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Acute tier-1 and tier-2 effect assessment approaches in the EFSA Aquatic Guidance Document: are they sufficiently protective for insecticides?

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Abstract

BACKGROUND: The objective of this paper is to evaluate whether the acute tier-1 and tier-2 methods as proposed by the Aquatic Guidance Document recently published by the European Food Safety Authority (EFSA) are appropriate for deriving regulatory acceptable concentrations (RACs) for insecticides. The tier-1 and tier-2 RACs were compared with RACs based on threshold concentrations from micro/mesocosm studies (ETO-RAC). A lower-tier RAC was considered as sufficiently protective, if less than the corresponding ETO-RAC.

RESULTS: ETO-RACs were calculated for repeated ($n = 13$) and/or single pulsed applications ($n = 17$) of 26 insecticides to micro/mesocosms, giving a maximum of 30 insecticide × application combinations (i.e. cases) for comparison. Acute tier-1 RACs (for 24 insecticides) were lower than the corresponding ETO-RACs in 27 out of 29 cases, while tier-2 Geom-RACs (for 23 insecticides) were lower in 24 out of 26 cases. The tier-2 SSD-RAC (for 21 insecticides) using HC_5/3 was lower than the ETO-RAC in 23 out of 27 cases, whereas the tier-2 SSD-RAC using HC_5/6 was protective in 25 out of 27 cases.

CONCLUSION: The tier-1 and tier-2 approaches proposed by EFSA for acute effect assessment are sufficiently protective for the majority of insecticides evaluated. Further evaluation may be needed for insecticides with more novel chemistries (neonicotinoids, biopesticides) and compounds that show delayed effects (insect growth regulators).
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Supporting information may be found in the online version of this article.

Keywords: effect assessment; insecticides; aquatic invertebrates; tiered approach; micro/mesocosms; evaluation

1 INTRODUCTION

Tiered approaches are the basis of environmental risk assessment schemes that support the registration of pesticides in Europe.[1] A tier is defined as a complete exposure or effect assessment resulting in a predicted environmental concentration (PEC) or a regulatory acceptable concentration (RAC). The principle of tiered approaches is to start with a simple conservative assessment and to do additional more complex work if necessary.[2] In the aquatic effect assessment for pesticides as described by EFSA,[1] tier 1 is based on the results of laboratory toxicity tests conducted with a limited number of standard test species and the application of an assessment factor (AF). Tier 2 also includes results of laboratory toxicity tests with additional test species, allowing the geometric mean (geomean) approach or the species sensitivity distribution (SSD) approach.[3–5] A third tier-2 option mentioned by EFSA[1] is the refined exposure test, which is not considered in the present paper because of limited availability of information for most compounds. Tier 3 comprises micro/mesocosm studies; for guidance on their conduct and interpretation, see, for example, Giddings *et al.*,[6] the OECD guidance document[7] and De Jong *et al.*[8] In addition, EFSA indicates that experimental higher-tier studies may be supplemented with food-web and/or population models, although guidance on the use of modelling approaches still needs to be developed by EFSA.[1,9–11]

In pesticide risk assessment under Regulation (EC) No. 1107/2009,[12] the basic data requirements for the tier-1 effect assessment are strictly defined.[13] The Aquatic Guidance Document[1] describes the procedures for RAC derivation on the basis of tier-1 (standard test species), tier-2 (geomean and SSD) and tier-3 (micro/mesocosms) approaches (Table 1). The adequacy of tier-1 and tier-2 RACs can be evaluated by comparing these with safe threshold concentrations set for aquatic environments as derived from aquatic micro/mesocosms as the highest experimental tier

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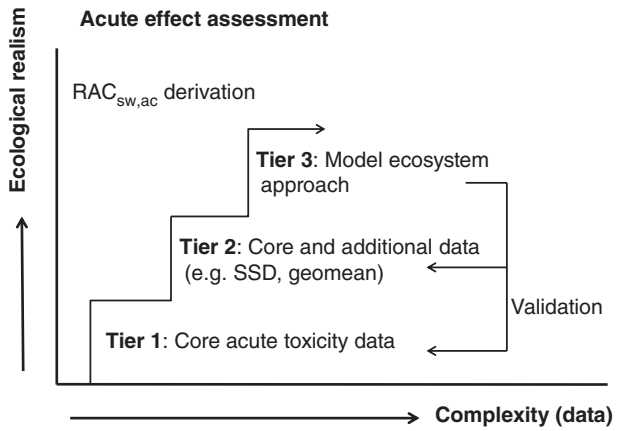

Figure 1. Schematic presentation of the validation of experimental lower-tier approaches with results of micro/mesocosms (redrafted after Solomon *et al.*[11]). RAC$_{sw,ac}$ = Regulatory Acceptable Concentration for surface water within the context of the acute effect assessment scheme; SSD = species sensitivity distribution approach; geomean = geometric mean approach.

available (Fig. 1). Field data (i.e. biomonitoring data) do not usually allow observed effects to be linked to a single active substance and thus are not appropriate for this purpose. In the aquatic effect assessment, micro/mesocosm studies are considered to be representative models to estimate safe threshold concentrations for edge-of-field ecosystems because they include a relatively high number of interacting species representing different trophic levels.[1,14–20] We therefore used estimates of ETO-RACs (RACs based on the ecological threshold option for the most sensitive measurement endpoint in micro/mesocosms) to validate the proposed tier-1 and tier-2 acute effect assessment procedures.

The present paper, which focuses on insecticides, is a follow-up to studies in which the tier-1 acute effect assessment and the tier-2 SSD approach were compared with results of micro/mesocosm tests.[15,20] In these studies, however, derivation of the threshold concentration for effects in micro/mesocosms was slightly different from what is finally proposed in the new Aquatic Guidance Document.[1] Furthermore, data have become available for several insecticides with a novel toxic mode of action (e.g. neonicotinoids, insect growth regulators) that were not considered in Maltby *et al.*[15] In the present paper we therefore re-evaluate the validation of the acute tier-1 and tier-2 SSD approach to comply with the EFSA Aquatic Guidance Document.

For tier 2, the proposal in the new Aquatic Guidance Document (EFSA)[1] is to apply the SSD approach if toxicity data are available for eight or more species of the sensitive taxonomic groups, and to apply the geomean approach when data are available for fewer than eight species (Table 1). In the case of insecticides, which are the subject of the present paper, aquatic arthropods can be considered to be the most sensitive taxonomic group.[1,15] To our knowledge, no publications exist that validate the geomean approach with results of micro/mesocosm studies. In the present paper we therefore also evaluate the tier-2 geomean approach.

2 MATERIALS AND METHODS

Single-species acute toxicity data and micro/mesocosm data were collected from existing toxicity databases [ECOTOX (www.epa.gov/ecotox/); Footprint (www.eu-footprint.org/ppdb.html); AGROTOX (www.agrotox.anses.fr)], open 'grey' literature including EU draft assessment reports or DARs (www//dar.efsa.europa.eu/dar-web/provision), RIVM reports (www.rivm.nl/bibliotheek/index-en.html), summary reports of EU member states (e.g. www.ctgb.nl) and scientific papers in the open literature (search program Web of Science from 1 January 1995 to 31 December 2012) (see Table 2). In addition, industry data that were provided to Alterra for use in the paper of Maltby *et al.*[15] were also used. Insecticides were allocated to one of the following categories: organophophates, carbamates, pyrethroids, insect growth regulators (IGRs), neonicotinoids, biopesticides and a remaining miscellaneous group (Table 2). The different compounds were made anonymous in the graphs to respect the confidentiality of some of the data.

Information from freshwater and saltwater arthropods (i.e. crustaceans and insects) was considered of interest. Criteria used to select single-species toxicity data were test endpoint and duration. Selected endpoints were the median effect concentrations for immobility and mortality (EC_{50}/LC_{50}), and the test durations selected were 48, 72 and 96 h. When more than one toxicity value within this timespan was reported for a species, then the geometric mean of these values was calculated when similar endpoints and similar timespans were involved. In the cases where either endpoints or timespans were different, the lowest toxicity value was selected.

Micro/mesocosm data were used to derive safe threshold concentrations. Each study was classified into one of two exposure categories, namely (1) a single pulse exposure regime or (2) a repeated exposure regime. In addition, responses observed for the most sensitive endpoint of a study were ascribed to effect classes in order to derive RACs for the most sensitive endpoint and the ecological threshold option (ETO-RAC).[8] For each compound and exposure regime, an ETO-RAC was derived using the guidance provided in EFSA.[1] For ETO-RAC derivation, effect class 1 and effect class 2 concentrations were used. Within a study, an effect class 1 concentration is the highest test concentration at which a NOEC could be derived for the most sensitive measurement endpoints, and an effect class 2 concentration is the lowest test concentration with statistically significant but slight/transient effects on an individual sampling occasion for the most sensitive measurement endpoint. When possible, separate ETO-RACs were derived for single and repeated treatment regimes for each compound. Construction of the ETO-RAC was as follows. When only effect class 1 values were available, half of the effect class 1 concentration was used as the ETO-RAC [(effect class 1 concentration)/2]. When only effect class 2 values were available, this value was divided by three [(effect class 2 concentration)/3]. When both effect class 1 and effect class 2 values were available, then the (effect class 1 concentration)/2 value was used as the ETO-RAC. In the exceptional case where more than one effect class 1 value was available for a compound and the same exposure regime (e.g. from different micro/mesocosm studies), then the geomean of these values was used for ETO-RAC derivation. In contrast, for effect class 2 values, the lowest class 2 value was chosen.

2.1 Tier-1 effect assessment

For each compound, tier-1 RACs were compared with model ecosystem threshold levels (ETO-RAC). Tier-1 RACs were based on the lowest acute toxicity value obtained from the required standard toxicity tests performed with *Daphnia magna*, *Americamysis bahia* and/or OECD-*Chironomus* sp. [i.e. *C. riparius*, *C. dilutus* (= *C.*

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Table 1. Derivation of acute tier-1, tier-2 and tier-3 RACs for insecticides using experimental methods proposed in the Aquatic Guidance Document,[1] assuming that aquatic arthropods are the most sensitive taxonomic group

	RAC derivation			
	Data		Endpoint	AF[a]
Tier 1	Core toxicity data: *Daphnia magna*, OECD-*Chironomus* sp. and/or *Americamysis bahia*		48 h EC_{50}	100
			96 h $L(E)C_{50}$	100
Tier 2	Core and additional toxicity data for aquatic arthropod species	n <8	Geomean $L(E)C_{50}$ (separately for insects and crustaceans)	100
	Core and additional toxicity data for aquatic arthropod species	n ≥8	Median acute HC_5	3-6
Tier 3	Micro/mesocosm experiment; ecological threshold option (ETO)		Effect class 1 (NOEC most sensitive endpoint)	2
			Effect class 2 (slight effect for most sensitive endpoint on individual sampling)	2–3

[a] AF: assessment factor.

tentans), *C. yoshimitsui*].[67] The lowest EC_{50}/LC_{50} value was divided by an AF of 100 to derive the tier-1 RAC.

2.2 Tier-2 geomean approach

The geomean approach can be used if more toxicity data are available than under tier 1 but less than required for the SSD approach. In tier 1, the preferred toxicity data are EC_{50} values for *D. magna* and an OECD-*Chironomus*. Therefore, the acute toxicity data of *Daphnia magna* (crustacean) and an OECD-*Chironomus* (insect) were first compared. The lowest EC_{50} value for these two standard test species was the starting point for adding acute toxicity data for an additional species to calculate the geometric mean. If *D. magna* was the most sensitive, acute toxicity data for a crustacean were added, but if *Chironomus* sp. was the most sensitive, then toxicity data of an insect were added. The new geomean was then compared for crustaceans and insects to decide whether to add further toxicity data to the crustacean geomean or the insect geomean. This procedure was repeated until a maximum of seven species in total were added (see supporting information Table S1 for an example).

The order in which species were added was determined by their frequency of testing across all 26 compounds evaluated (see supporting information Table S2), the rationale being that species with a high test frequency are more likely to occur in the dataset. For crustaceans, acute toxicity data of *A. bahia* were often available. As this is a standard test species, the toxicity data for this species were the first to be included to calculate the crustacean geomean.

The options of using the geomean of four taxa (including two or three standard species) and the geomean of seven taxa reflect the range that will normally be used when applying the geomean approach. Geomean tier-2 RACs were obtained by applying an AF of 100 to the geomean values of the most sensitive taxonomic group (insects or crustaceans).

2.3 Tier-2 SSD approach

Median HC_5 values (i.e. the median hazardous concentration that is estimated to affect 5% of the potentially sensitive species) were derived from SSDs constructed with acute EC_{50}/LC_{50} values for at least eight taxa using the computer program ETX v.2.0.[68] SSDs were based on the complete arthropod dataset for a compound if the Anderson–Darling test for normality was accepted at the 5% level. If the test for normality was not accepted, the extent to which the arthropod data gave a conservative fit through the data was evaluated. If the fit was conservative, then the HC_5 of the complete arthropod dataset was used. If the fit was not conservative, then the SSDs were constructed for crustaceans and insects separately (if at least eight toxicity values were available for the subgroup), and the lowest HC_5 of the two was used in the validation process. Median HC_5 values were divided by AFs of 3 or 6 to obtain SSD-RACs. These AFs represent the most lenient and the most stringent options in the proposed range of 3–6 (Table 1).

The derived tier-1 and tier-2 RACs were plotted against the corresponding ETO-RACs; compounds falling below the 1:1 line indicate that lower-tier RAC values are protective of ecological effects towards invertebrate populations and communities subjected to single or repeated pulsed treatment regimes of the compounds evaluated.

3 RESULTS

Lower-tier RACs and ETO-RAC values could be compared for 26 insecticides (Table 2). Tier-1 RACs could be constructed for 24 of these 26 compounds, and the tier-2 geomean approach could also be applied to 24 compounds (Table 2). The SSD approach could be used for 21 compounds (Table 2). ETO-RACs were calculated for repeated and/or single pulsed applications of 26 insecticides to micro/mesocosms, giving a maximum of 30 insecticide × application combinations (i.e. cases) for comparison (Table 2).

3.1 First tier

Derived tier-1 RACs (acute $EC_{50}/100$) based on the most sensitive available acute toxicity value for *D. magna*, *A. bahia* and/or OECD-*Chironomus* (EFSA, 2013) were protective for organophosphates (seven cases; single and repeated applications), carbamates (two cases; single applications) and pyrethroids (eight cases; single and multiple applications) (Fig. 2). By a 'case' we mean a single point in the figures representing a lower-tier RAC and its corresponding ETO-RAC, based on either a single application or repeated applications to micro/mesocosms. For neonicotinoids, tier-1 RACs were protective in three of the four cases (both single and multiple applications). In the remaining case (single application of thiacloprid) the line representing the 1:1 ratio was exceeded by a factor of 1.7 (Fig. 2). IGRs were represented by four cases. One IGR case (single application of fenoxycarb) exceeded the 1:1 ratio

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Table 2. Insecticides used in the tier-1 and tier-2 evaluation in relation to their corresponding ETO-RACs (tier 3) and related scientific papers in the open literature that were consulted in addition to the toxicity databases mentioned in Section 2[a]

			Tier 2[b]		Tier3[c]		
	Compound	Tier 1	Geom	SSD	Single	Mult	Open literature references
Organophosphates	Azinphos-methyl	×	×	×	×	×	15,21
	Chlorpyrifos	×	×	×	×	×	15,22–27
	Fenitrothion	×	×	×	×	–	15,21
	Parathion-ethyl	×	×	×	–	×	15,21,28
	Phosalone	×	–	–	×	–	
	Phosmeth	×	×	×	×	–	
Carbamates	Carbaryl	×	×	×	×	–	15,21,29
	Carbofuran	×	×	×	×	–	15,21
Pyrethroids	Cypermethrin	×	×	×	–	×	15,23,30,31
	Deltamethrin	×	×	×	–	×	15,32–34
	Esfenvalerate	×	×	×	×	–	33,35–39
	Fenvalerate	×	×	×	×	–	15,21,35
	Gamma-cyhalothrin	–[d]	×	×	–	×	40,41
	Lambda-cyhalothrin	×	×	×	–	×	42–46
	Bifenthrin	×	×	×	–	×	47,48
Benzylurea and other IGRs	Diflubenzuron	×	×	×	×	×	15,16,49
	Teflubenzuron	–[d]	×	–	–	×	50
	Fenoxycarb	×	×	–	×	–	51–54
	Pyriproxifen	×	–	–	×	–	55,56
Biopesticides	Abamectin	×	×	×	×	×	57–59
Neonicotinoids	Clothianidin	×	–	–	×	–	
	Imidacloprid	×	×	×	–	×	29,35,60–63
	Thiacloprid	×	×	×	×	–	35,64,65
	Thiamethoxam	×	×	×	×	–	66
Miscellaneous	Lindane	×	×	×	–	×	15,16
	Methoxychlor	×	×	×	×	–	15, 16

[a] ×: data sufficient for evaluation; —: data not sufficient/available for evaluation.
[b] Geom: geometric mean approach; SSD: SSD approach.
[c] Single: single application; Mult: multiple applications.
[d] Only one standard test species available.

by a factor of 161. For the other categories (biopesticides and miscellaneous; each with two cases) the tier-1 RACs were protective.

3.2 Geomean approach

Tier-2 RACs based on the geomean of four toxicity values ($n = 4$) were protective in 24 of 26 cases evaluated (Fig. 3A). One case where the tier-2 RAC was above the 1:1 line was an IGR (fenoxycarb) and one a neonicotinoid (thiacloprid), the same compounds as in the tier-1 RAC evaluation (Fig. 2).

Twenty-six cases could be evaluated using a dataset containing seven toxicity values (Fig. 3B). Using seven toxicity values instead of four seemed to result in a more or less similar protection level (compare Fig. 3A with Fig. 3B).

When using four toxicity values to calculate the tier-2 Geom-RAC, the tier-2 RACs for compounds below the 1:1 line were on average a factor of 25 lower than the corresponding tier-3 RAC. When using seven toxicity values to calculate the tier-2 RAC, this factor increased to 28. However, increasing the number of toxicity data from four to seven to calculate the geometric mean does not necessarily reduce the tier-2 RAC. For both cases that exceeded the 1:1 line, the exceedance was greatest when seven toxicity values were used to derive the RAC. The neonicotinoid thiacloprid exceeded the 1:1 line by a factor of 1.5 when four toxicity values were used, and by a factor of 1.9 when 7 toxicity values were used. For the IGR (fenoxycarb), deviation from the line increased from 317 ($n = 4$) to 567 ($n = 7$).

3.3 SSD approach

The SSD-RAC was based on the median acute HC_5 from arthropod SSDs. In general, the Anderson–Darling goodness-of-fit test was accepted at the 5% level for the arthropod sensitivity distributions (supporting information Table S3). In situations where this was not the case (i.e. chlorpyrifos, carbaryl, thiacloprid) the arthropod HC_5 values were very similar to HC_5 values derived for just crustaceans or just insects. Moreover, the model fits for arthropods in these three cases were conservative in the left-hand tail of the SSD.

The tier-2 RAC based on the $HC_5/3$ was shown to be protective in 23 of the 27 cases evaluated (Fig. 4A). The exceptions were two neonicotinoids (single applications of thiacloprid and thiamethoxam), which are positioned a factor of 3.7 and 1.1 above the 1:1 line, the biopesticide abamectin (multiple application), which was positioned a factor 2.8 above the 1:1 line, and one compound from the miscellaneous group (lindane; multiple application), which exceeded the 1:1 line by a factor of 1.5.

The tier-2 RAC based on the $HC_5/6$ was shown to be protective in 25 of the 27 cases evaluated (Fig. 4B). The exceptions were a neonicotinoid (thiacloprid; single application), which was positioned a

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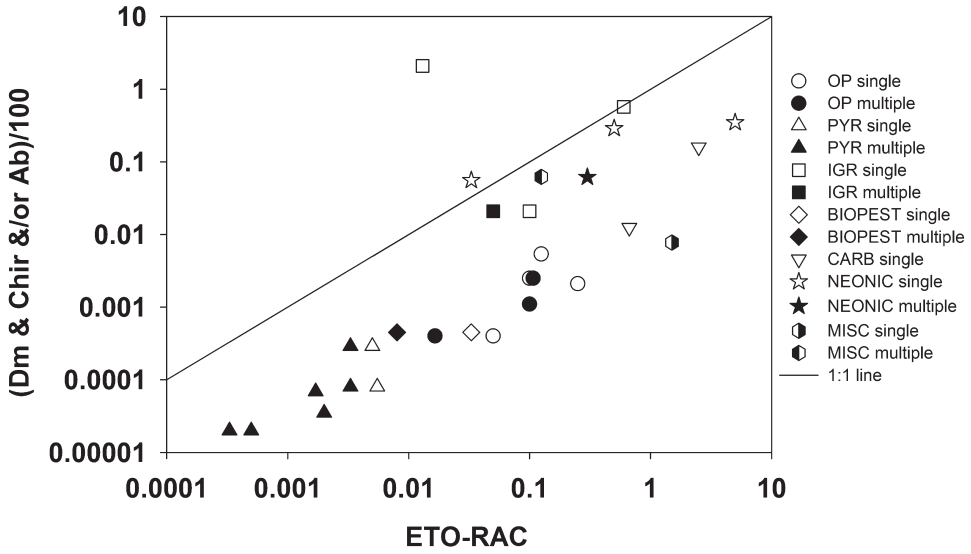

Figure 2. Plot of the acute tier-1 RACs for insecticides against the corresponding ETO-RACs as derived from aquatic micro/mesocosm studies. The line represents the 1:1 ratio (tier-1 RAC:ETO-RAC). Tier-1 RACs are exclusively based on the lowest acute toxicity value for the combination *Daphnia magna*, OECD-*Chironomus* and/or *Americamysis bahia* [(Dm & Chir &/or Ab)/100]. OP: organophosphates; PYR: pyrethroids; IGR: benzylurea/insect growth regulators; BIOPEST: biopesticides; CARB: carbamates; NEONIC: neonicotinoids; MISC: miscellaneous insecticides; single: single insecticide application in micro/mesocosm study; multiple: multiple insecticide applications in micro/mesocosm study.

factor of 1.8 above the 1:1 line, and a biopesticide (abamectin; multiple application), which was a factor of 1.4 above the 1:1 line. The IGR fenoxycarb, which exceeded the safe threshold in the tier-1 evaluation (Fig. 2) and the tier-2 geomean evaluation (Fig. 3), was not included in the current SSD evaluation because insufficient data were available.

4 DISCUSSION

Overall, the tier-1 and tier-2 approaches proposed by EFSA for acute effect assessment seem to be sufficiently protective for insecticides when compared with the corresponding ETO-RACs derived from micro/mesocosm experiments. Only a few tier-1 and tier-2 RACs were underprotective for effects observed in micro/mesocosms, and in all but one case only slightly underprotective. In the case of the IGR fenoxycarb, however, the tier-1 and tier-2 geomean-RAC exceeded the corresponding ETO-RAC by a factor of >100. It has already been concluded that fenoxycarb is an outlier, possibly owing to an overly conservative ETO-RAC.[20] The mesocosm study used (evaluated in Smit and Vonk)[53] showed a very broad range in effect class 2 concentrations (0.096–3.2 µg L^{-1}), and we used the lowest effect class 2 concentration (and an AF of 3) for ETO-RAC derivation. Another explanation is that the standard acute toxicity test time-window (48–96 h) may be insufficient to capture the incipient toxicity of this IGR. Growth regulators are known to exhibit latency of effects.[69] Therefore, the toxicity testing duration should be expanded in order to quantify adequately the effects caused by short-term exposures to compounds showing delayed effects. This is also recommended in the EFSA Aquatic Guidance Document.[1] Disregarding the results for fenoxycarb, the tier-1 approach was protective in 28 out of 29 cases, and the tier-2 geomean approach in 24 out of 25 cases.

An overall sufficient level of protection was achieved when deriving tier-1 RACs (acute EC_{50}/100) on the basis of the most sensitive available acute toxicity value for *D. magna*, OECD-*Chironomus* and/or *A. bahia* as proposed by the Aquatic Guidance Document.[1] This approach, in general, seems applicable to established chemistries (i.e. pyrethroids, organophosphates and carbamates), but exceptions were observed for a few compounds of more recently developed chemistries (i.e. one neonicotinoid and the IGR fenoxycarb in particular). The deviating neonicotinoid, however, was positioned slightly above the 1:1 line in Fig. 1.

The Aquatic Guidance Document recommends calculating geometric mean EC_{50} values for crustaceans and insects separately and selecting the lowest value to derive the acute RAC.[1] This seems to be a rather robust method, as the two evaluations presented, one with a low number of acute toxicity data for arthropods ($n = 4$) and one with the maximum number of data ($n = 7$), essentially produced a similar outcome (compare Figs 3A and B). Deriving the geomean of the most sensitive group produced tier-2 acute RACs that were protective for organophosphates, carbamates and pyrethroids, but not for all compounds with more novel modes of action, such as the neonicotinoid thiacloprid and the IGR fenoxycarb.

The SSD approach has been considered and evaluated previously for neurotoxic insecticides, herbicides and fungicides.[15–17] The aim of the present study was to extend this evaluation to include insecticides with more novel modes of action (e.g. neonicotinoids, IGRs, biopesticides). The data search, however, indicated that the necessary combination of toxicity data and micro/mesocosm studies were only available for a few compounds (one IGR, one biopesticide, three neonicotinoids and two in the miscellaneous group, compared with 14 cases of the neurotoxins organophosphates, carbamates and pyrethroids). Improvements of the validation are therefore dependent on more model ecosystem studies being conducted for compounds with novel modes of action. Based on the data that are available, the tier-2 SSD RAC was protective in 25 out of 27 cases when the HC_5/6 was used, and in 23 out of 27 cases when the less stringent HC_5/3 was used. In common with the tier-1 RAC and tier-2 Geom RAC evaluations, the SSD approach was underprotective for some neonicotinoids plus a biopesticide. In all cases, the level of underprotection was less than a factor of 2 when HC_5/6 was used, and thus the tier-2 SSD-RAC was slightly lower than the NOEC of the most sensitive measurement

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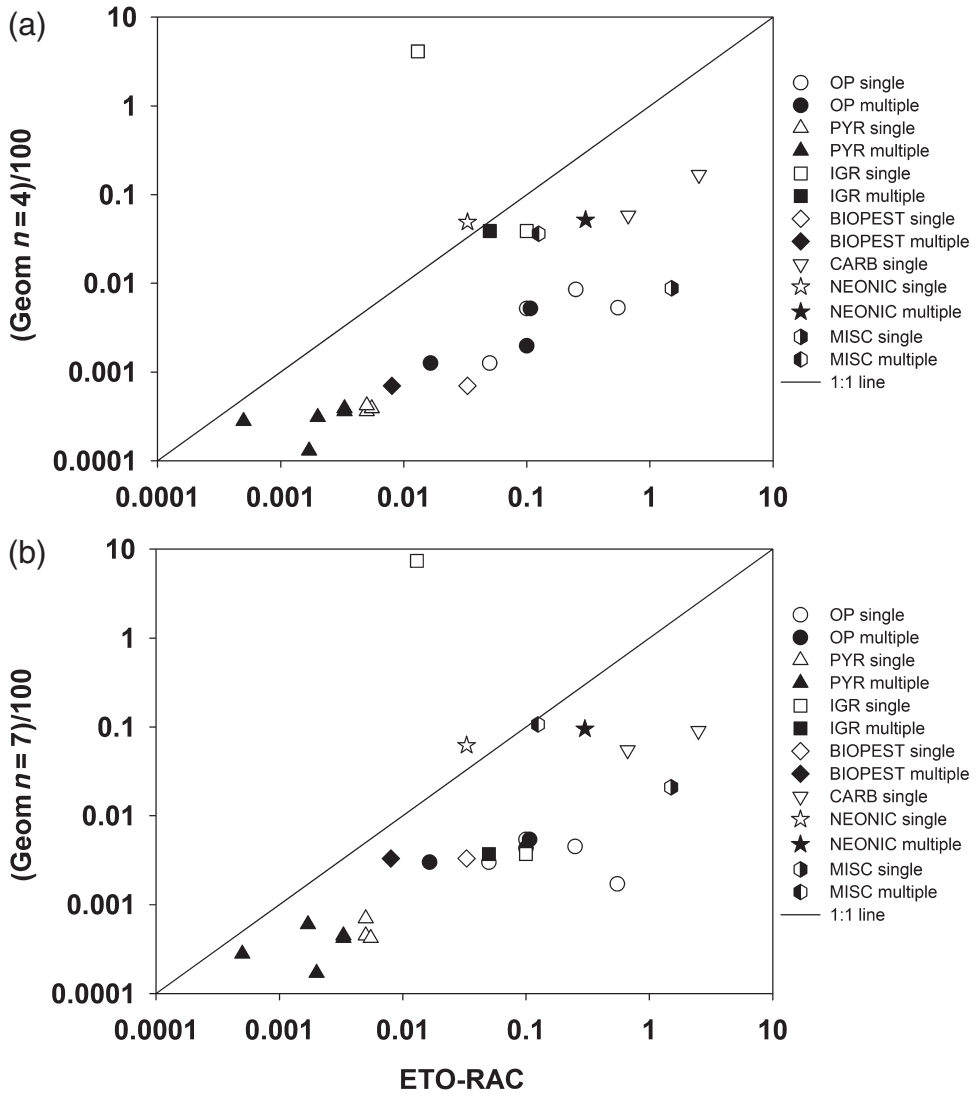

Figure 3. Plot of the acute tier-2 Geom-RACs for insecticides against the corresponding ETO-RACs derived from aquatic micro/mesocosm tests. The line represents the 1:1 ratio (Geom-RAC:ETO-RAC). Panel A: Geom-RACs based on the lowest geomean after splitting the arthropod acute toxicity dataset ($n = 4$) into sets for crustaceans and insects [(Geom $n = 4$)/100]. Panel B: Geom-RACs based on the lowest geomean after splitting the arthropod acute toxicity dataset ($n = 7$) into sets for crustaceans and insects [(Geom $n = 7$)/100]. For explanation of symbols, see Fig. 2.

endpoint of the corresponding micro/mesocosm study, because in the derivation of the ETO-RAC an AF of 2 was applied to this NOEC.

In the present paper we focused on tier-1 and tier-2 approaches for acute effect assessment, but ideally this analysis should also be performed for chronic effect assessment. One of the major challenges when analysing chronic toxicity data is whether, and when, to combine information on different endpoints. For acute toxicity, the combination of immobility and mortality endpoints can be considered acceptable, as ecologically they are expected to result in a more or less similar response. In the case of chronic studies, however, the validity of combining different effect endpoints (e.g. reproduction, growth, immobility, mortality) for different species in the geomean and SSD approaches is an important topic for future research. In the Aquatic Guidance Document[1] it is recommended to combine toxicity data of similar endpoints only when applying the geomean approach. Our search for chronic laboratory toxicity data for aquatic arthropods as part of this review yielded very few data, and, because the selection of similar effect endpoints is critical for deriving a valid geomean, this resulted in even less suitable study cases. Laboratory toxicity studies with insecticides that focus on chronic and/or long-term effects on aquatic crustaceans and insects are still sparse, and more extensive and robust datasets are necessary to enable the validation of the lower-tier chronic effect assessment procedures with results of micro/mesocosm experiments.

In the present paper, the RACs derived from lower tiers are compared with the ETO-RAC derived from microcosm/mesocosm studies. These microcosm/mesocosm studies are used as a surrogate reference tier; the real reference tier being the field situation. The EFSA Aquatic Guidance Document[1] states that it is important to note that communities and environmental conditions in micro/mesocosm represent only one of the many possible conditions of edge-of-field surface waters. Edge-of-field surface water bodies potentially at risk vary in community structure (including species composition and life cycle traits) and abiotic conditions. This should be accounted for in the effect assessment, e.g. by applying an appropriate AF for spatiotemporal extrapolation of the concentration–response relationships observed

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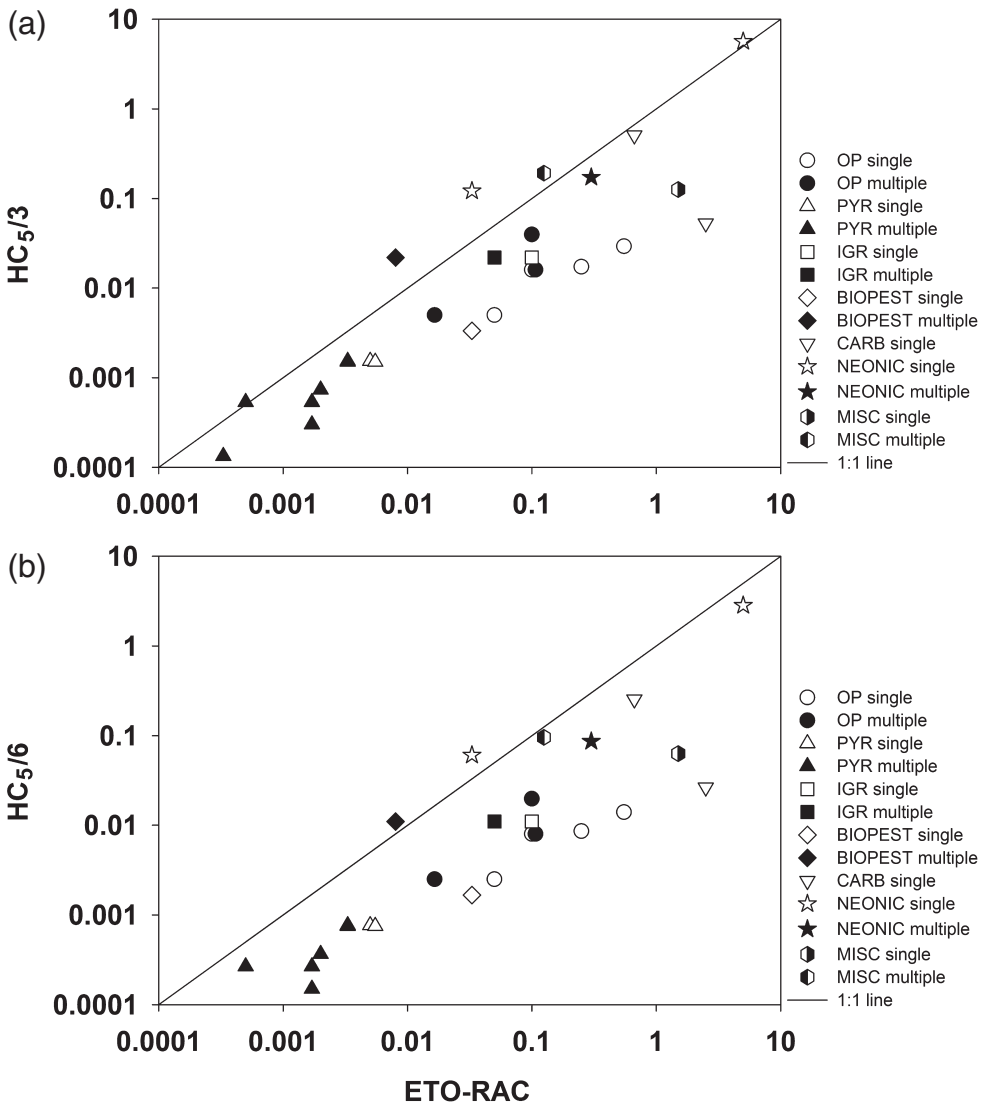

Figure 4. Plot of the acute tier-2 SSD-RACs for insecticides against the corresponding ETO-RACs derived from aquatic micro/mesocosm tests. The line represents the 1:1 ratio (SSD-RAC:ETO-RAC). Panel A: SSD-RACs based on the median HC5 values derived from acute toxicity SSDs for arthropods (i.e. crustaceans and insects) and an AF of 3 ($HC_5/3$). Panel B: SSD-RACs based on the median HC5 values derived from acute toxicity SSDs for arthropods and an AF of 6 ($HC_5/6$). For explanation of symbols, see Fig. 2.

in micro/mesocosms. In the present paper, we used the AF as proposed in the EFSA Aquatic Guidance Document[1] to extrapolate effect class 1 and effect class 2 concentrations to obtain an ETO-RAC.

5 CONCLUDING REMARKS

Compared with the neurotoxic organophosphates/carbamates and pyrethroids, far fewer cases for each of the other insecticide groups (i.e. neonicotinoids, IGRs, biopesticides) were available for the acute tier-1 and tier-2 validation. Nevertheless, several of the representatives of these other groups (except the neonicotinoid thiacloprid and the IGR fenoxycarb) conform to the pattern observed for the neurotoxins, giving some confidence that the acute tier-1 and tier-2 approaches as now proposed by the Aquatic Guidance Document[1] also hold for a broader scope of chemistries. This contention, however, needs further underpinning by both additional laboratory toxicity data and micro/mesocosm studies with compounds representing neonicotinoids, IGRs and biopesticides, particularly if delayed effects are expected. For compounds with delayed effects (e.g. IGRs) prolonged acute toxicity tests are requested by the EFSA Aquatic Guidance Document[1] to derive lower-tier RACs. In prolonged acute toxicity tests, the observation of treatment-related responses is continued after the test organisms are transferred to clean medium. Moreover, the evaluation presented in this paper has no *a priori* validity for non-tested modes of action. Besides the evaluation of the acute toxicity assessments, the chronic effect assessment scheme needs to be evaluated as well. However, validation of chronic lower tiers, and the geomean and SSD approaches in particular, is currently hampered by the limited availability of chronic toxicity data for many insecticides.

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SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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