The minimum detectable difference (MDD) and the interpretation of treatment-related effects of pesticides in experimental ecosystems

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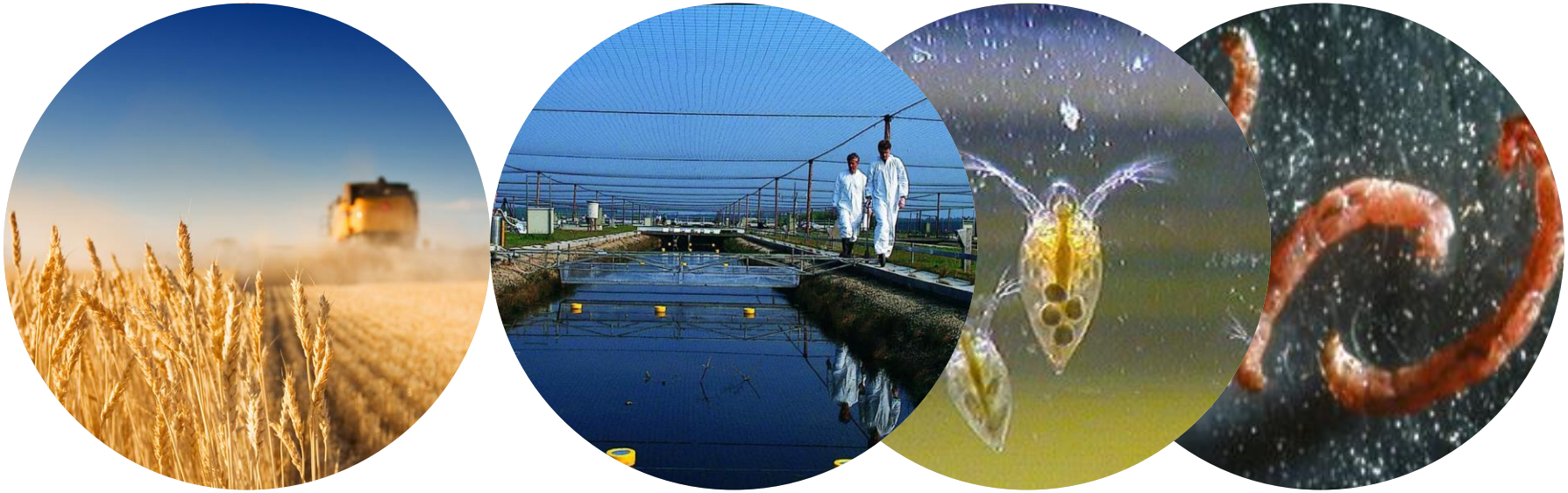

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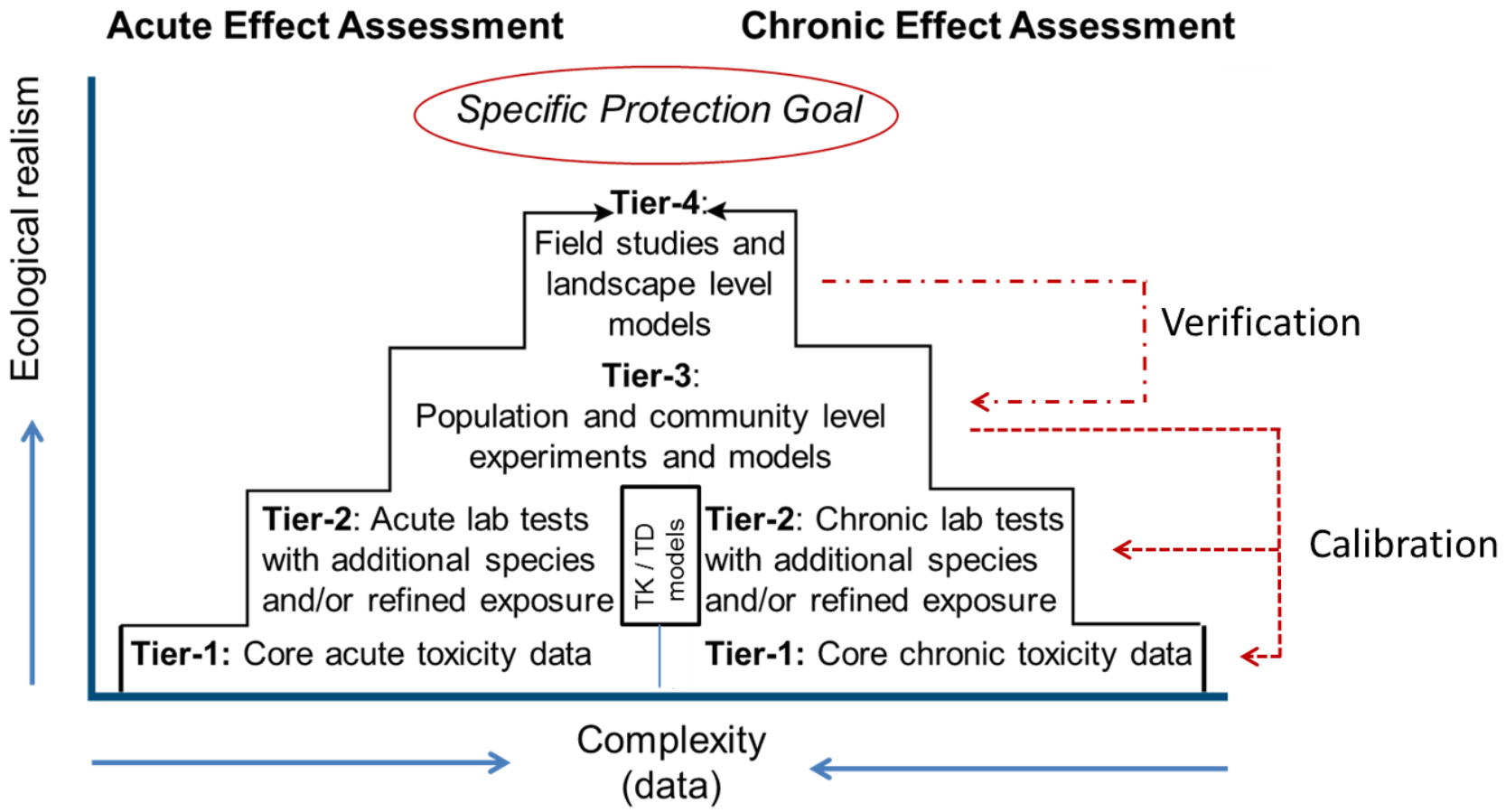
Aims of the presentation

Expand on the EFSA Aquatic Guidance Document (EFSA 2013)

- Explain the role of experimental ecosystem studies in the tiered approach of pesticide ERA
- Present the concept of the minimum detectable difference (MDD)
- Give suggestions how to decrease the MDD for measurement endpoints in micro-/mesocosm tests
- Present a procedure to report MDDs for NOECs derived from these tests
- Proposal how to use MDDs to facilitate the interpretation of micro-/mesocosm experiments
    - *Validity of the study for regulatory purposes*
    - *Derivation of Effect classes for RAC estimation*

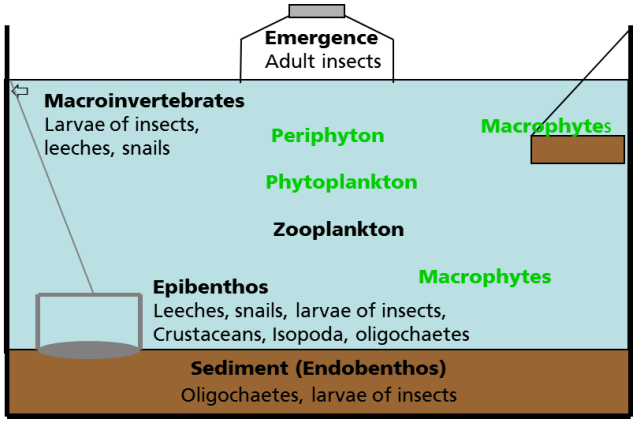
Micro-/mesocosm studies as highest experimental tier

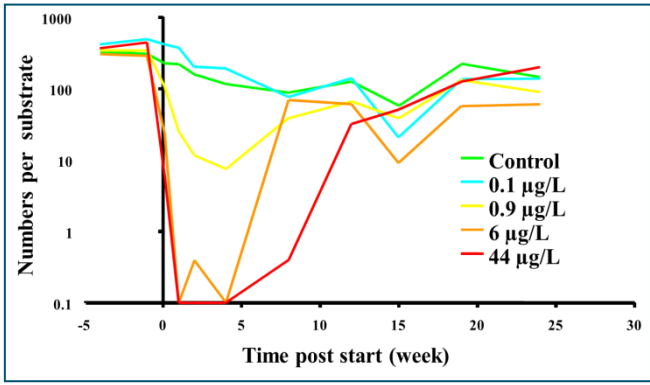


Micro-/mesocosms are test systems used as highest experimental tier (Tier 3) in the ERA for pesticides and as "surrogate reference" tier to calibrate lower tiers

Micro-/mesocosm experiments

- Allow to study treatment-related effects at the population and community level
  - Direct and indirect effects
  - Delayed effects and recovery
- Replicated test systems to facilitate statistical interpretation
  - Controls (n = 3-5)
  - 5 test concentrations (n= 2-3)
- Derivation of Tier 3 Regulatory Acceptable Concentrations (RACs)
  - Ecological Threshold Option (ETO)
  - Ecological Recovery Option (ERO)





Ecological Threshold Option (ETO)

- Accepting only negligible effects on populations of aquatic non-target organisms in edge-of-field
- Propagation of effects to the community, ecosystem and landscape will be less likely
- All tiers can address ETO

Ecological Recovery Option (ERO)

- Accepting some population level effects if ecological recovery takes place within an acceptable time
- Focus on vulnerable populations of aquatic organisms
- Reasonable option only if recovery is not hampered by multi-stress of pesticides
- ERO may be addressed by mesocosm experiments and effect models

ETO/ERO-RAC derivation in EFSA AGD

Ecological threshold option

	AF for ETO-RAC_{sw} derivation	Field exposure concentration to compare with the RAC_{sw}
Effect class 1 concentration	**2**	**Acute risk: $PEC_{sw;max}$** **Chronic risk: $PEC_{sw;max}$ or $PEC_{sw;twa}$**
Effect class 2 concentration	**2 – 3**	**Acute risk: $PEC_{sw;max}$** **Chronic risk: $PEC_{sw;max}$ or $PEC_{sw;twa}$**

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

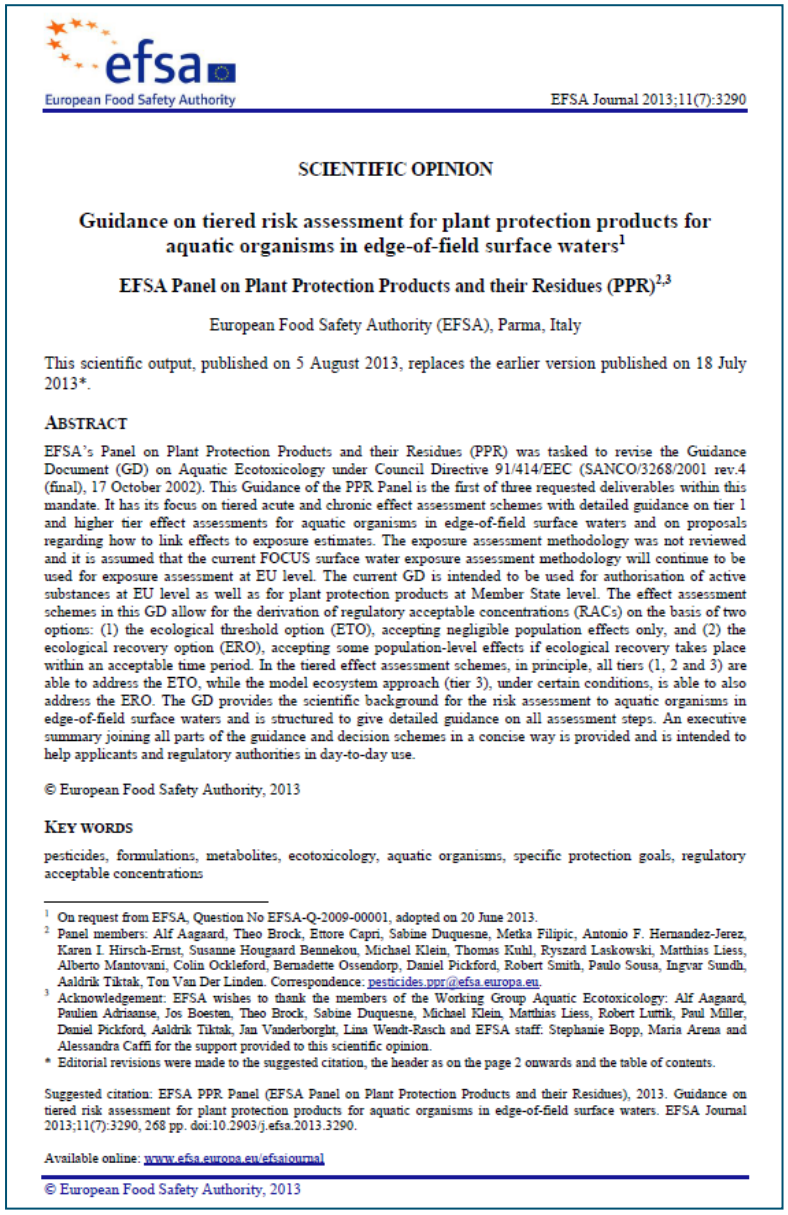
Ecological recovery option

	AF for ERO-RAC_{sw} derivation	Field exposure concentration to compare with the RAC_{sw}
Effect class 3A concentration	**3 – 4**	**Acute risk: $PEC_{sw;max}$** **Chronic risk: $PEC_{sw;max}$ or $PEC_{sw;twa}$**

Effect class 3A = pronounced short-term effect on most sensitive endpoint, total effect period < 8 weeks

Ecological Threshold (ETO)and Recovery Option (ERO)

- Concentration-response relationships for a sufficient number of **potentially sensitive populations** (≥8) (ETO) including representative **vulnerable taxa** (ERO)
- The **exposure** in the test system is **relatively worst case** to that predicted for edge-of-field surface water
- Information on the **Minimum Detectable Difference** (MDD) should be provided and used in the interpretation



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SCIENTIFIC OPINION

Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters[1]

EFSA Panel on Plant Protection Products and their Residues (PPR)[2,3]

European Food Safety Authority (EFSA), Parma, Italy

This scientific output, published on 5 August 2013, replaces the earlier version published on 18 July 2013*.

ABSTRACT

EFSA's Panel on Plant Protection Products and their Residues (PPR) was tasked to revise the Guidance Document (GD) on Aquatic Ecotoxicology under Council Directive 91/414/EEC (SANCO/3268/2001 rev.4 (final), 17 October 2002). This Guidance of the PPR Panel is the first of three requested deliverables within this mandate. It has its focus on tiered acute and chronic effect assessment schemes with detailed guidance on tier 1 and higher tier effect assessments for aquatic organisms in edge-of-field surface waters and on proposals regarding how to link effects to exposure estimates. The exposure assessment methodology was not reviewed and it is assumed that the current FOCUS surface water exposure assessment methodology will continue to be used for exposure assessment at EU level. The current GD is intended to be used for authorisation of active substances at EU level as well as for plant protection products at Member State level. The effect assessment schemes in this GD allow for the derivation of regulatory acceptable concentrations (RACs) on the basis of two options: (1) the ecological threshold option (ETO), accepting negligible population effects only, and (2) the ecological recovery option (ERO), accepting some population-level effects if ecological recovery takes place within an acceptable time period. In the tiered effect assessment schemes, in principle, all tiers (1, 2 and 3) are able to address the ETO, while the model ecosystem approach (tier 3), under certain conditions, is able to also address the ERO. The GD provides the scientific background for the risk assessment to aquatic organisms in edge-of-field surface waters and is structured to give detailed guidance on all assessment steps. An executive summary joining all parts of the guidance and decision schemes in a concise way is provided and is intended to help applicants and regulatory authorities in day-to-day use.

KEY WORDS

pesticides, formulations, metabolites, ecotoxicology, aquatic organisms, specific protection goals, regulatory acceptable concentrations

[1] On request from EFSA, Question No EFSA-Q-2009-00001, adopted on 20 June 2013.

[2] Panel members: Alf Aagaard, Theo Brock, Ettore Capri, Sabine Duquesne, Metka Filipic, Antonio F. Hernandez-Jerez, Karen I. Hirsch-Ernst, Susanne Hougaard Bennekou, Michael Klein, Thomas Kuhl, Ryszard Laskowski, Matthias Liess, Alberto Mantovani, Colin Ockleford, Bernadette Ossendorp, Daniel Pickford, Robert Smith, Paulo Sousa, Ingvar Sundh, Aaldrik Tiktak, Ton Van Der Linden. Correspondence: pesticides.ppr@efsa.europa.eu.

[3] Acknowledgement: EFSA wishes to thank the members of the Working Group Aquatic Ecotoxicology: Alf Aagaard, Paulien Adriaanse, Jos Boesten, Theo Brock, Sabine Duquesne, Michael Klein, Matthias Liess, Robert Luttik, Paul Miller, Daniel Pickford, Aaldrik Tiktak, Jan Vanderborght, Lina Wendt-Rasch and EFSA staff: Stephanie Bopp, Maria Arena and Alessandra Caffi for the support provided to this scientific opinion.

* Editorial revisions were made to the suggested citation, the header as on the page 2 onwards and the table of contents.

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Minimum Detectable Difference (MDD)

- The MDD defines the difference between the means of a treatment and the control that must exist to detect a statistically significant effect
- The lower the MDD, the less severe treatment-related declines in population abundance between controls and treatments need to be, to calculate a NOEC/LOEC

$$MDD = (\bar{x}_0 - \bar{x}\ )^* = t_{1-\alpha,df,k}\sqrt{\frac{s_0^2}{n_0} + \frac{s^2}{n}}$$

$$MDD = (\bar{x}_0 - \bar{x})^* = t_{1-\alpha,df,k}\, s\sqrt{\frac{1}{n_0} + \frac{1}{n}}$$

$(\bar{x}_0 - \bar{x})^*$ = corresponding difference between control and treatment mean

$t_{1-\alpha}$ = quantile of the t-distribution

df = degrees of freedom

k = number of comparisons

s^2 = residual variance one-way ANOVA

n_0, n = sample sizes

MDD in micro-/mesocosm experiments

$$MDD\% = MDD / \bar{x}_0 * 100$$

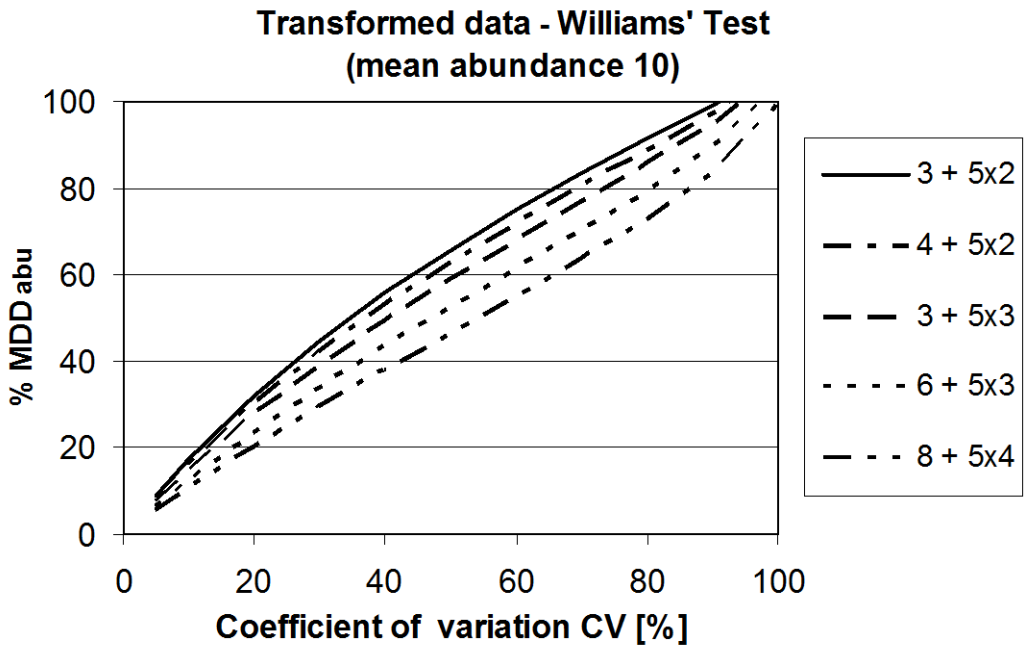
- The MDD usually is reported as a percentage of the control mean
- If in the statistical testing log-transformed abundance data are used, the MDD also relates to the transformed abundance data (= MDD_{ln} or $\%MDD_{ln}$)
- Since % effects on a log-scale are difficult to interpret we suggest to back-transform the MDD_{ln}, resulting in MDD_{abu} or $\%MDD_{abu}$





How to decrease the MDD

1. By selecting a higher error level α
   - No straightforward option; normal practise to select 0.05
2. Increasing the number of replicates of controls and treated systems
   - Increase from 2 to 4 treatment replicates will reduce the MDD_{abu} only by a maximum of 11% (at 60 % CV)



Currently five or more test concentrations and a control are recommended

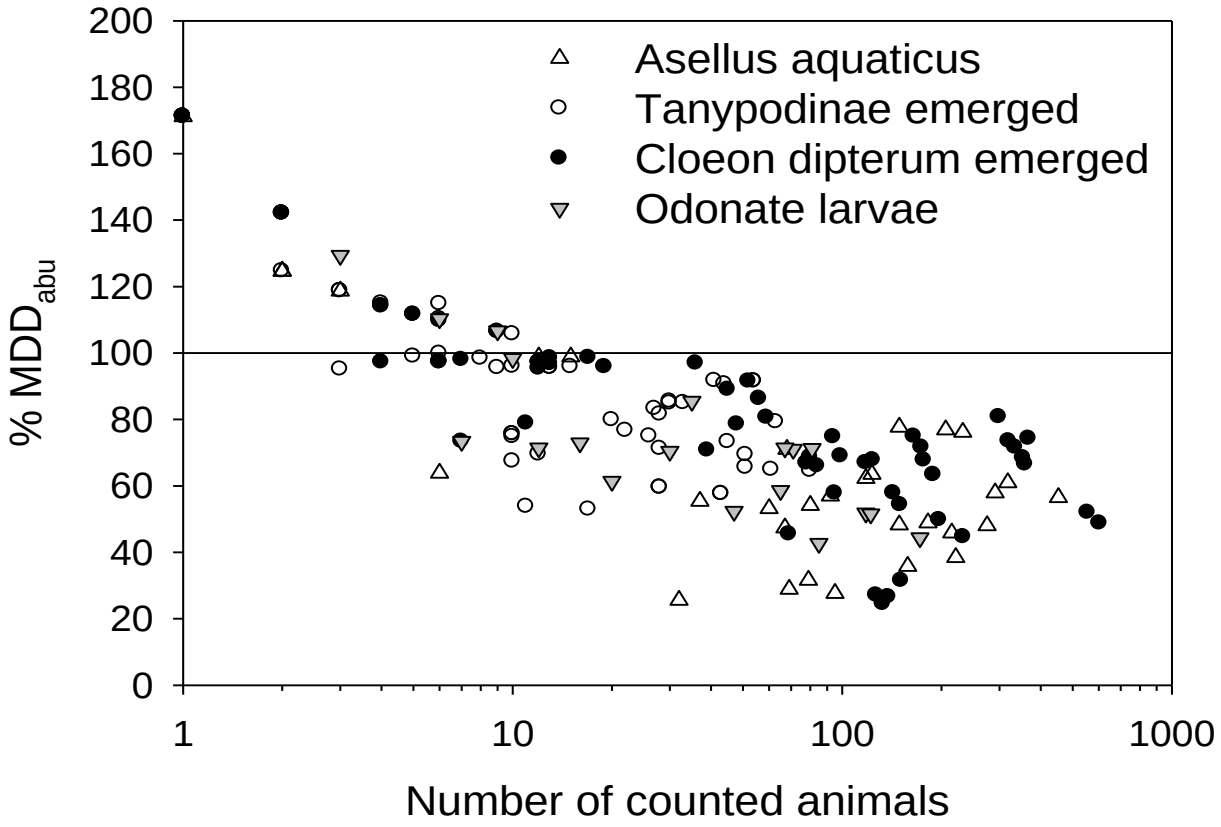
At least two replicates per treatment-level are required and 3 or 4 control replicates is common practise

For practical reasons the total number of test systems seldom exceeds 20 -30

How to decrease the MDD

3. Reducing the inherent variability between replicates
4. Reducing the variability caused by sampling methods

- Improving quantification methods may be very effective to decrease $\%MDD_{abu}$



How to report MDDs

Minimum Detectable Difference (MDD) should be reported in concert with NOEC/LOEC values

Geometric mean abundance of *Daphnia galeata*

day	Controls	2 µg/L	6 µg/L	18 µg/L	54 µg/L	162 µg/L	Williams	%MDD$_{abu}$
-5	94.3	93.3	88.8	139.3	86.2	108.5	- NOEC>=162 µg/L (incr.)	40.9
3	121.1	131.2	97.2	158.7	87.9	16.0	* NOEC=54 µg/L (decr.)	42.6
9	114.0	107.4	32.9	49.2	26.4	1.1	* NOEC=18 µg/L (decr.)	70.5
23	98.1	142.1	143.6	147.9	36.4	2.6	* NOEC=18 µg/L (decr.)	44.4
37	50.2	44.0	49.7	49.2	42.7	10.0	* NOEC=54 µg/L (decr.)	68.4
51	35.0	50.2	28.3	45.4	43.2	16.6	- NOEC>=162 µg/L (decr.)	57.6
65	35.0	87.9	29.2	32.9	108.5	18.6	- NOEC>=162 µg/L (decr.)	67.2
79	54.9	122.3	39.1	66.4	218.5	45.8	- NOEC>=162 µg/L (decr.)	82.9

The MDD$_{abu}$ needs to be below 100% to allow a statistical evaluation on treatment-related declines in abundance, and subsequent recovery.

The lower the MDD the larger is the power of the test.

How to report MDDs

Geometric mean abundance of *Stylaria lacustris*

day	Controls	2 µg/L	6 µg/L	18 µg/L	54 µg/L	162 µg/L	Williams	%MDD$_{abu}$
-5	7.9	5.0	13.8	15.3	6.1	6.2	- NOEC>=162 µg/L (incr.)	94.0
9	2.5	2.6	2.1	2.0	1.6	1.4	- NOEC>=162 µg/L (decr.)	107.1
23	5.3	4.5	5.3	6.0	3.8	2.4	* NOEC>=18 µg/L (decr.)	71.9
37	2.1	3.1	3.2	4.2	2.3	2.0	- NOEC>=162 µg/L (decr.)	104.9
51	0.5	1.0	1.5	2.2	0.5	0.0	- NOEC>=162 µg/L (decr.)	173.2
65	1.7	0.4	1.7	1.0	6.0	0.0	- NOEC>=162 µg/L (decr.)	114.8
79	0.8	1.0	1.5	0.4	5.2	1.4	- NOEC>=162 µg/L (incr.)	144.2

If the MDD is consistently larger than 100% then

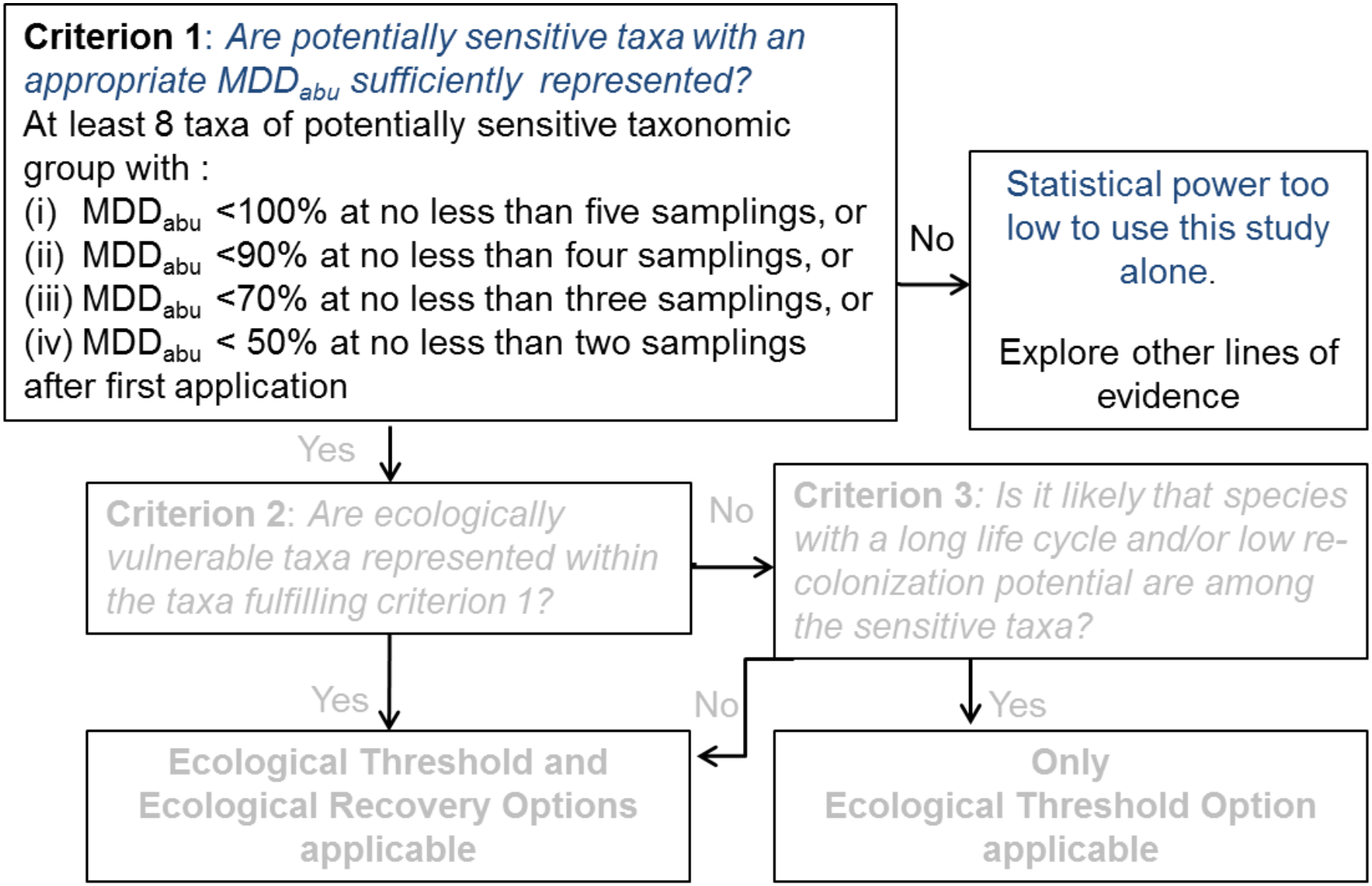
- the statistical power is too low to demonstrate treatment-related **declines**
- it will be difficult to draw firm conclusions on recovery if on isolated samplings a NOEC can be calculated

MDD classes as proposed by EFSA AGD

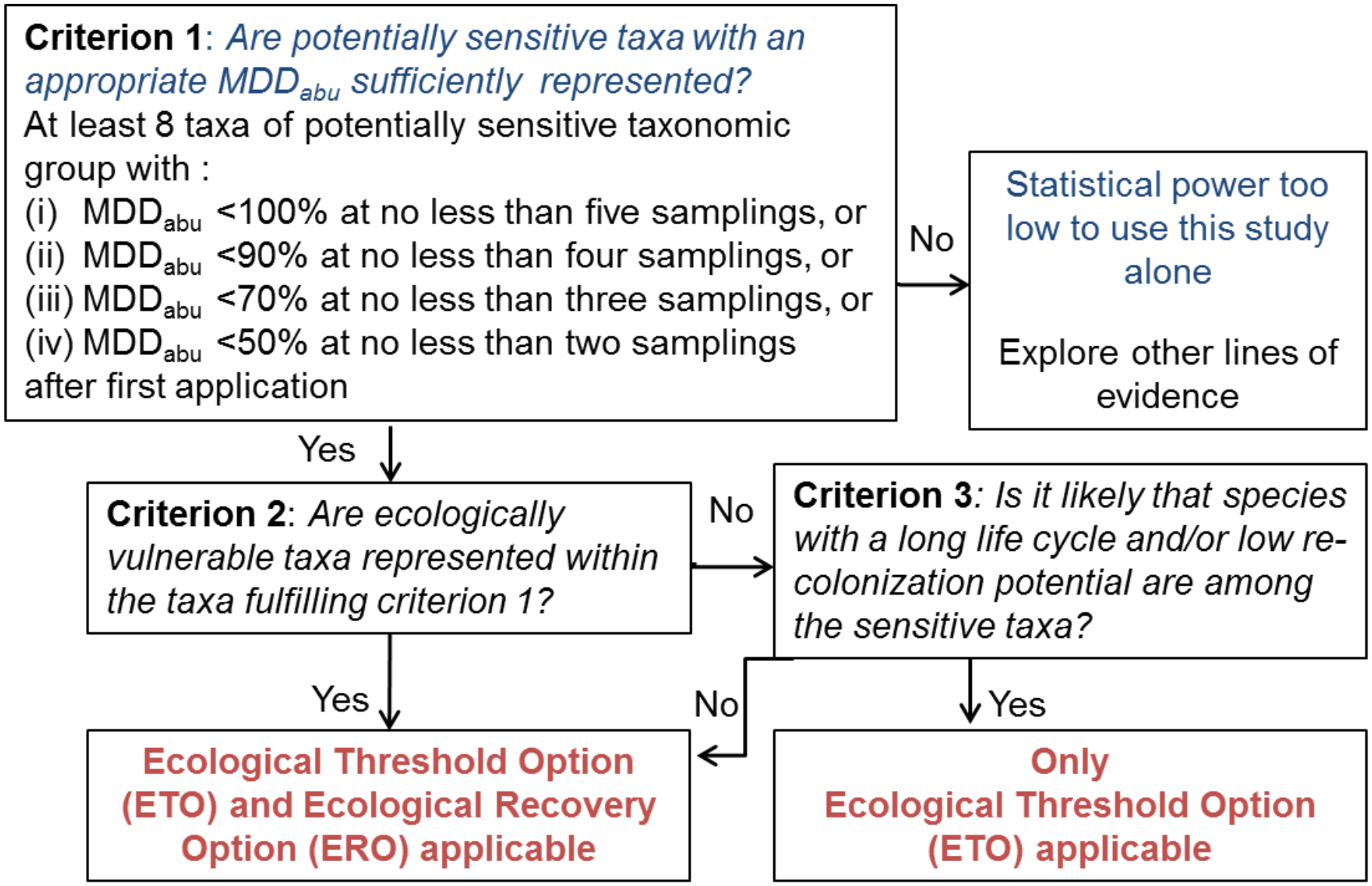
MDD Class	MDD%	Comment
0	>100%	No effects can be determined statistically
I	90-100%	Only large effects can be determined statistically
II	70-90 %	Large to medium effects can be determined statistically
III	50-70 %	Medium effects can be determined statistically
IV	< 50%	Small effects can be determined statistically

- We assume that the MDD as defined in the EFSA AGD refers to MDD_{abu}
- To demonstrate statistically significant reductions in abundance of taxa, the MDD_{abu} needs to be <100%
- To demonstrate statistically significant increases in abundance the MDD_{abu} may be smaller to larger than 100%

Regulatory reliability of a micro-/mesocosm study (a proposal)



Regulatory reliability of a micro-/mesocosm study (a proposal)



Effect classes to address the $\%MDD_{abu}$

Effect class 0: Treatment effects cannot be evaluated (overall high $\%MDD_{abu}$)

Effect class 1: No treatment-related effects demonstrated

Effect class 2: Slight effects (LOEC on individual sampling)

Effect class 3A: Pronounced short term effects (< 8 weeks)

Effect class 3B: Pronounced effects and recovery within 8 weeks post last application

Effect class 4A: Significant effect in short-term study so that recovery cannot be assessed

Effect class 4B: Significant effect and recovery cannot be assessed due to high $\%MDD_{abu}$ in recovery period

Effect class 5A: Pronounced long-term effects (> 8 weeks after last application) followed by recovery

Effect class 5B: Pronounced long-term effects no recovery

Decision scheme for RAC derivation

***For each taxon**:*

Is criterion 1 applicable ?

(i) MDD_{abu} < 100% on at least five samplings, or

(ii) MDD_{abu} < 90% on at least four samplings, or

(iii)MDD_{abu} < 70% on at least three samplings, or

(iv)MDD_{abu} < 50% on at least two samplings

or

Can on at least one sampling, a significant deviation relative to controls be calculated?

No → **Effect class 0**

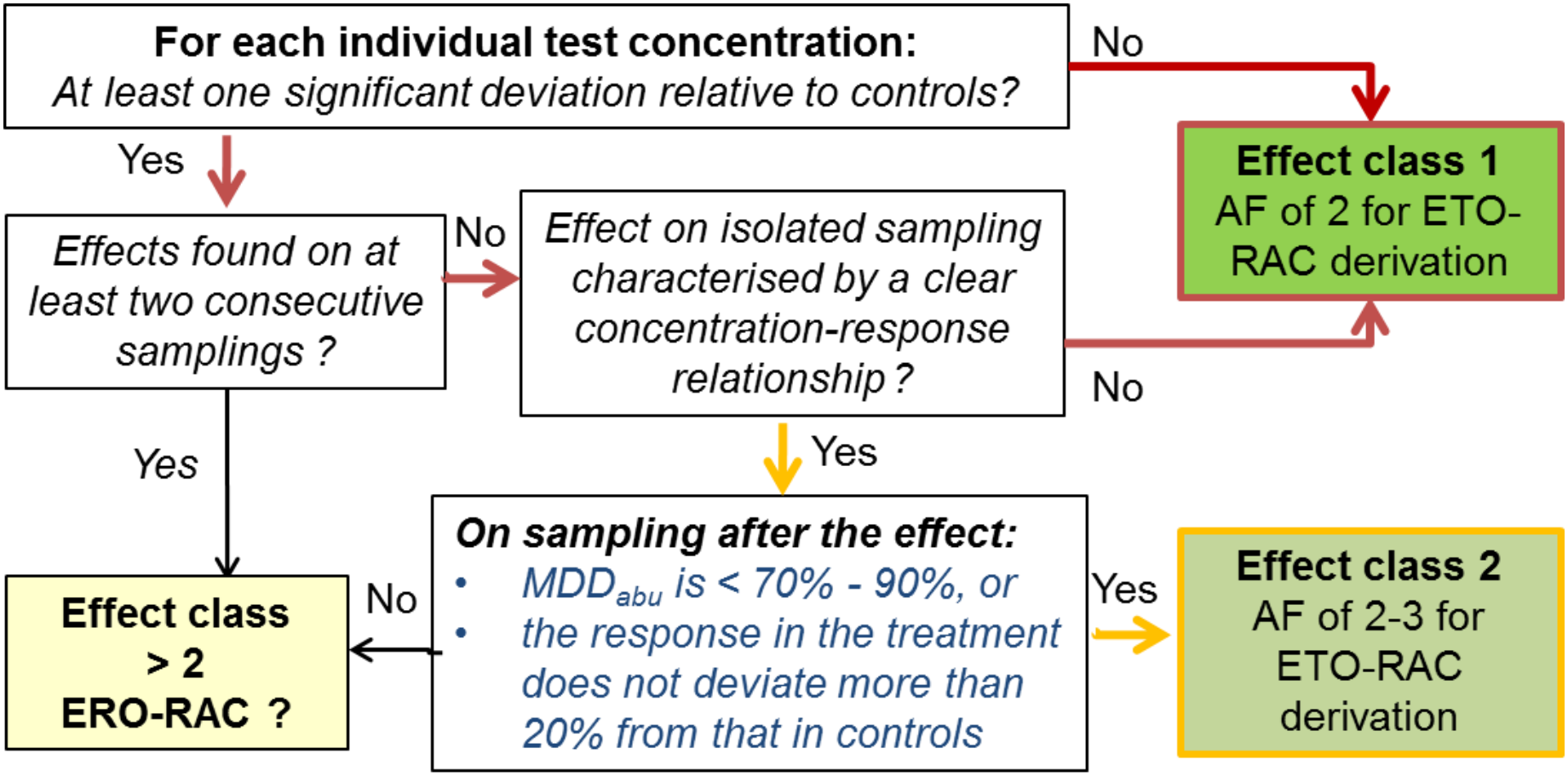
These taxa are excluded from the effect assessment

Yes ↓

Taxa that can be used in the effect assessment

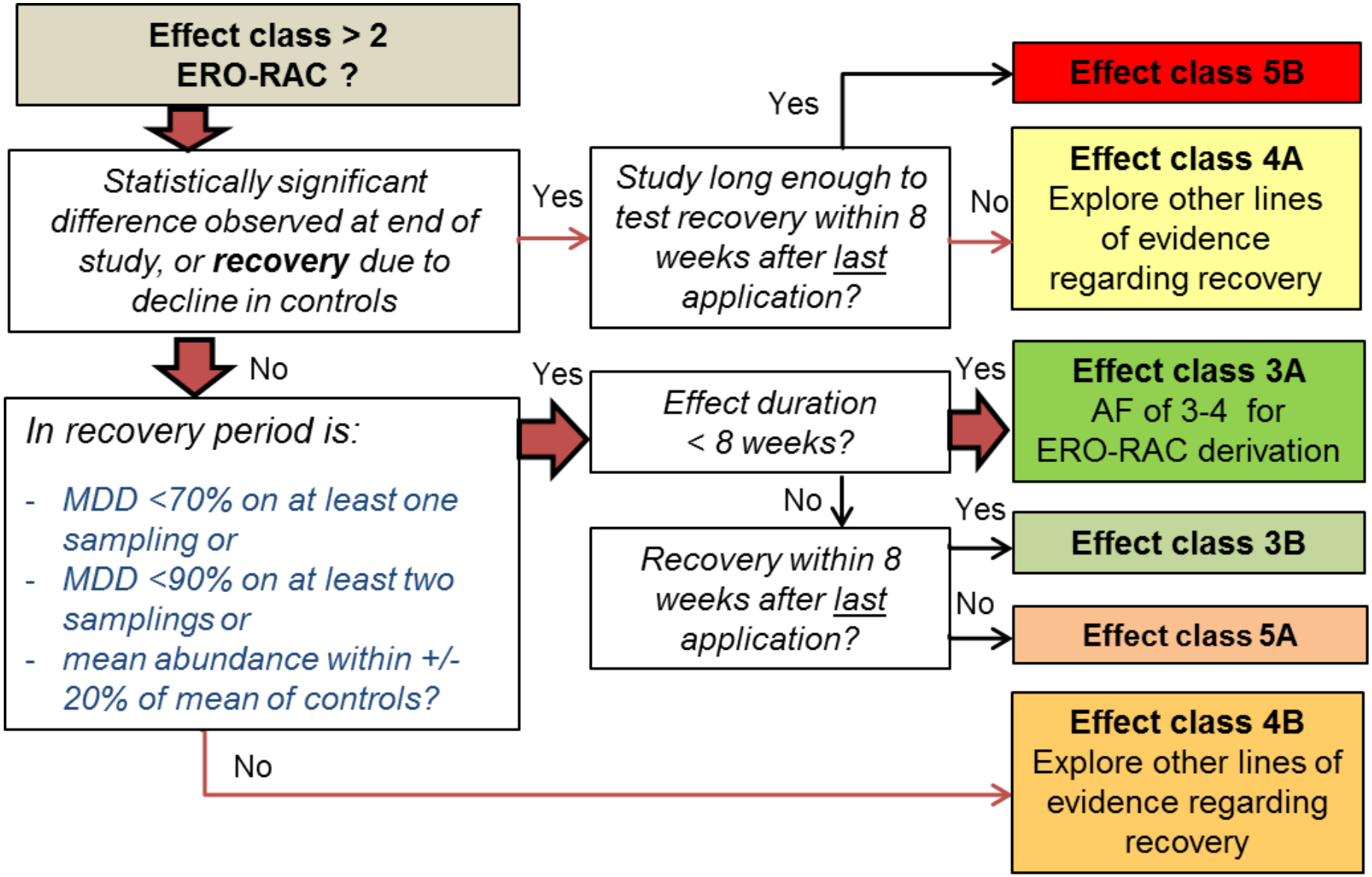
Decision scheme for ETO-RAC derivation

Taxa that can be used in the effect assessment



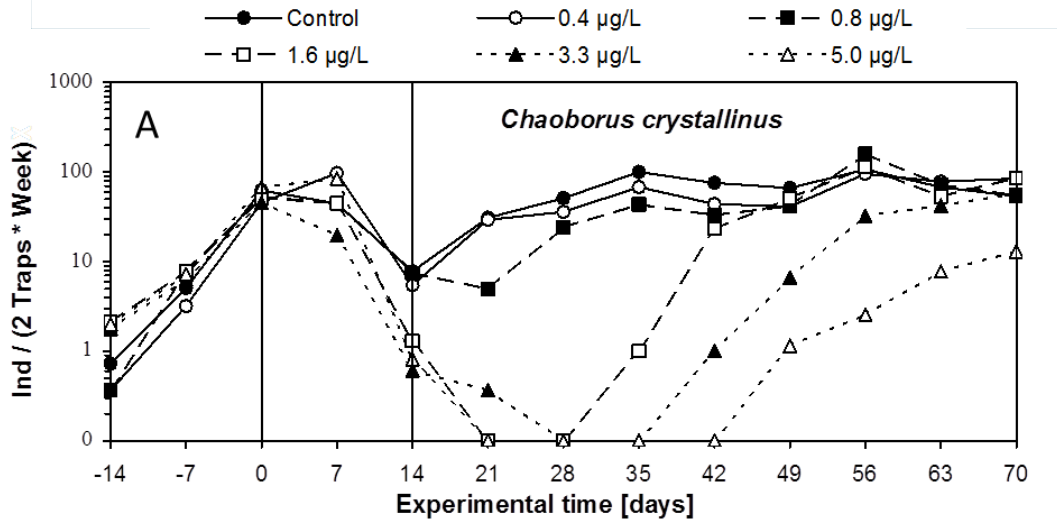
Decision scheme for ERO-RAC derivation

Taxa that can be used in the effect assessment



Examples for treatment-related declines

For Effect class derivation decision schemes can be used

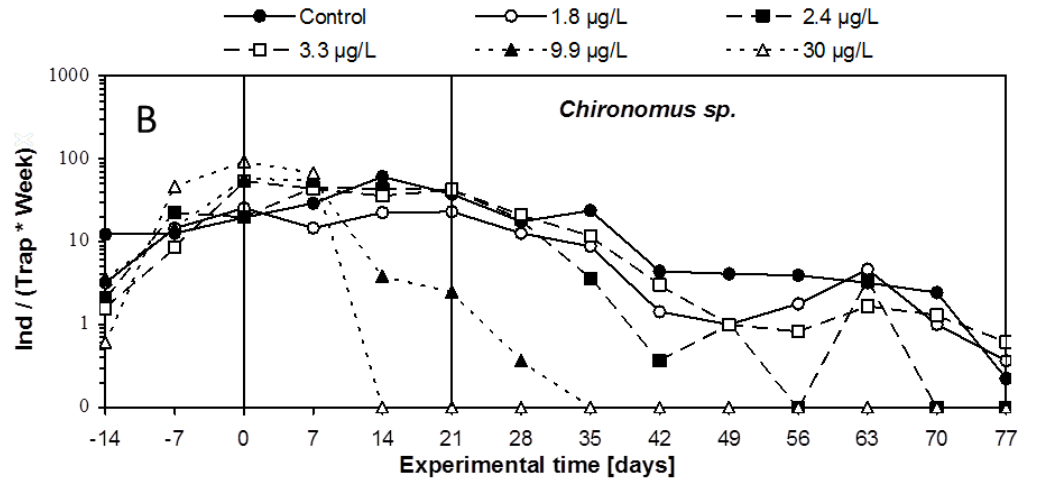


% MDD_{abu}	124	91	75	89	92	52	66	68	86	88	88	77	73
NOEC	(≥5.0)	≥5.0	≥5.0	≥5.0	≥5.0	0.4	0.8	0.8	1.6	1.6	3.3	3.3	3.3

Effect class 1 = 0.4 µg/L; Effect class 2 = 0.8 µg/L (ETO-RAC derivation)

Effect class 3A = 1.6 and 3.3 µg/L (ERO-RAC derivation)

Effect class 5B = 5.0 µg/L



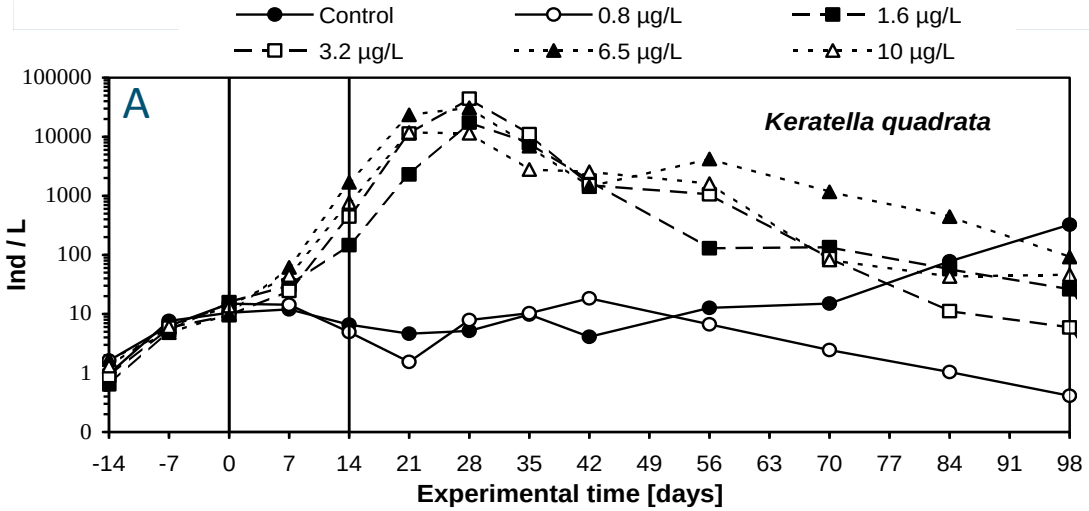
% MDD_{abu}	103	82	97	94	65	73	91	76	88	95	87	111	109	224
NOEC	(≥30)	≥30	≥30	≥30	3.3	3.3	3.3	3.3	3.3	3.3	1.8	(≥30)	(≥30)	(≥30)

Effect class 1 = 1.8 – 3.3 µg/L (ETO-RAC derivation)

Effect class 4B = 9.9 – 30 µg/L

Examples for treatment-related increases

Application of Effect classes requires more expert judgement



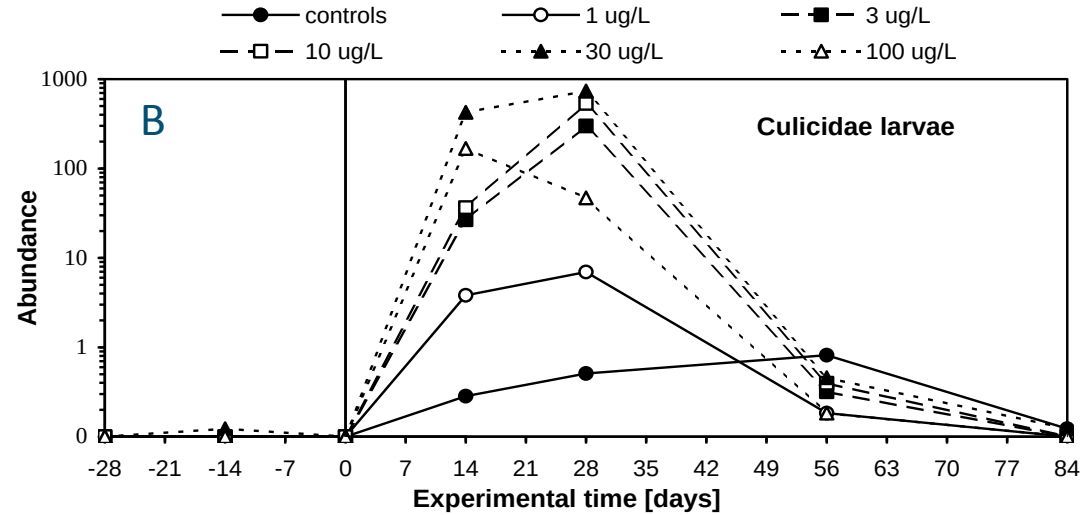
% MDD_{abu}	51	72	42	61	77	69	82	88	97	94	99	98	98
NOEC	≥10	≥10	≥10	3.2+	0.8+	0.8+	0.8+	0.8+	0.8+	1.6+	≥10	≥10	≥10

MDD_{abu} < 100%

Effect class 1 = 0.8 µg/L

Effect class 3A ↑ = 1.6 µg/L

Effect class 3B ↑ = 3.2 – 10 µg/L



% MDD_{abu}	-	n.c.	-	185	142	105	138
NOEC	-	(≥100)	-	3+	1+	(≥100)	(≥100)

MDD_{abu} > 100% or not calculable (n.c.)

Effect class 1 = 1.0 µg/L (ETO-RAC derivation)

Effect class 4A = 3 – 100 µg/L

Thank you for your attention
Questions ?

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