Practical 2 (Aquatic ERA): Perform a higher tier risk assessment for the insecticide Phantasithrin on basis of the predicted environmental exposure concentrations (PECs) and results of a mesocosm test

Evaluation criteria of mesocosm experiments

Minimum Detectable Difference (MDD)

The power of a mesocosm test is the probability of finding treatment-related differences that do exist, as opposed to the likelihood of declaring treatment-related effects that do not exist (which is known as a Type I error or "false positive"). An indicator of the statistical power of a mesocosm test can be estimated *a posteriori*: viz. the minimum detectable difference (MDD). The MDD defines the mean amount of difference between a treatment and the control that must exist in order to conclude that there is a significant effect. This means that the lower the MDD, the less severe a difference needs to be to result in a significant effect.

Note that MDD% can be calculated on the basis of the Ln transformed abundance data and on the basis of the absolute abundance data. The MDD% based on the Ln transformed data are by definition lower than those based on the absolute data. Although the NOEC calculations for invertebrate populations usually are based on the Ln transformed abundance data, the MDD% values used in the evaluation are based on absolute abundance data.

In the EFSA Aquatic Guidance Document MDD values are ranked as follows (EFSA 2013):

Table 2.1. *Classes of MDD values with associate effect detection for treatment-related declines in abundance/biomass (EFSA PPR, 2013)*

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

If for a specific taxon on a specific sampling day the MDD is <100%, in theory a treatment-related decline in abundance can be demonstrated. If the MDD is ≥100%, however, the power of the test is too low to demonstrate treatment-related declines in abundance. Note, however, that in some cases of treatment-related increases a statistically significant effect may be demonstrated if the MDD is ≥100%, particularly when the abundance in control test systems is very low.

Three categories of organisms on the basis of their MDD can be distinguished, namely:

1. Category 1 taxa: characterized by sufficient statistical power to demonstrate treatment-related declines in abundance. The MDD criterion as specified in Figure 2.1 is proposed by Brock et al. (2015). This category can be be used to evaluate the validity of the mesocosm study, with respect to the requirement that at least eight taxa of the potentially sensitive taxonomic groups should be present with a high enough MDD.
2. Category 2 taxa: these do not meet the MDD criterion mentioned above but for these taxa a NOEC could be calculated on at least one sampling. The NOECs calculated for both category 1 and category 2 taxa can be used to derive effect classes for RAC derivation.
3. Category 3 taxa: do not meet the MDD criterion mentioned above and no NOEC could be calculated on any of the samplings. These taxa cannot be used to derive effect classes for RAC derivation

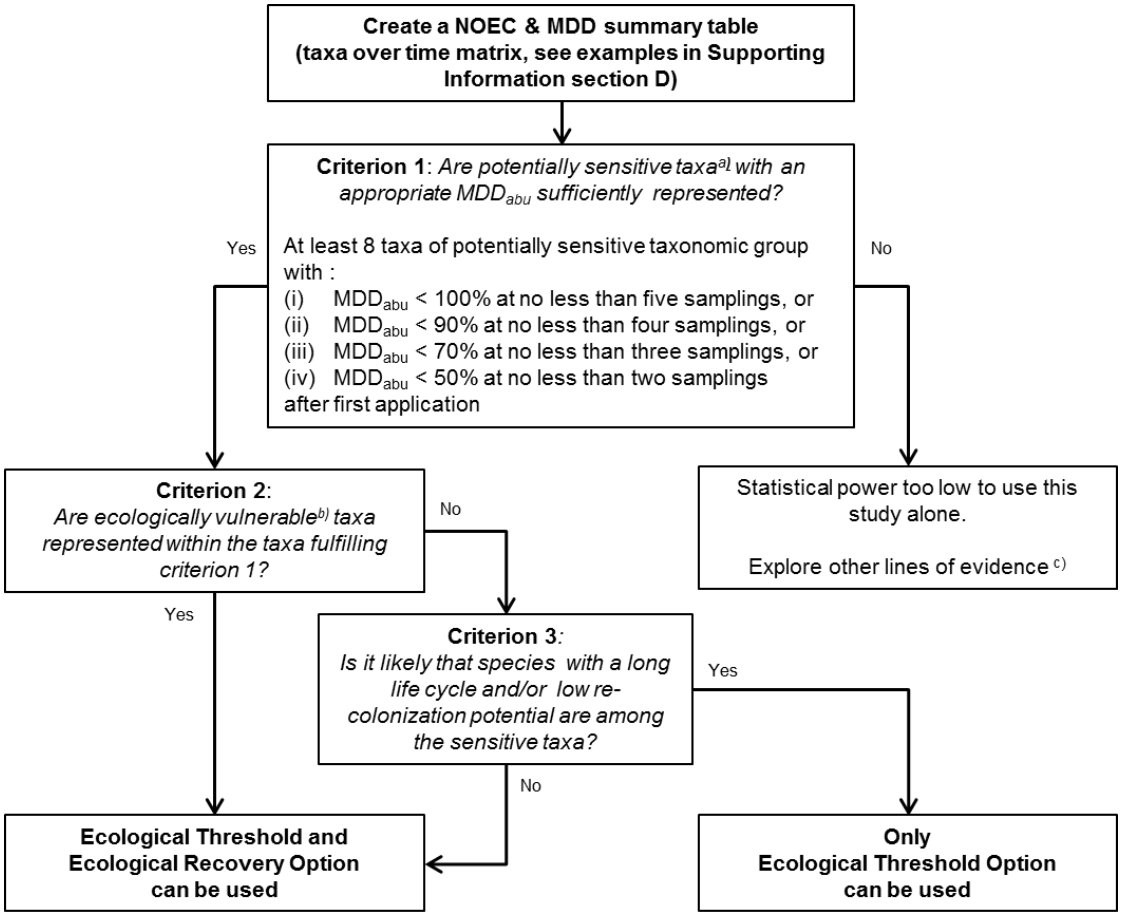

Figure 2.1*: Decision scheme 1 to assess the reliability of a mesocosm study to derive regulatory acceptable concentrations (RACs) on the basis of treatment-related effects of pesticide exposure (Brock et al., 2015).* [a)] *= Informed by e.g. available single species and semi-field tests and other read-across information.* [b)] *= Ecologically vulnerable due to potential intrinsic sensitivity to the test item, likelihood of exposure, long life-cycle (e.g. bi-, uni- or semivoltine) and/or low immigration potential.* [c)] *= For example, focued population-level and micro-/mesocosm studies addressing additional sensitive species or population modelling.*

Interpretation of biological and statistical significance

NOEC (No Observed Effect Concentration) estimations at taxon level ($p \leq 0.05$) and for each sampling day can be carried out using an appropriate univariate test (e.g. the Williams test; Williams, 1972).

The following criteria can beused to consider an observed effect as treatment-related:

- Statistically significant effects were demonstrated;
- Abundance values on the sampling day of an isolated NOEC were not too low (i.e. <3 individuals for macroinvertebrates and <10 individuals/L for zooplankton);
- A clear concentration-response relationships was observed on the date of the isolated NOEC;
- The response was not yet present in the pre-treatment period.

Items two and three are applied to exclude possible Type I errors. A Type I error occurs when the null hypothesis (no significant difference between controls and treatments) is rejected when it is true (i.e. the statistical results show a significant difference even though there is no treatment-related significant difference). It is anticipated that these Type I errors may be more prominent if isolated NOEC values coincide with low abundance values in test systems and/or non-linear concentration-response relationships.

In the EFSA aquatic guidance document it is stated that in the test systems at least eight populations of the potential sensitive group with an appropriate MDD should be present. For pyrethroid insecticides populations of aquatic arthropods (crustaceans and insects) can be considered the sensitive taxonomic group.

To evaluate the validity of the microcosm experiments the MDD classes as described in Table 2.1 and the MDD criterion as given in Figure 2.1 can be used.

Where statistically significant differences between treatments and controls are observed, and these are considered to be treatment-related and biologically significant, the responses can be categorized into Effect Classes as described in Figure 2.2 and adapted from EFSA (2013) to also address the MDD information when evaluating recovery. A more detailed description of the effect classes is given in Table 2.2 (see Brock *et al.*, 2015).

Note that the effect class concentrations 1 to 5B can only be used for endpoints with a sufficient MDD. It is important to realize that effect classes 4A and 4B indicate that insufficient data is available in the post-effect period to conclusively indicate recovery. Class 4A indicates that the study was too short, whereas class 4B indicates insufficient quality of the MDD's to reliably assess recovery. Hence, when the decision scheme indicates class 4A or 4B, it is important to additionally indicate what effect class (2 for demonstrated effects lasting only during a single sample date, or 3A for demonstrated effects lasting during at least 2 consecutive samplings) is substantiated by the available effects data.

Hence, any occurrence of effect class 4A or 4B in the analysis will be indicated as effect class (2 – 4A/4B) when transient effects during a single sampling were found, or as effect class (3A – 4A/4B) when effects on at least 2 consecutive samplings were observed (3A for effects lasting less than 8 weeks, possibly 3B when effects lasted longer than 8 weeks but recovery occurred within 8 weeks after the last application of the test substance).

Table 2.2. *Effect classes and their criteria.*

Effect class	**Description**	**Criteria**
0	Treatment-related effects cannot be evaluated statistically	Effect class 0 is used for all category 3 taxa
1	No treatment-related effects demonstrated ($NOEC_{population}$)	No (statistically and/or ecologically significant) effects observed as a result of the treatment. Observed differences between treatment and controls show no clear causal relationship.
2	Slight effects	Statistically significant effects concern short-term and/or quantitatively restricted responses usually observed at individual samplings only.
3A	Pronounced effects; total period of effects < 8 weeks followed by recovery	Clear response of sensitive endpoints, but full recovery of affected endpoints within 8 weeks after the first application or, in case of delayed responses and repeated applications, the duration of the effect period is less than 8 weeks and followed by full recovery. Treatment-related effects demonstrated on consecutive samplings. Note that recovery from treatment-related declines in abundance can only be considered if the MDD_{abu} values during the relevant recovery period were at least smaller than 100%, or, as indicated in Decision scheme 2, the % deviation from controls is less than 20%. If this is not the case an Effect class 4B has to be selected.

Effect class	Description	Criteria
3B	Pronounced effects that last longer than 8 weeks but recovery observed within 8 weeks post last application	Clear response of the endpoint in the micro-/mesocosm experiment repeatedly treated with the test substance and that lasts longer than 8 weeks (responses may already start in treatment period), but full recovery of affected endpoints within 8 weeks post last application. Note that recovery from treatment-related declines in abundance can only be considered if the MDD_{abu} values during the relevant recovery period were, as indicated in Decision scheme 2, at least smaller than 90%, or the % deviation from controls is less than 20%. If this is not the case an Effect class 4B has to be selected.
4A	Pronounced effect in short-term study	Clear effects (e.g. large reductions in densities of sensitive species) observed, but the study is too short to demonstrate complete recovery within 8 weeks after the (last) application.
4B	Pronounced short-term effects demonstrated but recovery cannot be properly evaluated	Clear effects (e.g. large reductions in densities of sensitive species) observed, statistically significant differences from controls last less than 8 weeks but recovery cannot be evaluated e.g. due to MDD_{abu} values > 100% or decline or absence also in controls in recovery period after a treatment-related decline.
5A	Pronounced long-term effect followed by recovery	Clear response of sensitive endpoint, effect period longer than 8 weeks and recovery did not yet occur within 8 weeks after the last application, but full recovery is demonstrated to occur in the year of application. Note that recovery after a treatment-related decline can only be considered if the MDD_{abu} values during the relevant recovery period were, as indicated in Decision scheme 2, at least smaller than 90%, or the % deviation from controls is less than 20%. If this is not the case an Effect class 5B has to be selected.
5B	Pronounced long-term effects without recovery	Clear response of sensitive endpoints (> 8 weeks post last application) and full recovery cannot be demonstrated before termination of the experiment or before the start of the winter period.

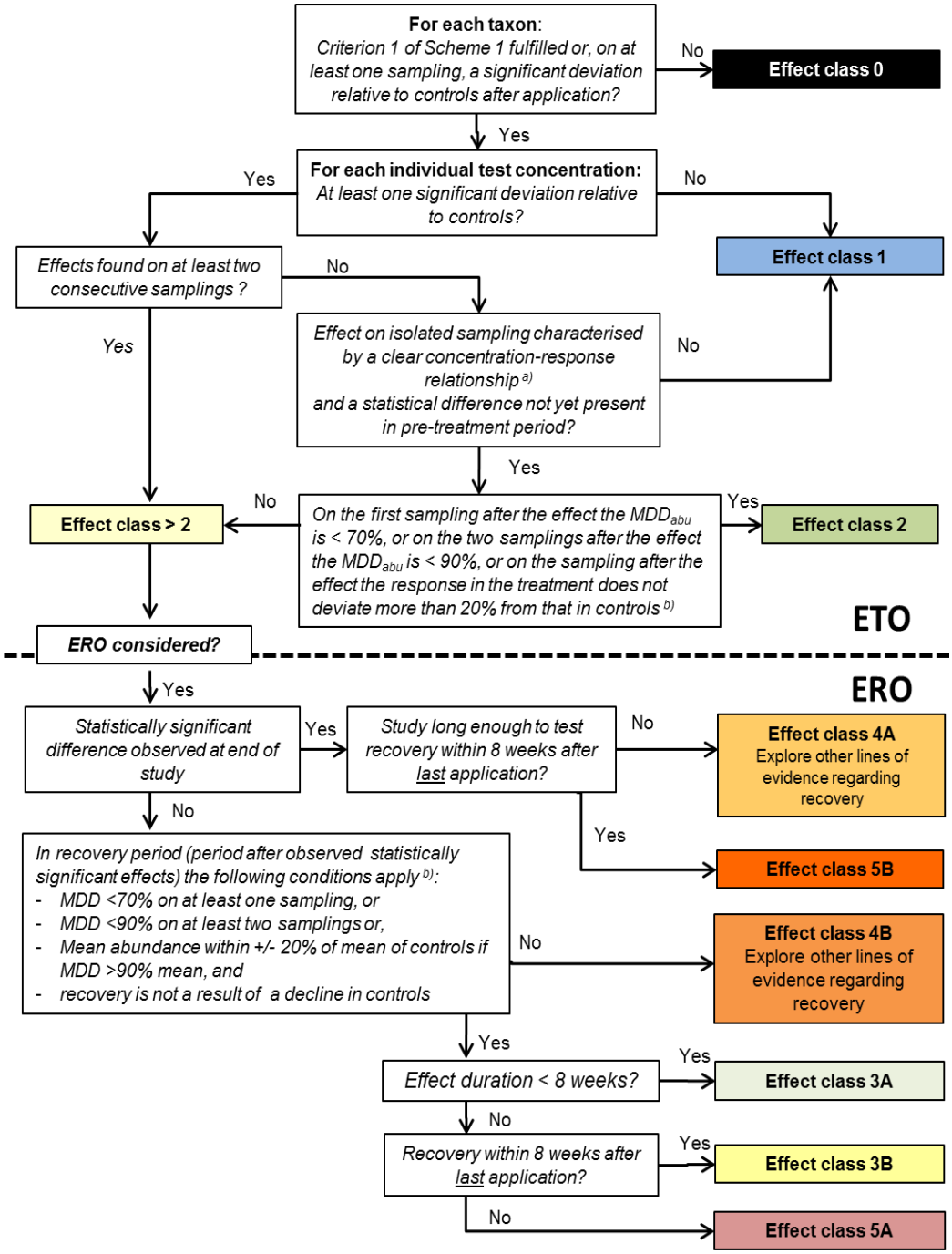

***Figure 2.2**: Decision scheme 2 for the derivation of Effect classes for treatment-related effects (focus on treatment-related declines) on population abundance from results of micro-/mesocosm studies (Brock et al., 2015). The MDD_{abu} values mentioned in the decision scheme are not applicable to indirect effects in the form of increases in population abundance if the NOECs of these treatment-related increases are associated with MDD_{abu} values >100% or if no MDD_{abu} can be calculated due to the absence of the taxon in control test systems (n.c.). a) = A clear concentration-response relationship for direct effects is characterised by a monotonous treatment-related decrease in abundance while in addition the statistical difference coincides with a high enough mean abundance of the taxon in controls. When selecting a certain minimum abundance for a taxon in controls the argumentation for this should be provided. If the significant effect is observed in the application period the next sampling should occur within a week. b) = If the high $\%MDD_{abu}$ in the post-effect period can be explained ecologically (e.g. emergence of insects) and a justification is given that this phenomenon will also occur under realistic field conditions, some flexibility of the MDD criterion is recommended.*

Mesocosm study with phatasithrin

In spring of the year 2004 a GLP study on the fate and ecological effects of the pyrethroid insecticide Phatasithrin in mesotrophic mesocosms was performed. In this study the formulated product KILLER (100 g Phantasithrin per L and as a capsule suspension) was used. The test systems used consisted of ditch enclosures (water depth 0.5 m; diameter enclosure 2 m) pressed into the sediment (15 cm deep). In total 19 (5 treatment levels in triplicate and 4 controls) enclosures were used. No fish were present in the enclosures. The study focussed on responses of invertebrates in zooplankton and macrofauna, but phytoplankton was studies as well. Phantasithrin was applied three times at one-week intervals and nominal treatment concentrations used to assess effects were 0, 25, 50, 100, 200 and 400 ng/L. The rate of dissipation (DT50) of Phantasithrin in the water column of the test systems was 1.2 days. Measured concentrations in water of the microcosms I hour post each application were 85 - 98% (average 90%) of the nominal concentration.

In Table 2.3 below, arthropod invertebrate taxa (the sensitive taxonomic group for pyrethroids) sampled in the test systems of the phantasithrin mesocosm experiment are presented for which at least on one sampling date the MDD was lower than 100%. Their lowest NOEC and their geometric mean number of individuals throughout the experiment in control test systems (Ncontrol) and in all test systems (Ntotal) are presented as well. MDDs are presented for each taxon and sampling day. If the lowest NOEC value is placed between brackets it concerns an isolated NOEC that is considered less reliable (n.c. = not calculated since taxon is not present in controls; - = taxa not sampled in any test system)

Table 2.3: *Invertebrate taxa samples in the mesocosm study and their MDD values*

Name	**Lowest NOEC (ng a.s./L)**	**Mean number of individuals (sample or L)**		**Minimum Detectable Difference (%)**									
				Day									
		$N_{control}$	N_{total}	**-11**	**3**	**11**	**17**	**24**	**32**	**39**	**46**	**54**	**61**
Arthropod taxa													
Anisoptera (Odonata)	(50↓)*	0.46	0.36	195	112	151	157	87	83	98	232	272	130
Asellus aquaticus (Isopoda)	200↓	34.41	15.19	38	59	61	44	62	54	49	56	38	57
Bosmina longirostris (Cladocera)	(100↓)*	0.49	0.4	136	98	101	182	198	208	116	197	111	147
Caenis horaria (Ephemeroptera)	100↓	5.46	2.17	104	n.c.	132	n.c.	287	64	77	84	76	76
Ceratopogonidae (Diptera)	-	1.37	0.93	95	74	179	126	162	114	100	75	81	89
Chaoborus sp. (Diptera)	25↓	66.89	15.25	65	45	65	54	57	41	41	71	34	52
Chironomini (Diptera)	200↑	50.42	41.73	50	57	78	48	51	40	54	53	39	44
Cloeon dipterum (Ephemeroptera)	100↓ (10↓)*	22.06	6.36	69	84	118	71	57	46	63	71	52	41
Cyclopoida (Copepoda)	50↓	6.37	3.93	95	74	179	126	162	114	100	75	81	89
Daphnia galeata (Cladocera)	200↓	57.56	48.77	82	58	67	44	52	43	72	54	56	49
Dytiscidae (Coleoptera)	(100↓)*	1.7	0.73	101	167	101	69	99	63	103	91	130	92
Gammarus pulex (Amphipoda)	50↓	28.31	18.4	36	41	68	58	59	66	54	46	46	63
Nauplii (Copepoda)	50↓	1152	987	86	69	67	51	74	81	104	144	160	236
Notonecta sp. (Hemiptera)	(25↓)*	0.49	0.4	136	98	101	182	198	208	116	197	111	147
Orthocladiinae sp. (Diptera)	(400↑)*	0.5	0.51	77	136	116	392	148	118	104	130	n.c.	167
Trianodes bicolor (Trichoptera)	(50↓)*	0.17	0.14	n.c.	259	139	148	105	120	n.c.	-	n.c.	n.c.

Question: Considering the information presented in Table 2.3 and Figure 2.1, how many category taxa are present in the mesocosm test systems?

Can this mesocosm study be used as a higher tier test to evaluate aquatic risks of phantasithrin?

Two most sensitive insect populations in the phantasithrin mesocosm study

In Tables 3 and 4 below, for each sampling day the geometric mean abundance values for larvae of the phantom midge *Chaoborus obscuripes* and the ephemeropteran *Cloeon dipterum* are presented for controls and all treatment levels, as well as the corresponding NOEC values (Williams test) and the $\%MDD_{abu}$ values (Minimum Detectable Differences). For controls also the minimum and maximum abundance values are given. The sampling day is expressed in terms of day after first application of phantasithrin to the mesocosms.

***Table 2.4**: Chaoborus obscuripes*

day	Min	Max	Controls	10 ng a.s./L	25 ng a.s./L	50 ng a.s./L	100 ng a.s./L	200 ng a.s./L	400 ng a.s./L	Williams	$\%MDD_{abu}$
-11	7.97	44.96	26.80	22.54	18.74	23.96	28.78	40.63	10.82	- NOEC(Wi)>=400 µg/L (decr.)	65
3	70.09	101.69	88.34	70.78	58.76	55.98	45.88	6.94	0.54	* NOEC(Wi)=100 µg/L (decr.)	45
11	50.25	125.57	79.89	75.22	65.98	19.52	16.06	2.98	0.73	* NOEC(Wi)=25 µg/L (decr.)	65
17	55.03	82.34	64.66	57.94	58.49	53.90	19.93	2.84	0.46	* NOEC(Wi)= 50 µg/L (decr.)	54
24	62.11	89.23	74.45	69.32	59.65	56.72	16.06	1.86	0.22	* NOEC(Wi)= 50 µg/L (decr.)	57
32	56.72	89.23	72.24	71.98	69.54	16.06	4.85	0.00	0.22	* NOEC(Wi)= 25 µg/L (decr.)	41
39	32.84	99.67	58.46	88.62	97.68	83.71	18.06	5.29	2.62	* NOEC(Wi)=50 µg/L (decr.)	41
46	30.90	119.42	69.39	74.54	64.66	55.03	39.32	3.19	4.39	* NOEC(Wi)=100 µg/L (decr.)	71
54	47.77	129.41	82.34	105.78	136.07	93.84	44.55	33.86	21.63	* NOEC(Wi)=100 µg/L (decr.)	34
61	57.87	115.88	82.34	125.58	132.04	109.10	77.87	51.89	19.52	* NOEC(Wi)=200 µg/L (decr.)	52
Effect class											

***Table 2.5**: Cloeon dipterum*

day	Min	Max	Controls	10 ng a.s./L	25 ng a.s./L	50 ng a.s./L	100 ng a.s./L	200 ng a.s./L	400 ng a.s./L	Williams	$\%MDD_{abu}$
-11	2.00	9.95	5.18	4.72	4.20	9.25	7.97	5.18	7.09	- NOEC(Wi)>=400 µg/L (decr.)	69
3	1.00	3.01	1.88	2.20	0.45	0.45	1.26	0.45	0.87	* NOEC(Wi)=10 µg/L (decr.)	84
11	0.00	4.01	0.49	0.03	1.03	0.22	0.22	0.00	0.22	- NOEC(Wi)>=400 µg/L (decr.)	118
17	17.98	68.69	27.91	26.63	13.19	18.28	2.13	0.00	0.00	* NOEC(Wi)=100 µg/L (decr.)	71
24	39.02	65.98	46.35	29.07	29.07	60.87	41.64	0.61	0.00	* NOEC(Wi)=200 µg/L (decr.)	57
32	47.77	123.08	89.23	71.63	71.63	81.86	7.89	0.54	0.00	* NOEC(Wi)= 100 µg/L (decr.)	46
39	31.86	108.01	57.87	105.86	105.86	22.08	14.04	1.28	0.36	* NOEC(Wi)=100 µg/L (decr.)	63
46	31.86	169.68	78.30	41.47	41.47	46.82	19.52	1.03	0.73	* NOEC(Wi)=100 µg/L (decr.)	71
54	35.98	145.97	54.47	101.69	101.69	32.84	49.24	15.73	24.55	* NOEC(Wi)=200 µg/L (decr.)	52
61	37.09	95.74	60.87	49.74	49.74	58.46	74.45	29.67	26.80	* NOEC(Wi)=200 µg/L (decr.)	41
Effect class											

Question: Based on the information presented in the tables 2.4 and 2.5 above please provide the corresponding Effect class values (see Table 2.2 and Fig. 2.2) for the two insect taxa and import the lowest values into Table 2.6 (category insects).

Deriving the Regulatory Acceptable Concentration from the phantasithrin mesocosm study

Effects on macroinvertebrates, zooplankton, phytoplankton, macrophytes and community metabolism were recorded and evaluated using univariate and multivariate statistical techniques.

Table 2.6 presents a summary of responses observed in the mesocosms. Insects (mainly *Chaoborus obscuripes*) comprised the most sensitive populations, but all affected insects showed recovery in the 50 and 100 ng/L treatments. The sensitive insect and crustacean taxa present in the mesocosms were bi-voltine or multi-voltine species. Of the macrocrustaceans it was *Gammarus pulex* that was the most sensitive population (no recovery in the highest treatments).

Table 2.6*: Summary of treatment-related effects in the mesocosms treated with the insecticide Phantasithrin (after Joke et al. 2005). The numbers in the table refer to the effect classes described above. The treatment-levels are expressed as nominal concentrations. ↓ = decrease , ↑ = increase. Within each category (e.g. Insects) the response of the most sensitive measurement endpoint was selected*

	25 ng/L	50 ng/L	100 ng/L	200 ng/L	400 ng/L
Population responses					
Macrocrustaceans	1	1↓	3A↓	5B↓	5B↓
Insects	?	?	?	?	?
Other macroinvertebrates	1	1	1	2↑	2↑
Microcrustaceans	1	1	2↓	3A↓	3A↓
Rotifers	1	1	2↑	3A↑	3A↑
Phytoplankton Chl-a	1	1	1	1	2↑
Macrophyte biomass	1	1	1	1	1
Community responses					
PRC macroinvertebrates	1	2	3A	3A	3A
PRC zooplankton	1	1	2	3A	3A
Community metabolism	1	1	1	1	1
Overall response	?	?	?	?	?

Question: Based on the model ecosystem approach and the results of the mesocosm data presented above, what is the Regulatory Acceptable Concentration (also considering the data for Phantasithrin presented in practical 1)