CONCLUSION ON PESTICIDE PEER REVIEW

Conclusion on the peer review of the pesticide risk assessment of the active substance azadirachtin[1]

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SUMMARY

Azadirachtin is one of the 295 substances of the fourth stage of the review programme covered by Commission Regulation (EC) No 2229/2004[3] , as amended by Commission Regulation (EC) No 1095/2007[4] . In accordance with the Regulation, at the request of the Commission of the European Communities (hereafter referred to as 'the Commission'), the EFSA organised a peer review of the initial evaluation, i.e. the Draft Assessment Report (DAR), provided by Germany, being the designated rapporteur Member State (RMS). The peer review process was subsequently terminated following the applicants' decision, in accordance with Article 24e, to withdraw support for the inclusion of azadirachtin in Annex I to Council Directive 91/414/EEC.

Following the Commission Decision of 2008/941/EC[5] concerning the non-inclusion of azadirachtin in Annex I to Council Directive 91/414/EEC and the withdrawal of authorisations for plant protection products containing that substance, the applicants Trifolio-M GmbH, Sipcam S.p.A, and Mitsui AgriScience International S.A/B.V made a resubmission application for the inclusion of azadirachtin in Annex I in accordance with the provisions laid down in Chapter III of Commission Regulation (EC) No. 33/2008[6] . The resubmission dossier included further data in response to the issues identified in the DAR.

In accordance with Article 18 of Commission Regulation (EC) No. 33/2008, Germany, being the designated RMS, submitted an evaluation of the additional data in the format of an Additional Report. The Additional Report was received by the EFSA on 10 December 2009.

In accordance with Article 19 of Commission Regulation (EC) No. 33/2008, the EFSA distributed the Additional Report to Member States and the applicants for comments on 11 December 2009. The EFSA collated and forwarded all comments received to the Commission on 25 January 2010.

In accordance with Article 20, following consideration of the Additional Report, the comments received, and where necessary the DAR, the Commission requested the EFSA to conduct a focused peer review in the areas of mammalian toxicology, residues, fate and behaviour, and ecotoxicology and to deliver its conclusions on azadirachtin.

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[1] On request from the European Commission, Question No EFSA-Q-2010-00134, issued on 11 October 2010.
[2] Correspondence: praper@efsa.europa.eu
[3] OJ L 379, 24.12.2004, p.13
[4] OJ L 246, 21.9.2007, p. 19
[5] OJ L 335, 13.12.2008, p.91
[6] OJ L 15, 18.01.2008, p.5

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The conclusions laid down in this report were reached on the basis of the evaluation of the representative use of azadirachtin as an insecticide on potato, as proposed by the applicants. Full details of the representative use can be found in Appendix A.

Azadirachtin A was the proposed lead substance, however this was not accepted and the content of the total biologically active extract is not yet defined.

A general data gap is identified for data in the area of identity, physical/chemical/technical properties and methods of analysis for the other biologically active components, as the original proposal that azadirachtin A is the lead compound was not accepted by the experts' meeting on mammalian toxicology (see section 2). Pending on the residue definitions in all compartments, data gaps might be identified for monitoring analytical methods.

Data gaps were identified in the toxicology section to address the toxicological equivalence of the Mitsui source (ATI 720) to the Trifolio-M (Neem Azal) and Sipcam (Fortune Aza) sources, therefore no reference values could be set for the Mitsui source. Insufficient information is available on the batches used in the toxicological studies conducted with the Mitsui source to conclude if they are representative of the respective technical specification. Regarding the specification, the relevance of the impurities/by-products of azadirachtin extracts from the three sources is not addressed (except for the aflatoxins, which are known relevant impurities).

There is no conclusion in the residues area. The nature of residues in plants is unknown and a critical area of concern is identified.

Data gaps for all or some of the known active components of azadirachtin extract and its metabolites have been identified for all the environmental compartments. A critical area of concern has been identified since contamination of groundwater above the regulatory limits cannot be excluded. Degradation of the polycyclic structure common to all known active components of azadirachtin in the environment has not been demonstrated.

Azadirachtin is very toxic to aquatic organisms. The risk to aquatic organisms was assessed as low for the majority of FOCUS scenarios. The risk assessment is not finalised for the run-off scenario R1 (stream). An initial impact on populations of sensitive arthropod species can be expected based on the observations in laboratory studies. However higher tier data suggest that recolonisation of the in-field area is possible within one year. An in-field no-spray buffer zone of 5m was suggested to protect sensitive arthropod populations in the off-field area. The risk to soil-dwelling organisms was assessed as low for the azadirachtin extract and for azadirachtin A. However the risk assessment for the individual compounds of the extract and potential degradation products could not be finalised. The risk to birds and mammals, bees, non-target plants and biological methods of sewage treatment was assessed as low.

KEY WORDS

Azadirachtin, peer review, risk assessment, pesticide, insecticide.

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BACKGROUND

Legislative framework

Commission Regulation (EC) No 2229/2004[7], as amended by Commission Regulation (EC) No 1095/2007[8] , lays down the detailed rules for the implementation of the fourth stage of the work programme referred to in Article 8(2) of Council Directive 91/414/EEC. This regulates for the European Food Safety Authority (EFSA) the procedure for organising, upon request of the Commission of the European Communities (hereafter referred to as 'the Commission'), a peer review of the initial evaluation, i.e. the Draft Assessment Report (DAR), provided by the designated rapporteur Member State.

Commission Regulation (EC) No 33/2008[9] lays down the detailed rules for the application of Council Directive 91/414/EEC for a regular and accelerated procedure for the assessment of active substances which were part of the programme of work referred to in Article 8(2) of Council Directive 91/414/EEC but which were not included in Annex I. This regulates for the EFSA the procedure for organising the consultation of Member States and the applicant(s) for comments on the Additional Report provided by the designated RMS, and upon request of the Commission the organisation of a peer review and/or delivery of its conclusions on the active substance.

Peer review conducted in accordance with Commission Regulation (EC) No 2229/2004

Azadirachtin is one of the 295 substances of the fourth stage of the review programme covered by Commission Regulation (EC) No 2229/2004, as amended by Commission Regulation (EC) No 1095/2007. In accordance with the Regulation, at the request of the Commission, the EFSA organised a peer review of the DAR provided by the designated rapporteur Member State, Germany, which was received by the EFSA on 4 January 2008 (Germany, 2007).

The peer review was initiated on 18 February 2008 by dispatching the DAR to Member States and the applicants Trifolio-M GmbH, Sipcam S.p.A, and Mitsui AgriScience International S.A/B.V for consultation and comments.

The peer review process was subsequently terminated following the applicants' decision, in accordance with Article 24e, to withdraw support for the inclusion of azadirachtin in Annex I to Council Directive 91/414/EEC.

Peer review conducted in accordance with Commission Regulation (EC) No 33/2008

Following the Commission Decision of 2008/941/EC[10] concerning the non-inclusion of azadirachtin in Annex I to Council Directive 91/414/EEC and the withdrawal of authorisations for plant protection products containing that substance, the applicants Trifolio-M GmbH, Sipcam S.p.A, and Mitsui AgriScience International S.A/B.V made a resubmission application for the inclusion of azadirachtin in Annex I in accordance with the provisions laid down in Chapter III of Commission Regulation (EC) No. 33/2008. The resubmission dossier included further data in response to the issues identified in the DAR.

In accordance with Article 18, Germany, being the designated RMS, submitted an evaluation of the additional data in the format of an Additional Report. The Additional Report was received by the EFSA on 10 December 2009 (Germany, 2009).

In accordance with Article 19, the EFSA distributed the Additional Report to Member States and the applicants for comments on 11 December 2009. In addition, the EFSA conducted a public consultation

[7] OJ L 379, 24.12.2004, p.13
[8] OJ L 246, 21.9.2007, p.19
[9] OJ L 15, 18.01.2008, p.5
[10] OJ L 335, 13.12.2008, p.91

on the Additional Report and the DAR. The EFSA collated and forwarded all comments received to the Commission on 25 January 2009. At the same time, the collated comments on both the DAR and the Additional Report were forwarded to the RMS for compilation in the format of a Reporting Table. The applicants were invited to respond to the comments in column 3 of the Reporting Table. The comments and the applicants' response were evaluated by the RMS in column 3.

In accordance with Article 20, following consideration of the Additional Report, the comments received, and where necessary the DAR, the Commission decided to further consult the EFSA. By written request, received by the EFSA on 22 February 2010, the Commission requested the EFSA to arrange a consultation with Member State experts as appropriate and deliver its conclusions on azadirachtin within 6 months of the date of receipt of the request, subject to an extension of a maximum of 90 days where further information were required to be submitted by the applicants in accordance with Article 20(2).

The scope of the peer review and the necessity for additional information, not concerning new studies, to be submitted by the applicants in accordance with Article 20(2), was considered in a telephone conference between the EFSA, the RMS, and the Commission on 23 February 2010; the applicants were also invited to give their view on the need for additional information. On the basis of the comments received, the applicants' response to the comments, and the RMS' subsequent evaluation thereof, it was concluded that the EFSA should organise a consultation with Member State experts in the areas of mammalian toxicology, residues, fate and behaviour, and ecotoxicology and that further information should be requested from the applicants in the areas of physical chemical properties and fate and behaviour.

The outcome of the telephone conference, together with EFSA's further consideration of the comments is reflected in the conclusions set out in column 4 of the Reporting Table. All points that were identified as unresolved at the end of the comment evaluation phase and which required further consideration, including those issues to be considered in consultation with Member State experts, and the additional information to be submitted by the applicants, were compiled by the EFSA in the format of an Evaluation Table.

The conclusions arising from the consideration by the EFSA, and as appropriate by the RMS, of the points identified in the Evaluation Table, together with the outcome of the expert discussions where these took place, were reported in the final column of the Evaluation Table.

A final consultation on the conclusions arising from the peer review of the risk assessment took place with Member States via a written procedure in September 2010.

This conclusion report summarises the outcome of the peer review of the risk assessment on the active substance and the representative formulation evaluated on the basis of the representative uses as an insecticide and acaricide on potato, as proposed by the applicants. A list of the relevant end points for the active substance as well as the formulation is provided in Appendix A. In addition, a key supporting document to this conclusion is the Peer Review Report, which is a compilation of the documentation developed to evaluate and address all issues raised in the peer review, from the initial commenting phase to the conclusion. The Peer Review Report (EFSA, 2010) comprises the following documents:

- the comments received,
- the Reporting Table (revision rev 1-1; 25 February 2010),
- the Evaluation Table (11 October 2010),
- the reports of the scientific consultation with Member State experts (where relevant).

Given the importance of the DAR and the Additional Report including its addendum (compiled version of August 2010 containing all individually submitted addenda (Germany, 2010)) and the Peer Review Report, both documents are considered respectively as background documents A and B to this conclusion.

THE ACTIVE SUBSTANCE AND THE FORMULATED PRODUCT

Azadirachtin is a common name for an extract from seed kernels of the tropical neem tree *Azadirachta indica*. Azadirachtin A was proposed as the lead substance, however this was not accepted by the experts' meeting on mammalian toxicology (see section 2). There is no ISO common name for this extract. Azadirachtin A is a common name for dimethyl (2a*R*,3*S*,4*S*,4a*R*,5*S*,7a*S*,8*S*,10*R*,10a*S*,10b*R*)-10-acetoxy-3,5-dihydroxy-4-[(1a*R*,2*S*,3a*S*,6a*S*,7*S*,7a*S*)-6a-hydroxy-7a-methyl-3a,6a,7,7a-tetrahydro-2,7-methanofuro[2,3-*b*]oxireno[*e*]oxepin-1a(2*H*)-yl]-4-methyl-8-{[(2*E*)-2-methylbut-2-enoyl]oxy}octahydro-1*H*-naphtho[1,8*a*-*c*:4,5-*b'c'*]difuran-5,10a(8*H*)-dicarboxylate (IUPAC).

The representative formulated products for the evaluation were 'NeemAzal-T/S', and 'Oikos' both emulsifiable concentrates (EC) containing 10 g/l and 26 g/l of azadirachtin A, respectively, although the content of the total biologically active extract is not yet defined.

The representative use evaluated comprises application by spraying to control Colorado beetle on potato. Full details of the GAP can be found in the list of end points in Appendix A. It should be emphasized however, that the application rate is expressed on the basis of azadirachtin A content only, and the application rate of the biologically active components of the extract is not yet defined.

CONCLUSIONS OF THE EVALUATION

1. Identity, physical/chemical/technical properties and methods of analysis

Besides azadirachtin A, azadirachtin contains other compounds that also have biological activity. It is concluded by EFSA that azadirachtin A is not a sufficient marker to identify the different materials. It should be emphasized that the manufacturing process has a strong influence on the composition of the technical concentrate (TK) and it is necessary to link the specification of the technical concentrates to their respective manufacturing processes. As the three technical concentrates are not chemically equivalent, and there is a significant difference in the azadirachtin A content of the Trifolio-M source compared to the Mitsui and Sipcam sources, it is proposed to consider the active substance as the sum of all biologically active identified compounds in the specification. A concentration range should be proposed accordingly. On this basis, a general data gap is identified for data in the area of identity, physical/chemical/technical properties and methods of analysis for the other biologically active components. .

The azadirachtin A content of the technical concentrates are 250 – 500 g/kg (Trifolio-M), 120 – 180 g/kg (Mitsui) and 111 – 180 g/kg (Sipcam). The azadirachtin A content in the FAO specification 627/TK (May 2006), applicable to materials from Trifolio-M and EID Parry, is above 250 g/kg up to 500 g/kg and the content of aflatoxins (sum of aflatoxins B_1, B_2, G_1, and G_2) is maximum 0.00003% (300 μg/kg) of the azadirachtin A content. All three technical concentrates meet the requirements of the aflatoxins content of the FAO specification.

The assessment of the data package revealed no issues that need to be included as critical areas of concern with respect to the identity and technical properties of azadirachtin or the respective formulations. However, as stated above, there is a general data gap with respect to the other biologically active components of the extract. The main data regarding the identity of azadirachtin A and its physical and chemical properties are given in Appendix A.

Analytical methods are available for the determination of azadirachtin A and the relevant impurities in the technical concentrates and in the representative formulations. It should be noted that CIPAC methods also exist for the determination of azadirachtin A in the TK and EC formulations. Analytical

methods are available for the determination of residues of azadirachtin A in food of plant origin and in the environmental matrices. No methods are available for food of animal origin. As the residue definitions are not concluded on in any of the compartments, pending on the final residue definitions, data gaps might be identified for enforcement analytical methods. Analytical methods for residues in body fluids and tissues are not required since the neem extract is not classified as toxic or very toxic.

2. Mammalian toxicity

Azadirachtin was discussed at the PRAPeR Experts' Meeting on mammalian toxicology (PRAPeR 79). The batches used in the toxicological studies performed with the Trifolio-M source are within the range of the technical specification proposed for this extract (Neem Azal), however, it is noted that some uncertainty remains as to whether the whole range of the specification would be covered. Insufficient information is available to conclude on the technical specification for the Mitsui (ATI 720) source, and a data gap was identified for information on the composition of the batches used in the toxicological studies conducted with this source. The Trifolio-M and Sipcam sources are toxicologically equivalent, however equivalence cannot be established based on the azadirachtin A compound. A data gap was identified to conclude on the toxicological equivalence of the Mitsui extract with the other two sources. Regarding the specification, with the exception of the aflatoxins, which are known relevant impurities, the relevance of the other impurities/by-products could not be established, and a data gap was identified.

There is no information on bioavailability as no study could be performed on toxicokinetics and metabolism with azadirachtin. The three sources of azadirachtin extract present low acute toxicity when administered either by the oral, dermal or inhalation routes, they are not skin or eye irritants, but a potential for skin sensitisation is observed with the three sources. Upon short-term exposure the liver is the main target organ, the relevant NOAEL is 32 mg/kg bw/day; the three sources presented similar NOAEL values. The two long-term studies submitted are not adequate to conclude on the long-term toxicity or carcinogenicity. The three extracts were clastogenic *in vitro* in chromosomal aberration tests in cultured human lymphocytes. *In vivo* studies with the Trifolio-M and Sipcam extracts did not confirm these positive results and no potential for genotoxicity *in vivo* is attributed to these two extracts of azadirachtin; as no *in vivo* study was submitted with the Mitsui source, no conclusion could be reached on this extract and this was identified as a data gap. Fertility and reproductive performance were not impaired by azadirachtin in a valid multigeneration study in rat; reproductive effects observed in humans in the open literature are not relevant to this dossier as the raw material and extraction type are not comparable between the different extracts (the open literature reports on oily extracts or different parts of neem tree other than neem seed kernel). No developmental effects were observed in rats with the Trifolio-M and Sipcam sources; the developmental study provided with the Mitsui source could not be included in the overall assessment as toxicological equivalence was not established between the three sources. No neurotoxic potential is attributed to azadirachtin.

The acceptable daily intake (ADI) of azadirachtin extracts is 0.1 mg/kg bw/day, based on the 90-day study in rat, applying a safety factor of 300 - an additional safety factor of 3 due to the missing toxicological information on long-term, carcinogenicity and rabbit developmental study. The acceptable operator exposure level (AOEL) is 0.1 mg/kg bw/day based on the 90-day rat study, applying the same safety factor of 300 considering the missing information on the bioavailability and rabbit developmental study. The acute reference dose (ARfD) is 0.75 mg/kg bw based on the developmental study in rat with a maternal NOAEL of 225 mg/kg bw/day, and applying a safety factor of 300 due to the missing rabbit developmental study. The reference values are expressed in terms of whole extract and not in terms of the azadirachtin A compound, and they apply to the Trifolio-M and Sipcam extracts, but not to the Mitsui source.

The exposure risk assessment is based on the assumption that the 'Neem Azal T/S' formulation is to be applied at amounts of 2.5 L/ha corresponding to amounts of 25 g Azadirachtin A/ha, which would correspond to about 75 g/ha Neem Azal technical (Trifolio-M source). The 'Oikos' formulation is to be applied at amounts corresponding to 25 g azadirachtin A/ha (1 L/ha) corresponding to about 250 g/ha Fortune Aza technical (Sipcam source). However, these values do not cover the whole range of

concentrations stated in the technical specifications, and therefore this approach introduces further uncertainty.

According to these assumptions, the estimated operator exposure is below the AOEL when no personal protective equipment (PPE) is worn for both the 'Neem Azal T/S' and 'Oikos' formulations according to the German model for field crop, tractor-mounted applications, and according to the UK POEM for home garden sprayers. Worker exposure was estimated to remain below the AOEL for both formulations without the use of PPE. Bystander exposure was considered negligible.

3. Residues

The issue of plant metabolism data was raised in the commenting period by both EFSA and a Member State. An expert teleconference discussion was held in PRAPeR TC 33, where it was agreed that the nature of the residue in plants had not been elucidated. It was agreed that without further data no conclusion can be drawn. On this basis a valid risk assessment cannot be conducted and a critical area of concern is identified. Subject to the data gap for elucidation of the relevant residue in plants, all of the other data in the residues area will have to be re-assessed.

4. Environmental fate and behaviour

There is only one study available that provides some information on the route of degradation of some of the components of azadirachtin extracts in soil, performed with non-radiolabelled material containing AzA, AzB, and component 2.2 listed in section C.1.1.2.1 of Volume 4 of the DAR (and other azadirachtins not relevant for the specifications of the products examined) as identified components. In this study, the only degradation proven is that resulting from the hydrolysis of acetyl at C3 group in AzA (to yield major metabolite Azadirachtin H* [max 63 %]). A minor route of transformation produces azadirachtin by internal cyclisation. None of the products identified show any major transformation on the polycyclic structure of azadirachtin and therefore all known degradation products may be presumed to retain, at least in part, the biological properties attributed to this family of compounds. A data gap has been identified for further investigation of the route of degradation of the azadirachtin extract active components to at least demonstrate that the polycyclic structure, common to all the active components, is broken down in soil under environmental conditions.

Sufficient information is available on the rate of degradation of azadirachtin A in soil under aerobic conditions (six soils). Under these conditions azadirachtin A exhibits low to moderate persistence. Information on the rate of degradation of azadirachtin B in soil under aerobic conditions is only available for three soils. Azadiractin B exhibits low to moderate persistence in these experiments (on average slightly more persistent that azadirachtin A). Information on the rate of degradation of the major metabolite azadirachtin H* is not sufficient to conclude on its persistence. Formation and degradation of this metabolite in soil has only been investigated in one soil. A data gap was identified to investigate the formation and degradation of this metabolite in at least two additional soils.

No data are available on the route and rate of degradation of all the other known active components of azadirachtin extract: components 2.2, 3, 4, 5, and 6 listed in section C.1.1.2.1 of Volume 4 of the DAR. It should be noted that, for example, component 6 could be applied at levels close to 20 g / ha with products in compliance with the specifications and according to the representative GAP. A data gap was therefore identified to address the rate of degradation of these other components in soil. No data on the degradation of azadirachtin extract components in soil under anaerobic conditions are available. These data are not deemed necessary to assess the representative use on potato. No data on the photolytic degradation of the azadirachtin extract active components in soil are available, and a data gap was identified.

Sufficient data on the adsorption/desorption of azadirachtin A in soil are available. The study performed to derive Freundlich behaviour used only three concentrations. Therefore, all available adsorption/desorption data have been retained to derive a Koc to be used in the exposure assessment. According to these data azadirachtin A may be classified as exhibiting low to very high mobility. The

adsorption desorption end points for azadirachtin A may be used in the exposure assessment for azadirachtin B and component 2.2 listed in section C.1.1.2.1 of Volume 4 of the DAR, but not in the exposure assessment for metabolite azadirachtin H*,which is envisaged to be more mobile. A data gap was identified for a soil batch adsorption/desorption study with metabolite azadirachtin H* in at least three soils. No information is available for the rest of the azadirachtin extract components, and therefore a data gap was identified to address the mobility in soil of the azadirachtin extract active components: components 3, 4, 5 and 6 listed in section C.1.1.2.1 of Volume 4 of the DAR.

Both azadirachtin A and B hydrolyse in water (likely to form the C3 hydroxyl derivative, azadirachtin H*) at environmental pHs (pH 4-8). Hydrolysis is faster at more alkaline pHs. No information is available on the hydrolysis of the other known active azadirachtin extract components, and therefore a data gap was identified. Similarly, no experimental information is available on the aqueous photolysis of any of the known active components of azadirachtin extract, and therefore a data gap was identified.

No guideline water/sediment study is available for the azadirachtin extract active components. A natural water degradation study is available for azadirachtin A and azadirachtin B, and an outdoor study with a water/forest sediment system is available for azadirachtin A. The degradation half-life in the water system was used as the end point to represent dissipation from water in the calculation of PEC_{sw} for azadirachtin A. These calculations were done following the FOCUS SW (FOCUS 2001)[11] scheme up to step 4 assuming mitigation of spray drift and run-off. According to the EFSA PPR panel opinion, vegetative buffer strips may not be effective to mitigate run-off of mobile substances such as azadirachtin A (FOCUS 2007). No data on the fate and behaviour of the other known active components of azadirachtin extract are available, and no PEC_{sw} were derived for these other components. Since the relationship between azadirachtin A and the other azadirachtin extract components is not fully known, it is only possible to extrapolate the exposure assessment performed for azadirachtin A to the whole azadirachtin when the peak concentration is the initial one resulting for the spray drift event. Neither data nor assessments are available for potential metabolites. In particular, neither data nor an aquatic exposure assessment are available for the major soil metabolite azadirachtin H* (likely to be also produced by hydrolysis in water). A data gap was identified to address the water exposure assessment to the known active components of azadirachtin extract other than azadirachtin A and their environmental metabolites, in particular for major soil metabolite azadirachtin H*.

The potential for groundwater contamination by azadirachtin A was addressed by standard FOCUS GW calculations (FOCUS, 2000)[12] with the PEARL and PELMO models. The limit of 0.1 µg / L is not exceeded for any of the simulated scenarios. Potential groundwater contamination by the major soil metabolite azadirachtin H* has only been preliminarily assessed by the RMS on the basis of a single soil half-life and an assumed K_{oc} = 10 mL/g with FOCUS GW (using PEARL and PELMO). The values obtained do not enable the potential for leaching to be excluded and confirm the need for further data to finalize the groundwater exposure assessment for this metabolite. Potential groundwater contamination by the other known active components of azadirachtin and its metabolites has not been assessed, and therefore a data gap was identified. Taking into account the active substance definition, it is not clear to EFSA if the parametric drinking water limit of 0.1 µg / L applies to each of the individual active components or to the sum of all of them. In the case that it is decided that 0.1 µg / L is applicable to the individual components, then Council Directive 98/83/EC[13] prescribes that the limit of 0.5 µg / L would need to be taken into consideration for the sum of all the active components.

[11] Simulations utilised a Q10 of 2.58 (EFSA 2007) and Walker Equation coefficient of 0.7
[12] Simulations complied with EFSA (2004) and utilised a Q10 of 2.58 (EFSA 2007) and Walker Equation coefficient of 0.7
[13] Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption, OJ L 330 5.12, 1998. p.32

5. Ecotoxicology

Azadirachtin was discussed in the PRAPeR 77 ecotoxicology experts' meeting in June 2010.

A data gap was identified by EFSA after the experts' meeting to assess the compliance of the batches used in the ecotoxicology tests with the technical specification of azadirachtin. The RMS does not support this data gap. The RMS is of the opinion that since azadirachtin is a plant extract and the concentration of the individual components will vary from year to year it is not possible to have an analytically clearly defined batch and azadirachtin A should be used as an indicative marker of the ecotoxicological profile. EFSA is of the opinion that the current risk assessment should be regarded as provisional until it has been firmly established that azadirachtin A can be used as an indicative marker of the ecotoxicological profile of the whole extract.

The acute and long-term risk to birds and mammals was assessed as low in a first-tier risk assessment according to SANCO/4145/2000 (European Commission, 2002). The long-term reproduction endpoint from the 2-generation rat study (NOEL = 13.7 mg azadirachtin A/kg bw/d) was used in the original risk assessment for mammals. This was questioned during the peer-review since a lower endpoint was observed in a teratogenicity study. In the meeting of experts (PRAPeR 77) it was decided that the lower endpoint from the teratogenicity study (NOEL = 8.3 mg azadirachtin A/kg bw/d) should be used in the risk assessment. The re-calculated TERs were well above the Annex VI trigger of 5. The risk was assessed according to SANCO/4145/2000 (European Commission, 2002) based on a medium herbivorous mammal. It was noted in the meeting that shrews may also be found in potato fields. It can be expected that TERs for shrews would exceed the Annex VI trigger and hence are covered by the available risk assessment. Overall the risk to birds and mammals is expected to be low for the representative use evaluated.

Azadirachtin was very toxic to aquatic organisms. The lowest endpoints were observed for fish (acute LC_{50} = 0.048 mg azadirachtin A/L, chronic NOEC = 0.0047 mg azadirachtin A/L) and aquatic insects (chronic NOEC = 0.0016 mg azadirachtin A/L). The TERs exceeded the Annex VI trigger for all $FOCUS_{sw}$ step 3 scenarios except the part scenario R1 stream. A $FOCUS_{sw}$ step 4 calculation including a 10 m no-spray buffer zone resulted in a TER of 15 for the part scenario R1 stream. Vegetative buffer strips may not be effective to mitigate run-off of mobile substances such as azadirachtin A (see section 4). Overall it was concluded that the risk from azadarachtin A to the aquatic environment was low, except for the part scenario R1 stream, for which risk mitigation was suggested. The RMS disagreed with the EFSA view that the aquatic risk assessment is not finalised for the other compounds of the extract. EFSA acknowledges that the studies with aquatic organisms were conducted with the different extracts and therefore the aquatic risk assessment can be finalised for FOCUS scenarios where the initial peak concentrations are from entry via spray drift. EFSA has calculated FOCUS step3 PECsw and TERs for the different extracts for the scenarios D3 (ditch), D4 (pond), D4 (stream). The TERs for the most sensitive organism *Chironomus riparius* exceeded the trigger of 10 for all three extracts for all scenarios except for the extract Azatin (Mitsui) where a TER of <10 was observed in D3 (ditch). EFSA maintains its position that the risk assessment is not finalised for the run-off scenario R1 (stream) since no information is available on the degradation pattern and ecotoxicity of the individual compounds of the extract. However, overall it is concluded that the risk to aquatic organisms was demonstrated to be low in the majority of the scenarios for the extracts Fortune Aza (Sipcam) and Neem Azal (Trifolio).

The risk assessment for non-target arthropods was discussed in the expert meeting (PRAPeR 77). The risk was assessed as low for *Typhlodromus pyri, Aphidius rhopalosiphi* and *Poecilus cupreus. Coccinella septempunctata* and *Chrysoperla carnea* were clearly more sensitive, and an initial impact on populations of sensitive arthropod species can be expected based on the observations in laboratory studies. However, higher tier data suggest that recolonisation of the in-field area is possible within one year. An in-field no-spray buffer zone of 5m is required to protect sensitive arthropod populations in the off-field area.

The Member State experts discussed whether the effects of potential degradation products of azadirachtin would be covered by the study with the soil-dwelling mite *Hypoaspis aculeifer*. The mites were exposed for 14 days to fresh treated soil (9794 mg NeemAzal/kg soil corresponding to 3000 mg azadirachtin A/kg soil) and after ageing of the treated soil. Significant adverse effects were observed after exposure to fresh residues and after 2 days of ageing, but no adverse effects on mortality or reproduction were observed after 7 days of ageing of residues. The experts considered it likely that degradation products were present after ageing of residues and that the residues would not pose a high risk to soil-dwelling mites. Uncertainty remains since no measurements of residues were performed. However, since the tested concentrations were more than 4 orders of magnitude greater than the initial PECsoil a large margin of safety is indicated and the risk to soil-dwelling mites was considered to be low.

The risk to earthworms and soil macro-organisms from azadirachtin A and the extracts was assessed as low on the basis of initial PEC_{soil} values. However, no information was available for the individual compounds of the extract or degradation products, which adds uncertainty to the outcome of the risk assessment on a long-term time scale. The study with soil-dwelling mites gave an indication that ageing of residues would not lead to an increase of the risk. Overall it is considered as unlikely that the risk to soil-dwelling organisms would be high. Information on the fate and behaviour and toxicity of the individual compounds is needed to confirm the risk assessment for soil-dwelling organisms and to finalise the relevance assessment of metabolites. This need for further information was not agreed by the RMS.

The risk to bees, soil micro-organisms, non-target plants and biological methods of sewage treatment was assessed as low.

6. Overview of the risk assessment of compounds listed in residue definitions triggering assessment of effects data for the environmental compartments

6.1. Soil

Compound (name and/or code)	Persistence	Ecotoxicology
Azadirachtin A	Low to moderate ($DT_{50\ 20°C}$ = 1.7 – 25 d)	The risk to earthworms, soil-dwelling micro- and macro-organisms was assessed as low.
Azadirachtin B	Low to moderate ($DT_{50\ 20°C}$ = 5.9 – 34 d)	No data available. Data Gap.
Components 2.1 and 2.2 listed in section C.1.1.2.1 of Volume 4 of the DAR [in principle only for Trifolio-M source according the latest specifications].	No data available	No data available. Data Gap.
Component 3 listed in section C.1.1.2.1 of Volume 4 of the DAR	No data available	No data available. Data Gap.
Component 4 listed in section C.1.1.2.1 of Volume 4 of the DAR	No data available	No data available. Data Gap.
Component 5 listed in section C.1.1.2.1 of Volume 4 of the DAR	No data available	No data available. Data Gap.
Component 6 listed in section C.1.1.2.1 of Volume 4 of the DAR	No data available	No data available. Data Gap.
Azadirachtin H*	Available data are not sufficient to finalize the risk assessment.	No data available. Data Gap.
Other components and metabolites.	No data available	No data available. Data Gap.

The active substance or its components are given in bold.

6.2. Groundwater

Compound (name and/or code)	Mobility in soil	>0.1 µg/L 1m depth for the representative uses (at least one FOCUS scenario or relevant lysimeter)	Pesticidal activity	Toxicological relevance	Ecotoxicological activity
Azadirachtin (whole) extract	Not possible to assess as the known components only cover up to a maximum of 71 % for Trifolio-M (Neem Aza) and only up to 35-37 % of Mitsui (ATI 720) and Sipcam products (Fortune Aza). The available Information for the known components is not complete.	Due to the limited information available for some of the components, it cannot be excluded that the sum of active components found in azadirachtin extract exceeds 0.1 µg/L or 0.5 µg/L**.** It is not possible to assess how the amounts of individual components that could be found in groundwater have to be related to the toxicological end points that are set on the basis of the bulk material. Data gap.	Yes	Yes	Very toxic to aquatic organisms. No risk assessment was conducted for situations where groundwater becomes surface water.
Azadirachtin A	low to very high mobile (K_{oc} = 20.6 – 875.1 mL/g)	FOCUS GW: no	Yes	No data available; not possible to assess the toxicity of azadirachtin A *per se*	Very toxic to aquatic organisms. No risk assessment was conducted for situations where groundwater becomes surface water. No further assessment needed because of the expected low concentrations in groundwater.

Azadirachtin B	No information available. Azadirachtin A adsorption/desorption end points are considered applicable to azadirachtin B	No data available. Data gap.	No data available. Data gap.	No data available; not possible to assess the toxicity of azadirachtin B *per se*	No data available. Data gap.
Components 2.1 and 2.2 listed in section C.1.1.2.1 of Volume 4 of the DAR [in principle only for Trifolio-M source according to the latest specifications].	No information available Azadirachtin A adsorption/desorption end points are considered applicable to components 2.1 and 2.2.	No data available. Data gap.	No data available. Data gap.	No data available; not possible to assess the toxicity of components 2.1 and 2.2. *per se*	No data available. Data gap.
Component 3 listed in section C.1.1.2.1 of Volume 4 of the DAR	No information available. Data gap identified.	No data available. Data gap.	No data available. Data gap.	No data available; not possible to assess the toxicity of component 3 *per se*	No data available. Data gap.
Component 4 listed in section C.1.1.2.1 of Volume 4 of the DAR	No information available Data gap identified.	No data available. Data gap.	No data available. Data gap..	No data available; not possible to assess the toxicity of component 4 *per se*	No data available. Data gap.
Component 5 listed in section C.1.1.2.1 of Volume 4 of the DAR	No information available Data gap identified.	No data available. Data gap.	No data available. Data gap.	No data available; not possible to assess the toxicity of component 5 *per se*	No data available. Data gap.
Component 6 listed in section C.1.1.2.1 of Volume 4 of the DAR	No information available Data gap identified.	No data available. Data gap.	No data available. Data gap.	No data available; not possible to assess the toxicity of component 6 *per se*	No data available. Data gap.

Azadirachtin H*	No information available Data gap identified. Assumed to be more mobile than azadirachtin A. Koc = 10 mL/g has been used as default for a preliminary assessment.	Preliminary data available. Data gap. The FOCUS preliminary assessment shows that groundwater concentrations are not expected to be negligible and that safe use may not be presumed without further data.	No data available. Data gap.	No data available; not possible to assess the toxicity of azadirachtin H* *per se*	No data available. Data gap.
Other components and metabolites.	No information available	No data available. Data gap.	No data available. Data gap.	No data available	No data available. Data gap.

The active substance or its components are given in bold.

6.3. Surface water and sediment

Compound (name and/or code)	**Ecotoxicology**
Azadirachtin A	Very toxic to aquatic organisms (fish LC_{50} = 0.048 mg azadirachtin A/L and aquatic insects chronic NOEC = 0.0016 mg azadirachtin A/L). The lowest TERs were above the Annex VI triggers for 2 out of 3 full FOCUS step 3 scenarios. One part scenario (R1 stream) needed risk mitigation comparable to a 10m no-spray buffer zone.
Azadirachtin B	No studies with azadirachtin B were submitted. Data gap.
Components 2.1 and 2.2 listed in section C.1.1.2.1 of Volume 4 of the DAR [in principle only for Trifolio-M source according to the latest specifications].	No studies with components 2.1 and 2.2 were submitted. Data gap.
Component 3 listed in section C.1.1.2.1 of Volume 4 of the DAR	No studies with component 3 were submitted. Data gap.

Component 4 listed in section C.1.1.2.1 of Volume 4 of the DAR	No studies with component 4 were submitted. Data gap.
Component 5 listed in section C.1.1.2.1 of Volume 4 of the DAR	No studies with component 5 were submitted. Data gap.
Component 6 listed in section C.1.1.2.1 of Volume 4 of the DAR	No studies with component 6 were submitted. Data gap.
Azadirachtin H* (from soil)	No studies with azadirachtin H* were submitted. Data gap.
Other components and metabolites.	No studies with other components and metabolites were submitted. Data gap.

The active substance or its components are given in bold.

6.4. Air

Compound (name and/or code)	**Toxicology**
Azadirachtin extract active components (No conversion factor from azadiracthin A (used as a marker for analytical purposes) and the other components to the bulk azadirachtin extract is available. Such a conversion factor would need to consider the different specifications proposed for the different technical materials.)	Rat LC_{50} inhalation > 0.72 mg Trifolio-M extract/L air (4 h, whole body) – no classification proposed Rat LC_{50} inhalation > 2.45 mg Sipcam extract/L air (4 h, whole body) – no classification proposed

LIST OF STUDIES TO BE GENERATED, STILL ONGOING OR AVAILABLE BUT NOT PEER REVIEWED

- For each source it is necessary to identify fingerprint compounds so that the identity of the active substance can be verified (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see section 1).

- Data on the other biologically active components of the neem extract in the area of identity, physical/chemical/technical properties and methods of analysis (relevant for all applicants and representative uses evaluated; submission date proposed by the applicants: unknown; see section 1).

- Information on the composition of the batches used in the toxicological studies conducted with the Mitsui source (relevant for all representative uses evaluated with the Mitsui source; submission date proposed by the applicant: unknown; see section 2).

- Toxicological information to assess the equivalence of the Mitsui source to the other two extracts (Trifolio-M and Sipcam sources), which would mean at least an *in vivo* genotoxicity study with this extract (relevant for all representative uses evaluated with the Mitsui source; submission date proposed by the applicant: unknown; see section 2).

- Information on the toxicological profile/relevance of the different components/impurities/by-products present in the technical specification of the three azadirachtin extracts (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see section 2).

- Elucidate the nature of the residue in plants from the application of the neem extracts (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see section 3).

- Further investigation of the route and rate of degradation of the azadirachtin extract active components to identify other potential major metabolites and to at least demonstrate that the polycyclic structure, common to all the active components, is broken down in soil under environmental conditions (relevant for all representative uses; submission date proposed by the applicant: unknown; see section 4).

- The formation and degradation of azadirachtin H* (major soil metabolite product of desacetylation of azadirachtin A) to be investigated in two additional soils (relevant for all representative uses; submission date proposed by the applicant: unknown; see section 4).

- Information on the photolysis of the azadirachtin extract active components in soil (relevant for all representative uses; submission date proposed by the applicant: unknown; see section 4).

- Adsorption/desorption study in at least three soils with major soil metabolite azadirachtin H* (relevant for all representative uses; submission date proposed by the applicant: unknown; see section 4).

- The mobility in soil of the azadirachtin extract active components: components 3, 4, 5 and 6 listed in section C.1.1.2.1 of Volume 4 of the DAR to be addressed (relevant for all representative uses; submission date proposed by the applicant: unknown; see section 4).

- The aqueous hydrolysis of the azadirachtin extract active components: components 2.2, 3, 4, 5 and 6 listed in section C.1.1.2.1 of Volume 4 of the DAR to be addressed (relevant for all representative uses; submission date proposed by the applicant: unknown; see section 4).

- The aqueous photolysis of the azadirachtin extract active components to be addressed (relevant for all representative uses; submission date proposed by the applicant: unknown; see section 4).

- Water exposure assessment for the known active components of azadirachtin extract, other than azadirachtin A, and their environmental metabolites, in particular for major soil metabolite azadirachtin H* (relevant for all representative uses; submission date proposed by the applicant: unknown; see section 4).

- Groundwater exposure assessment for the known active components of azadirachtin extract and their metabolites (including azadirachtin H*), including an assessment of pesticidal activity and ecotoxicological activity to finalise the groundwater metabolite relevance assessment (relevant for all representative uses; submission date proposed by the applicant: unknown; see section 4).

- In order that azadirachtin A can be considered the lead compound in the environmental risk assessment, the relationship between azadirachtin A and the rest of the active components in the neem extract technical material needs to be established. This relationship (with respect to amount, biological activity and persistence) is needed to bridge the results and assessment obtained on the basis of azadirachtin A to the whole active substance (relevant for all the representative uses; submission date proposed by the applicant: unknown; see sections 4 and 5).

- An assessment of the equivalence of the ecotoxicological test batches with the technical specification of all sources (relevant for all representative uses evaluated with all source; submission date proposed by the applicant: unknown; see section 5).

PARTICULAR CONDITIONS PROPOSED TO BE TAKEN INTO ACCOUNT TO MANAGE THE RISK(S) IDENTIFIED

- An in-field no-spray buffer zone of 5m is needed to protect sensitive arthropod species (see section 5).

- Risk mitigation comparable to a no-spray buffer zone of 10m is necessary to protect aquatic species from exposure to azadirachtin A under environmental conditions represented by FOCUS scenario R1 stream (see section 5).

ISSUES THAT COULD NOT BE FINALISED

- No conclusion could be reached on the Mitsui extract (no reference values could be set) as it is not demonstrated to be toxicologically equivalent to the other two sources, and the toxicological information submitted for this extract is insufficient by itself.

- There is no information to conclude if the batches used in the toxicological studies with the Mitsui source are representative of the respective technical specification.

- The relevance of the impurities and by-products of the three azadirachtin extracts (from the Trifolio-M, Sipcam and Mitsui sources) are unknown; the main compound(s) responsible for the toxicological properties of the azadirachtin extracts were not identified.

- The environmental exposure assessment including, groundwater exposure, cannot been finalized.

- There is no assessment to conclude if the batches used in the ecotoxicological studies with any of the sources are representative of the technical specification.

- The risk assessment for soil-dwelling organisms and the relevance of the metabolites could not be finalised, pending further information on the fate and behaviour and toxicity of the individual compounds.

CRITICAL AREAS OF CONCERN

- The nature of residues in plants from application of the neem extracts is unknown. It is therefore not possible to conduct a valid consumer risk assessment.

- Potential groundwater contamination by some of the active components of azadirachtin extract or its metabolites cannot be excluded with the available information.

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[14] For further guidance documents see http://ec.europa.eu/food/plant/protection/resources/publications_en.htm#council (EC) or http://www.oecd.org/document/59/0,3343,en_2649_34383_1916347_1_1_1_1,00.html (OECD)

APPENDICES

APPENDIX A – LIST OF END POINTS FOR THE ACTIVE SUBSTANCE AND THE REPRESENTATIVE FORMULATION

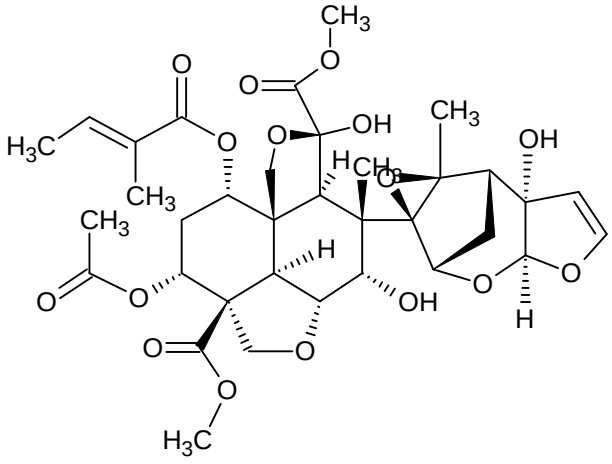
Identity, Physical and Chemical Properties, Details of Uses, Further Information

All end points are open for the other biologically active components of the neem extracts.

Active substance (ISO Common Name) ‡	Azadirachtin A (no ISO common name allocated). Open for all other biologically active components of the neem extracts.
Function (*e.g.* fungicide)	Insecticide
Rapporteur Member State	Federal Republic of Germany
Co-rapporteur Member State	none

Identity (Annex IIA, point 1)

Chemical name (IUPAC) ‡	Azadirachtin A: dimethyl (2a*R*,3*S*,4*S*,4a*R*,5*S*,7a*S*,8*S*,10*R*,10a*S*,10b*R*)-10-acetoxy-3,5-dihydroxy-4-[(1a*R*,2*S*,3a*S*,6a*S*,7*S*,7a*S*)-6a-hydroxy-7a-methyl-3a,6a,7,7a-tetrahydro-2,7-methanofuro[2,3-*b*]oxireno[*e*]oxepin-1a(2*H*)-yl]-4-methyl-8-{[(2*E*)-2-methylbut-2-enoyl]oxy}octahydro-1*H*-naphtho[1,8*a*-*c*:4,5-*b*′*c*′]difuran-5,10a(8*H*)-dicarboxylate. Open for all other biologically active components of the neem extracts.
Chemical name (CA) ‡	Azadirachtin A: dimethyl (2a*R*,3*S*,4*S*,4a*R*,5*S*,7a*S*,8*S*,10*R*,10a*S*,10b*R*)-10-(acetyloxy)octahydro-3,5-dihydroxy-4-methyl-8-[[(2*E*)-2-methyl-1-oxo-2-butenyl]oxy]-4-[(1a*R*,2*S*,3a*S*,6a*S*,7*S*,7a*S*)-3a,6a,7,7a-tetrahydro-6a-hydroxy-7a-methyl-2,7-methanofuro[2,3-b]oxireno[e]oxepin-1a(2*H*)-yl]-1*H*,7*H*-naphtho[1,8-bc:4,4a-c′]difuran-5,10a(8*H*)-dicarboxylate Open for all other biologically active components of the neem extracts.
CIPAC No ‡	Azadirachtin A: 627 Open for all other biologically active components of the neem extracts.
CAS No ‡	Azadirachtin A: 11141-17-6

	Open for all other biologically active components of the neem extracts.
EC No (EINECS or ELINCS) ‡	Not available
FAO Specification (including year of publication) ‡	627/TK (May 2006) above 250 g/kg up to 500 g/kg ± 15 % of the declared azadirachtin A content. aflatoxins (sum of aflatoxins B_1, B_2, G_1 and G_2 max 0.00003 % of the azadirachtin A content. The specification is related to Trifolio-M and EID Parry.
Minimum purity of the active substance as manufactured ‡	Trifolio-M 250 – 500 g/kg azadirachtin A (TK) Mitsui 120 – 180 g/kg azadirachtin A (TK) Sipcam 111 – 180 g/kg azadirachtin A (TK) Open for all other biologically active components of the neem extracts.
Identity of relevant impurities (of toxicological, ecotoxicological and/or environmental concern) in the active substance as manufactured	Sum of aflatoxin B_1, B_2, G_1, G_2 = 300 µg/kg azadirachtin A (TC) Open for others – data gap
Molecular formula ‡	Azadirachtin A: $C_{35}H_{44}O_{16}$ Open for all other biologically active components of the neem extracts.
Molecular mass ‡	Azadirachtin A: 720.7 g/mol Open for all other biologically active components of the neem extracts.
Structural formula ‡	 (azadirachtin A) Open for all other biologically active components of the neem extracts.

Physical and chemical properties (Annex IIA, point 2)

Melting point (state purity) ‡	154-158 °C azadirachtin A (Merck index) > 120 °C azadirachtin technical (30 % azadirachtin A) (TRF) 76 – 111 °C azadirachtin technical (Mitsui/Sipcam, NPI 720)
Boiling point (state purity) ‡	no boiling until decomposition
Temperature of decomposition (state purity)	above 200°C azadirachtin technical (30 % azadirachtin A) (TRF)
Appearance (state purity) ‡	yellow to light brown powder, garlic odour (TRF) (30 % azadirachtin A) light yellow to red-brown amorphous solid, distinct of sulfur containing compounds (Mitsui/Sipcam) (Mitsui/Sipcam, NPI 720)
Vapour pressure (state temperature, state purity) ‡	3.6×10^{-13} Pa (20 °C) azadirachtin A (extrapolation) (TRF) 1.9×10^{-20} Pa (25 °C) azadirachtin A (calculation) (Mitsui/Sipcam)
Henry's law constant ‡	10^{-14} -10^{-19} Pa m^3 $mole^{-1}$
Solubility in water (state temperature, state purity and pH) ‡	No effect of pH because no dissociation occurs 2.9 g/L azadirachtin A (TRF) (30 % azadirachtin A) 2 – 4.25 g/L azadirachtin A (Mitsui/Sipcam) (calculation) 0.116 g/L azadirachtin A (Mitsui/Sipcam) (WSKOW WIN calculation)
Solubility in organic solvents ‡ (state temperature, state purity)	azadirachtin A (Mitsui/Sipcam) (20 °C) (30 % azadirachtin A) toluene: 65.0 g/L dichlormethane: 79.5 g/L methanol: 92.9 g/L acetone: 77.9 g/L ethyl acetate: 75.1 g/L *n*-hexane: 1.7 mg/L (LOQ)
Surface tension ‡ (state concentration and temperature, state purity)	56.4 mN/m (20 °C) azadirachtin technical (36 % azadirachtin A) (TRF) 52.1 mN/m (25 °C) azadirachtin technical (Mitsui/Sipcam) 48.5 mN/m (20 °C) azadirachtin technical (17 % azadirachtin A, 4.8 % azadirachtin B) (Mitsui/Sipcam, Mitsui) Technical azadirachtin can be considered as surface active.

Partition co-efficient ‡ (state temperature, pH and purity)	No effect of pH because no dissociation occurs 0.99 azadirachtin A at 20 °C (TRF) (30 % azadirachtin A) 0.56 azadirachtin A (Oikos) (estimation, additional information) 0.85 – 0.95 azadirachtin A (Oikos) 1.29 azadirachtin B at 20 °C (TRF) 0.68 component 2.2 (listed in section C.1.1.2.1 of Volume 4 of the DAR) at 20 °C (TRF) 1.09 azadirachtin at 25 °C (Oikos, NPI 100)
Dissociation constant (state purity) ‡	Not applicable, azadirachtin A does not dissociate.
UV/VIS absorption (max.) incl. ε ‡ (state purity, pH)	Maximum UV absorption (neutral pH): 211.5 nm (ε = 12145 L*mol-1*cm-1) At 290 nm: ε = 70 L*mol-1*cm^{-1} (97.2 azadirachtin A)
Flammability ‡ (state purity)	The technical material is not highly flammable.
Explosive properties ‡ (state purity)	The technical material has no explosive properties.
Oxidising properties ‡ (state purity)	The technical material has no oxidising properties

Summary of representative uses evaluated (*name of active substance or the respective variant*)*

Crop and/ or situation / Country	Product name	Field, glasshouse or indoor use	Pests or group of pest controlled	Formulation		Application				Application rate per treatment			PHI (days) ***	Remarks
				Type	Conc. of *as (g/L)	Method kind	Growth stage & season	Number per growing season (max)	Interval between applications (days)	kg *as/hL	Water (L/ha)	kg *as/ha		
Northern Europe Potato	NeemAzal-T/S	Field (professional and home garden)	Colorado beetle	EC	10	Spray	During the vegetation period (irrespective of growth stage)	1	-	0.0042-0.0083	300-600	0.025	4	Treatment at beginning infestation: 5 days after hatching of young larvae [1] [2] [3] [4]
Northern Europe Potato	Oikos	Field (professional and home garden)	Colorado beetle	EC	26	Spray	During the vegetation period (independent from growth stage)	1	-	0.0042-0.0083	300-600	0.025	4	Treatment at beginning of infestation (ca. 5 days after hatching of young larvae) [1] [2] [3] [4]

[1] The relevance of the impurities and by-products of the three azadirachtin extracts is unknown.
[2] A consumer risk assessment cannot be conducted because the residue definition cannot be defined.
[3] The environmental exposure assessment could not be finalised. Potential groundwater contamination by active components of the azadirachtin extract or its metabolites cannot be excluded with the available information.
[4] There is no assessment to conclude if the batches used in the ecotoxicological studies are representative of the technical specifications. The risk assessment for soil-dwelling organisms could not be finalised pending further information on the fate and behaviour and toxicity of the individual compounds.
*as refers to the compound azadirachtin A

Remarks:
(a) For crops, the EU and Codex classifications (both) should be used; where relevant, the use situation should be described (e.g. fumigation of a structure)
(b) Outdoor or field use (F), glasshouse application (G) or indoor application (I)
(c) e.g. biting and sucking insects, soil borne insects, foliar fungi, weeds
(d) e.g. wettable powder (WP), emulsifiable concentrate (EC), granule (GR)
(h) Kind, e.g. overall, broadcast, aerial spraying, row, individual plant, between the plants - type of equipment used must be indicated
(i) g/kg or g/L
(j) Growth stage at last treatment (BBCH Monograph, growth stages of plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant information on season at time of application
(k) The minimum and maximum number of applications possible under practical conditions of use must be provided

(e) GCPF Codes - GIFAP Technical Monograph No. 2, 1989
(f) All abbreviations must be explained
(g) Method, e.g. high volume spraying, low volume spraying, spreading, dusting, drench
(l) PHI - minimum pre-harvest interval
(m) Remarks may include: Extent of use/ economic importance/restrictions

For uses where the column "Remarks" is marked in grey further consideration is necessary. Uses should be crossed out when the notifier no longer supports this use(s). (a) For crops, the EU and Codex classifications (both) should be taken into account; where relevant, the use situation should be described (e.g. fumigation of a structure) (b) Outdoor or field use (F), greenhouse application (G) or indoor application (I) (c) *e.g.* biting and suckling insects, soil born insects, foliar fungi, weeds (d) *e.g.* wettable powder (WP), emulsifiable concentrate (EC), granule (GR) (e) GCPF Codes - GIFAP Technical Monograph No 2, 1989 (f) All abbreviations used must be explained (g) Method, e.g. high volume spraying, low volume spraying, spreading, dusting, drench (h) Kind, *e.g.* overall, broadcast, aerial spraying, row, individual plant, between the plant- type of equipment used must be indicated	(i) g/kg or g/L. Normally the rate should be given for the active substance (according to ISO) and not for the variant in order to compare the rate for same active substances used in different variants (e.g. fluoroxypyr). **In certain cases, where only one variant is synthesised, it is more appropriate to give the rate for the variant (e.g. benthiavalicarb-isopropyl).** (j) Growth stage at last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application (k) Indicate the minimum and maximum number of application possible under practical conditions of use (l) The values should be given in g or kg whatever gives the more manageable number (e.g. 200 kg/ha instead of 200 000 g/ha or 12.5 g/ha instead of 0.0125 kg/ha (m) PHI - minimum pre-harvest interval

Uses for which the risk assessment can not be concluded are marked grey.

Analytical methods for the active substance (Annex IIA, point 4.1)

Technical as (analytical technique)	**Trifolio-M GmbH** HPLC - UV	**Trifolio-M GmbH** HPLC - UV
Impurities in technical as (analytical technique)	The analytical methods determine components and impurities.	
	Trifolio-M GmbH HPLC – UV, extraction-evaporation, Karl-Fischer titration, HPLC-FLD	**Trifolio-M GmbH** HPLC – UV, extraction-evaporation, Karl-Fischer titration, HPLC-FLD
Plant protection product (analytical technique)	HPLC-UV	

Analytical methods for residues (Annex IIA, point 4.2)

Residue definitions for monitoring purposes

Food of plant origin		Not defined
Food of animal origin		Not defined
Soil		Not defined
Water	surface	Not defined
	drinking/ground	Not defined
Air		Not defined

Monitoring/Enforcement methods

Food/feed of plant origin (analytical technique and LOQ for methods for monitoring purposes)	Open. azadirachtin A LC-MS/MS 0.02 mg/kg (beans, cabbage, lettuce, cucumber, melon, peaches, strawberries grapes, peppers, orange); TRF LC-MS/MS 0.02 mg/kg (cucumber, lemon balm); TRF HPLC-UV 0.01 mg/kg (potato); TRF HPLC-UV 0.01 mg/kg (tomato); TRF HPLC-UV 0.1 mg/kg (spinach); TRF azadirachtinA and azadirachtin B HPLC-UV 0.02 mg/kg (apple); SIP Note: All HPLC-UV methods are not validated in an independent laboratory. However, the LC-MS/MS ones are validated in an independent laboratory.
Food/feed of animal origin (analytical technique and LOQ for methods for monitoring purposes)	Open.
Soil (analytical technique and LOQ)	Open. azadirachtin A LC-MS/MS 0.02 mg/kg (standard soil); TRF

Water (analytical technique and LOQ)	Open. azadirachtin A HPLC-UV 1 µg/L (surface water); TRF LC-MS/MS 0.05 µg/L (drinking water, surface water); TRF, SIP, Certis
Air (analytical technique and LOQ)	Open. azadirachtin A LC-MS/MS 3 µg/m³ (ambient air, warm humid air); TRF, SIP, Certis
Body fluids and tissues (analytical technique and LOQ)	Not relevant as not classified as toxic or very toxic

Classification and proposed labelling with regard to physical and chemical data (Annex IIA, point 10)

	RMS/peer review proposal
Active substance	None

Impact on Human and Animal Health

Absorption, distribution, excretion and metabolism (toxicokinetics) (Annex IIA, point 5.1)

Rate and extent of oral absorption ‡ Distribution ‡ Potential for accumulation ‡ Rate and extent of excretion ‡ Metabolism in animals ‡	Azadirachtin technical is a mixture of several different limonoids and other compounds extracted from the seed kernels of the Neem tree. It is therefore not feasible to perform ADME studies with azadirachtin technical. It is furthermore also not possible to perform such a study for its several components (azadirachtin A and others) due to the unavailability of chemically synthesised and radioactively labelled components, since it can be obtained by extraction and cleanup of the seed kernels of the Neem tree only. Therefore it is not possible to obtain radioactive labelled material.
Toxicologically relevant compounds ‡ (animals and plants)	Azadirachtin extract (as whole extract)
Toxicologically relevant compounds ‡ (environment)	Azadirachtin extract (as whole extract)

Acute toxicity (Annex IIA, point 5.2)

Rat LD_{50} oral ‡	> 5000 mg Trifolio extract/kg bw > 5000 mg Sipcam extract/kg bw > 5000 mg Mitsui extract/kg bw	
Rat LD_{50} dermal ‡	> 2000 mg Trifolio extract/kg bw > 2000 mg Sipcam extract/kg bw > 2000 mg Mitsui extract/kg bw	
Rat LC_{50} inhalation ‡	All studies: highest attainable concentration > 0.72 mg Trifolio extract/L air (4 h, whole body) > 2.45 mg Sipcam extract/L air (4 h, whole body)	
Skin irritation ‡	Not irritating (Trifolio, Sipcam, Mitsui extracts)	
Eye irritation ‡	Not irritating (Trifolio, Sipcam, Mitsui extracts)	
Skin sensitisation ‡	Sensitising (M&K, Trifolio, Sipcam extracts) Sensitising (Buehler, Mitsui extract)	**R43**

Short term toxicity (Annex IIA, point 5.3)

Target / critical effect ‡	Rat: Liver, thyroid (organ weight, clinical chemistry) No study in dog submitted, not required	
Relevant oral NOAEL ‡	Rat, 90-day: 32 mg Trifolio extract/kg bw/day Rat, 90-day: 33 mg Sipcam extract/kg bw/day Rat, 90-day: 35 mg Mitsui extract/kg bw/day	
Relevant dermal NOAEL ‡	No data - not required	
Relevant inhalation NOAEL ‡	No data - not required	

Genotoxicity ‡ (Annex IIA, point 5.4)

	Positive *in vitro* (chromosome aberration) (Trifolio, Sipcam, Mitsui extracts); Negative *in vivo* (Trifolio, Sipcam); Overall unlikely to present a genotoxic potential to humans (Trifolio, Sipcam); No conclusion on the Mitsui source.	

Long term toxicity and carcinogenicity (Annex IIA, point 5.5)

Target/critical effect ‡	No reliable study submitted	
Relevant NOAEL ‡	No reliable study submitted	
Carcinogenicity ‡	No reliable study submitted	

Reproductive toxicity (Annex IIA, point 5.6)

Reproduction toxicity

Reproduction target / critical effect ‡	No evidence of effects on reproduction by Trifolio extract. No effects observed on parents and offspring	
Relevant parental NOAEL ‡	50 mg Trifolio extract/kg bw/day	
Relevant reproductive NOAEL ‡	50 mg Trifolio extract/kg bw/day	
Relevant offspring NOAEL ‡	50 mg Trifolio extract/kg bw/day	

Developmental toxicity

Developmental target / critical effect ‡	Rat, Trifolio extract: ↑ incidence of supernumerary ribs at maternally toxic doses (↓ body weight gain, liver toxicity expected from the results of the 90-day study). Rat, Sipcam extract no effects on foetuses; ↓ body weight gain in dams. Rabbit, Mitsui extract: ↓ number of viable litters and of live foetuses per dam, ↑ number of *in utero* deaths in maternal toxic doses (↓ body weight).	
Relevant maternal NOAEL ‡	Rat: 225 mg Trifolio extract/kg bw/day Rat: 300 mg Sipcam extract/kg bw/day Rabbit: 20 mg Mitsui extract/kg bw/day	
Relevant developmental NOAEL ‡	Rat: 225 mg Trifolio extract/kg bw/day Rat: 1000 mg Sipcam extract/kg bw/day Rabbit: 100 mg Mitsui extract/kg bw/day	

Neurotoxicity (Annex IIA, point 5.7)

Acute neurotoxicity ‡	No data - not required	

Repeated neurotoxicity ‡	No data - not required	
Delayed neurotoxicity ‡	No valid study submitted – not required	

Other toxicological studies (Annex IIA, point 5.8)

Mechanism studies ‡	No data - not required
Studies performed on metabolites or impurities ‡	No data - not required

Medical data ‡ (Annex IIA, point 5.9)

No effects in manufacturing staff reported. Reports in literature of infant intoxications and mortalities after oral administration of neem oil (extracts of neem tree fruits or neem tree seeds, which are chemically distinct from azadirachtin technical extracts used for PPP).

Summary (Annex IIA, point 5.10)

Trifolio, Sipcam extracts	Value	Study	Safety factor
ADI ‡	0.1 mg/kg bw/day	Rat, 90-day (Trifolio, Sipcam extracts)	300[(1)]
AOEL ‡	0.1 mg/kg bw/day	Rat, 90-day (Trifolio, Sipcam extracts)	300[(2)]
ARfD ‡	0.75 mg/kg bw	Rat, teratogenicity (Trifolio extract)	300[(3)]

[(1)] higher safety factor to account for missing long term/carcinogenicity studies and developmental study in rabbit with Trifolio and Sipcam sources
[(2)] higher safety factor to account for missing information on oral absorption and missing developmental study in rabbit with Trifolio and Sipcam sources
[(3)] higher safety factor to account for missing developmental study in rabbit with Trifolio and Sipcam sources

For Mitsui extract no reference values could be established.

Dermal absorption ‡ (Annex IIIA, point 7.3)

Formulation (Neem Azal-T/S, Oikos)	10 % absorption based on expert judgement

Exposure scenarios (Annex IIIA, point 7.2)

Operator	**Estimated exposure for field crop (tractor mounted boom sprayer)** approximate application rate: 75 g Neem Azal-T/S/ha and 250 g Oikos/ha % of systemic AOEL German model: Without PPE 9.5 % (NeemAzal-T/S) 31.8 % (Oikos,) UK-POEM: Without PPE 50.1 % (NeemAzal-T/S) 166.9 % (Oikos) With PPE (gloves during M/L) 69.4 % (Oikos) **Home garden sprayer:** UK-POEM: Without PPE 7.3 % (NeemAzal-T/S 24.3 % (Oikos)
Workers	Estimated exposure, worst case: 25.7 % and 85.7 % of systemic AOEL for Trifolio and Sipcam extracts, respectively, without PPE
Bystanders	Estimated exposure, worst case: 0.04 % and 0.12 % of systemic AOEL for Trifolio and Sipcam extracts, respectively

Classification and proposed labelling with regard to toxicological data (Annex IIA, point 10)

Substance classified (azadirachtin technical extracts from Trifolio and Sipcam sources)	RMS/peer review proposal
according to the criteria in Dir. 67/548/EEC	**Xi** "Irritant" **R43** "May cause sensitization by skin contact"
according to the criteria in Regulation (EC) No 1272/2008	Skin Sens. 1, H317 (May cause an allergic skin reaction)

Metabolism in plants (Annex IIA, point 6.1 and 6.7, Annex IIIA, point 8.1 and 8.6)

Plant groups covered	No data available on the nature of residues in plants.
Rotational crops	No data available on the nature of residues in plants.
Metabolism in rotational crops similar to metabolism in primary crops?	No data available on the nature of residues in soil.
Processed commodities	No data available on the nature of residues in plants.
Residue pattern in processed commodities similar to residue pattern in raw commodities?	No data available on the nature of residues in plants.
Plant residue definition for monitoring	No data available on the nature of residues in plants.
Plant residue definition for risk assessment	No data available on the nature of residues in plants.
Conversion factor (monitoring to risk assessment)	No data available on the nature of residues in plants.

Metabolism in livestock (Annex IIA, point 6.2 and 6.7, Annex IIIA, point 8.1 and 8.6)

Animals covered	No data available on the nature of residues in plants.
Time needed to reach a plateau concentration in milk and eggs	-
Animal residue definition for monitoring	-
Animal residue definition for risk assessment	-
Conversion factor (monitoring to risk assessment)	-
Metabolism in rat and ruminant similar (yes/no)	-
Fat soluble residue: (yes/no)	-

Residues in succeeding crops (Annex IIA, point 6.6, Annex IIIA, point 8.5)

No data available on the nature of residues in soil.

Stability of residues (Annex IIA, point 6 Introduction, Annex IIIA, point 8 Introduction)

No data available on the nature of residues in plants.

Residues from livestock feeding studies (Annex IIA, point 6.4, Annex IIIA, point 8.3)

	Ruminant:	Poultry:	Pig:
	Conditions of requirement of feeding studies		
Expected intakes by livestock ≥ 0.1 mg/kg diet (dry weight basis) (yes/no - If yes, specify the level)	Open	Open	Open

Summary of residues data according to the representative uses on raw agricultural commodities and feedingstuffs (Annex IIA, point 6.3, Annex IIIA, point 8.2)

Crop	Northern or Mediterranean Region, field or glasshouse, and any other useful information	Trials results relevant to the representative uses (a)	Recommendation/comments	MRL estimated from trials according to the representative use	HR (c)	STMR (b)
Open						

(a) Numbers of trials in which particular residue levels were reported *e.g.* 3 x < 0.01, 1 x 0.01, 6 x 0.02, 1 x 0.04, 1 x 0.08, 2 x 0.1, 2 x 0.15, 1 x 0.17
(b) Supervised Trials Median Residue *i.e.* the median residue level estimated on the basis of supervised trials relating to the representative use
(c) Highest residue

Consumer risk assessment (Annex IIA, point 6.9, Annex IIIA, point 8.8)

ADI	For Trifolio and Sipcam extract 0.1 mg/kg bw/day
TMDI (% ADI) according to WHO European diet	Open
TMDI (% ADI) according to national (to be specified) diets	Open
IEDI (WHO European Diet) (% ADI)	Open
NEDI (specify diet) (% ADI)	Open
Factors included in IEDI and NEDI	Open
ARfD	For Trifolio and Sipcam extract 0.75 mg/kg bw
IESTI (% ARfD)	Open
NESTI (% ARfD) according to national (to be specified) large portion consumption data	Open
Factors included in IESTI and NESTI	Open

Proposed MRLs (Annex IIA, point 6.7, Annex IIIA, point 8.6)

Open

When the MRL is proposed at the LOQ, this should be annotated by an asterisk after the figure.

Route of degradation (aerobic) in soil (Annex IIA, point 7.1.1.1.1)

Mineralisation after 100 days ‡	Quantification technically not possible in the available studies since no radiolabelled material was employed. Total chemical synthesis of the components of azadirachtin would be theoretically possible but extremely costly and laborious. Partial synthesis from precursors obtained by partial degradation of naturally obtained compounds and/or cell cultures fed with radiolabelled precursors have not been explored as more feasible ways to obtain radiolabelled components. Breaking down of the policyclic structure of azadirachtin (common to all the active components) in soil is not demonstrated in the available studies.
Non-extractable residues after 100 days ‡	Quantification technically not possible in the available studies since no radiolabelled material was employed. Total chemical synthesis of the components of azadirachtin would be theoretically possible but extremely costly and laborious. Partial synthesis from precursors obtained by partial degradation of naturally obtained compounds and/or cell cultures fed with radiolabelled precursors have not been explored as more feasible ways to obtain radiolabelled components.
Metabolites requiring further consideration ‡ - name and/or code, % of applied (range and maximum)	Azadirachtin H* (desacetyl Azadirachtin A) metabolite of Azadirachtin A (max 63 % of applied Azadirachtin A). No data on the degradation of other components of azadirachtin extract (components 3, 4, 5 and 6 listed in section C.1.1.2.1 of Volume 4 of the DAR) are available. Data available on the route of degradation of azadirachtin extract components in soil are not sufficient to conclude on the potential metabolites that may require further assessment. Data gap identified.

Route of degradation in soil - Supplemental studies (Annex IIA, point 7.1.1.1.2)

Anaerobic degradation ‡

Mineralisation after 100 days	Insecticides based on azadirachtin are recommended to be applied to potatoes during the vegetation period. Situations where conditions are likely to be anaerobic are improbable to occur. Therefore no studies under anaerobic conditions are required.
Non-extractable residues after 100 days	Not applicable
Metabolites that may require further consideration for risk assessment - name and/or code, % of applied (range and maximum)	Not applicable

Soil photolysis ‡

Soil photolysis ‡ Mineralisation after 30 d non-extractable residues after 30 d	No data available. Data gap.
Metabolites that may require further consideration for risk assessment - name and/or code, % of applied (range and maximum)	No data available. Data gap.

Rate of degradation in soil (Annex IIA, point 7.1.1.2, Annex IIIA, point 9.1.1)

Laboratory studies ‡

Parent components

Azadirachtin A	Aerobic conditions						
Soil type (site)	X[15]	pH	t. °C / % MWHC	DT_{50} /DT_{90} (d) (study, recalculated SFO)*	DT_{50} (d)** 20°C pF2/10 kPa	St. (r^2)	Model, Kinetics; method of calculation
Sand (LUFA 2.1)		6.0	20 / 40	3.3/11	3.2	0.997	SFO
Loamy sand (LUFA 2.2)		5.8	20 / 40	4.0 / 13.4	4.0	0.989	SFO
Sandy loam (LUFA 2.3)		6.6	20 / 40	2.0 / 6.6	1.7	0.955	SFO
Loamy sand (LUFA 2.2)		5.6	20 / 40	1.9 / 6.5	1.9	0.993	SFO
Silty clay (soil B)		8.0	25/40-50	25.6 / 85	25.0	0.986	SFO
Loam (soil C)		5.9	25/40-50	10.6 / 35	11.2	0.998	SFO
Geometric mean (DT_{50}):					**4.76**		
Median (DT_{50}):					3.6		
Maximum (DT_{50}):					25.0		

* original data from study referred to pseudo 1st order

** normalised values using a Q_{10} = 2.2 (temperature correction factor)

[15] X This column is reserved for any other property that is considered to have a particular impact on the degradation rate.

Azadirachtin B	Aerobic conditions						
Soil type (site)	X[16]	pH	t. °C / % MWHC	DT_{50} /DT_{90} (d) (study, recalculated SFO)*	DT_{50} (d)** 20°C pF2/10 kPa	St. (r^2)	Model, Kinetics; method of calculation
Sand (LUFA 2.1)		6.0	20 / 40	6.0/19.9	5.86	0.96	SFO
Silty clay (soil B)		8.0	~~20~~ 25/40-50	33.4 / 110	34.4	0.99	SFO
Loam (soil C)		5.9	~~20~~ 25/40-50	30.6 / 101	15.0	0.93	SFO
Geometric mean (DT_{50}):					**14.5**		
Maximum (DT_{50}):					34.4		

* original data from study referred to pseudo 1st order

** normalised values using a Q_{10} = 2.2 (temperature correction factor)

Data gaps are identified for the other known active components: Components 2.2, 3, 4, 5 and 6 listed in section C.1.1.2.1 of Volume 4 of the DAR. In particular component 6 may be applied at levels comparable to azadirachtin A for some of the technical neem extracts under assessment when the proposed GAPS are followed.

Metabolites

Azadirachtin H*	Aerobic conditions						
Soil type (site)	pH	t. °C / % MWHC	DT_{50} /DT_{90} (d) (study, recalculated SFO)*	DT_{50} (d)** 20°C pF2/10 kPa	f. f.	St. (chi^2)	Model, Kinetics; method of calculation
Sand (LUFA 2.1)	6.0	20 / 40	9.8	9.6	0.85	10.0	SFO

* original data from study referred to kinetic model (consecutive first order)

** For moisture normalisation the maximum water holding capacity (MWHC) derived from the study and B=0.7 were used.

Data gap identified for experiments to determine the rate of degradation of Azadirachtin H* in two additional soils.

Further information may be needed once the route of degradation in soil is adequately investigated.

[16] X This column is reserved for any other property that is considered to have a particular impact on the degradation rate.

Laboratory studies ‡

Azadirachtin A	Aerobic conditions (10° C)				
Soil type (site)	X[17]	pH	t. °C / % MWHC	DT_{50} (d) * 10°C, pF2/10 kPa	Model, Kinetics; method of calculation
Sand (LUFA 2.1)		6.0	20/40	6.2	SFO
Loamy sand (LUFA 2.2)		5.8	20/40	8.8	SFO
Sandy loam (LUFA 2.3)		6.6	20/40	3.7	SFO
Loamy sand (LUFA 2.2)		5.6	20/40	4.2	SFO
Silty clay (soil B)		8.0	25/40-50	54.8	SFO
Loam (soil C)		5.9	25/40-50	24.8	SFO

* calculated values using a Q_{10} = 2.2 (temperature correction factor)

Azadirachtin B	Aerobic conditions (10° C)			
Soil type (site)	pH	t. °C / % MWHC	DT_{50} (d) * 10°C, pF2/10 kPa	Model, Kinetics; method of calculation
Sand (LUFA 2.1)	6.0	20/40	13.0	SFO
Silty clay (soil B)	8.0	25/40-50	75.4	SFO
Loam (soil C)	5.9	25/40-50	33.2	SFO

Field studies ‡

Parent	As the maximum DT_{50} value of 25 days at 20 °C, pF 2 of azadirachtin A and Azadirachtin B derived from the laboratory studies does not exceed 60 days, a soil dissipation testing for these components is not required. The DT_{50} values of azadirachtin A calculated for 10 °C also confirm these findings. The respective maximum value amounted to 75.4 days and does not exceed the trigger value of 90 days.

pH dependence ‡ (yes / no) (if yes type of dependence)	no
Soil accumulation and plateau concentration ‡	Due to the rapid degradation of azadirachtin A, a soil accumulation test is not required. The average DT_{50} value (calculated for 20 °C, pF 2) of the laboratory studies was 4.7 days. The respective DT_{90} value was calculated to be 15.6 days, being far below the trigger value for soil dissipation studies of 1 year. However, this may need to be revised once information on the route of degradation is completed, since persistence in soil of some of the components is still unknown and the break down of the polycyclic structure (common to all the active components) has not been proven.

Soil adsorption/desorption (Annex IIA, point 7.1.2)

Parent ‡

[17] X This column is reserved for any other property that is considered to have a particular impact on the degradation rate.

Soil Type	OC %	Soil pH	K_d	$K_{d\text{-}oc}$	K_f	K_{foc}	1/n
silty clay (soil A) [a]	1.86	8.1	2.26 *	121.5	3.13	168	0.87
silty clay (soil B) [a]	0.47	8.0	4.11 *	875.1	5.07	1079	0.93
Loam (soil C) [a]	3.32	5.9	2.51 *	75.8	3.33	99	0.91
silt loam (soil D) [a]	1.36	6.8	1.02 *	75.1	2.43	179	0.73
sand (LUFA 2.1) [b]	0.62	5.9	0.405	65.4	–	–	1.0 [1]
loamy sand (LUFA 2.2) [b]	2.32	5.6	0.479	20.6	–	–	1.0 [1]
loamy sand (LUFA 2.3) [b]	1.22	6.4	0.373	30.6	–	–	1.0 [1]
			Arithmetic mean	180	3.49	381.3	0.92
			Median	**75**	3.23	173.5	**0.93**
			pH dependence (Yes or No)	No			

[a] Test material: Azadiractin A TEC
[b] Test material: NeemAzal
* K_d calculated as the mean of three individual data points
[1] default value = 1.0

Adsorption / desorption data obtained for azadirachtin A may be extrapolated to azadirachtin B but not to other known active components of azadirachtin extract (components 3, 4, 5 and 6 listed in section C.1.1.2.1 of Volume 4 of the DAR) or to the major metabolite azadirachtin H*.

Mobility in soil (Annex IIA, point 7.1.3, Annex IIIA, point 9.1.2)

Column leaching ‡	No study available, no study required.
Aged residues leaching ‡	No study available, no study required.
Lysimeter/ field leaching studies ‡	No study available, no study required.

PEC (soil) (Annex IIIA, point 9.1.3)

Parent components	Azadirachtin A
Method of calculation	DT_{50} (d): **25.0** days (maximum, n = 6, laboratory data 20°C, pF2) Kinetics: SFO

Application data	Crop: potato Depth of soil layer: 5 cm Soil bulk density: 1.5 g/cm^3 % plant interception: 15 Number of applications: 1 Interval (d): - Application rate(s): 25 g as/ha

PEC$_{(s)}$ (mg/kg)		Single application Actual	Single application Time weighted average
Initial		0.0283	
Short term	24 h	0.0276	0.0279
	2 d	0.0268	0.0276
	4 d	0.0254	0.0268
Long term	7 d	0.0233	0.0258
	28 d	0.0130	0.0197
	50 d	0.0071	0.0153
	100 d	0.0018	0.0096
Plateau concentration		not necessary	

No calculation has been provided for any of the other components. Initial PEC soil for the whole azadirachtin extract will range from a 0.045-0.09 mg / kg (Trifolio, Neem Azal) to 0.14 – 0.24 mg / Kg (SIPCAM , Fortune Aza).

Route and rate of degradation in water (Annex IIA, point 7.2.1)

Hydrolytic degradation of the active substance and metabolites > 10 % ‡	Azadirachtin A (TRF) pH 4: 49.9 d (1198 h) at 20 °C pH 7: 19.5 d (467 h) at 20 °C pH 8: 4.4 d (106 h) at 20 °C Azadirachtin A (Mitsui/Sipcam) pH 4: 18.1 d (434.4 h) at 25 °C pH 7: 9.6 d (230.4 h) at 25 °C pH 10: < 1 d (< 24 h) at 25 °C Azadirachtin B (Mitsui/Sipcam) pH 4: 24.0 d (576 h) at 25 °C pH 7: 12.3 d (295.2 h) at 25 °C pH 10: < 1 d (< 24 h) at 25 °C No data are available for the other known active components of azadirachtin extracts. Data gap identified.
Photolytic degradation of active substance	Only QSAR calculation available. Results of ABIWAS2.0-Simulations, Natural light, 55°N 1) Simulation with Φ=0.000555 Month – Half life January 52.3 d February 24.1 d March 11.8 d April 6.78 d May 5.25 d June 4.63 d July 5.2 d August 5.41 d September 9.46 d October 17.9 d November 43.0 d December 83.5 d 2) Simulation with Φ=0.00094 Month – Half life January 336 d February 156 d March 77.6 d April 45.3 d May 35.4 d June 31.3 d July 35.2 d August 36.5 d September 63.2 d October 118 d November 279 d December 1.47 y Data gap for the experimental measurement of the aqueous photolysis half-life is identified.
Quantum yield of direct phototransformation in water at λ > 290 nm	1.Study (Werle, 1995): 5.55 x 10^{-4} mol · Einstein $^{-1}$ 2.Study (Hennecke, 2007): 9.4 x 10^{-4} mol · Einstein $^{-1}$

Readily biodegradable ‡ (yes/no)	no (based on data, four different test items were used)

Degradation water / sediment system	A guideline water/sediment study was not carried out, since radio-labelled components of azadirachtin extract were not available. A cold hydrolysis study in natural water was provided instead with an extract containing 85.4 g/kg of Az A and 26 g/kg Az B (content of Az A is well below the specified content for the commercial extracts). In this study, investigating the behaviour of azadirachtin TEC in river water samples of a single system, a rapid disappearance of azadirachtin A from the water phase was found (DT_{50} = 13.7 d). Additionally an outdoor water sediment system with forest sediment has been provided. Identification of metabolites has not been attempted.

Provided information on the fate of azadirachtin A in aquatic systems

water/sediment system	pH water phase	pH sedi-ment	T °C	DT_{50}/DT_{90} whole system (days)	DT_{50}/DT_{90} water (days)	r^2	DT_{50}/DT_{90} sediment (days)	Method of calculation/ kinetic
substance:	azadirachtin A							
water system (river)	7.58	n.d.	25	n.d.	8.82 d	0.997	n.d.	Pseudo 1st
			25		9.3 d	0.9986		SFO
			20		13.7 d*			
Outdoor water/sediment system (stream, forest)	6.32	6.21	n.d.	n.d.	8-13 d	n.d.	2-3 d	n.d.

* recalculated DT_{50} value of one water metabolism study used for modelling in surface waters

Azadirachtin B

water/sediment system	pH water phase	pH sedi-ment	T °C	DT_{50}/DT_{90} whole system (days)	DT_{50}/DT_{90} water (days)	r^2	DT_{50}/DT_{90} sediment (days)	Method of calculation/ kinetic
substance:	azadirachtin A							
water system (river)	7.58	n.d.	25	n.d.	12.6 d	0.9835	n.d.	Pseudo 1st
			20		19.51 d*	0.9986		SFO

No data on the other components of Azadirachtin are available. No data on metabolites formed from these components in water/sediment systems are available.
Data available on the route and rate of degradation of Azadirachtin extract active components in aquatic systems are not sufficient to finalize the exposure assessment. Data gap for further data is identified.

PEC surface water and PEC sediment (Annex IIIA, point 9.2.3)

Parent components. Parameters used in $FOCUS_{sw}$ step 1 and 2	Whole azadirachtin extract: due to the lack of knowledge on the properties of known active azadirachtin extract components with respect to azadirachtin A conversion of results calculated for the lead compound to the whole active substance is only possible for-situations were the peak maximum concentration is expected to occur as a result of spray drift event. Azadirachtin A Modelling using STEPS 1-2 in FOCUS Version 1.1 Input parameters of Azadirachtin A: Molecular weight (g/mol): 720.7 (Azadirachtin A) Water solubility (mg/L): 2900 at 20°C K_{OC} (L/kg): 121 (10^{th} percentile; n=4) DT_{50} soil (d): 4.7days (geometric mean, n=6, normalisation to 10 kPa or pF2 and 20 °C with Q10 of 2.2) DT_{50} water/sediment system (d): 13.7 days (no standard water/sediment study is provided, value from water metabolism study with river water, n=1, first order, 20 °C) DT_{50} water (d): 13.7 (n=1, first order; 20°C) DT_{50} sediment (d): 1000 (default, no standard water/ sediment study is provided) Crop interception (%): 15 Azadirachtin B, No assessment performed Component 3 listed in section C.1.1.2.1 of Volume 4 of the DAR No assessment performed. No input parameters available. Component 4 listed in section C.1.1.2.1 of Volume 4 of the DAR No assessment performed. No input parameters available. Component 5 listed in section C.1.1.2.1 of Volume 4 of the DAR No assessment performed. No input parameters available. Component 6 listed in section C.1.1.2.1 of Volume 4 of the DAR No assessment performed. No input parameters available. Metabolite identification not available. Input parameters

	not available.
Parameters used in $FOCUS_{sw}$ step 3 and 4	Whole azadirachtin extract: Due to the lack of knowledge on the properties of known active azadirachtin extract components with respect to azadirachtin A conversion of results calculated for the lead compound to the whole active substance is only possible for-situations were the peak maximum concentration is expected to occur as a result of spray drift event. Azadirachtin A Modelling using FOCUS_TOXWAv2.21 software with Driftcalculator 1(spray drift); MACRO 4.4.2 (drainage) and PRZM 1.5.6 (runoff) with appropriate $FOCUS_{sw}$ scenarios according to FOCUS guidance Input parameters of azadirachtin A: Vapour pressure: 1.9 x 10^{-20} Pa (25 °C Water solubility: 0.29 x 10^{4} (20° C) DT_{50} soil (d): 4,7 (geometric mean, n=6, normalisation to 10 kPa or pF2 and 20 °C with Q10 of 2.2) K_{OC} (L/kg): 121 (10^{th} percentile; n=4), 1/n = 0,861 (arithmetic mean; n=4) DT_{50} water/sediment system (d): 13.7 days (no standard water/sediment study is provided, value from water metabolism study with river water, n=1, first order, 20 °C) DT_{50} water (d): 13.7 (n=1, first order; 20°C) DT_{50} sediment (d): 1000 (default, no standard water/ sediment study is provided) Q_{10} value (used for simulations) = 2.58 Azadirachtin B, No assessment performed Component 3 listed in section C.1.1.2.1 of Volume 4 of the DAR No assessment performed. No input parameters available. Component 4 listed in section C.1.1.2.1 of Volume 4 of the DAR No assessment performed. No input parameters available. Component 5 listed in section C.1.1.2.1 of Volume 4 of the DAR No assessment performed. No input parameters available.

	Component 6 listed in section C.1.1.2.1 of Volume 4 of the DAR No assessment performed. No input parameters available. Azadirachtin H* (major soil metabolite). No assessment performed. No input parameters available. Identification of other aquatic or soil metabolites is not available. Input parameters not available.
Application rate	Step 3 and 4 Crop: potato Number of applications: 1 Application rate(s): 25 g as/ha Application window: beginning of emergence to 30 days after emergence Application method: spray (drainage: ground spray; runoff: ground spray, CAM2) Step 4 buffer zone (Step 4): 10 m (reduction of runoff volume and flux: 60 % reduction of erosion mass and flux: 85 %)

PECsw – FOCUS modelling results (Azadirachtin A maximum predicted actual concentrations after application in potatoes)

FOCUS STEP	Szenario/ water body	Drift	Maximal PEC_{SW} (µg/L)		PEC_{SED} (µg/kg)	
		%-of appl. rate	Actual	TWA	Actual	TWA
STEP 3	D3 ditch	1.5940	0.131	0.100	0.043	0.041
	D4 pond	0.2122	0.005	0.005	0.009	0.009
	D4 stream	1.4900	0.111	0.009	0.006	0.004
	R1 pond	0.2122	0.014	0.013	0.030	0.030
	R1 stream	1.4900	0.239	0.122	0.072	0.057
STEP 4	R1 stream	0.3320	0.108	0.055	0.033	0.026

Note: the values calculated for Azadirachtin A cannot be generally extrapolated or converted to PEC SW for the other parent compound components or for the whole azadirachtin extract due to the lack of knowledge on the properties of the other known active azadirachtin extract components with respect to azadirachtin A. Potential surface water contamination by major soil metabolite azadirachtin H* has not been addressed.
Further calculations may be needed once the route of degradation in soil and the degradation / dissipation in water sediment systems is adequately investigated.
Only when the peak maximum is the result of a spray drift event can the initial amount of whole extract reaching the surface water be estimated. The results for the different extracts are presented below following the latest technical specifications proposed by the different applicants.

Azadirachtin A **SIPCAM 1** **MITSUI (ATI 720)** **NeemAzal**

	(µgAzA / L)	(FortuneAza) (µgFortune / L)	(µgATI720 / L)	(µgNeemAzal / L)	
D3 Ditch	0.131	1.179	1.090	0.524	drift peak
D4 Pond	0.005	0.045	0.042	0.020	drift peak
D4 Stream	0.111	0.999	0.923	0.444	drift peak

PEC ground water (Annex IIIA, point 9.2.1)	
Method of calculation and type of study (*e.g.* modelling, field leaching, lysimeter)	Modelling using FOCUSPELMO 3.3.2 and FOCUS PEARL 3.3.3 with appropriate $FOCUS_{gw}$ scenarios according to FOCUS guidance. Crop: potatoes Scenarios: Châteaudun, Hamburg, Jokioinen, Kremsmünster, Okehampton Parent components **Azadirachtin A** (parent): DT_{50} (d): 4.7 d (geometric mean, n=6, SFO, 20 °C (Q_{10}= 2.2), pF2) K_{OC} (L/kg): 75.2; 1/n = 0.93 (median, n=7) Q_{10} value (used for simulations) = 2.58 Moisture exponent: 0.7 Azadirachtin B, No assessment performed Component 3 listed in section C.1.1.2.1 of Volume 4 of the DAR No assessment performed. No input parameters available. Component 4 listed in section C.1.1.2.1 of Volume 4 of the DAR No assessment performed. No input parameters available. Component 5 listed in section C.1.1.2.1 of Volume 4 of the DAR No assessment performed. No input parameters available. Component 6 listed in section C.1.1.2.1 of Volume 4 of the DAR No assessment performed. No input parameters available. Metabolites: **Azadirachtin H*** **Note:** Data available for this major metabolite of Azadirachtin A is not sufficient to satisfy the regulatory requirements. Data gaps have been identified for further degradation experiments in soil and measured batch adsorption desorption experiments. **The results have been maintained in the LoEP solely for illustrative purposes.** DT_{50} (d): 9.6 d (n=1, sand, LUFA 2.1, SFO, 20°C, pF 2) Formation fraction: 0.85

	K_{OC} (L/kg): 10 (default); 1/n = 1.0 (default) Q_{10} value (used for simulations) = 2.58 Moisture exponent: 0.7 Data gap for the route of degradation is identified; further metabolites may be identified that require groundwater assessment.
Application data	Application rate: 0.025 kg a.s./ha Crop interception: 15 % (resulting application rate as used in model: 0.0213 kg/ha) Number of applications: 1/year Region of use: North-EU Application timing: 1 day after emergence depend on the scenario

PEC_{gw} - FOCUS modelling results (80^{th} percentile annual average concentration at 1 m)

Crop	Scenario	Parent (µg/L): Azadirachtin A	Metabolite (µg/L):		
			Azadirachtin H*	-	-
FOCUS PELMO 3.3.2					
potatoes	Châteaudun	<<0.001	0.005	-	-
	Hamburg	<<0.001	0.017	-	-
	Jokioinen	<<0.001	0.062	-	-
	Kremsmünster	<<0.001	0.007	-	-
	Okehampton	<<0.001	0.016	-	-
FOCUS PEARL 3.3.3					
potatoes	Châteaudun	<<0.001	0.0388	-	-
	Hamburg	<<0.001	0.0497	-	-
	Jokioinen	<<0.001	0.0617	-	-
	Kremsmünster	<<0.001	0.0246	-	-
	Okehampton	<<0.001	0.0390	-	-

Note: Data available for the Azadirachtin H* (major metabolite of Azadirachtin A) is not sufficient to satisfy the regulatory requirements. Data gaps have been identified for further degradation experiments in soil and measured batch adsorption desorption experiments. The results have been maintained in the LoEP solely for illustrative purposes. The values obtained for Azadiractin H* are not negligible and further assessment is needed to exclude exceedance of the limit of 0.1µg/L . Exceedance of the limit of 0.1 µg/L cannot be excluded for the other known components of azadiractin extract (components 2.2, 3, 4, 5, and 6 listed in section C.1.1.2.1 of Volume 4 of the DAR.

Values obtained for Azadirachtin A cannot be extrapolated or converted to the whole azadirachtin extract equivalents due to the lack of knowledge on the properties of the other known active azadirachtin extract components with respect to azadirachtin A. This conversion would be necessary to perform a risk assessment since toxicological end points are determined on the basis of the whole extract. The toxicological evaluation has indicated that human toxicity of azadirachtin extract seems not to be directly related to the content of Azadirachtin A.

$PEC_{(gw)}$ from lysimeter / field studies

Compound	1^{st} year	2^{nd} year	3^{rd} year
Annual average (µg/L)	no studies performed	no studies performed	no studies performed

Fate and behaviour in air (Annex IIA, point 7.2.2, Annex III, point 9.3)

Direct photolysis in air ‡	Not studied - no data requested
Quantum yield of direct phototransformation	Azadirachtin A: 5.55 * 10^{-4}
Photochemical oxidative degradation in air ‡	DT_{50} of 1.696 hours derived by the Atkinson model (version 1.91). OH (24 h day) concentration assumed = 0.5E6 OH/cm^3
Volatilisation ‡	from plant surfaces (BBA guideline): not required
	from soil surfaces (BBA guideline): not required
Metabolites	No experimental measurements available. Not required.

PEC_{air}

Method of calculation	No calculated. Not required.

$PEC_{(a)}$

Maximum concentration	Not calculated. Not required.

Residues requiring further assessment

Environmental occurring metabolite requiring further assessment by other disciplines (toxicology and ecotoxicology).	3) **Soil:** Azadirachtin A, Azadirachtin B, components 2.1 and 2.2 listed in section C.1.1.2.1 of Volume 4 of the DAR [in principle only for Trifolio, Neem Aza according the latest specifications], components 3, 4, 5 and 6 listed in section C.1.1.2.1 of Volume 4 of the DAR, Azadirachtin H*, other components and metabolites yet to be identified. 4) **Surface water / sediment:** Azadirachtin A, Azadirachtin B, components 2.1 and 2.2 listed in section C.1.1.2.1 of Volume 4 of the DAR [in principle only for Trifolio, Neem Aza according the latest specifications], components 3, 4, 5 and 6 listed in section C.1.1.2.1 of Volume 4 of the DAR, Azadirachtin H*, other components and metabolites yet to be identified. 5) **Ground water:** Azadirachtin A, Azadirachtin B, components 2.1 and 2.2 listed in section C.1.1.2.1 of Volume 4 of the DAR [in principle only for Trifolio, Neem Aza according the latest specifications], components 3, 4, 5 and 6 listed in section C.1.1.2.1 of Volume 4 of the DAR, Azadirachtin H*, other components and metabolites yet to be identified. **Air:** Azadirachtin active components.

Monitoring data, if available (Annex IIA, point 7.4)

Soil (indicate location and type of study)	not available

Surface water (indicate location and type of study)	not available
Ground water (indicate location and type of study)	not available
Air (indicate location and type of study)	not available

Points pertinent to the classification and proposed labelling with regard to fate and behaviour data

Candidate for R53

Effects on terrestrial vertebrates (Annex IIA, point 8.1, Annex IIIA, points 10.1 and 10.3)

Species	Test substance	Time scale	End point (mg/kg bw/day)	End point (mg/kg feed)
Birds ‡				
Colinus virginianus	a.s. NeemAzal technical (Trifolio M-GmbH)	Acute (LD_{50})	>1000 azadirachtin A >4000 extract	not relevant
Colinus virginianus	a.s. NPI-720 (Mitsui)	Acute (LD_{50})	>225 azadirachtin A >2250 extract	not relevant
Colinus virginianus	a.s. Azadirachtin technical (Sipcam)	Acute (LD_{50})	>320 azadirachtin A. >2000 extract	not relevant
Colinus virginianus	a.s. NeemAzal technical (Trifolio M-GmbH)	Short-term (LC_{50})	>269.5 azadirachtin A >1078 extract	>1300 azadirachtin A >5200 extract
Colinus virginianus	a.s. NPI-720 (Mitsui)	Short-term (LC_{50})	>139.9 azadirachtin A >1398.8 extract	>562 azadirachtin A >5620 extract
Colinus virginianus	a.s. Azadirachtin techn. (Sipcam)	Long-term (NOEC)	8.4 azadirachtin A 71.2 extract	118 azadirachtin A 1000 extract

Mammals ‡				
Mouse	a.s.: NeemAzal technical (Trifolio M-GmbH)	Acute (LD_{50})	> 841 azadirachtin A > 3365 extract	not relevant
Rat	a.s.: Azadirachtin technical Fortune Aza (Sipcam)	Acute (LD_{50})	> 330 azadirachtin A > 5000 extract	not relevant
	a.s.: Azadirachtin technical Fortune Aza (Sipcam)	Short-term, 90 d *	3.4 azadirachtin A 33 extract	not relevant
	Preparation	Acute	no data submitted, not required	
	a.s.: NeemAzal technical (Trifolio M-GmbH)	Long-term, reproduction (NOEC)	≥ 13.7 azadirachtin A** ≥ 50.0 extract**	≥ 206 azadirachtin A ≥ 750 extract
	ATI-720	Long-term, teratogenicity (NOAEL)	8.3 azadirachtin A	
Additional higher tier studies not submitted				

Toxicity/exposure ratios for terrestrial vertebrates (Annex IIIA, points 10.1 and 10.3)

Crop: potatoes
Application rate: 0.025 kg azadirachtin A/ha

Indicator species/Category[2]	Time scale	ETE	TER[1]	Annex VI Trigger[3]
Tier 1 – uptake via diet (Birds)				
insectivorous bird	Acute	1,35	> 167	10
medium herbivorous bird	Acute	1,65	> 136	10
insectivorous bird	Short-term	0,75	> 187	10
medium herbivorous bird	Short-term	0,76	> 184	10
insectivorous bird	Long-term	0,75	11.2	5
medium herbivorous bird	Long-term	0.40	20.8	5
Higher tier refinement – uptake via diet (Birds) not required				
Tier 1– uptake via drinking water (Birds) not required				
Tier 1 – secondary poisoning (Birds) not required				
Tier 1– uptake via diet (Mammals)				
Medium herbivorous mammal	Acute	0,61	> 541	10
Medium herbivorous mammal	Long-term	0.15	55.3	5
Higher tier refinement – uptake via diet (Mammals) not required				
Tier 1– uptake via drinking water (Mammals) not required				
Tier 1 – secondary poisoning (Mammals) not required				

[1] in higher tier refinement provide brief details of any refinements used (e.g., residues, PT, PD or AV)
[2] for cereals indicate if it is early or late crop stage
[3] If the Annex VI Trigger value has been adjusted during the risk assessment of the active substance (e.g. many single species data), it should appear in this column.

Toxicity data for aquatic species (most sensitive species of each group) (Annex IIA, point 8.2, Annex IIIA, point 10.2)

Group	Test substance	Time-scale (Test type)	End point	Toxicity[1] (mg/L)
Laboratory tests ‡				
Fish				
Oncorhynchus mykiss	a.s. NeemAzal (Trifolio)	96 hr (flow-through)	Mortality, EC_{50}	> 2.219 azadirachtin A_{mm} > 6.18 extract $_{mm}$
Oncorhynchus mykiss	a.s. NPI-720 (Mitsui)	96 hr (flow-through)	Mortality, EC_{50}	0.048 azadirachtin A_{mm} 0.48 extract $_{mm}$
Oncorhynchus mykiss	a.s. Fortune Aza tech. (Sipcam)	96 hr (static)	Mortality, EC_{50}	0.086 azadirachtin A $_{mm}$ 0.73 extract $_{mm}$

Group	Test substance	Time-scale (Test type)	End point	Toxicity[1] (mg/L)
Oncorhynchus mykiss	a.s. azadirachtin techn. (Sipcam)	28 d (flow-through)	Growth NOEC	0.0047 azadirachtin A_{nom} 0.04 extract $_{nom}$
Danio rerio	a.s. NeemAzal (Trifolio M-GmbH)	174 d FLC (flow-through)	Growth NOEC	not valid 1.9 azadirachtin A_{mm} 6.4 extract $_{mm}$
Oncorhynchus mykiss	Preparation NeemAzal-TS (Trifolio M-GmbH)	96 hr (semi-static)	Mortality, EC_{50}	1.41 azadirachtin A_{mm} 141 product $_{mm}$
Oncorhynchus mykiss	Preparation Oikos (Sipcam)	96 hr (static)	Mortality, EC_{50}	0.077 azadirachtin A $_{mm}$ 2.96 product $_{mm}$
Oncorhynchus mykiss	Preparation NeemAzal-TS (Trifolio M-GmbH)	28 d (flow-through)	Growth NOEC	0.712 azadirachtin A_{mm} 63.6 product $_{mm}$
Aquatic invertebrate				
Daphnia magna	a.s. NeemAzal (Trifolio M-GmbH)	48 h (static)	Mortality, EC_{50}	3.54 azadirachtin A_{mm} 10.6 extract $_{mm}$
Daphnia magna	a.s. NPI-720 (Mitsui)	48 h (flow-through)	Mortality, EC_{50}	1 azadirachtin A_{mm} 10 extract $_{mm}$
Daphnia magna	a.s. NeemAzal (Trifolio M-GmbH)	21 d (semi-static)	Reproduction, NOEC	0.615 azadirachtin A_{mm} 1.84 extract $_{mm}$
Daphnia magna	a.s. Azadirachtin techn. (Sipcam)	21 d (semi-static)	Reproduction, NOEC	0.27 azadirachtin A_{mm} 2.3 extract $_{mm}$
Daphnia magna	Preparation NeemAzal-TS (Trifolio M-GmbH)	48 h (static)	Mortality, EC_{50}	>8 azadirachtin A_{mm} >800 product $_{mm}$
Daphnia magna	Preparation NeemAzal-TS (Trifolio M-GmbH)	21 d (semi-static)	Reproduction, NOEC	0.038 azadirachtin A_{mm} 3.4 product $_{mm}$
Sediment dwelling organisms				
Chironomus riparius	NeemAzal batch 134	28 d (static)	NOEC	0.0037_{mm} (azadirachtin A) 0.011 extract $_{mm}$

Group	Test substance	Time-scale (Test type)	End point	Toxicity[1] (mg/L)
Chironomus riparius	Azatin Technical-grade Active Ingredient AZ/148/06-07	28 d (static)	NOEC	0.0016_{mm} (azadirachtin A) 0.01 extract $_{mm}$
Chironomus riparius	Fortune 11004062007	28 d (static)	NOEC	0.0033_{mm} (azadirachtin A) 0.0245 extract $_{mm}$
Chironomus riparius	OIKOS, batch G249	28 d (static)	NOEC	0.0036_{mm} (azadirachtin A) 0.144 product $_{mm}$
Chironomus riparius	NeemAzal-T/S batch 240707M	28 d (static)	NOEC	0.0029_{mm} (azadirachtin A) 0.262 product $_{mm}$
Algae				
Pseudokirchneriella subcapitata	a.s. Azadirachtin techn. (Sipcam)	72 h (static)	Biomass: E_bC_{50} Growth rate E_rC_{50}	>5.76 azadirachtin A_{mm} >36 extract $_{mm}$
Desmodesmus subspicatus	Preparation NeemAzal-TS (Trifolio M-GmbH)	72 h (static)	Biomass: E_bC_{50} Growth rate E_rC_{50}	>27.4 azadirachtin $A_{ini\ nom}$ >2494 product $_{ini\ nom}$
Higher plant no data submitted				
Microcosm or mesocosm tests				
Not required				

[1] indicate whether based on nominal ($_{nom}$) or mean measured concentrations ($_{mm}$). In the case of preparations indicate whether end points are presented as units of preparation or a.s.

Toxicity/exposure ratios for the most sensitive aquatic organisms (Annex IIIA, point 10.2)

Maximum PEC_{sw} values and TER values for azadirachtin – application to potato at 25 g as/ha

Scenario	PEC global max (µg L)	PEC twa, 28d* (µg L)	fish acute	fish prolonged	Daphnia acute	Daphnia prolonged	Algae acute	Higher plant	Sed. dweller prolonged	Microcosm / Mesocosm
			O. mykiss	***O. mykiss***	***Daphnia magna***	***Daphnia magna***	***P. subcapitata***	***Lemna* sp.**	***C. riparius***	
			LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}	E_rC_{50}	NOEC	NOEC
			48 µg/L	4.7 µg/L	1000 µg/L	38 µg/L	>5760 µg/L	no data	1.6 µg/L	no data
FOCUS Step 1	7.406		6.5	0.6	135	5.1	> 777		0.2	
FOCUS Step 2										
Northern Europe	0.848		56.6	5.5		44.8			1.9	
FOCUS Step 3										
D3 / ditch	0.131		366.4	36		290			12	
D4 / pond	0.005		9600	940		7600			320	
D4 / stream	0.111		432.4	42		342			14	
D5 / pond										
D5 / stream										
R1 / pond	0.014		3428.6	336		2714			114	
R1 / stream	0.239		200.8	20		159			7	
R2 / stream										
R3 / stream										
R4 / stream										
Annex VI Trigger**			100	10	100	10	10	10	10	5

* 28 d-PECtwa to be used in connection with the 34 d-NOEC from the ELS with *P. promelas*.

** If the Annex VI Trigger value has been adjusted during the risk assessment of the active substance, it should appear as a footnote. E.g. if it is agreed during the risk assessment of mesocosm, that a trigger value of 5 is required, it should appear as a minimum requirement to MS in relation to product approval

$FOCUS_{sw}$ step 4

TER calculation for the most critical endpoint including different mitigation options for FOCUS Step 4 Scenario – application to potato at 25 g as/ha.

Mitigation options	10 m non-spray buffer zone		Xx % input reduction required – all scenarios.		Max drift reduction (95 %)		Max run-off reduction (90%)		Max drainage reduction (90%)	
	PECsw	TER	PECsw	TER	PECsw	TER	PECsw	TER	PECsw	TER
FOCUS Step 4**										
D3 / ditch										
D4 / pond										
D4 / stream										
D5 / pond										
D5 / stream										
R1 / pond										
R1 / stream	0.108	15								
R2 / stream										
R3 / stream										
R4 / stream										

* 30 m or less as required obtaining acceptable trigger levels
** (Only scenarios where the Annex VI trigger is not met at FOCUSsw step 3 should be included in step 4).

The TERs were calculated by EFSA for the most sensitive organism (*Chironomus riparius*) for scenarios where the peak concentration is from spray drift to assess the risk from the different extracts. The PECsw values and the endpoint for Chironomus is based on the concentration of the extracts.
The table was added by EFSA after the peer-review.

Scenario FOCUS step 3	Source	Extract	PEC Extract µg extract/L	*Chironomus r.* NOEC µg extract/L	TER
D3 (ditch)	Sipcam	Fortune	1.179	24.5	20.8
	Mitsui	Azatin	1.09	10	**9.2**
	Trifolio	Neem Azal	0.524	11	21
D4 (pond)	Sipcam	Fortune	0.045	24.5	544.4
	Mitsui	Azatin	0.042	10	238.1
	Trifolio	Neem Azal	0.02	11	550
D4 (stream)	Sipcam	Fortune	0.999	24.5	24.5
	Mitsui	Azatin	0.923	10	10.8
	Trifolio	Neem Azal	0.444	11	24.8

Bioconcentration				
	Active substance	Metabolite1	Metabolite2	Metabolite3
$\log P_{O/W}$	0,56 – 0,99 azadirachtin A at 20 °C			
Bioconcentration factor (BCF)[1] ‡	not required: log P_{OW}<3			
Annex VI Trigger for the bioconcentration factor				
Clearance time (days) (CT_{50})				
(CT_{90})				
Level and nature of residues (%) in organisms after the 14 day depuration phase				

[1] only required if log $P_{O/W}$ >3.
* based on total ^{14}C or on specific compounds

Effects on honeybees (Annex IIA, point 8.3.1, Annex IIIA, point 10.4)

Test substance	Acute oral toxicity (LD_{50} µg/bee)	Acute contact toxicity (LD_{50} µg/bee)
a.s. ‡	> 8.1 (azadirachtin A)	> 11.81 (azadirachtin A)
Preparation[1] NeemAzal-T/S	> 5.9 (azadirachtin A)	> 21.0 (azadirachtin A)
Metabolite 1	-	-
Field or semi-field tests		
In a tunnel test NeemAzal-T/S applied during bee flight at a high rate of 6.0 L/ha had no harmful effects on the brood development and on adult honey bees. Therefore the risk to honey bees is acceptable.		

[1] for preparations indicate whether end point is expressed in units of a.s. or preparation

Hazard quotients for honey bees (Annex IIIA, point 10.4)

Potatoes, 0.025 kg azadirachtin A/ha

Test substance	Route	Hazard quotient	Annex VI Trigger
a.s.	Contact	< 2.1	50
a.s.	oral	< 3.5	50
Preparation	Contact	< 1.2	50
Preparation	oral	< 4.2	50

Effects on other arthropod species (Annex IIA, point 8.3.2, Annex IIIA, point 10.5)

Laboratory tests with standard sensitive species

Species	Test Substance	End point	Effect (LR_{50} g/ha[1])
Typhlodromus pyri ‡	Preparation Aza-dirachtin 3% (Sipcam)	Mortality Reproduction red.	Limit, 100 g azadirachtin A/ha: 50 % (corr.) Limit, 100 g azadirachtin A/ha: 92.7 %
Aphidius rhopalosiphi ‡	Preparation Aza-dirachtin 3%	Mortality	Limit, 100 g azadirachtin A/ha: 65.7 % (corr.)

Species	Test Substance	End point	Effect (LR_{50} g/ha[1])
	(Sipcam)	Reproduction red.	Limit, 100 g azadirachtin A/ha: 54 %
Aphidius rhopalosiphi ‡	Preparation NeemAzal-T/S 1% (Trifolio M-GmbH)	Mortality	Limit, 57.6 g azadirachtin A/ha: 100 % (corr.)

[1] for preparations indicate whether end point is expressed in units of a.s. or preparation

Potatoe with 25 g as/ha

Test substance	Species	Effect (LR_{50} g/ha)	HQ in-field	HQ off-field[1]	Trigger
Azadirachtin A in NeemAzal T/S	*Typhlodromus pyri*	100	0.25	0.014 (1 m)	2
Azadirachtin A in NeemAzal T/S	*Aphidius rhopalosiphi*	No suitable LR_{50} available	-	-	2

[1] indicate distance assumed to calculate the drift rate

Further laboratory and extended laboratory studies ‡

Species	Life stage	Test substance, substrate and duration	Dose (g/ha)[1,2]	End point	% effect[3]	Trigger value
Chrysoperla carnea	larvae	Preparation Azadirachtin 3% (Sipcam), lab glass, 14 d, Limit	100 as initial	Mortality corr.	Whole unit sprayed: 79 % Half unit sprayed, food on sprayed part: 63 % Half unit sprayed, food on clean part: 0 % (-21 %)	50 %
Chrysoperla carnea 2-D	larvae	NeemAzal 34 %, extended lab, 26 d, exposure on detached apple leaves, dose response test	6.4 – 150 (Azadirach-tin A)	Mortality (corr.) No reproduction assessment, because > 50 % of the adults died	100 % (total mortality) LR_{50} < 6.4g a.s./ha	50 %

Species	Life stage	Test substance, substrate and duration	Dose (g/ha)[1,2]	End point	% effect[3]	Trigger value
Chrysoperla carnea 2-D	larvae	Azatin Technical-grade Active Ingredient 15.6 %, extended lab, 36 d, exposure on detached apple leaves, dose response test	6.4 – 150 (Azadirach-tin A)	Mortality (corr.) Reproduction (hatching rate) 14.1 g a.s./ha other conc. not tested	100 % (total mortality) LR_{50}< 6.4 g a.s/ha 9.11 % (compared to solvent control)	50 %
Chrysoperla carnea 2-D	larvae	Fortune 13.6 %, extended lab, 36 d, exposure on detached apple leaves, dose response test	6.40 – 150 (Azadirach-tin A)	Mortality (corr.) Reproduction (hatching rate) 6.40 g a.s./ha other conc. not tested	96.3 % (total mortality) LR_{50}< 6.4 g a.s./ha 28.56 % (compared to solvent control)	50 %
Chrysoperla carnea 2-D	larvae	Preparation NeemAzal-T/S 1.09 %, extended lab, 34 d, exposure on detached apple leaves, dose response test	0.77 – 30 (Azadirach-tin A)	Mortality (corr.) Reproduction (hatching rate) 0.77 g a.s./ha other conc. not tested	100 % (for 1.9 a.s./ha) LR_{50} ~ 0.77 g a.s./ha - 0.12 % (compared to control, value unreproducible)	50 %
Chrysoperla carnea 2-D	larvae	Preparation NeemAzal-T/S 1.09 %, extended lab, 34 d, exposure on detached apple leaves, dose response test	0.05 – 1.9 (Azadirach-tin A)	Mortality (corr.) Reproduction (hatching rate) 0.05 g a.s./ha 0.12 g a.s./ha 0.30 g a.s./ha 0.76 g a.s./ha 1.90 g a.s./ha	4.0 % (max. value**) LR_{50} > 1.9 g a.s./ha - 0.1 % - 7.8 % - 2.6% - 8.8 % - 7.0 % (compared to control)	50 %

Species	Life stage	Test substance, substrate and duration	Dose (g/ha)[1,2]	End point	% effect[3]	Trigger value
Chrysoperla carnea 3-D	larvae	Preparation NeemAzal-T/S 1.09 %, extended lab, 38 d, exposure on detached sweet pepper leaves, freshly applied and aged residues	48.4 (Azadirach-tin A) freshly applied 14 DAA 28 DAA 42 DAA	Mortality (corr.) Reproduction (reduction) 14 DAA 28 DAA freshly applied and 42 DAA not tested	96.2 % (max. value*) ~~45.3~~10.3 % ~~24.9~~3.3 % (compared to control)	50 %
Chrysoperla carnea 3-D	larvae	Preparation NeemAzal-T/S 1.09 %, extended lab, 49 d, exposure on detached sweet pepper leaves, freshly applied and aged residues	20.1 (Azadirach-tin A) freshly applied 21 DAA	Mortality (corr.) Reproduction (reduction) 21 DAA freshly applied not tested	57.7 % (max. value*) ~~12.8~~3.7 % (compared to control)	50 %
Chrysoperla carnea 2-D	larvae	Preparation OIKOS 2.53 %, extended lab, 34 d, exposure on detached apple leaves, dose response test	0.77 – 30.0 (Azadirach-tin A)	Mortality (corr.) Reproduction (hatching rate) 0.77 g a.s./ha 1.90 g a.s./ha 4.80 g a.s./ha 12 and 30 g a.is/ha not tested	100 % (max. value*) LR_{50}: 12.44 g a.s/ha - 52.67 % - 30.11 % - 41.01 % (compared to control)	50 %

Species	Life stage	Test substance, substrate and duration	Dose (g/ha)[1,2]	End point	% effect[3]	Trigger value
Chrysoperla carnea	larvae	Preparation OIKOS 2.9 %, extended lab, 34 d, exposure on detached apple leaves, dose response test	0.02 – 0.77 (Azadirachtin A)	Mortality (corr.) Reproduction (hatching rate) 0.02 g a.s./ha 0.05 g a.s./ha 0.12 g a.s./ha 0.31 g a.s./ha 0.77 g a.s./ha	- 11.5 % (max. value*) LR_{50} > 0.77 g a.s/ha - 3.9 % - 4.1 % - 2.9% - 2.7 % - 4.4 % (compared to control)	50 %
Chrysoperla carnea 3-D	larvae	Preparation OIKOS 2.53 %, extended lab, 38 d, exposure on detached sweet pepper leaves, freshly applied and aged residues	48.4 (Azadirachtin A) freshly applied 14 DAA 28 DAA 42 DAA	Mortality (corr.) Reproduction (reduction) 14 DAA 28 DAA freshly applied and 42 DAA not tested	84.6 % (max. value*) ~~16.6~~20.7 % ~~17.4~~0 % (compared to control)	50 %
Chrysoperla carnea 3-D	larvae	Preparation OIKOS 2.53 %, extended lab, 49 d, exposure on detached sweet pepper leaves, freshly applied and aged residues	20.1 (Azadirachtin A) freshly applied 21 DAA	Mortality (corr.) Reproduction (reduction) 21 DAA freshly applied not tested	57.7 % (max. value*) ~~0~~10.3 % (compared to control)	50 %
Poecilus cupreus	adults	Preparation Azadirachtin 3 % (Sipcam), lab sand, 14 d, Limit	100 as initial	Mortality corr.	0 %	50 %
Poecilus cupreus	adults	Preparation NeemAzal-T/S 0.4 % [a] (Trifolio M-GmbH), lab sand, 14 d, Limit	8 [a] as initial	Mortality corr.	3.3 %	50 %
Coccinella septempunctata	larvae	Preparation NeemAzal-T/S 0.4 % [a] (Trifolio M-GmbH), lab glass, 65 d, Limit	12 [a] as initial	Mortality corr. Reproduction reduction	10.2 % 17 %	50 %

Species	Life stage	Test substance, substrate and duration	Dose (g/ha)[1,2]	End point	% effect[3]	Trigger value
Coccinella septempunctata 2-D	larvae	NeemAzal 34 %, extended lab, 42 d, exposure on detached sweet pepper leaves, dose response test	2.6 – 100 (Azadirach-tin A)	Mortality (corr.) Reproduction reduction 2.6 g a.s./ha 6.4 g a.s./ha other conc. not tested	83.3 % (max. value*) LR_{50}: 15.8 g a.s./ha 62.6 % 78.3 %	50 %
Coccinella septempunctata 2-D	larvae	Azatin 15 % Technical product, extended lab, 42 d, exposure on detached sweet pepper leaves, dose response test	2.6 – 100 (Azadirachti n A)	Mortality (corr.) Reproduction reduction 2.6 g a.s./ha 6.4 g a.s./ha 16 g a.s./ha	76.6 % (max. value*) LR_{50}: 25.4 g a.s./ha 50.7 % 60.6 % 73.4 %	50 %
Coccinella septempunctata 2-D	larvae	Fortune Aza Technical Powder 13.6 %, extended lab, 42 d, exposure on detached sweet pepper leaves, dose response test	2.6 – 100 (Azadirach-tin A)	Mortality (corr.) Reproduction reduction 2.6 g a.s./ha 6.4 g a.s./ha 16 g a.s./ha	73.3 % (max. value*) LR_{50}: 26.2 g a.s./ha 43.3 % 49.7 % 74 %	50 %
Coccinella septempunctata 3-D	larvae	Preparation NeemAzal-T/S 1.09 %, extended lab, 47 d, exposure on detached bean leaves, dose response test	0.08 – 7.00 (Azadirach-tin A)	Mortality (corr.) Reproduction reduction 0.08 g a.s./ha 0.23 g a.s./ha 0.73 g a.s./ha 2.26 g a.s./ha 7 g a.s./ha not tested	100 % (max. value*) LR_{50}: 1.94 g a.s./ha 34.58 % 18.12 % - 79.53 % - 108.71 %	50 %

Species	Life stage	Test substance, substrate and duration	Dose (g/ha)[1,2]	End point	% effect[3]	Trigger value
Coccinella septempunctata 3-D	larvae	Preparation OIKOS 2.53 %, extended lab, 50 d, exposure on detached bean leaves, dose response test	0.15 – 14.00 (Azadirach-tin A)	Mortality (corr.) Reproduction reduction 0.15 g a.s./ha 0.47 g a.s./ha 1.46 g a.s./ha 4.52 g a.s./ha 14 g a.s/ha not tested	53.33 % (max. value*) LR_{50}: 10.45 g a.s./ha 9.26 % 45.37 % - 2.08 % - 13.59 %	50 %
Aphidius rhopalosiphi	adults	Preparation Oikos 2.16 % (Sipcam), extended lab, barley, 2 d, 11.3 – 118.4 g a.s./ha	118.4 as initial	Mortality corr.	6.9 % (max. value)	50 %
				Reproduction reduction	12 % (max. value)	
Aphidius rhopalosiphi	adults	Preparation NeemAzal-T/S 1 % (Trifolio M-GmbH), extended lab, oat, 2 d, Limit	57.6 as initial	Mortality corr.	15 %	50 %
				Reproduction reduction	5 %	
				beneficial capacity red.	19.3 %	
Typhlodromus pyri	adults	Preparation Oikos 2.16 % (Sipcam), extended lab, apple leaves, 7 d, 11.3 – 118.4 g as/ha	11.3 as initial	Mortality corr.	1.8 % (max. value) [b]	50 %
			NOEC Reproduction: 65.8 g azadirachtin A/ha initial			

[1] indicate whether initial or aged residues
[2] for preparations indicate whether dose is expressed in units of as or preparation
[3] indicate if positive percentages relate to adverse effects or not
a: purity: 4 g/kg – no clear indications whether sum of azadirachtins or amount of azadirachtin A
b: no clear concentration-response-relationship
*: corresponding to the highest test concentration

Field or semi-field tests						
Episyrphus balteatus	larvae	Preparation NeemAzal-T/S 1 % (Trifolio M-GmbH), semi-field, bean – gauze tent, 42 d, Limit	57.6 a.s. initial	Mortality corr.	49 %	50 %
				Reproduction reduction *	100 %	
				beneficial capacity red. *	100 %	

Typhlodromus pyri	adults	Preparation NeemAzal-T/S 1 % (Trifolio M-GmbH), field – viniculture, 42 d, Limit, 2 applications [a]	12.9 azadirachtin A initial	1. application - 58.7 % damage after 7 d
			18.9 azadirachtin A initial	2. application [a] - no damage after 42 d (inclusive time after 1st application)

*: 100 % mortality during phase of reproduction
a: 2nd application (18.9 g a.s./ha) 14 d after 1st application (12.9 g a.s./ha)

Effects on earthworms, other soil macro-organisms and soil micro-organisms (Annex IIA points 8.4 and 8.5. Annex IIIA, points, 10.6 and 10.7)

Test organism	Test substance	Time scale	End point[1]
Earthworms			
Eisenia foetida	Extract: Azadirachtin techn. (Sipcam)	Acute 14 d	>1000 mg azadirachtin A/kg d.w.soil $_{nom}$ >8880 mg/kg d.w.soil extract $_{nom}$
		Chronic 56 d	Not required
Eisenia foetida	Preparation NeemAzal-TS (Trifolio M-GmbH)	Acute 14 d	content of azadirachtin A in the formulation is not derivable. >1000 mg/kg d.w.soil $_{nom}$ product
		Chronic 56 d	Not required
	Metabolite	Acute 14 d	Not required
	Metabolite	Chronic 56 d	Not required
Other soil macro-organisms			
Hypoaspis aculeifer	NeemAzal	14 d	0 % effect mortality 29 – 34.9 % effect reprod.
	Preparation		
	Metabolite 1		
Collembola			
	a.s. ‡	Chronic	NOEC mg a.s./kg d.w.soil (mg a.s/ha)
	Preparation		
	Metabolite 1		
Soil micro-organisms no data submitted, not required			
Nitrogen mineralisation	Azadirachtin technical **Purity:** 170 g Azadirachtin A+B /kg Approx. 100 g azadirachtin A	Loamy sand, ph: 6.03	5.5 % effect at day 28 (< 25 %) at 480 g azadirachtin technical/ha at 82 g azadirachtin A+B /ha

Test organism	Test substance	Time scale	End point[1]
	Preparation NeemAzal-TS (1 % azadirachthin A)	Silty sand, pH: 6	9.0 % effect at day 28 (< 25 %) at 30 L/ha at 333 g as/ha
Carbon mineralisation	Azadirachtin technical **Purity:** 170 g Azadirachtin A+B /kg Approx. 100 g azadirachtin A	Loamy sand, ph: 6.03	5.3 % effect at day 28 (< 25%) at 480 g azadirachtin technical/ha at 82 g azadirachtin A+B /ha
	NeemAzal-TS (1% azadirachthin A)	Silty sand, pH: 6	13.0 % effect at day 28 (< 25%) at 30 L/ha at 333 g azadirachtin A/ha
Field studies[2]			
Earthworm, field, 13 months - no valid data submitted			

[1] indicate where end point has been corrected due to log P_{ow} >2.0 (e.g. LC_{50corr})
[2] litter bag, field arthropod studies not included at 8.3.2/10.5 above, and earthworm field studies

Toxicity/exposure ratios for soil organisms

Crop and application rate

Test organism	Test substance	Time scale	Soil PEC[2]	TER	Trigger
Earthworms					
Eisenia foetida	as ‡ azadirachtin A	Acute	1 x single Application PEC_i 0.033 mg as/kg	> 30303	10
	as ‡	Chronic	Not required		5
Eisenia foetida	Preparation NeemAzal-TS (Trifolio M-GmbH)	Acute	1 x single Application PEC_i 3.3	> 303	10
	Preparation	Chronic	Not required		5
	Metabolite 1	Acute	Not required		10
	Metabolite 1	Chronic	Not required		5
Other soil macro-organisms not required					
Soil mite	a.s. ‡		Not required		
	Preparation		Not required		
	Metabolite 1		Not required		
Collembola	a.s. ‡		Not required		
	Preparation		Not required		
	Metabolite 1		Not required		

[1] to be completed where first Tier triggers are breached

[2] indicate which PEC soil was used (e.g. plateau PEC)

Effects on non target plants (Annex IIA, point 8.6, Annex IIIA, point 10.8)

Preliminary screening data

Preparation NeemAzal-TS (Trifolio M-GmbH), limit-test (single application rate), 6 species, 22 d, *Zea mays*: 30.9 g azadirachtin A/ha $_{nom}$ (2940 g/ha product $_{nom}$) cause 21.1% effect in reduction biomass (fw). Threshold value is 50 % at single application rate. No phytotoxicity obtained.

Laboratory dose response tests

Most sensitive species	Test substance	ER_{50} (g/ha)[2] vegetative vigour	ER_{50} (g/ha)[2] emergence	Exposure[1] (g/ha)[2]	TER	Trigger
No additional data submitted, not required						

[1] explanation of how exposure has been estimated should be provided (e.g. based on Ganzelmeier drift data)
[2] for preparations indicate whether dose is expressed in units of a.s. or preparation

Additional studies (e.g. semi-field or field studies)

No additional data submitted, not required

Effects on biological methods for sewage treatment (Annex IIA 8.7)

Test type/organism	end point
Activated sludge	NOEC= 1000 mg Neem Azal (34% azadirachtin A)/ L
Pseudomonas sp	degradation >25% after 14d (100 mg azadirachtin A /L + 100 mg Na- benzoate /L)

Ecotoxicologically relevant compounds (consider parent and all relevant metabolites requiring further assessment from the fate section)

Compartment	
soil	Azadirachtin A
water	Azadirachtin A
sediment	Azadirachtin A
groundwater	Azadirachtin A

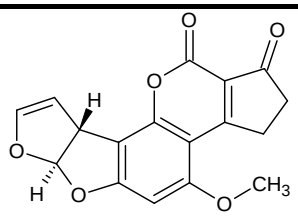
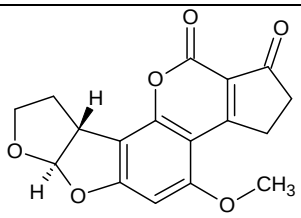
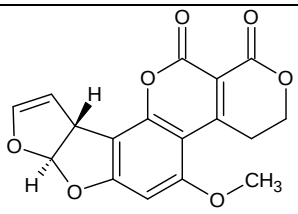
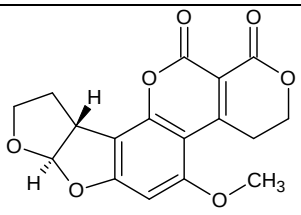
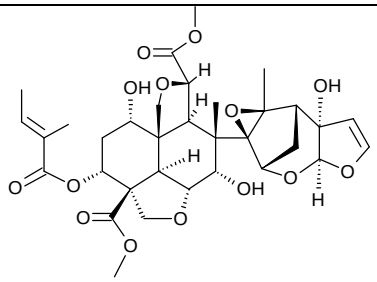
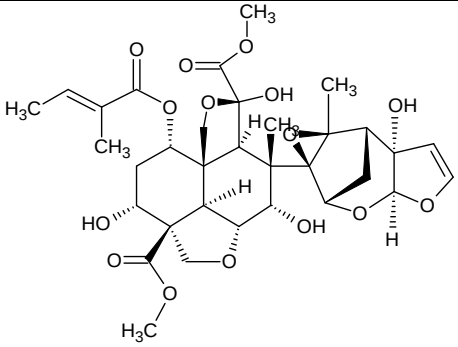
Classification and proposed labelling with regard to ecotoxicological data (Annex IIA, point 10 and Annex IIIA, point 12.3)

RMS/peer review proposal

Active substance	Azadirachtin technical from SIPCAM and Mitsui: N, R50/53 Dangerous for the environment, very toxic to aquatic organisms, may cause long-term effects in the aquatic environment Azadirachtin technical NeemAzal (TRIFOLIO): N, R51/53 Dangerous for the environment, toxic to aquatic organisms, may cause long-term effects in the aquatic environment

	RMS/peer review proposal
Preparation	OIKOS from SIPCAM: N, R51/53 Dangerous for the environment, toxic to aquatic organisms, may cause long-term effects in the aquatic environment NeemAzal-T/S from TRIFOLIO: N, R51/53 Dangerous for the environment, toxic to aquatic organisms, may cause long-term effects in the aquatic environment

APPENDIX B – USED COMPOUND CODE(S)

Code/Trivial name	Chemical name*	Structural formula*
aflatoxin B_1	(6a*R*,9a*R*)-4-methoxy-2,3,6a,9a-tetrahydrocyclopenta[*c*]furo[3',2':4,5]furo[2,3-*h*]chromene-1,11-dione	
aflatoxin B_2	(6a*R*,9a*R*)-4-methoxy-2,3,6a,8,9,9a-hexahydrocyclopenta[*c*]furo[3',2':4,5]furo[2,3-*h*]chromene-1,11-dione	
aflatoxin G_1	(7a*R*,10a*R*)-5-methoxy-3,4,7a,10a-tetrahydro-1*H*,12*H*-furo[3',2':4,5]furo[2,3-*h*]pyrano[3,4-*c*]chromene-1,12-dione	
aflatoxin G_2	(7a*R*,10a*R*)-5-methoxy-3,4,7a,9,10,10a-hexahydro-1*H*,12*H*-furo[3',2':4,5]furo[2,3-*h*]pyrano[3,4-*c*]chromene-1,12-dione	
azadirachtin B	dimethyl (2a*R*,3*S*,4*S*,4a*R*,5*S*,7a*S*,8*S*,10*R*,10a*S*,10b*R*)-3,8-dihydroxy-4-[(1a*R*,2*S*,3a*S*,6a*S*,7*S*,7a*S*)-6a-hydroxy-7a-methyl-3a,6a,7,7a-tetrahydro-2,7-methanofuro[2,3-*b*]oxireno[*e*]oxepin-1a(2*H*)-yl]-4-methyl-10-{[(2*E*)-2-methylbut-2-enoyl]oxy}octahydro-1*H*-naphtho[1,8a-*c*:4,5-*b*′*c*′]difuran-5,10a(8*H*)-dicarboxylate	
Azadirachtin H* (from soil)	dimethyl (2a*R*,3*S*,4*S*,4a*R*,5*S*,7a*S*,8*S*,10*R*,10a*S*,10b*R*)-3,5,10-trihydroxy-4-[(1a*R*,2*S*,3a*S*,6a*S*,7*S*,7a*S*)-6a-hydroxy-7a-methyl-3a,6a,7,7a-tetrahydro-2,7-methanofuro[2,3-b]oxireno[*e*]oxepin-1a(2*H*)-yl]-4-methyl-8-{[(2*E*)-2-methylbut-2-enoyl]oxy}octahydro-1*H*-naphtho[1,8a-*c*:4,5-*b*′*c*′]difuran-5,10a(8*H*)-dicarboxylate	

* ACD/ChemSketch, Advanced Chemistry Development, Inc., ACD/Labs Release: 12.00 Product version: 12.00 (Build 29305, 25 Nov 2008).

ABBREVIATIONS

1/n	slope of Freundlich isotherm
ε	decadic molar extinction coefficient
°C	degree Celsius (centigrade)
µg	microgram
µm	micrometer (micron)
a.s.	active substance
AChE	acetylcholinesterase
ADE	actual dermal exposure
ADI	acceptable daily intake
AF	assessment factor
AOEL	acceptable operator exposure level
AP	alkaline phosphatase
AR	applied radioactivity
ARfD	acute reference dose
AST	aspartate aminotransferase (SGOT)
AV	avoidance factor
BCF	bioconcentration factor
BUN	blood urea nitrogen
bw	body weight
CAS	Chemical Abstract Service
CFU	colony forming units
ChE	cholinesterase
CI	confidence interval
CIPAC	Collaborative International Pesticides Analytical Council Limited
CL	confidence limits
d	day
DAA	days after application
DAR	draft assessment report
DAT	days after treatment
DM	dry matter
DT_{50}	period required for 50 percent disappearance (define method of estimation)
DT_{90}	period required for 90 percent disappearance (define method of estimation)
dw	dry weight
EbC_{50}	effective concentration (biomass)
EC_{50}	effective concentration
ECHA	European Chemical Agency
EEC	European Economic Community
EINECS	European Inventory of Existing Commercial Chemical Substances
ELINCS	European List of New Chemical Substances
EMDI	estimated maximum daily intake
ER_{50}	emergence rate/effective rate, median
ErC_{50}	effective concentration (growth rate)
ETE	estimated theoretical exposure
EU	European Union
EUROPOEM	European Predictive Operator Exposure Model
f(twa)	time weighted average factor
FAO	Food and Agriculture Organisation of the United Nations
FIR	Food intake rate
FOB	functional observation battery
FOCUS	Forum for the Co-ordination of Pesticide Fate Models and their Use
g	gram
GAP	good agricultural practice
GC	gas chromatography

GCPF	Global Crop Protection Federation (formerly known as GIFAP)
GGT	gamma glutamyl transferase
GM	geometric mean
GS	growth stage
GSH	glutathion
h	hour(s)
ha	hectare
Hb	haemoglobin
Hct	haematocrit
hL	hectolitre
HPLC	high pressure liquid chromatography or high performance liquid chromatography
HPLC-FLD	high performance liquid chromatography with fluorescence detection
HPLC-MS	high performance liquid chromatography – mass spectrometry
HPLC-UV	high performance liquid chromatography with ultra violet detection
HQ	hazard quotient
IEDI	international estimated daily intake
IESTI	international estimated short-term intake
ISO	International Organisation for Standardisation
IUPAC	International Union of Pure and Applied Chemistry
JMPR	Joint Meeting on the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Expert Group on Pesticide Residues (Joint Meeting on Pesticide Residues)
K_{doc}	organic carbon linear adsorption coefficient
kg	kilogram
K_{Foc}	Freundlich organic carbon adsorption coefficient
L	litre
LC	liquid chromatography
LC_{50}	lethal concentration, median
LC-MS	liquid chromatography-mass spectrometry
LC-MS-MS	liquid chromatography with tandem mass spectrometry
LD_{50}	lethal dose, median; dosis letalis media
LDH	lactate dehydrogenase
LOAEL	lowest observable adverse effect level
LOD	limit of detection
LOQ	limit of quantification (determination)
m	metre
M/L	mixing and loading
MAF	multiple application factor
MCH	mean corpuscular haemoglobin
MCHC	mean corpuscular haemoglobin concentration
MCV	mean corpuscular volume
mg	milligram
mL	millilitre
mm	millimetre
MRL	maximum residue limit or level
MS	mass spectrometry
MSDS	material safety data sheet
MTD	maximum tolerated dose
MWHC	maximum water holding capacity
NESTI	national estimated short-term intake
ng	nanogram
NOAEC	no observed adverse effect concentration
NOAEL	no observed adverse effect level
NOEC	no observed effect concentration

NOEL	no observed effect level
OM	organic matter content
Pa	Pascal
PD	proportion of different food types
PEC	predicted environmental concentration
PEC_{air}	predicted environmental concentration in air
PEC_{gw}	predicted environmental concentration in ground water
PEC_{sed}	predicted environmental concentration in sediment
PEC_{soil}	predicted environmental concentration in soil
PEC_{sw}	predicted environmental concentration in surface water
pH	pH-value
PHED	pesticide handler's exposure data
PHI	pre-harvest interval
PIE	potential inhalation exposure
pK_a	negative logarithm (to the base 10) of the dissociation constant
POEM	Predictive Operator Exposure Model
P_{ow}	partition coefficient between *n*-octanol and water
PPE	personal protective equipment
ppm	parts per million (10^{-6})
ppp	plant protection product
PT	proportion of diet obtained in the treated area
PTT	partial thromboplastin time
QSAR	quantitative structure-activity relationship
r^2	coefficient of determination
RPE	respiratory protective equipment
RUD	residue per unit dose
SC	suspension concentrate
SD	standard deviation
SFO	single first-order
SSD	species sensitivity distribution
STMR	supervised trials median residue
$t_{1/2}$	half-life (define method of estimation)
TER	toxicity exposure ratio
TER_A	toxicity exposure ratio for acute exposure
TER_{LT}	toxicity exposure ratio following chronic exposure
TER_{ST}	toxicity exposure ratio following repeated exposure
TK	technical concentrate
TLV	threshold limit value
TMDI	theoretical maximum daily intake
TRR	total radioactive residue
TSH	thyroid stimulating hormone (thyrotropin)
TWA	time weighted average
UDS	unscheduled DNA synthesis
UV	ultraviolet
W/S	water/sediment
w/v	weight per volume
w/w	weight per weight
WBC	white blood cell
WG	water dispersible granule
WHO	World Health Organisation
wk	week
yr	year