Introduction to the Tier 3 effect and risk assessment (model ecosystem approach) in the new Aquatic Guidance Document

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Model ecosystems (microcosms, mesocosms)

- Constructed artificially with samples from, or portions of, natural ecosystems
- Housed in artificial containers or enclosures
- Reduction in size and complexity when compared with natural ecosystems
- Include an assemblage of organisms representing several trophic levels (*primary producers, herbivores, carnivores, decomposers*)
- Community should be “adapted” to and in “equilibrium” with its ambient environment

Model ecosystems (microcosms, mesocosms)

Most experience with lentic freshwater ecosystems

Model ecosystems *versus* natural ecosystems

PROS

- Experimental control and replication (*exclusion confounding factors; statistical evaluation*)
- Their flexibility make them useful to study the fate and effects of different types of PPPs and exposure regimes
- Less ethical to perform tests in natural ecosystems

CONS

- No perfect analogs of edge-of-field ecosystems (*e.g. lack of larger predators; wall effects*)
- A microcosm/mesocosm test systems represent one type of edge-of-field community only

Cause-and-effect relationships in micro/mesocosms

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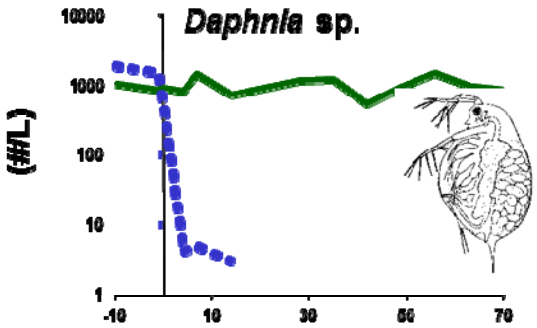
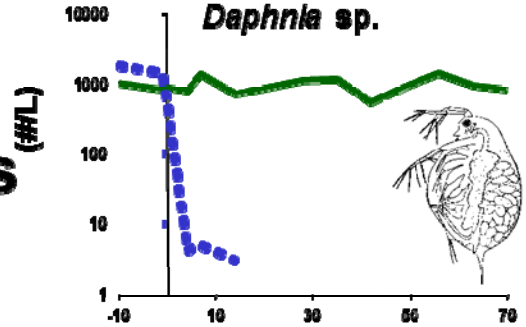
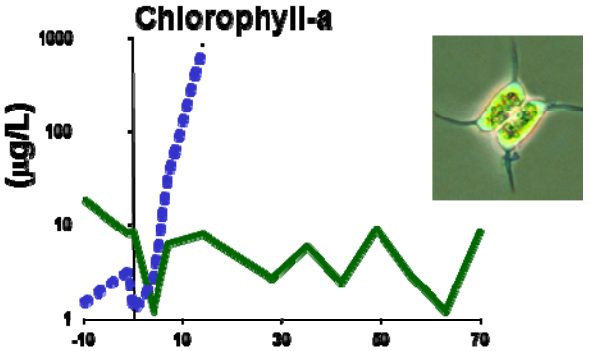
Sensitivity



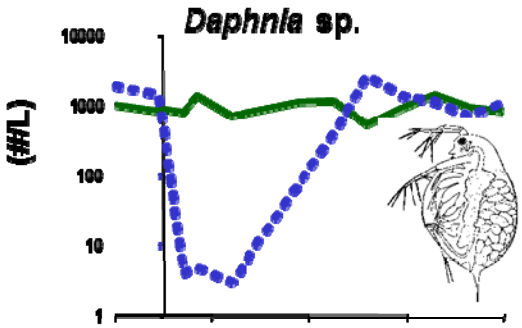
Control
Exposed

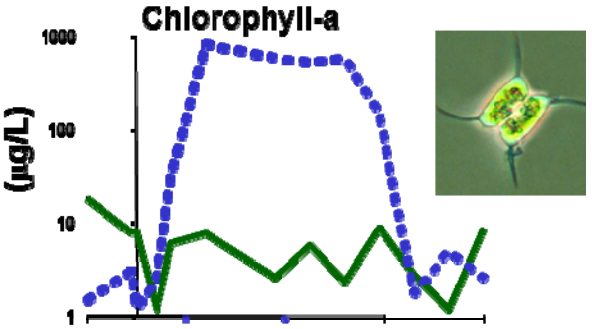
Ecosystem interactions





Recovery





Days post application

Cause-and-effect relations in micro/mesocosms

Direct or primary effects

- Toxicological effects on abundance/biomass of potentially sensitive populations (*link to protection goals*)
- Laboratory toxicity tests facilitate the interpretation (*link to lower tiers*)

Indirect or secondary effect

- Ecosystem/community changes that result from a reduction of (toxicant) susceptible populations (*link to food web ecology*)

Delayed effects and biological recovery

- Long-term observations to monitor possible chronic effects (including latency) effects and recovery of affected populations (*link to protection goals*)

Community level responses

- Functional endpoints
- Multivariate or trait-based approaches

Herbicide example (Brock et al 2004)

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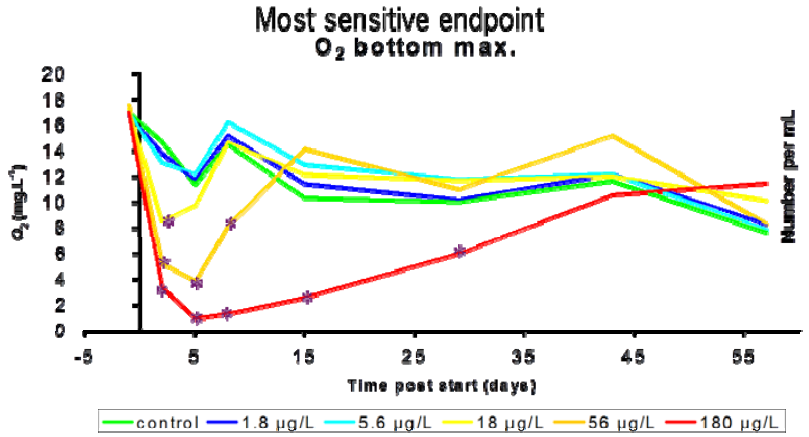
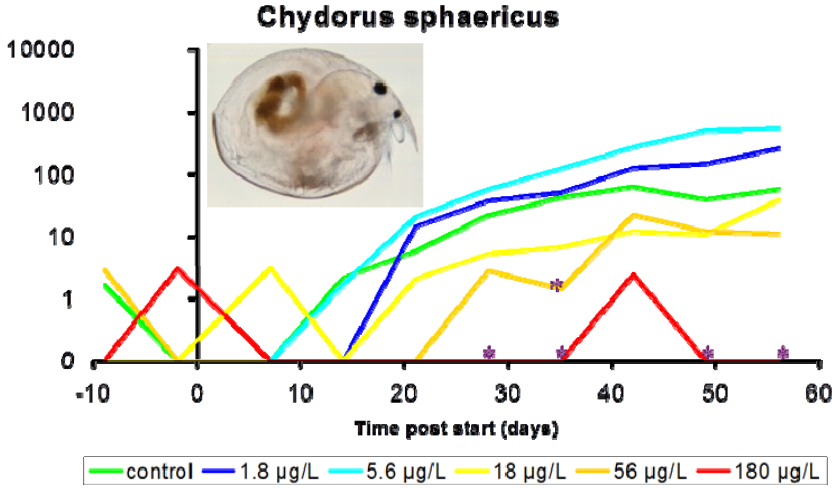
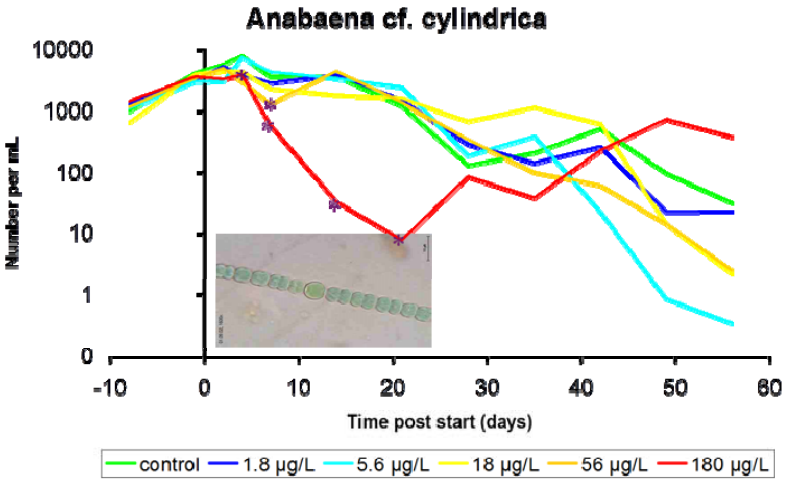
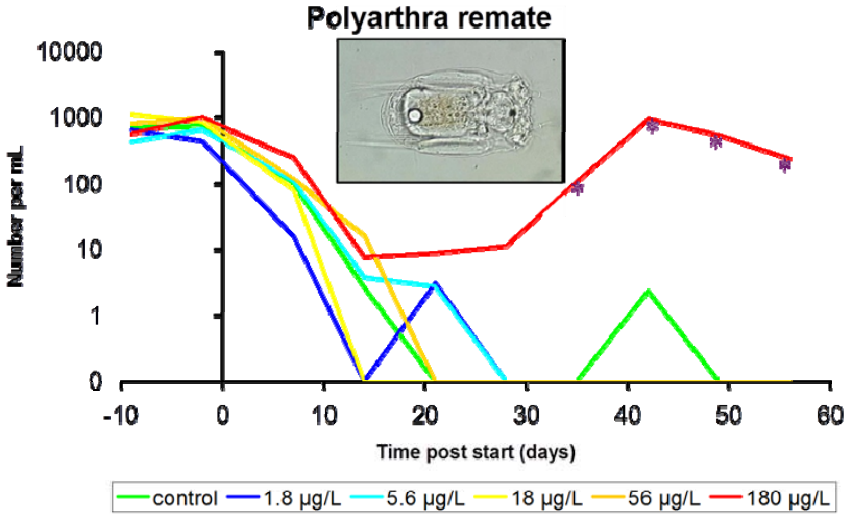
Direct effects



Indirect effects







Functional endpoint more sensitive and indirect effects more persistent

Designing mico-/mesocosm experiments

1. Establishment of a representative aquatic community
2. The appropriate exposure regime to simulate
3. Exposure regime and replicate test systems per treatment
4. Selection of measurement endpoints
5. Statistical and ecological evaluation

Guidance on the conduct and interpretation of micro-/mesocom tests: ***SETAC*** (Arnold et al 1991; Hill et al. 1993; Campbell et al. 1999; Giddings et al 2002), ***OECD*** (2006) and ***RIVM*** (De Jong et al. 2008)

Aquatic community in mesocosms (new requirements)

Ecological Threshold Option

- ≥ 8 different populations of sensitive taxonomic group(s) present, for which statistical evaluation is possible (*MDD*)

Identification of sensitive taxonomic group(s):

- Lower Tier information
- Read-across information for substances with a similar MoA
  - Insecticides (*insects; crustaceans*)
  - Herbicides (*algae; macrophytes*)
  - Fungicides (*often wide array of taxa*)

Aquatic community in mesocosms (new requirements)

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Ecological Recovery Option

- Populations of sensitive taxonomic group(s) for which statistical evaluation is possible also comprise **vulnerable taxa**

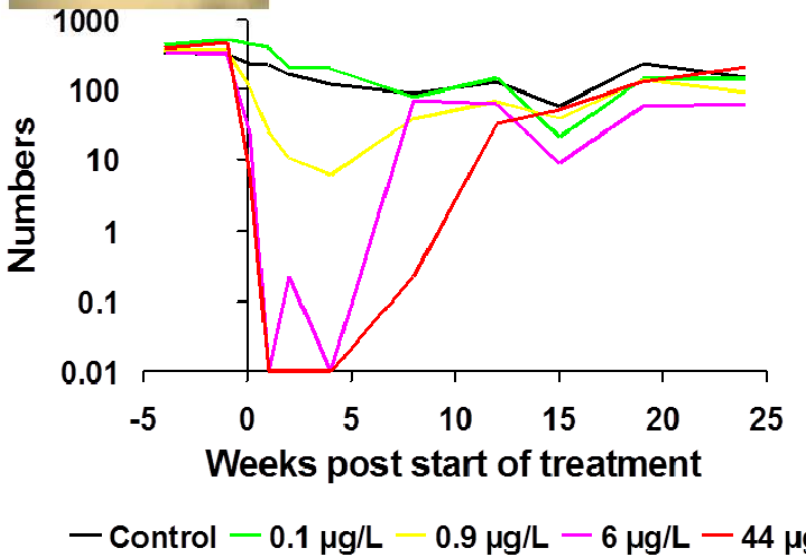
Identification of vulnerable taxa

- Requires ecological scenarios for edge-of-field surface waters
  - Length life cycle (*e.g. univoltine insects*)
  - Low dispersal ability (*e.g. macro-crustaceans in isolated experimental ponds*)
  - Slow growth rate (*e.g. certain rooted submerged macrophytes*)

Chlorpyrifos exp.: sensitivity and recovery (Van den Brink et al. 1996)

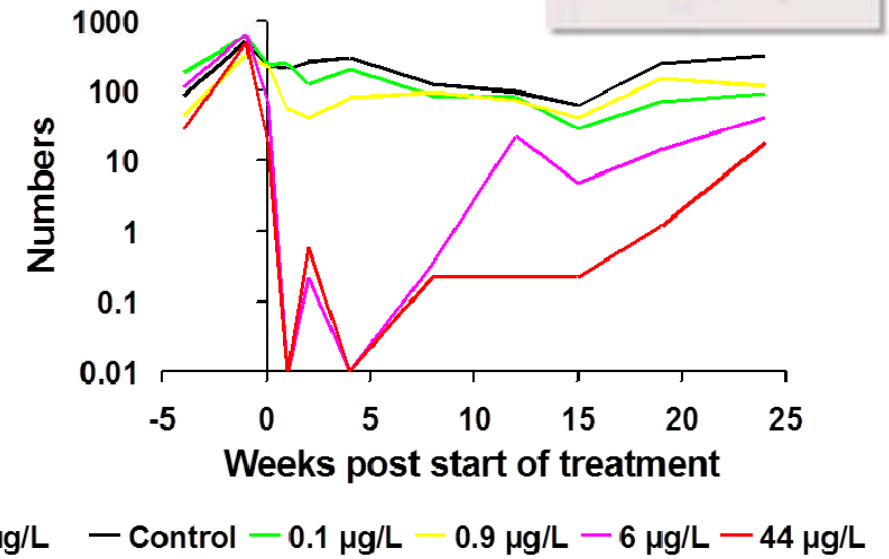
Two mayfly species

Cloeon dipterum
NOEC = 0.1 µg/L



Relatively fast recovery
- appr. 3 generations/year
- egg deposition by terrestrial adults

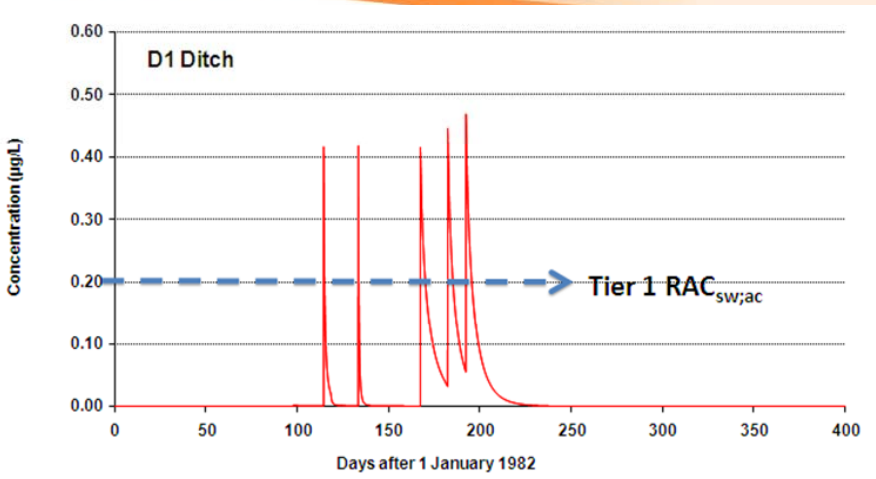
Caenis horaria
NOEC = 0.1 µg/L



Relatively slow recovery
- 1 to 2 generations/year
- egg deposition by terrestrial adults

Rate of recovery is affected by number of generations per year (Appendix D of AGD provides life cycle information)

Selecting the appropriate exposure regime (lessons from ELINK)



- The exposure regime simulated in test system should be realistic to worst-case relative to predicted exposure profile (*e.g. FOCUS sw*)
- A repeated pulse exposure needs to be tested if the predicted pulses in the field exposure profile are considered to be toxicologically dependent
- Minimum number of pulses to be tested may be informed by the duration of the chronic test of the Tier-1 species that triggered the risk (*time to onset of effects*)
- Exposure-response experimental design with preferably five or more concentrations (*to derive ETO & ERO RAC*)

Statistical evaluation (new requirements)

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%
-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 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.

Statistical evaluation (new requirements)

Minimum Detectable Difference (MDD)

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%
-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% the statistical power is too low to derive firm conclusions on concentration-response relationships, including recovery

MDD in new GD

Proposal on classes of minimal detectable differences (MDD) due to treatment-related declines in abundance/biomass

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

New Effect class 0 in AGD

Evaluation of different measurement endpoints

Effect class 0: Treatment effects cannot be evaluated

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 4: Pronounced effect in short-term study

Effect class 5A: Pronounced long-term effects followed by recovery

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

Overall assessment on basis of most sensitive endpoints

RAC derivation from micro-/mesocosms

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

Motivation of AFs (Appendix E)

Chronic exposure to carbendazim

Exposure regime	Effect class 1	Effect class 2	Effect class 3	Effect class 4	Type of test system	Reference; Country
Chronic	3.3 µg/L	-	-	33.0 µg/L	Indoor microcosms	Cuppen et al. 2000; Van den Brink et al. 2000; NL
Chronic	2.6 µg/L	-	-	26.4 µg/L	Outdoor microcosms	Daam et al. 2009a Thailand
Chronic	2.2 µg/L	-	-	20.7 µg/L	Outdoor mesocosms	Slijkerman et al. 2004, NL

Effect class 1 concentrations of most sensitive endpoint fairly similar between different micro-/mesocosms studies

Motivation of AFs (Appendix E)

Short-term exposure to chlorpyrifos

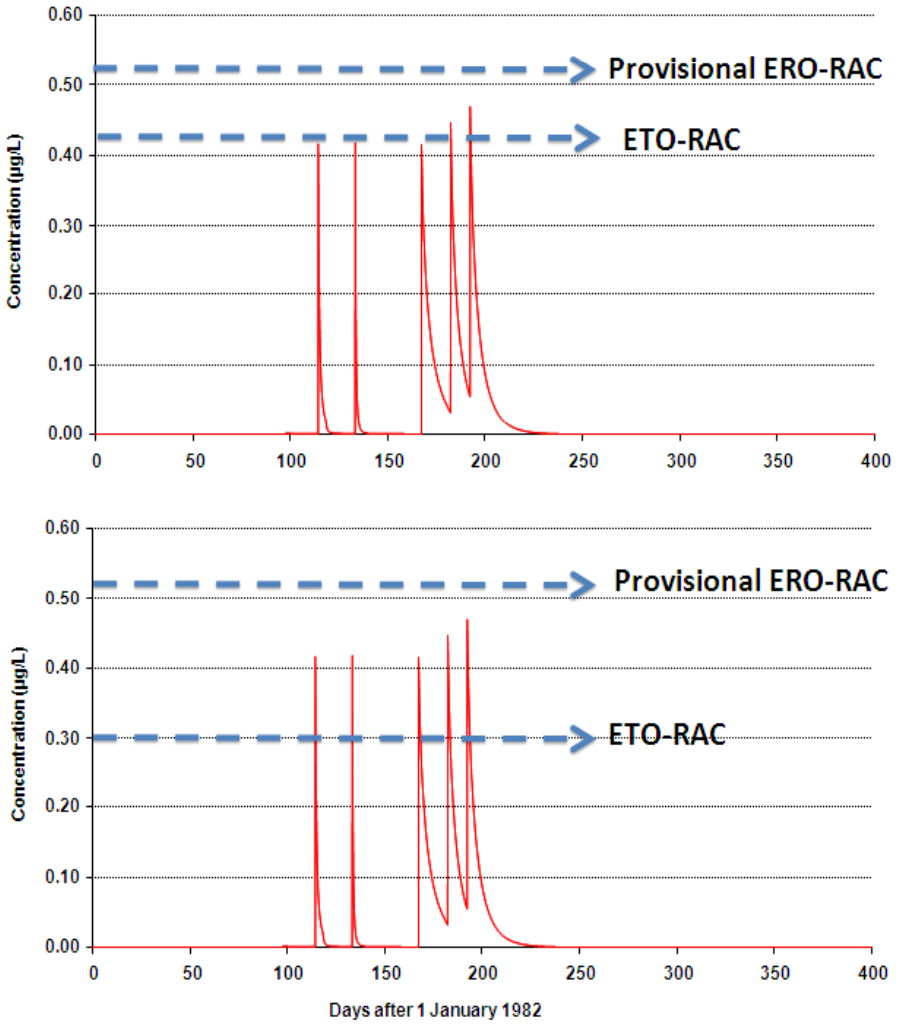
Application regime	Class 1 NOEC	Class 2 LOEC	Class 3A LOEC	Type of test system	Location Reference
6 h pulse	0.1 µg/L	-	(5.0 µg/L)	Experimental streams	Australia Pusey et al. 1994
Single	0.1 µg/L	0.3 µg/L	1.0 µg/L	Outdoor microcosms	USA, Kansas Bieveret al. 1994
Single	-	0.1 µg/L	<0.9 µg/L	Experimental ditches	Netherlands Van den Brink et al. 1996
Single	0.1 µg/L	--	1.0 µg/L	Lab microcosms Cool,Mesotr	Netherlands Van Wijngaarden et al. 2005
Single	0.1 µg/L	-	≤1.0 µg/L	Lab microcosms Warm, Eutrophic	Netherlands Van Wijngaarden et al. 2005
Single	0.1 µg/L	-	1.0 µg/L	Outdoor mesocosms	Spain López-Mancisidoet al. 2005
Single	0.1 µg/L	-	1.0 µg/L	Outdoor microcosms	Thailand Daamet al. 2008
Single	-	-	0.5 µg/L	Pond enclosures	USA, Minnesota Siefertet al. 1989

Threshold levels for effects can be extrapolated with lower uncertainty than responses caused by higher exposures

ERO-RAC derivation

If study addressed lower number of pulse exposures than present in field exposure profile

- Plot ETO-RAC and provisional ERO-RAC on field exposure profile
- Consider exposure period above ETO-RAC (ecological threshold)
- Assess the total effect period that might be expected for the taxa at risk
- If effect period is acceptable, upgrade the provisional ERO-RAC to an official ERO-RAC



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Thank you !