Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

to the Opinion proposing harmonised classification and labelling at EU level of

emamectin benzoate (ISO); (4''R)-4''-deoxy-4''-(methylamino)avermectin B1 benzoate

EC Number: -
CAS Number: 155569-91-8
(formerly 13751274-4 and 179607-18-2)

CLH-O-0000006712-75-01/F

Adopted

20 September 2019

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON EMAMECTIN BENZOATE (ISO); (4''R)-4''-DEOXY-4''-(METHYLAMINO)AVERMECTIN B1 BENZOATE

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during public consultation are made available in the table below as submitted through the web form. Any attachments received are referred to in this table and listed underneath, or have been copied directly into the table.

All comments and attachments including confidential information received during the public consultation have been provided in full to the dossier submitter (Member State Competent Authority), the Committees and to the European Commission. Non-confidential attachments that have not been copied into the table directly are published after the public consultation and are also published together with the opinion (after adoption) on ECHA's website. Dossier submitters who are manufacturers, importers or downstream users, will only receive the comments and non-confidential attachments, and not the confidential information received from other parties.

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Substance name: emamectin benzoate (ISO); (4''R)-4''-deoxy-4''-(methylamino)avermectin B1 benzoate
EC number: -
CAS number: 155569-91-8 (formerly 13751274-4 and 179607-18-2)
Dossier submitter: The Netherlands

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
08.01.2019	Germany		MemberState	1
Comment received				
Generally, we agree with the proposals made by the DS. With regard to Reproductive Toxicity comments are added below. Some clarifications are needed and the rationale should be revised, because a classification as Repr. 2 H361f and Repr. 2 H361d might be indicated.				
Dossier Submitter's Response				
Thank you for your comment. Please see our response to comment 3.				
RAC's response				
Thank you for your comments. RAC is mandated to compare the available data against the current CLP criteria and use the current CLP guidance. As to developmental toxicity and effects on sexual function and fertility, RAC has provided an extensive analysis taken into account the CLH report as well as comments and data submitted during the public consultation.				

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
08.01.2019	United Kingdom	Syngenta	Company-Manufacturer	2
Comment received				
Syngenta agree with the dossier submitter that no classification is required for reproductive effects. The apparent effects on fertility/fecundity in the rat at high dose can be explained by impaired mating due to neurotoxicity. See attached document - RAC Public Comments rat fecundity.				

The effects in the neonatal rat are considered to be direct toxicity, and not a maternally mediated effect. See attached document - RAC Public Comments neonatal rat neurotoxicity ECHA note – An attachment was submitted with the comment above. Refer to public attachment Emamectin benzoate RAC Public Comments Jan 19.zip
Dossier Submitter's Response
Thank you for your support and elaborate documents on reduced rat fecundity and neonatal neurotoxicity.
RAC's response
RAC considers the lower fertility/fecundity index at the top dose as an adverse effect in its own right (cf. CLP, Annex I, 3.7.1.3). See RAC's response to comment 3.

Date	Country	Organisation	Type of Organisation	Comment number
08.01.2019	Germany		MemberState	3
Comment received				
Adverse effects on sexual function and fertility It can be taken from the CLH report (p. 38, chapter 10.10.1, table 38) that a treatment-related decrease of the fecundity index (defined as pregnant females/mated females) occurred in the high dose of the 2-generation study. In chapter 10.10.2 (p. 39) the observed effect is generalized as reduced fecundity and fertility and it is stated by the DS that "reduced fecundity and fertility at the high dose level is considered to be treatment related and to be a secondary consequence of neurotoxicity to the male leading to ineffective copulation" and in chapter 10.10.3 (p. 40) that "mating behaviour is considered to be influenced by parental effects not directly related to reproduction (e.g. neurotoxicity).Therefore the effects on the mating behaviour may not warrant classification". The term "fertility index" is defined in the CLP Regulation (and the guidance) as "no. animals with implants/no. of matings x 100" while the term "fecundity index" is not mentioned there. With respect to reproductive indices in animal studies more precise definitions for both terms are given in the textbook "Developmental and Reproductive Toxicology: A Practical Approach, ed. RD Hood 2006, p 440/441". "Fertility index" is defined as number of pregnant females/number of cohabited females x 100) and "fecundity index" as number of pregnant females/number of females with confirmed mating x 100. According to the original study report "The number of 'infertile' males was comparable across all groups (2-3-1-1). This limited analysis to determine the affected sex suggests that there was a treatment-related effect on a small proportion of females involving an inhibition of some reproductive processes that normally occur after successful mating." This information indicates that a classification as Repr. 2 H361f might be appropriate, as there could be some "evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development (…)" (Annex I: 3.7.2.1.1, Table 3.7.1 (a)). It would be helpful to clarify, whether mating was confirmed by detecting parameters like vaginal plugs and smears. Using the definitions of Hood (2006) marked neurological disorders could induce a reduced fertility index, because animals are hindered from				

mating. A reduced fecundity index is more difficult to explain by neurological disorders since copulation occurred in the reference group.

Adverse effects on development

In the range finding study in rabbits (tested dose levels were 2, 4, 6, 8 mg/kg bw/d), one foetus showed at 8 mg/kg bw/d cleft palate and hydrocephalus, another foetus from another litter showed hydrocephalus. These malformations are not mentioned in the Summary Table on page 41 and should be added.

The DS argues that "these foetuses were (…) from females with the greatest body weight loss and/or tremors. The effects were therefore considered to be secondary to maternal toxicity." However, it is stated in the CLP Regulation that "classification shall not automatically be discounted for substances that produce developmental toxicity only in association with maternal toxicity" (Annex I: 3.7.2.4.3).

The decrease in body weight (gain) is considered remarkable. However, teratogenic effects are not a common secondary effect of decreased food intake and body weight (gain) in rabbits (Nitzsche, D. Regulatory Toxicology and Pharmacology 90 (2017) 95-103). It would be helpful to compare the incidence of these malformations with historical control data.

In the main study in rabbits, dose levels of 1.5, 3 and 6 mg/kg bw/d were tested. At 6 mg/kg bw/d, 2 foetuses from separate litters showed lumbar vertebra malformations, 2 other foetuses from separate litters showed sternebral malformations, and 2 other foetuses from the same litter showed cervical rib variations (none in the control group). This information is not given in the CLH-report, but can be found in the corresponding annex 1 (CLH_REP_Annex1_NL_SPS-014459-18).

There it reads (p. 79): "Slight increases in the number of skeletal malformations and variations were found in the high dose group, like malformations of lumbar vertebra and of sternebra and a slightly higher number of cervical rib (variant). According to the study authors the incidences of these anomalies were within the historical control data from the laboratory concerned. In table B.6.6.2-3 the incidences from the study and from the historical control data are shown. Although no extensive historical control data is available, the present reviewer can agree with the study authors that the increased incidences are not treatment-related."
Only the highest incidence values are given in the corresponding table on page 80, not the range.

In the study on developmental neurotoxicity in rats a reduced body weight gain of pre-weaning and post-weaning pubs (e. g. 42 % and 40 % below control, day 21) and neurotoxicity in pubs were observed in the high dose. It is stated in chapter 10.10.6, (p. 45): "These findings were observed in the presence of maternal toxicity and not considered sufficient for classification." However no relevant maternal toxicity is described and the highest dose is set as maternal NOAEL in the CLH-report. Furthermore, only in chapter 10.12 (Specific target organ toxicity-repeated exposure) it is stated on page 67, that effects observed in neonatal rats during lactation are considered not relevant for human risk assessment, because neonatal rats have limited P-glycoprotein expression until 20 days after birth. This information should also be mentioned with regard to the evaluation of the study on developmental neurotoxicity.

Overall, the above mentioned data deserve some more attention to clarify whether a

classification as Repr. 2 H361d is indicated. RAC adopted the opinion that abamectin/avermectin B1a, a substance similar to emamectin, should be classified as Repr.2 H361d (https://echa.europa.eu/documents/10162/bae6ac0b-7da6-c49d-3f6c-de4196db0427)

Dossier Submitter's Response

Thank you for your comments.

Adverse effects on sexual function and fertility

Please be referred to the public attachment Emamectin benzoate RAC Public Comments Jan 19.zip. The DS agrees with the conclusions drawn by the notifier on reduced rat fecundity/ fertility as observed in a rat 2-generation reproduction study. In short, reduced fecundity/ fertility was only observed in the high dose group which reaches statistical significance only at the high dose F1-animals upon generating the F2-generation. These findings are consistent with the neurological effects noted at the high dose group (3.6 mg/kg bw/day). Upon lowering the high dose level, the incidence of the observed neurological effects were reduced. In fact, as indicated in the footnote to table 10.1.2-1 (i.e. page 65 of Annex I to the CLH-report) in all F1a litters of the high dose group (3.6 mg/kg bw/day) a number of pups displayed head or whole body tremors, hindlimb extensions, and limited use of hind limbs, usually starting between LD 7-12. In the high dose F1b litters, where the dose was lowered from 3.6 to 1.8 mg/kg bw/day from GD 0 to LD 21, tremors and limited use of hindlimbs was only observed in 5 and 7 litters, respectively. In F2 pups, in the high dose group (1.8 mg/kg bw/day) from GD 0 to LD 21, tremors and limited use of hindlimbs was observed in 1 litter only. Moreover, at the high dose there was an increase of females showing evidence of pseudopregnancy since a characteristic body weight gain was observed in females, e.g. increased weight gain up to day 12 and a weight loss on the subsequent days. These effects are likely caused by ineffective copulation caused by neurological effects in male rats. Recalculating the fecundity/ fertility index by excluding pseudopregnancies shows that both fecundity and fertility are not affected at all doses tested. Therefore, the DS is of the opinion that the observed effects on fecundity/ fertility are secondary to neurotoxicity and that classification is thus not warranted.

Adverse effects on development

Unfortunately we cannot alter the CLH report after public consultation to include additional information such as historical control data. However, we will consider your comment for future CLH proposals. As we argued in the discussion of the CLH report, the DS is of the opinion that the observed effects do not warrant classification.

With regard to the range finding study showing cleft palate and hydrocephalus at 8 mg/kg bw in rabbits, this information was indeed only added to the text and not to the respective table. However, the malformations observed at 8 mg/kg bw (one fetus had cleft palate and hydrocephaly, the other fetus had hydrocephaly) were considered to be incidental/ secondary to maternal toxicity. This was concluded since these affected fetuses were from dams which showed the greatest body weight losses during the dosing period and/or had tremors. According to the study report, the single incidence of cleft palate (fetal incidence 1.59%) in rabbits was within the laboratory historical control for this finding (max. fetal incidence 1.85%). However, any details on these historical control data (e.g. specific strain and time period) are not available to the DS. There are no historical control data for hydrocephaly from this laboratory. In the main developmental rabbit study these effects were not confirmed and there was no evidence of a teratogenic

effect in the rabbit, and no embryo and/or foetotoxic effects were observed at any dose level.The only effect observed was a slight increase in foetal weight (mid dose group) in the absence of a dose relation.
With respect to the historical control data on the foetal anomalies (lumbar vertebra, sternebral malformation, cervical rib variation) in the main rabbit developmental toxicity study: as indicated in a footnote to table 3.10.1.5-2 (i.e. page 80 of the CLH-report) only the highest incidence values were reported in the study report and no further data are available. Nevertheless, the comparison with the historical control data show that the incidences of foetal anomalies are below the upper limit of the HCD, thereby reducing the concern for classification.

With regard to the limited P-glycoprotein expression until 20 days after birth, the DS agrees that this could have been described in the CLH-report in the evaluation of the study on developmental neurotoxicity. Considering that the low P-glycoprotein levels and that the blood-brain barrier is not completely formed at birth in neonatal rats (in contrast to man), the effects observed in neonatal rats are not considered relevant for human hazard evaluation. Rat neonates are considered more susceptible to neurotoxicity induced by emamectin compared to human neonates.

As indicated by Germany, the closely related substance abamectin has a harmonised classification for reproductive toxicity as Repr. 2 (H361d). However, the conclusion of RAC for abamectin is based on the observation of a slightly higher incidence of clubbed forefoot in rabbit foetuses in the high dose group compared to the control (5 vs 1). The increased incidence was small but considered as evidence of developmental toxicity, although not being clear evidence. Further, RAC considered that these effects were observed in presence of only slight maternal toxicity, and concluded that this was unlikely to be related to the increased incidence in malformations. There were no such findings in either the main rabbit developmental toxicity study with emamectin. The malformations seen in the range finding developmental rabbit study are in the presence of clear maternal toxicity.

Please note that based on the same data package (except the range finding rabbit study) also the JMPR concluded that emamectin was not teratogenic in rats or rabbits. (http://www.fao.org/fileadmin/templates/agphome/documents/Pests_Pesticides/JMPR/Report11/Emamectin.pdf)

RAC's response

Adverse effects on sexual function and fertility

Overall, RAC considers the lower fertility/fecundity index at the top dose as an adverse effect in its own right (cf. CLP, Annex I, 3.7.1.3). The definitions provided by the DE CA is correct and has been taken into account by RAC. The low fertility/fecundity index is sufficient to raise concern regarding the capability of treated females with emamectin benzoate to become pregnant. The fecundity/fertility effects were more marked in the F1 adults that had previously been exposed to emamectin postnatally and had shown increased hypersensitivity to emamectin in the form of neurological effects. Although a clear dose response relationship was absent during the first and second mating producing F1 animals, a steep dose response relationship was observed at the highest dose in animals producing the F2 generation. However, these changes did not achieve statistical significance at any dose level. Furthermore, given the lack of a coherent dose-related response and the fact that the fertility index in all test substance administered groups was within the historical control range provided during public consultation, the relationship to emamectin treatment is considered equivocal.

RAC finds more relevant that, in the second cohabitation initiated to investigate the cause of the significant decreased in fecundity index, several non-pregnant F0 females mated with known fertile males failed to become pregnant. RAC stresses that pseudo-pregnancy may have occurred in some of the mated females but this hypothesis is difficult to ascertain in the absence of additional data e.g. hormone levels. Pseudopregnancy can occur in receptive (pro-oestrous/oestrous) females after sterile or inadequate mating. RAC stresses that there was no available data to indicate that emamectin benzoate altered progesterone/prolactin levels over time or affected other neuroendocrine mechanisms.

A pseudopregnant mating can indicate inadequacy of the male. Physical impairment of the top dose male rats may have been a key factor contributing to inadequate copulation, increased numbers of pseudopregnant females with a concomitant reduction in the fecundity index. Re-calculation of fecundity index by the manufacturer following exclusion of animals concluded to be pseudo-pregnant suggested that there is indeed no significant effect on fecundity at any dose level in the F1a litter generation. Pseudo-pregnancy was only assessed by individual body weight gains. RAC notes that increased body weight gains mimicking pregnancy have occurred in female rats on other studies with emamectin benzoate independently of mating, due to a possible interference with the energy metabolism.

RAC however notes also that in a developmental neurotoxicity study with rats, reproductive performance, as assessed by implantation rate, live litters, duration of gestation, post-implantation survival and pup viability at birth, was unaffected at all dose levels.

Overall, RAC agrees with the DS that neurotoxicity in males may have played a key role and that pseudo-pregnancy is a combination of neurotoxicity effects in males and females, which is already covered by the classification as STOT RE 1 (nervous system). In addition, substantial delays in BPS and VO in rats are considered not substance-related per se but secondary to the decreased pup post-natal body weight gain. Overall, RAC agrees with the DS and proposes no classification for adverse effects on sexual function and fertility.

Adverse effects on development

Overall, RAC agrees with the comment by the DE CA that the malformations (cleft palate and hydrocephalus) in rats and rabbits, the increased number of resorptions in rats and the alterations in ossification and variations in rats raise concern. Sections 3.7.2.4.1 and 3.7.2.4.2 of Annex I to the CLP Regulation acknowledge that the assessment of whether the development of offspring throughout gestation and during the early postnatal stages can be influenced by toxic effects in the mother is a complex issue because of uncertainties surrounding the relationship between maternal toxicity and developmental toxicity. Section 3.7.2.4.2 of Annex I to the CLP Regulation provides that d*evelopmental effects which occur even in the presence of maternal toxicity are considered to be evidence of developmental toxicity, unless it can be unequivocally demonstrated on a case-by-case basis that the developmental effects are secondary to maternal toxicity*". Section 3.7.2.4.3 of Annex I to the CLP Regulation provides that "*classification shall not automatically be discounted for substances that produce developmental toxicity only in association with maternal toxicity*".

RAC notes that late in the opinion process industry supplied further data in support of considering the lower relevance of hydrocephaly in the rabbit developmental studies. This

data clarified the spontaneous incidence of hydrocephaly in this strain and colony of rabbit. The highest foetal/litter incidence of spontaneously occurring hydrocephaly recorded during 1984-1990 was 3 foetuses (2.7 %) from 2 litters (11.8 %). The findings in the rabbit dose-range finding study are compromised by considerable toxicity in the two affected females (9.1 mg/kg bw/day), and as the top dose was decreased in the full study (to 6.8 mg/kg bw/day) the findings (for cleft palate) could not be reproduced.

The fact that cleft palates were also reported for abamectin and ivermectin certainly increases the concern for human health. It is worth noting that classification of abamectin by RAC for development (cat. 2) was not based on the occurrence of cleft palate but rather on other treatment-related malformations at very low incidence (club fore-foot in rabbits). Other effects of lesser concern include resorptions in rats as well as delayed ossification and increased variations. The weight of evidence is considered borderline to support the classification of emamectin benzoate for developmental toxicity. At doses where no clear maternal neurotoxicity was seen, the only effect considered to be due to emamectin benzoate treatment was slight retardation in ossification. Overall, RAC acknowledges difficulties related to the developmental toxicity profile and uncertainties associated with the sporadic occurrence of rare malformations. Because the data failed to demonstrate a consistent, reproducible relationship to treatment between and across species and that there was maternal (neuro-)toxicity in at least one species, RAC concludes that there was insufficient evidence to classify emamectin benzoate for developmental toxicity.

Developmental neurotoxicity

RAC agrees with the comment from the DE CA that developmental neurotoxicity should be assessed separately, since the substance emamectin benzoate induces neurotoxicity during the post-natal development phase of the offspring. Overall, RAC considers that, in line with the RAC opinion for Abamectin (ISO) (RAC, 2010) and EFSA (2012), neurotoxicity of emamectin benzoate in CF-1 mice should be excluded and that effects in neonatal rats shall be considered with caution for classification purposes. RAC acknowledges that since the MoA is not fully established and no recent studies have been provided by the applicant, there are also uncertainties to be taken into account in the weight of evidence approach for the hazard assessment. With regard to the limited P-glycoprotein expression until 20 days after birth, considering that the low P-glycoprotein levels and that the blood-brain barrier is not completely formed at birth in neonatal rats (in contrast to man), RAC agrees that the effects observed in neonatal rats are equivocal and not considered fully relevant for human hazard evaluation. Although no clear increased sensitivity was observed in developmental toxicity studies in rats and rabbits, increased qualitative and/or quantitative sensitivity of rat pups was seen in the reproductive toxicity and in the developmental neurotoxicity studies. RAC considers that these effects should be covered under STOT RE 1 and are therefore not considered for classification under developmental toxicity.

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment

Date	Country	Organisation	Type of Organisation	Comment number
09.01.2019	France		MemberState	4
Comment received				
France agreed with the classification proposed for emamectine benzoate (H400 with acute M factor of 10,000 and H410 with chronic M factor of 1,000)				

Dossier Submitter's Response
Thank you for your support. Please refer to comment 5.
RAC's response
Noted. Please see RAC's response to Comment 5.

Date	Country	Organisation	Type of Organisation	Comment number
11.01.2019	United Kingdom		MemberState	5

Comment received

Emamectin benzoate CAS: 155569-91-8
We note that the most acutely sensitive test organism was Mysidopsis bahia. As a chronic study using this species is not available we feel the surrogate approach should be considered for chronic classification. This approach indicates Chronic 1, M-factor 10,000 for a non-rapidly degradable substance is appropriate.

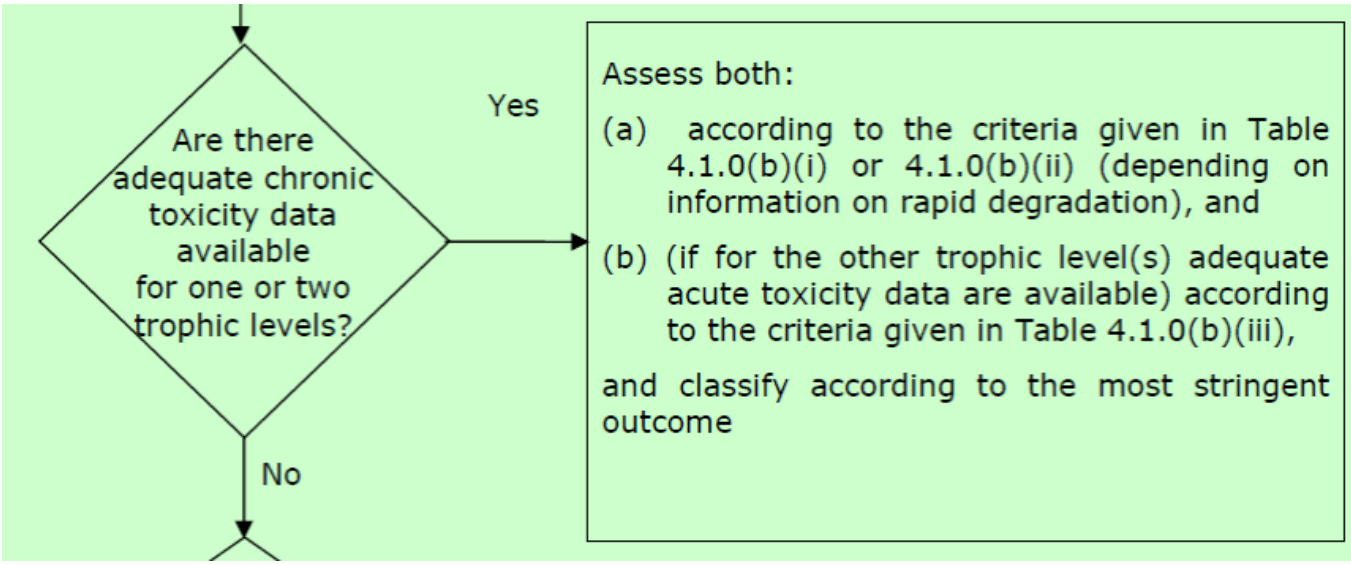
Dossier Submitter's Response

Thank you for your comment.

Initially we considered chronic toxicity data in defining the long-term hazard category for emamectin because adequate toxicity data exists for the three trophic levels. However, we believe the UK makes a good point. The acute M-factor is based on the most sensitive endpoint observed in Mysidopsis bahia which is 10x higher when compared to the chronic M-factor which is based on Daphna magna.

According to the CLP guidance (Section 4.1.3.3.1), "*chronic toxicity data would normally override acute data for long-term hazard classification. However, when assessing the adequacy there may be some cases (such as data poor substances) where the chronic data do not represent the species that is considered the most sensitive in available short-term tests. In such cases the classification should be based on the data (acute or chronic) that gives the most strict classification and M-factor.*"

According to figure 4.1.1 of the CLP regulation, the following applies for emamectin benzoate:



Ad a) One long-term guideline study in fish is available in which a NOEC of 0.012 mg/L was derived. The NOEC for crustacea was found to be 0.000088 mg/L. A NOEC of 0.00125 mg/kg sediment was determined for midge larvae (Chironomus riparius). However, this NOEC cannot be used for classification puroposes as the NOEC was determined for exposure via the sediment compartment and not the water compartment. For algae and aquatic plants the lowest NOEC was determined to be ≥ 0.0039 mg/L. Considering the lowest chronic value of 0.000088 mg/L derived from the three trophic levels, classification for chronic toxicity is warranted as Aquatic Chronic 1; H 410, with an M-factor of 1000 (This text is included in the current draft CLH report). Ad b) In the available studies performed with fish, the lowest LC50 value was found to be 0.174 mg/L and is thus lower than 1 mg/L. The toxicity to crustacea, oyster embryos, algae and aquatic plants was also below 1 mg/L. Based on the lowest EC50 of 0.000040 mg/L for mortality observed in Mysidopsis bahia (salt water), emamectin should be classified as Aquatic Acute 1; H 400, with an M-factor of 10000 We agree that the most stringent outcome is determined for b) as the substance is considered not rapidly degradable. Resulting classification of the substance: Acute (short-term) aquatic hazard: category Acute 1, M-factor: 10000. Long-term aquatic hazard: category Chronic 1, M-factor: 10000.
RAC's response
RAC agrees with the MSCA comment and with the DS amendment of the classification proposal.

Date	Country	Organisation	Type of Organisation	Comment number
08.01.2019	Germany		MemberState	6
Comment received				
We agree with the proposal of classification for environmental hazards as Aquatic acute 1 (H400), Aquatic chronic 1 (H410) and the acute/chronic M-factor of 10000/1000.				
Dossier Submitter's Response				
Thank you for your support. Please refer to comment 5, based on the comment of the UK a chronic M-factor of 10000 is considered more relevant.				
RAC's response				
Noted.				

PUBLIC ATTACHMENTS

1. Emamectin benzoate RAC Public Comments Jan 19.zip [Please refer to comment No. 2]