposure route?
- food, water, skin?

What conditions?
- quality and quantity of sediment/soil/water/food

# Production of toxicity data

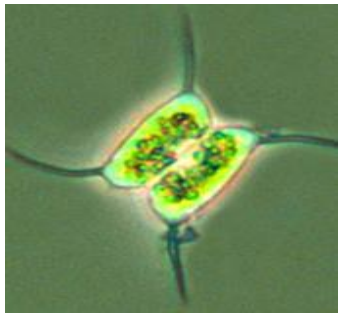
- Ideal test should be replicable, repeatable and relevant.
- For regulation, replicability and repeatability are the most important.
- Results in standardised tests with clear end points.

# Toxicity tests for ERA

- Test guidelines produced by international (e.g. OECD) and national (e.g. US EPA) bodies.

- Acute studies focus on mortality and immobility.

- Chronic test consider sub-lethal endpoints like reproduction and growth.



- Standard species representing specific trophic levels.

# Standard tests with freshwater fish

- Widely used species is *Oncorhynchus mykiss*
- Several other cold and warm water species frequently tested (e.g. *Pimephales promelas; Lepomis macrochirus*)
- Acute toxicity (96h); OECD test guideline 203
  - semi-static
- Long-term / chronic tests for continuous or repeated exposure; ELS, juvenile growth, full life cycle test. (OECD test guideline 210; OECD 2008)
  - flow-through
- For animal welfare reasons test with vertebrates should be minimized where possible. In EU only one standard test species of fish is required

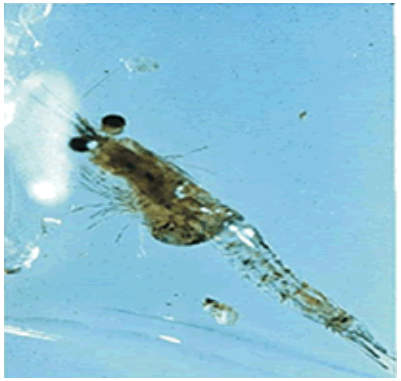
  (*Oncorhynchus mykiss*)

# Standard tests with aquatic invertebrates

*Daphnia magna*

- Acute 48 h (OECD test guideline 202)
  - Mortality; immobility
- Chronic 21 days (OECD  test guideline 211)
  - Reproduction

*Americamysis bahia*



- Acute 96 h (US EPA, 1996)
  - Mortality; immobility
- Chronic 28 days (US EPA, 1996)
  - Growth; mortality; reproduction

# Standard tests with benthic invertebrates

*Hyalella azteca* (ASTM E1706)

- Acute, water (48 h to 96 h); mortality
- Semi-chronic, sediment-spiked (10d); mortality
- Chronic, sediment spiked (28 – 42 d); growth

*Chironomus riparius / dilutus* (OECD 218; 233)

- Acute, water (48 h to 96 h); mortality
- Semi-chronic, sediment-spiked (10d); mortality
- Chronic, water/sediment-spiked 28-65 d; emergence, growth

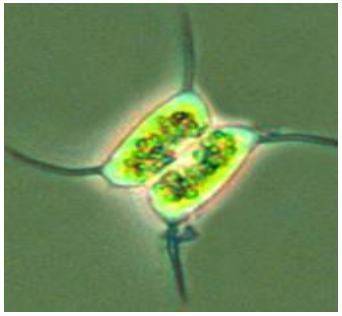
*Lumbriculus variegatus* (OECD 225)

*Tubifex tubifex* (ASTM E1706)

- Semi-chronic, sediment-spiked (10d); mortality
- Chronic, water/sediment-spiked 28 d; growth

# Standard tests with primary producers

- Unicellular algae (e.g. *Pseudokirchneriella subcapitata; Navicula pelliculosa*)
  - OECD guideline 201
  - 72 h to 120 h
  - Population growth (cell number, biomass)
- Floating macrophytes (*Lemna*)
  - OECD guideline 221
  - 7 to 14 d
  - Population growth (front numbers, biomass)
- Rooted macrophytes (*Myriophyllum*)
  - OECD guideline 239
  - Biomass, length, number of shoots / roots
  - Cultures or field collection.

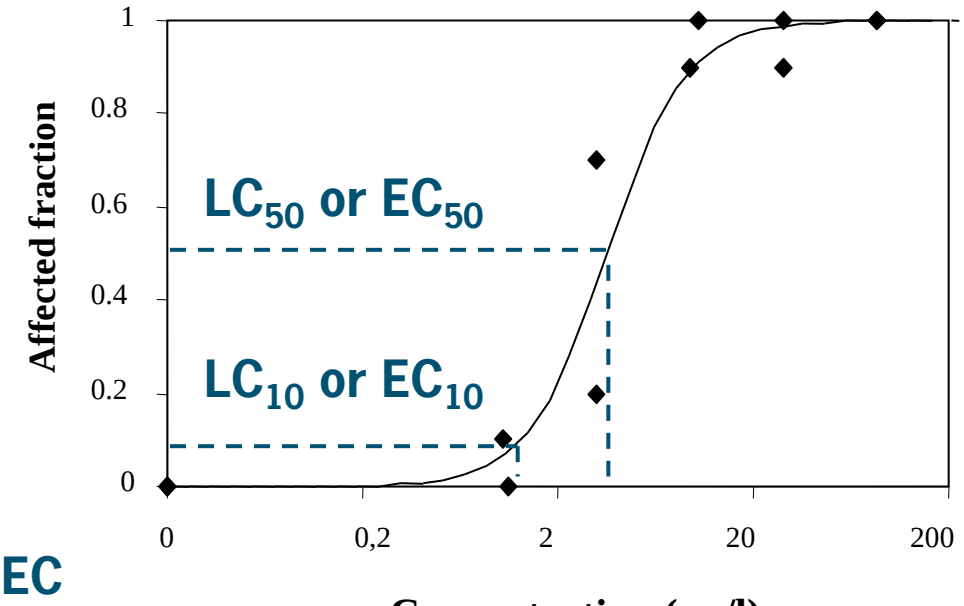


# Conduct of single species toxicity tests

TOXICITY STUDIES WITH AQUATIC ORGANISMS

| CONCENTRATION (µg/l) | % KILLED AT 4 DAYS | |
|---|---|---|
| 0 | 0 | |
| 0,5 | 0 | |
| 1 | 0 | ← NOEC |
| 2,5 | 30 | |
| 6 | 80 | |
| 12 | 90 | |

RESULT: LC50 = 3.8 µg/l




# Factors influencing toxicity

- Extrinsic
  - Exposure period
  - Temperature
  - Hardness, pH

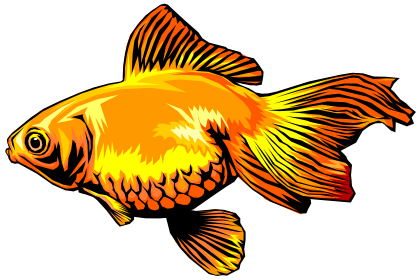
- Intrinsic
  - Species
  - Population
  - Life-history stage

Modify exposure

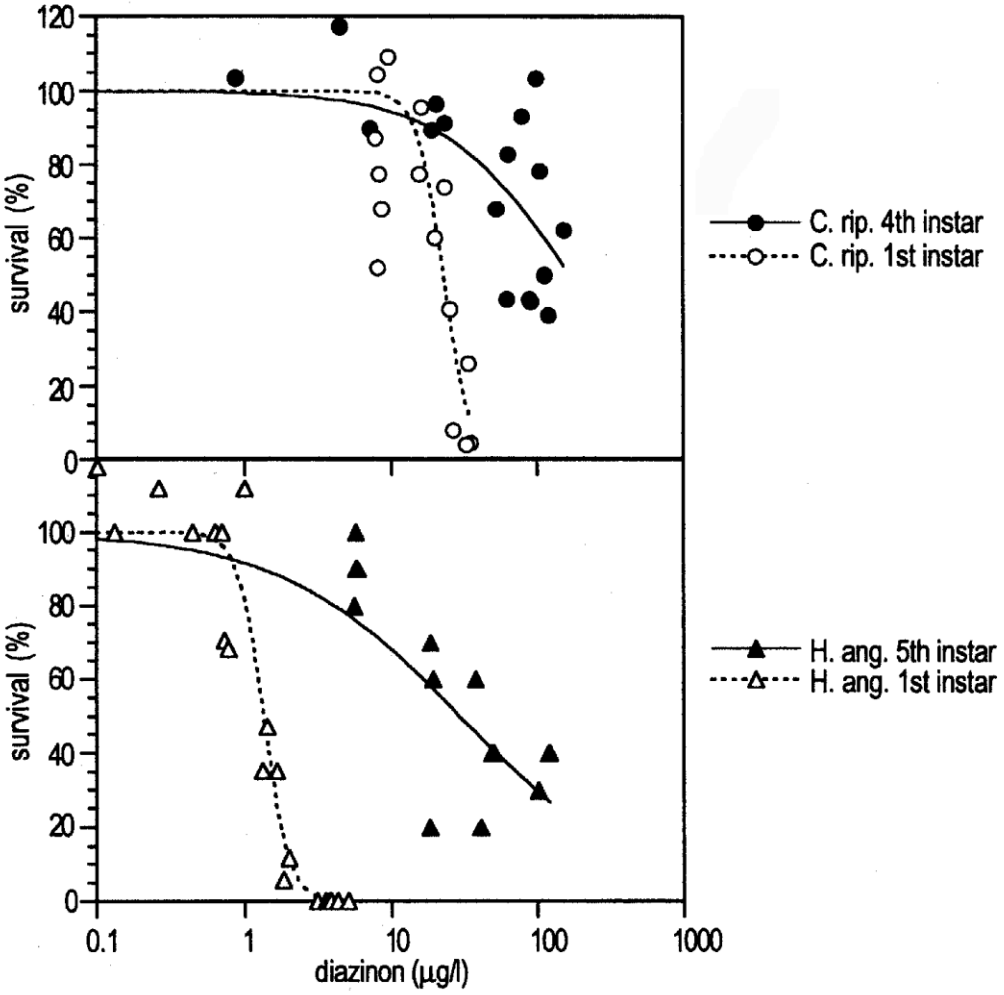
Modify effects

# Exposure period & temperature

## Hydrogen sulphide toxicity



# Life stage and sensitivity to the insecticide diazinon

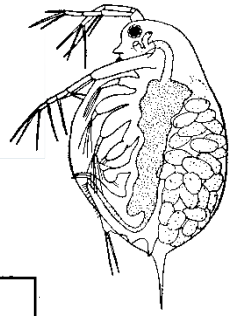


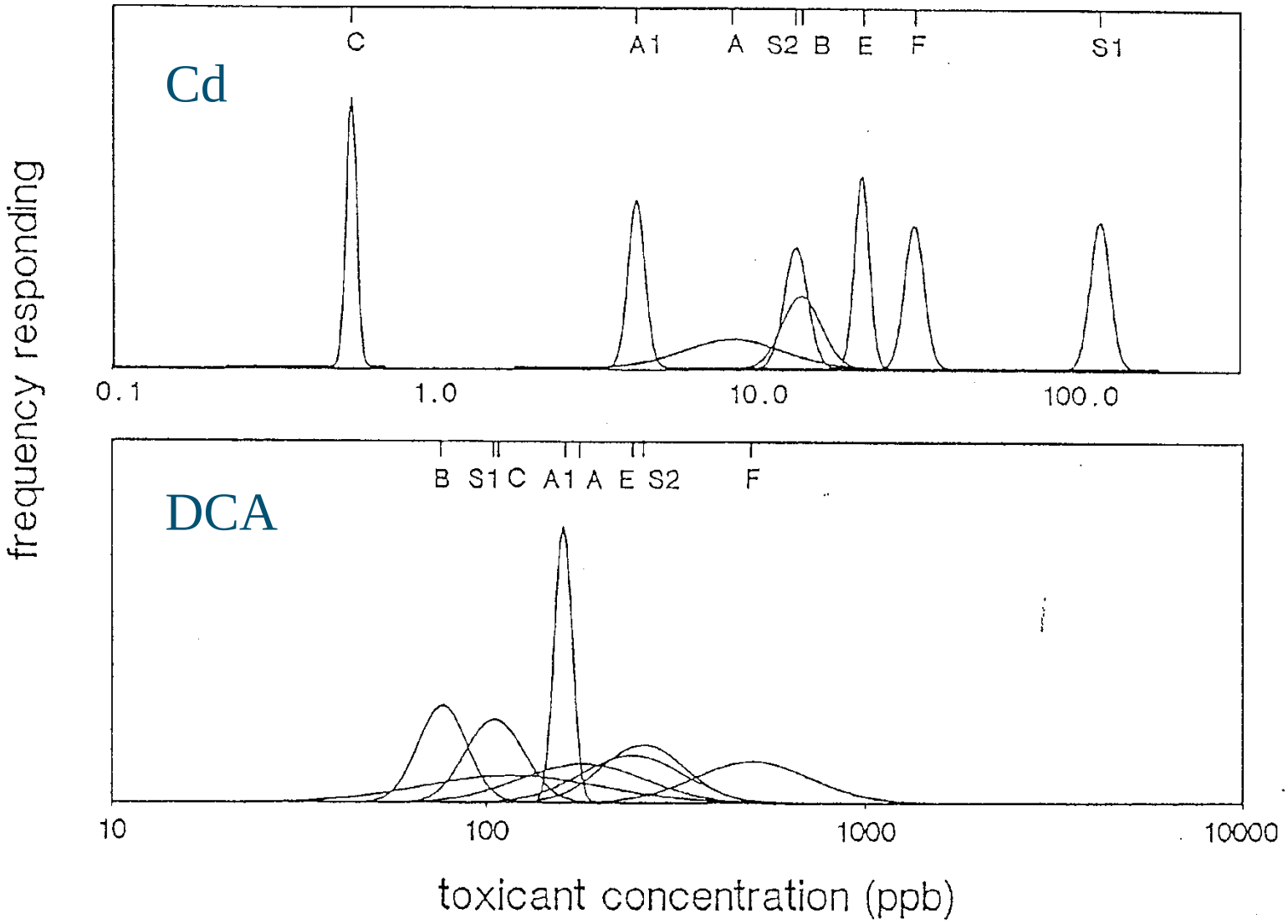

*Chironomus riparius*

*Hydropsyche angustipennis*

*Stuijfzand 99*

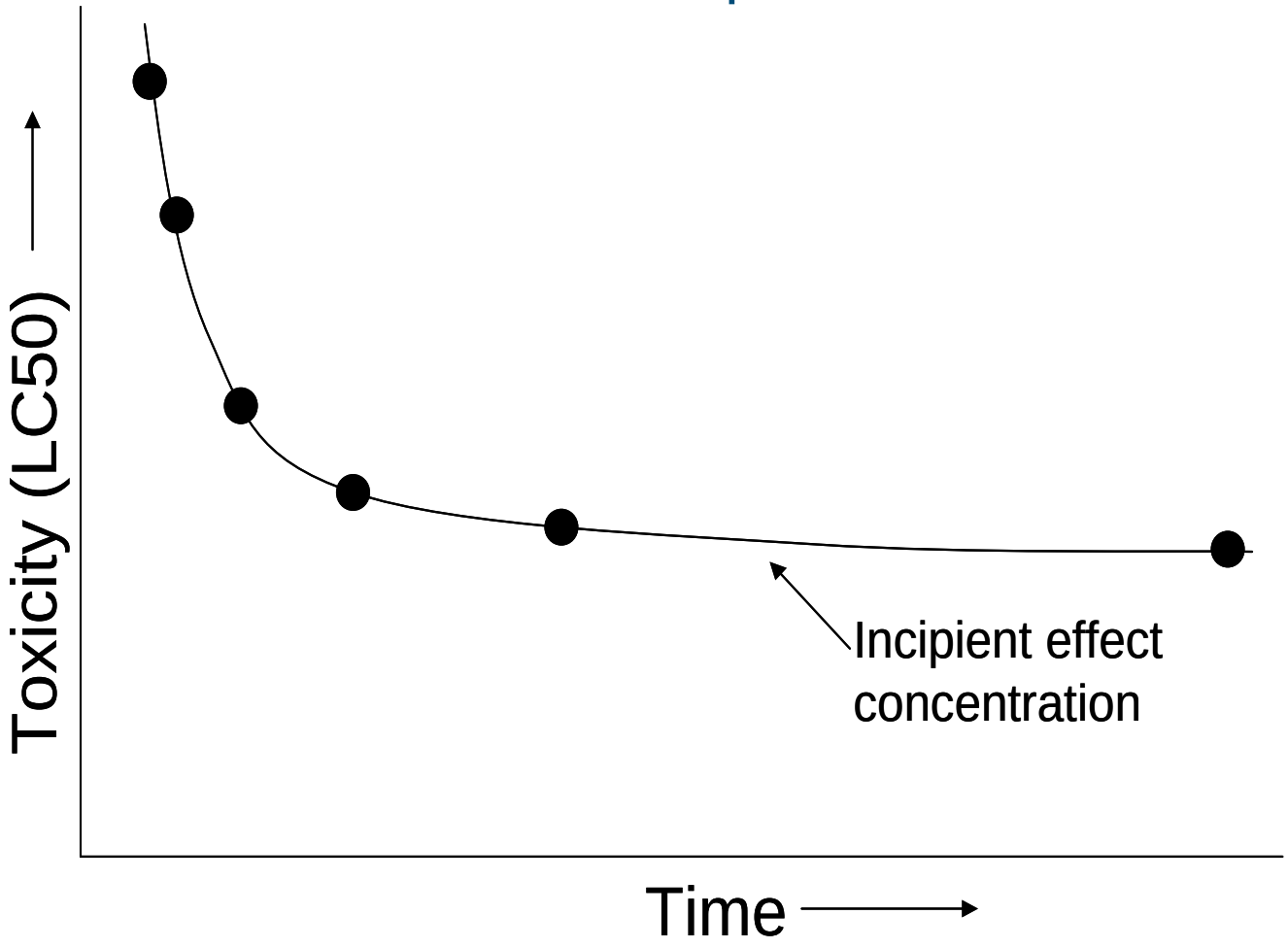
# Between-individual variation





# Organism chemical interaction

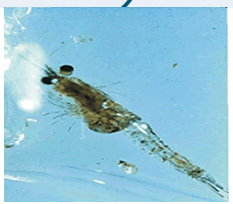
## The time needed for the expression of the effects

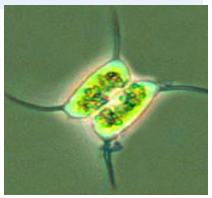


# Tier-1 effect assessment insecticides (EU)
## Pelagic organisms (EFSA Aquatic Guidance Document)

| | Standard test species | Duration | Endpoint | RAC |
|---|---|---|---|---|
| **Acute effect assessment** (link to $PEC_{sw;max}$) | - *Daphnia* sp. | 48 h | $EC_{50}$ | $EC_{50}/100$ |
| | - Second arthropod (e.g. *Chironomus* or *A. bahia*) | 48 h | $EC_{50}$ | $EC_{50}/100$ |
| | - *Oncorhynchus mykiss* | 96 h | $LC_{50}$ | $LC_{50}/100$ |
| **Chronic effect assessment** (link to $PEC_{sw;max}$ or $PEC_{sw;twa}$) | - *Daphnia* sp. or additional arthropod | 21 d | $EC_{10}$ (NOEC) | $EC_{10}/10$ |
| | - *Chironomus* sp. | 20-28 d | $EC_{10}$ (NOEC) | $EC_{10}/10$ |
| | - Early life stage test (ELS) or full life-cycle test (FLC) with fish | Up to 60 d<br>Egg to egg | $EC_{10}$ (NOEC) | $EC_{10}/10$ |
| | - Green alga (e.g. *Pseudokirchneriella subcapitata)* | 72(-96) h | $E_rC_{50}$ | $E_rC_{50}/10$ |







# Tier-1 effect assessment insecticides (EU)

## Benthic organisms

| | Standard test species | Duration | Endpoint | $RAC_{sed}$ |
|---|---|---|---|---|
| **Chronic effect assessment** (link to $PEC_{sed;max}$ or $PEC_{sed;twa}$) | - *Chironomus* sp. | 20 - 28 d | $EC_{10}$ (NOEC) | $EC_{10}/10$ |
| | - *Hyalella azteca* * | (28-)42 d | $EC_{10}$ (NOEC) | $EC_{10}/10$ |

*Recent EFSA proposal (EFSA PPR, 2015)

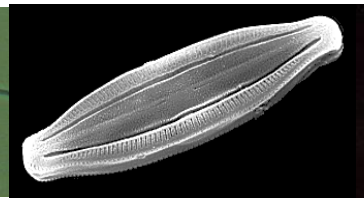
Sediment-spiked tests are preferred but water-spiked tests in a water-sediment test system also possible

Toxicity estimates should be expressed in terms total sediment concentrations (dry weight; normalised for OC content) and, if possible, pore water concentrations

# Tier-1 effect assessment herbicides (EU)
## Pelagic organisms (EFSA Aquatic Guidance Document)

| | Standard test species | Duration | Endpoint | RAC |
|---|---|---|---|---|
| **Acute effect assessment** | - *Daphnia* sp. | 48 h | $EC_{50}$ | $EC_{50}/100$ |
| | - *Oncorhynchus mykiss* | 96 h | $LC_{50}$ | $LC_{50}/100$ |
| **Chronic effect assessment** (link to $PEC_{sw;max}$ or $PEC_{sw;twa}$) | - Green alga (e.g. *Pseudokirchneriella subcapitata)* | 72(-96) h | $E_rC_{50}$ | $E_rC_{50}/10$ |
| | - Additional non-green alga (e.g. *Navicula pelliculosa*) | 72(-96) h | $E_rC_{50}$ | $E_rC_{50}/10$ |
| | - *Lemna* sp or *Myriophyllum sp.* | 7-14 d | $E_rC_{50}$ | $E_rC_{50}/10$ |
| | - *Daphnia* sp. | 21 d | $EC_{10}$ (NOEC) | $EC_{10}/10$ |
| | - Early life stage test (ELS) or full life-cycle test (FLC) with fish | Up to 60 d<br>Egg to egg | $EC_{10}$ (NOEC) | $EC_{10}/10$ |



# Tier-1 effect assessment herbicides (EU)
## Benthic organisms

| | Standard test species | Duration | Endpoint | $RAC_{sed}$ |
|---|---|---|---|---|
| **Chronic effect assessment** (link to $PEC_{sed;max}$ or $PEC_{sed;twa}$) | - *Myriophyllum* sp. | 14 d | $EC_{50}$ | $EC_{50}/10$ |
| | - *Chironomus* sp. or *Hyalella azteca* * | 28-42 d | $EC_{10}$ (NOEC) | $EC_{10}/10$ |

*Recent EFSA proposal (EFSA PPR, 2015)

Sediment-spiked tests are preferred but water-spiked tests in a water-sediment test system also possible

Toxicity estimates should be expressed in terms total sediment concentrations (dry weight; normalised for OC content) and, if possible, pore water concentrations

# Tier-1 effect assessment fungicides (EU)
## Pelagic organisms (EFSA Aquatic Guidance Document)

| | Standard test species | Duration | Endpoint | RAC |
|---|---|---|---|---|
| **Acute effect assessment** (link to $PEC_{sw;max}$) | - *Daphnia* sp. | 48 h | $EC_{50}$ | $EC_{50}/100$ |
| | - *Oncorhynchus mykiss* | 96 h | $LC_{50}$ | $LC_{50}/100$ |
| **Chronic effect assessment** (link to $PEC_{sw;max}$ or $PEC_{sw;twa}$) | - Green alga (e.g. *Pseudokirchneriella subcapitata)* | 72(-96) h | $E_rC_{50}$ | $E_rC_{50}/10$ |
| | - *Daphnia* sp. | 21 d | $EC_{10}$ (NOEC) | $EC_{10}/10$ |
| | - Early life stage test (ELS) or full life-cycle test (FLC) with fish | Up to 60 d<br>Egg to egg | $EC_{10}$ (NOEC) | $EC_{10}/10$ |

# Tier-1 effect assessment fungicides (EU)
## Benthic organisms

| | Standard test species | Duration | Endpoint | $RAC_{sed}$ |
|---|---|---|---|---|
| **Chronic effect assessment** (link to $PEC_{sed;max}$ or $PEC_{sed;twa}$) | - *Lumbriculus* sp. | 14 d | $EC_{50}$ | $EC_{50}/10$ |
| | - *Chironomus* sp. or other relevant species* | 28-42 d | $EC_{10}$ (NOEC) | $EC_{10}/10$ |

*Recent EFSA proposal (EFSA PPR, 2015)

Sediment-spiked tests are preferred but water-spiked tests in a water-sediment test system also possible

Toxicity estimates should be expressed in terms total sediment concentrations (dry weight; normalised for OC content) and, if possible, pore water concentrations

# Calibration of Tier-1 RAC for insecticides

Standard single species

?

field

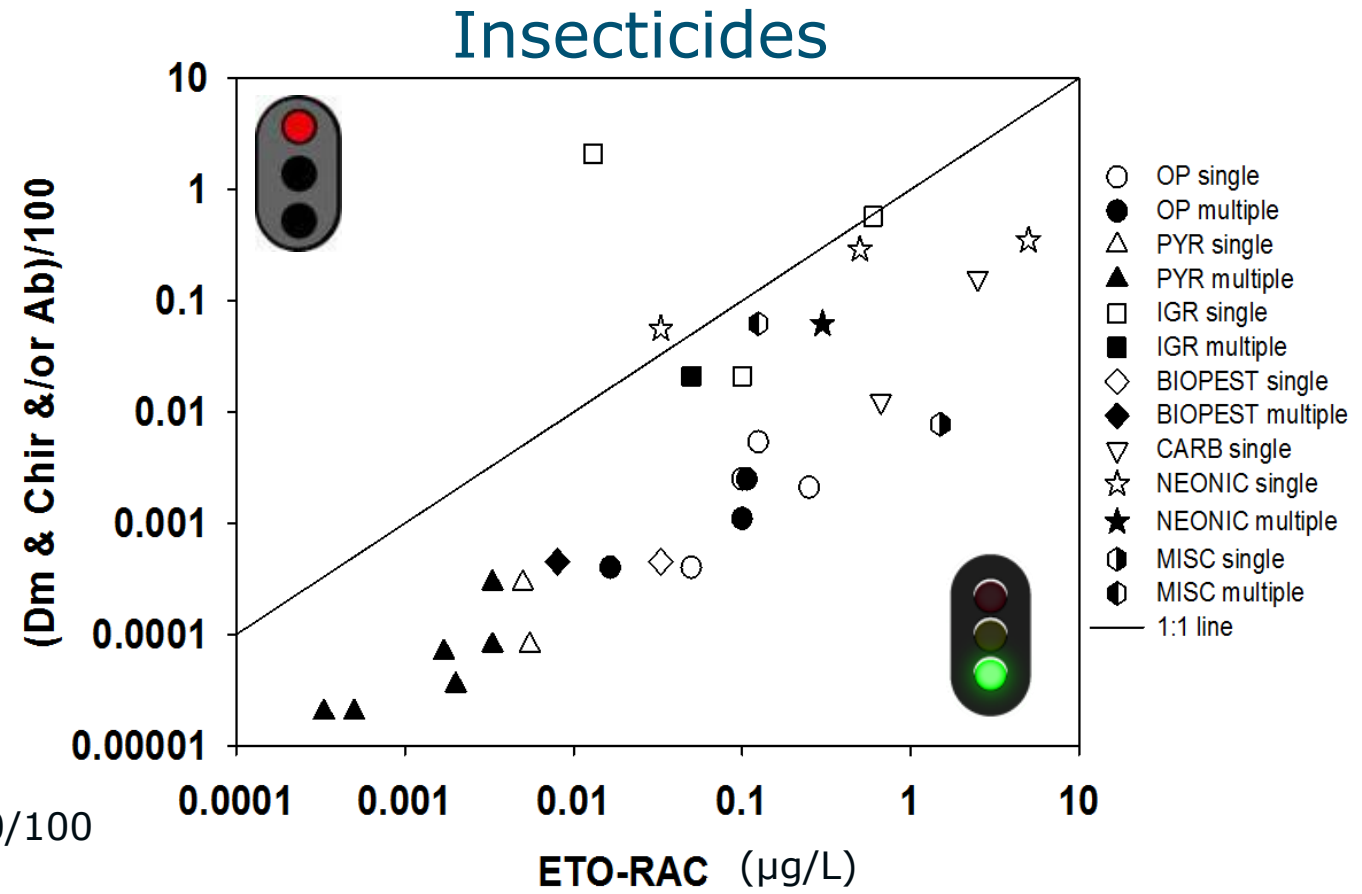
Tier-1 $RAC_{sw}$ =
Laboratory toxicity (e.g. NOEC or $EC_{50}$) divided by AF (e.g. 10 or 100)

Tier-3 $RAC_{sw}$ =
Threshold concentration most sensitive endpoint in mesocosms

- Effect class 1 divided by 2
- Effect class 2 divided by 3

Effect class 1 = no treatment-related effect on sensitive endpoints
Effect class 2 = slight effect (isolated sampling) on most sensitive endpoint

# Calibration/verification acute Tier-1




RAC = lowest 48-h EC50/100

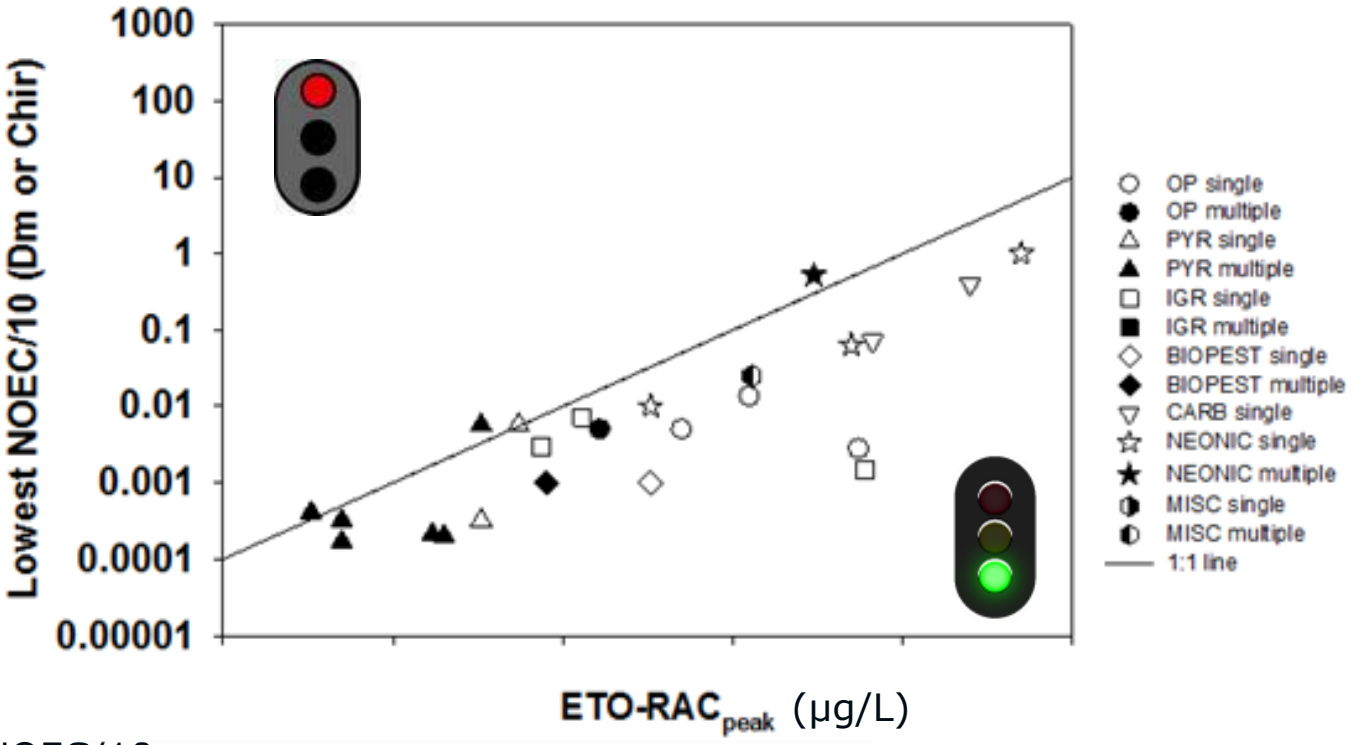
In 28 out of 30 insecticide cases the acute tier-1 RAC is protective

Particularly the IGR fenoxicarb is exception (wide range in Effect class 2 concentrations in mesocosms) and to a lesser extent the neonicotinoid thiacloprid (less than a factor of 2)

Van Wijngaarden, Maltby & Brock (2014)

# Calibration/verification chronic Tier-1

## Insecticides

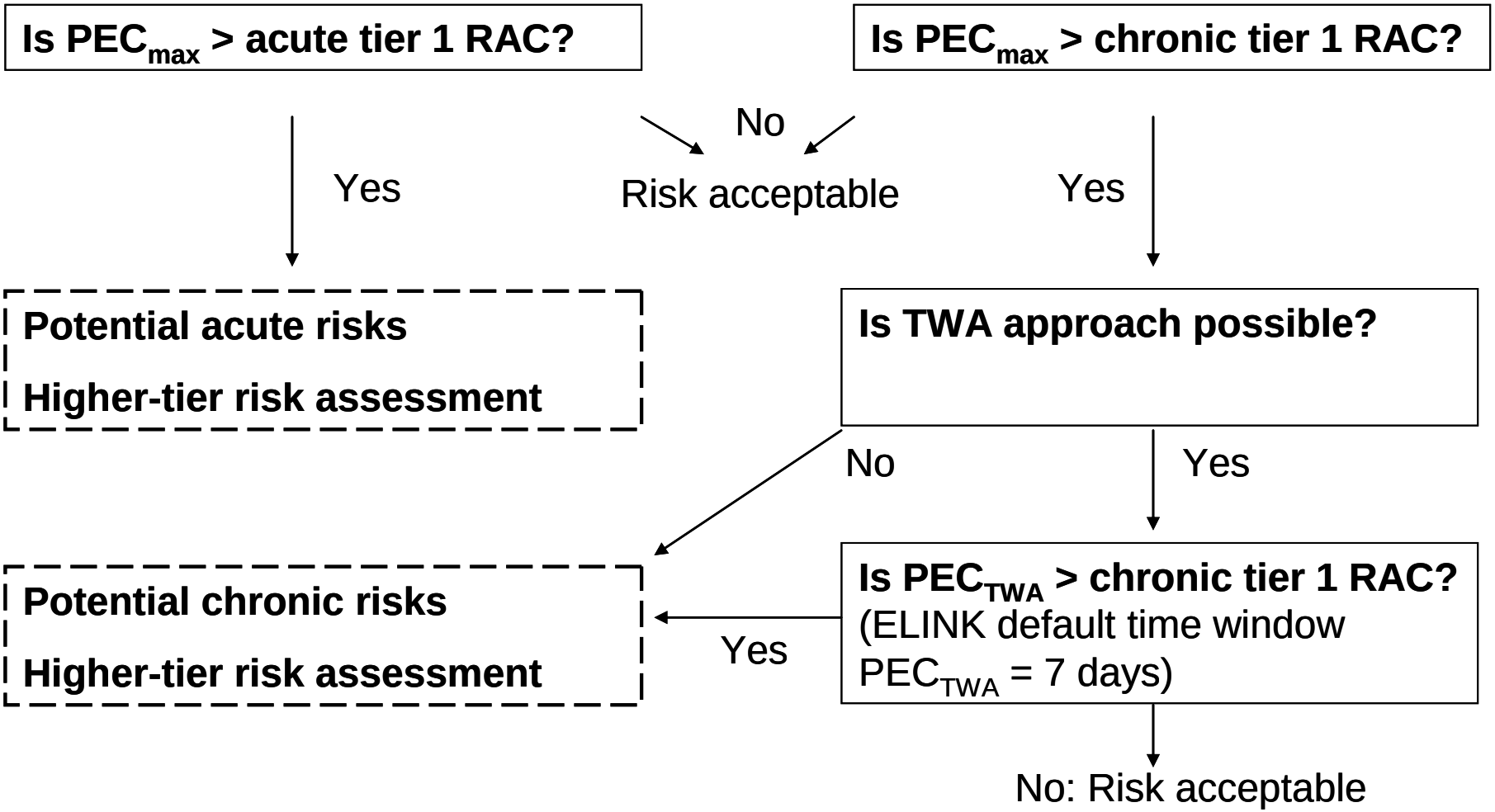



RAC = lowest 21d/28d NOEC/10

In 21 out of 24 insecticide cases the chronic tier-1 RAC is protective

Brock et al. (in prep)

# Risks due to toxicity

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# Thank you for your attention
# Questions ?

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