Expert Statement

Re-evaluation of exposure concentrations of azadirachtin to *Chironomus riparius* in OECD 219 studies

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TABLE OF CONTENTS

TABLE OF CONTENTS 2

1 SUMMARY 3

2 BACKGROUND 4

2.1 Terminology 4
2.2 Aim of the expert statement 4

3 EU POSITION PUBLISHED IN ADDENDUM TO DAR FROM 2009 6

4 SCOPE AND RESTRICTIONS OF OECD 219 (2004) 8

5 CONSIDERATION OF THE DISTRIBUTION OF AZADIRACHTIN IN WATER AND SEDIMENT 10

6 CONCLUSION 15

7 REFERENCES 16

1 SUMMARY

Chironomus riparius studies according to OECD 219 (2004) have been performed for the EU registration of azadirachtin, considering different extracts (i.a. NeemAzal) and formulated products (i.a. NeemAzal-T/S) by spiking the respective substance to the water phase. According to the analytical measurements in water, porewater and sediment during the studies, test substance concentrations could only be measured one hour after application, whereas after 7 and 28 days all concentrations were below LOQ. The RMS concluded that the nominal exposure of the chironomids was not given throughout the study and therefore, with reference to SANCO/3268/2001, the endpoints were based on the mean measured concentrations recovered from the water and porewater instead of the initially measured concentrations as recommended by OECD 219.

This statement aims to (i) outline and critically evaluate the EU position during the review of azadirachtin, (ii) elucidate the scope and restrictions of testing under OECD 219 and (iii) demonstrate the realistic distribution of azadirachtin in a water-sediment system.

Firstly, the evaluation of the original position during EU review revealed that the reference to SANCO/3268/2001 does not fully justify the use of mean measured over initially measured concentrations in the case of OECD 219, since the SANCO document only recommends to use mean measured concentrations for semi-static or flow-through systems where a gradual degradation to < 80% was observed. This does not apply to the studies performed on azadirachtin under OECD 219.

In addition, the scope and restrictions of OECD 219 clarify that the test system is intended for singular exposure events such as drift or drainage after rainfall and not for continuous exposure over the whole life cycle of chironomids. Due to the changing exposure during the different life stages from egg over larvae to pupae and adults, chironomids are not only exposed to the water phase but also, and even more, to the sediment top layer which is exactly the surface to which substances such as NeemAzal tend to be translocated.

Due to the lack of data from a water-sediment study, the predicted distribution of azadirachtin between water and sediment was calculated under consideration of the K_{OC} value and the organic carbon content of the sediment in the study. The results indicate that with a distribution coefficient (K_d) of 1.62 more NeemAzal will be distributed into the sediment compared to the water phase. The lack of verification via analytical measurements in the sediment during the *C. riparius* studies might be explained by the way of sediment sampling. For sampling, the whole sediment is mixed and the average concentration in the whole bulk is determined instead of the concentration in the top sediment layer where NeemAzal is actually expected to be present at much higher concentrations. Further calculations presented in this document confirm that concentrations above the LOQ could have been found when only taking the upper 0.2 cm of the sediment into account. Publications (e.g. Brock, 2013) support the assumption that for sediment dweller only the upper sediment layer should be considered to determine the realistic worst-case environmentally relevant concentration (ERC) based on their ecology.

In conclusion, it was demonstrated that chironomids during the tests with NeemAzal and NeemAzal-T/S were sufficiently exposed to the test items. Consequently, based on the life cycle of chironomids and the intention of the test system to represent a single exposure event (drift, drainage), it is considered most reasonable to use the initially measured concentrations instead of the mean measured concentrations for the determination of the NOEC. An additional safety factor, next to the regulatory safety factor of 10, via adaptation of the endpoint is not considered justified as this will be part of the risk assessment.

2 BACKGROUND

2.1 Terminology

First, a short clarification of the different substances mentioned in this statement shall be given:

Azadirachtin:	Common name for an extract from seed kernels of the tropical neem tree *Azadirachta indica*, comprising different components such as azadirachtin A, azadirachtin B, 11-epi-azadirachtin D, etc.
Azadirachtin A:	Proposed lead substance of azadirachtin extracts; major biologically active component of NeemAzal
NeemAzal:	Specific azadirachtin extract from the applicant Trifolio-M, also referred to as Margosa Extract-in the biocidal context, containing mainly azadirachtin A (~ 34%)
NeemAzal-T/S:	Formulated product from the applicant Trifolio-M, containing 1% azadirachtin A
Azatin and Fortune:	Specific azadirachtin extract and formulated product from the applicant Mitsui
OIKOS:	Formulated product from the applicant Sipcam

All substance specific data used in the following document, such as DT_{50} values, adsorption values and others are always related to azadirachtin A, unless stated otherwise.

2.2 Aim of the expert statement

According to EC Regulation 283/2013, studies with sediment dwelling organisms that include sediment in the test system are required for registration of active substances in case the active substance is either expected to be an insect growth regulator and/or accumulation in aquatic sediments is indicated or predicted from environmental fate studies. Azadirachtin A, which is the major biologically active component of NeemAzal, is predicted to degrade quickly in water (DT_{50} ~ 10 days according to Molinari, 2002a) and also the half-life in soil is stated to be less than 10 days. Based on these considerations, accumulation of azadirachtin A in sediment is expected to be low. However, NeemAzal, the extract of Trifolio-M containing mainly azadirachtin A, acts via growth regulation and inhibits moult. Therefore, *Chironomus riparius* was tested with NeemAzal (extract; Gonsior, 2008a, Ref. IIA 8.5.2/01) and NeemAzal-T/S (formulated product; Gonsior, 2008e, Ref. IIIA 10.2.6.2/02) in water/sediment-studies according to OECD 219 (2004) during the EU review of azadirachtin. Further studies from the EU review on *C. riparius* are available with Azatin (extract of the notifier Mitsui with different amounts of azadirachtin A and B; Gonsior, 2008b, Ref. IIA 8.5.2/02), Fortune (formulated product of the notifier Mitsui; Gonsior, 2008c, Ref. IIA 8.5.2/03) and OIKOS (formulated product of the notifier Sipcam; Gonsior, 2008d, Ref. IIIA 10.2.6.2/01).

Shortly summarized, all studies were performed with first instar larvae of *C. riparius* in a dose-response static water/sediment system over 28 days. The artificial sediment contained 75-76% sand, 20% kaolin clay and 4-5% peat resulting into an organic carbon content of 2 ± 0.5% depending on the final percentages of sand and peat. An amount of ~ 350 g sediment (wet weight) was placed into 2 L glass beakers (12 cm diameter) forming an approximate depth of 3 cm. The sediment was overlayed

with a water column of ~ 12 cm test medium. Test vessels were aerated and each day 1 mg of fish food (TetraMin) per larvae (25 larvae per test vessel) was added. Analytical measurements were performed at test start (1 hour after application), after 7 days and after 28 days in the overlaying water, the pore water and the sediment from additional test vessels that were set up in the same way but not used for biological data evaluation. Behaviour, emergence and development were monitored and the EC_{50} as well as the NOEC and LOEC were determined.

According to OECD 219 (2004), endpoints shall be determined based on the measured concentrations at the beginning of the test, i.e. the initially measured concentration. However, during EU review of the studies on *C. riparius*, all endpoints were determined based on geometric mean measured concentrations since the test items were assumed to have degraded very quickly due to measured concentrations below LOQ (0.00625 mg/L for NeemAzal (Gonsior, 2008a) and 0.183 mg/L for NeemAzal-T/S (Gonsior, 2008e)) after 7 and 28 days. This was the case in all *C. riparius* studies with azadirachtin in the EU review, although different LOQs were determined, however the focus here is on the two studies with NeemAzal and NeemAzal-T/S since in the other studies test items with different (lower) amounts of azadirachtin A were used.
In the studies the geometric mean measured concentrations were calculated using the geometric mean between the nominal concentration (as surrogate for the concentration at test start) and half of the LOQ (as estimated surrogate of the concentration at test end) according to the recommendations by OECD 23 (2000). The RMS' justification for using the geometric mean concentration instead of the initial or nominal concentration to determine the endpoints was the assumption that the larvae of *C. riparius* were not exposed to the nominal/initial concentration over the whole test period.

In the following chapters it is aimed to:

1. **outline and critically evaluate the original position during the EU review of azadirachtin**
2. **elucidate the scope and restrictions of testing under OECD 219 (2004)**
3. **demonstrate the realistic distribution of azadirachtin A in a water-sediment system**

3 EU POSITION PUBLISHED IN ADDENDUM TO DAR FROM 2009

As mentioned above, several studies with azadirachtin extracts and formulated products were performed, however, only the relevant studies from the sponsor will be reviewed here, i.e. Gonsior, (2008a and 2008e). For Gonsior (2008a) in the Additional Report to the DAR for Azadirachtin – Volume 3-B9 from December 2009 (page 191) it was stated:

'*Conclusion: The initially measured concentrations of NeemAzal were > 80 % and < 120 % of nominal for six of the eight tested concentrations. For 0.147 and 0.294 mg NeemAzal/L the actual concentrations were below 80 % of the nominal concentrations already at test start. This is acceptable in this case because > 80 % of nominal were reached in the concentration range of the NOEC. The analytical measurements after 7 and 28 days showed a degradation of the test substance below the limit of quantification (LOQ) of 0.00625 mg NeemAzal/L for water and pore water and 0.0156 mg/kg for sediment. In the sediment the NeemAzal concentrations did not exceed the LOQ during the whole study. Consequently, the chironomids were not exposed to the nominal concentrations over the whole time. According to the Guidance Document on Aquatic Ecotoxicology SANCO/3268/2001 rev. 4 (final, chapter 2.1.4) the toxicity endpoints (EC_{50}, NOEC and LOEC) should be additionally presented based on measured concentrations in this case. This was not done, thus the given endpoints were determined based on the geometric mean concentrations. Therefore the mean of the NOEC based on nominal concentrations and the LOQ (for water and pore water, because no test substance was found in the sediment) divided by 2 was calculated. The NOEC based on the geometric mean concentration was calculated to be 0.011 mg NeemAzal/L.*'

Two points need to be mentioned here, first, the calculation of 0.011 mg NeemAzal/L was not the <u>geometric mean</u> concentration although stated as such in the DAR but instead the <u>arithmetic mean</u> concentration was determined.

Second, it is noted that although the EU agreed endpoint was 0.011 mg NeemAzal/L, different endpoints were determined by the study director (0.0184 mg/L, nominal concentration) and the Federal Institute for Occupational Safety and Health (0.0075 mg/L, geometric mean concentration). This shows that at the time of evaluation no consistent approach was used by the different authorities.

A similar conclusion was also drawn for Gonsior (2008e), again with reference to SANCO/3268/2001.

Chapter 2.1.4 of the SANCO/3268/2001 document, however, indicates:

'2.1.4 Calculation of test endpoints

Toxicity endpoints (LC/EC_{50}, NOEC, etc.) should usually be calculated using nominal concentrations. This is because nominal concentrations are the most suitable for calculating TER values with maximum predicted environmental concentrations (PEC_{max}). In studies where the initial measured concentrations are < 80 % or > 120% of the nominal, toxicity values should be presented as measured initial concentrations. These can then be used in the risk assessment in the same way as nominal concentrations. This approach is especially relevant for static tests.

If the measured concentrations are < 80 % or >120 % of nominal ones during the test, toxicity values should be presented additionally as measured concentrations and the mean measured concentration for the relevant test period should be used to express toxicity. If the measured concentrations are very low compared to nominal ones, the validity of the test might be questionable, and a justification for using such study should be required. In short summary, the following rules apply in general:

- *if measured concentrations are > 80 %, then the nominal concentration can be used to express toxicity,*
- *if initial measured concentrations are < 80 %, then toxicity values should be expressed as initial measured concentrations,*
- *if measured concentrations in semi-static and flow-through systems fall graduately below 80 % during the test, then toxicity values should be expressed as mean measured concentrations.*'

Accordingly, SANCO/3268/2001 does recommend the calculation of endpoints based on initially measured concentrations for static tests and also the risk assessment can be based upon those endpoints. In contrast, mean measured concentrations are recommended for semi-static and flow-through tests in case the concentrations fall gradually below 80%. The chironomid test according to OECD 219 (2004) is neither a semi-static or flow-through test nor has gradual degradation been observed. Consequently, a risk assessment based on mean measured endpoints instead of initially measured endpoints is not considered justified based on SANCO/3268/2001.

4 SCOPE AND RESTRICTIONS OF OECD 219 (2004)

OECD 219 (2004) gives a clear description of the scope and functionality of the chironomid, spiked water test in its paragraphs 2 and 3:

'*2. The exposure scenario used in this guideline is water spiking. The selection of the appropriate exposure scenario depends on the intended application of the test. The water exposure scenario, involving spiking of the water column, is intended to simulate a pesticide spray drift event and covers the initial peak of concentrations in pore water. It is also useful for other types of exposure (including chemical spills) except accumulation processes lasting longer than the test period.*
3. Substances that need to be tested towards sediment-dwelling organisms usually persist in this compartment over long time periods. The sediment-dwelling organisms may be exposed via a number of routes. The relative importance of each exposure route, and the time taken for each to contribute to the overall toxic effects, is dependent on the physical-chemical properties of the chemical concerned. For strongly adsorbing substances (e.g. with log Kow > 5) or for substances covalently binding to sediment, ingestion of contaminated food may be a significant exposure route. In order not to underestimate the toxicity of highly lipophilic substances, the use of food added to the sediment before application of the test substance may be considered. In order to take all potential routes of exposure into account the focus of this Guideline is on long-term exposure. The test duration is in the range of 20 - 28 days for C. riparius and C. yoshimatsui, and 28 - 65 days for C. tentans. If short-term data are required for a specific purpose, for example to investigate the effects of unstable chemicals, additional replicates may be removed after a ten-day period.'

Accordingly, it is clearly stated in OECD 219 (2004) that the water exposure scenario shall simulate a spray drift event or a similar single peak exposure pattern such as drainage after rainfall or spillage. It is not intended to reflect continuous exposure over the whole life cycle of chironomids, which would anyway be unreasonable since the exposure of chironomids in water-sediment systems is constantly changing throughout their life cycle. After hatching from the egg mass, first-instar larvae are mainly located in and on the sediment during their growth period, i.e. are exposed to a test substance via contact with the sediment. After the growth period, chironomid larvae start their metamorphosis which mainly takes place at the water surface (exposure via water phase) and subsequently the adult midges emerge. Consequently, two main life stages (larva and pupa) are exposed to the test item during the test. This exposition is not limited to azadirachtin A and other identified components typical for the extract NeemAzal, it also allows the estimation of the risk for the whole extract including potentially highly adsorbing ingredients.

Based on the K_{OC} values of azadirachtin A and the remaining components of NeemAzal ranging from 28.8 to 7413 L/kg (Mori, 2013), significant translocation from the water phase into the sediment is to be expected. Accordingly, the worst-case exposure for chironomids to NeemAzal occurs via the top sediment layer and other adsorbing surfaces such as food particles present on the sediment. It therefore seems unreasonable to base the endpoint on the mean measured concentration in the water phase. The initially measured concentration is the more reasonable one for the calculation of a reliable endpoint since it considers the differences in exposure during the various life stages of chironomids. Additional safety factors outside the principles of the risk assessment procedure are scientifically and regulatory unjustified.

In addition, according to paragraph 38 the analytical measurements proposed by OECD 219 (2004) are actually intended to prove that a similar distribution compared to the water-sediment study can be observed, i.e. a similar sediment etc. was used, and not primarily for re-calculation of endpoints:

'*38. As a minimum, samples of the overlying water, the pore water and the sediment must be analysed at the start (preferably one hour after application of test substance) and at the end of the test, at the highest concentration and a lower one. These determinations of test substance concentration inform*

on the behaviour/partitioning of the tested chemical in the water-sediment system. […] Measurements in sediment might not be necessary if the partitioning of the test substance between water and sediment has been clearly determined in a water/sediment study under comparable conditions (e.g. sediment to water ratio, type of application, organic carbon content of sediment).'

5 CONSIDERATION OF THE DISTRIBUTION OF AZADIRACHTIN IN WATER AND SEDIMENT

During the EU evaluation, no water/sediment study was submitted since radio-labelled components of the azadirachtin extract are technically not feasible to provide. Instead, as a surrogate, a water metabolism study was performed (Molinari, 2002a) which determined the concentrations of azadirachtin (A and B) in natural Po river water after 0, 1, 3, 6, 10, 15, 20, 30 and 60 days of incubation under dark conditions at 25°C. The resulting DT_{50} of azadirachtin A in water was 8.82 days and, after correction to 20°C, 13.7 days. No sediment was included in the study; therefore the study does not reflect the conditions in the *C. riparius* studies, however, from the water metabolism it can be concluded that the low analytical recovery in the *C. riparius* studies is not only a result of fast degradation of the test item but other physical and chemical processes need to be taken into account.

To predict the distribution of azadirachtin in the *C. riparius* studies, the physical/chemical properties of azadirachtin are further investigated subsequently. In general, the substance might attach to various compartments in the test system such as the water phase, the sediment, the food particles, the glass surface of the test vessels or even to the chironomids themselves.

The tendency of azadirachtin to attach to soil or sediment can be expressed via the distribution coefficient K_d which determines the ratio between the content of a substance in the soil phase and the mass concentration of the substance in the aqueous solution after reaching adsorption equilibrium. Values greater than 1 indicate that more of the substance will be distributed into the soil/sediment compared to the water phase:

$$K_d = \frac{C_s^{ads}\ (eq)}{C_{aq}^{ads}\ (eq)}$$

With: C_s^{ads} (eq) Content of substance adsorbed to the soil at adsorption equilibrium
C_{aq}^{ads} (eq) Mass concentration of the substance in the aqueous phase at adsorption equilibrium

Since often adsorption to soil occurs mainly by partitioning into the organic matter, the content of organic carbon plays an important role and therefore the K_d can be normalized for the organic carbon content resulting into the K_{OC} value.

$$K_{OC} = K_d * \frac{100}{\%oc}$$

With: %oc Percentage of organic carbon in the soil sample

The adsorption of azadirachtin (as azadirachtin A or NeemAzal) was determined during EU review in several studies including seven different soils (Troß, 1996 and Molinari, 2002b). A further study was available from the confirmatory data (Mori, 2013) which determined the adsorption coefficients and partition coefficients of various components of the azadirachtin extract. The resulting K_{OC} and log P_{OW} values are also considered for this expert statement. The overall geometric mean K_{OC} from studies in eight soils was determined to be 79.8 L/kg (refer to Table 5-1 below). To determine the distribution of azadirachtin A and the resulting predicted concentrations in the water phase and the sediment in the *C. riparius* studies, the K_{OC} value is re-calculated to the K_d value under consideration of 2.03% organic carbon which was measured in the sediment of the NeemAzal and NeemAzal-T/S studies (Gonsior, 2008a and e). The resulting K_d value is 1.62, indicating that more azadirachtin A is distributed into the sediment compared to the water phase.

Table 5-1: K_d values from the EU review and the confirmatory data on azadirachtin (azadirachtin A or NeemAzal)

Soil type	K_d [L/kg]	Organic carbon content [%]	K_{OC} [L/kg]	Reference
Silty clay (soil A) [a)]	2.26	1.86	121.5	Molinari, 2002b
Silty clay (soil B) [a)]	4.11	0.47	875.1	Molinari, 2002b
Loam (soil C) [a)]	2.51	3.32	75.8	Molinari, 2002b
Silt loam (soil D) [a)]	1.02	1.36	75.1	Molinari, 2002b
Sand (LUFA 2.1) [b)]	0.405	0.62	65.4	Troß, 1996
Loamy sand (LUFA 2.2) [b)]	0.479	2.32	20.6	Troß, 1996
Loamy sand (LUFA 2.3) [b)]	0.373	1.22	30.6	Troß, 1996
- (HPLC determination)	-	-	66.1	Mori, 2013
Geometric mean value			79.8	

[a)] Test material: Azadirachtin A TEC
[b)] Test material: NeemAzal

Furthermore, the partition coefficient (log P_{OW}) of azadirachtin A was determined as 1.37 (Mori, 2013) indicating hydrophobicity. Azadirachtin A is therefore not expected to stay in the water phase but to attach to the sediment and other organic particles such as the fish food which is added to the system (also considered relevant by OECD 219) and also to the chironomids themselves, which were, however, not analytically measured.

An additional aspect which is worth to be taken into account is the distribution of azadirachtin A in the sediment compared to the sampling of the sediment for analytical measurements. The test items NeemAzal and NeemAzal-T/S, with the lead component azadirachtin A, were not mixed into the sediment but spiked to the water phase of the test system which correlates with the expected route of entry into the environment as a spray drift or drainage event. Azadirachtin A is consequently assumed to settle and stay in the top layer of the sediment since distribution into deeper layers is unlikely to have occurred during the study period. This assumption is supported by the EFSA Scientific Opinion on Sediment Dweller (2015) who cite data from Crum and Brock (1994) and Brock (2013) indicating that particularly for compounds that easily sorb to sediment particles, the upper sediment layer (0-1 cm) may have much higher concentrations than deeper sediment layers (> 1 cm; refer to Figure 5-1). In addition, the upper sediment layer is more favourable for benthic organisms with respect to food and oxygen conditions and should therefore be considered for a realistic worst-case environmentally relevant concentration (ERC).

Figure 5-1: Stratification of the insecticide chlorpyrifos in different sediment layers seven days after a single spray drift application (nominal concentration 44 µg/L) to an experimental ditch (data derived from Crum and Brock, 1994; Brock, 2013). The figure was prepared by the PPR WG. Source: EFSA Scientific Opinion (2015).

In contrast to the assumptions above, the samples for the analytical determination in the *C. riparius* studies with NeemAzal and NeemAzal-T/S were not taken from the top sediment layer but from the whole sediment. Afterwards, the analytically determined concentration was re-calculated to the initially introduced amount (350 g) of sediment into the test system. Therefore only a small amount of azadirachtin A is assumed to have been present in the sample and consequently the measured azadirachtin A concentration was below the LOQ which might easily be misinterpreted as a lack of exposure.
To demonstrate the actual concentration in the sediment when only considering the first 2 mm of the top layer, calculations were performed taking into account the predicted distribution into the sediment based on the K_d value determined for azadirachtin A and NeemAzal (with main component azadirachtin A).

The following formula from OECD Guideline No. 106 (2000) is the basis for the calculations:

$$K_d = \frac{C_s^{ads}\,(eq)}{C_{aq}^{ads}\,(eq)} = \frac{m_s^{ads}\,(eq)}{m_{aq}^{ads}\,(eq)} * \frac{V_0}{m_{soil}} \qquad \left[\frac{mL}{g}\right]$$

With:	K_d	Distribution coefficient [mL/g]
	C_s^{ads} (eq)	Content of substance adsorbed to the soil at adsorption equilibrium [µg/g]
	C_{aq}^{ads} (eq)	Mass concentration of the substance in the aqueous phase at adsorption equilibrium [µg/mL]
	m_s^{ads} (eq)	Mass of substance adsorbed to the soil at adsorption equilibrium [µg]
	m_{aq}^{ads} (eq)	Mass of substance in the solution at adsorption equilibrium [µg]
	m_{soil}	Quantity of the soil phase expressed in dry mass of soil [g]
	V_0	Initial volume of the aqueous phase in contact with soil [mL]

The actual mass of substance which is adsorbed to the soil shall be determined, therefore the formula was converted (details can be found in the appendix). Since no data for m_{aq}^{ads} was available, this term was substituted:

$$m_{aq}^{ads} = m_0 - m_s^{ads}$$

With:	m_0	Initial mass of substance in the aqueous phase (i.e. nominal mass) [µg]

The formula converts to:

$$K_d * m_0 = m_s^{ads} * \left(\frac{V_0}{m_{soil}} + K_d\right)$$

For K_d, the geometric mean value as calculated in Table 5-1 above was inserted, although it is recognized that this value was determined from mixed data on azadirachtin A and NeemAzal and therefore is only used as a surrogate for NeemAzal for this calculation. However, the K_d value for NeemAzal is expected to be even higher, i.e. more NeemAzal will adsorb to the sediment, since the remaining ingredients of the NeemAzal extract have either similar or much higher K_{OC} values (between 28.8 and 7413 L/kg; Mori, 2013).
For m_0 the nominal mass in the test vessels of the NeemAzal study was inserted as a surrogate amounting to 18.4 µg NeemAzal.
The initial water volume in the study was 1000 mL and for m_{soil} 23.3 g were inserted. The value for m_{soil} was calculated from the total amount of soil (350 g) which was distributed into 3 cm depth and the consideration of a realistic distribution of azadirachtin in the first 0.2 cm depth. Therefore, the soil mass of the first 0.2 cm soil amounts to 23.3 g.

The resulting value for m_s^{ads} is 0.669 µg in 23.3 g sediment, i.e. 0.0287 mg NeemAzal/kg sediment. The LOQ of the study was 0.0156 mg NeemAzal/kg sediment. It is therefore concluded that the concentration of NeemAzal would have been determinable in the sediment if only the top layer of the sediment instead of the whole sediment would have been sampled. This would have confirmed the exposure of *C. riparius* larvae, which are mainly located on the sediment, i.e. are in direct contact with the top sediment layer. This proves that exposure of the chironomids was present and consequently, endpoints from the studies should be expressed based on initially measured concentrations instead of geometric mean measured concentrations.

The use of initially measured instead of geometric mean measured concentrations is also supported by the EFSA technical report (2015, chapter 3.1). This report tackles general recurring issues in ecotoxicology and picked up the topic on the basis that '*During the peer review of the active substances, it was highlighted several times that section 2.1.4 of European Commission (2002a) is not sufficiently clear and it is open for different interpretations. Indeed, by analysing assessment reports (DAR/RAR) prepared by a rapporteur Member State (RMS), EFSA noted that no common understanding exists on this part of the guidance.*' and furthermore it is stated that '*There are no specific recommendations in the European Commission (2002a) for studies performed in the presence of sediment (e.g. for sediment dwelling organisms). Relevant OECD test guidelines, such as OECD 218, 219 or 233 prefer to express the endpoint as measured initial, without particular consideration of the potential disappearance of the test item from the system that may occur by the end of the study.*'
The EFSA technical report (2015) concludes that the way to express toxicity endpoints for tier 1 studies should depend on the actual exposure throughout the study. In the case at hand it was demonstrated above that exposure can be expected to occur via sediment and food particles based on the physical/chemical parameters of azadirachtin such as distribution coefficient (K_d), adsorption coefficient (K_{OC}) and partition coefficient (log P_{OW}). However, EFSA (2015) also states that in case test concentrations were not maintained over the test period and significant residues were not present at the end of the exposure period, the validity of the study should be questioned. In the case of the chironomid studies, the problem is that there is no other way to expose the chironomids more realistically. Mixing of the test substance into the sediment would likely result into good recoveries after 28 days, however the exposure is neither realistic nor worst-case, since chironomids will not dig throughout the whole sediment and therefore exposure via the top sediment layer (as it was performed in the studies) represents the realistic exposure. Another option to obtain good recoveries of the test item at the end of the exposure would be a semi-static or flow-through design as it was performed for chronic fish and daphnia studies, however, this is not feasible for sediment-containing test systems and

normally, since these studies are mainly performed for substances that actually persist in the sediment, not required for azadirachtin.

Even in case the geometric mean concentration would have been reasonable to determine the endpoints, EFSA (2015, chapter 3.1) states that the use of half LOQ as foreseen in OECD 23 for difficult substances is not supported in case no concentrations were detected at study end.

6 CONCLUSION

The aim of the *C. riparius* study is the simulation of toxicity caused by singular exposure events like drift and drainage after rainfall to a sediment dwelling organism in the aerobic layer of the sediment. Therefore, the peak exposure promoted by this static test design is fairly representing the toxicological effects to be expected. A direct comparison of the NOEC (based on initial concentrations) of the *Chironomus* test according to OECD 219 versus $PEC_{initial}$ (spray drift) or $PEC_{maximum}$ considering the regulatory assessment factor of 10 is justified for the risk assessment. In addition, the initially measured concentrations better reflect the varying exposure of chironomids during their life cycle. Finally, based on the lack of persistence of azadirachtin and the remaining natural components of NeemAzal extract under test and natural environment conditions, any adjustment of the NOEC based on mean measured concentrations seems to be an unjustified overestimation of the risk.

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